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Occurrence and distribution of *Salmonella* Dublin in Tyrolean dairy farms with alpine pasture grazing

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Summary

Salmonella (*S.*) Dublin is an important pathogen in dairy farms and is regularly detected in Austria. We report an investigation of the occurrence and distribution of *S.* Dublin in Tyrolean dairy farms, with a focus on alpine pastures, where cattle from various farms are herded together, and on the potential role of surface water as a source of infection and mode of transmission.

We examined twelve dairy farms (440 cattle) with known high positive bulk milk antibody results (percent positivity PP ≥ 100 %) for *S.* Dublin in Tyrol at individual and herd levels. We also examined six alpine pastures, where cattle of these twelve farms were herded, before, in the middle and at the end of the pasture season.

At the herd level, bulk milk antibody testing (80.0 % positive) seems to be more suitable than boot swab testing (75.0 % positive) to survey the infection status. The seroprevalence in calves was 5.4 %, in youngstock animals 4.1 % and in dairy cows 24.3 %. In contrast, 50.0 % of dairy cows exhibited antibodies in individual milk samples. Bacteriologically, faecal cultures were positive for 35.0 % of dairy cows, 13.3 % of which were chronic shedders with 60.0 % exhibiting antibodies in serum and milk. Boot swabs and bulk milk tested positive on all pastures. Of the 47 water samples taken, three (6.4 %) tested positive for *S.* Dublin on two different pastures.

The results show that bulk milk antibody testing is a suitable method for screening at herd level. Due to the

Zusammenfassung

Vorkommen und Verbreitung von *Salmonella* Dublin in Tiroler Milchviehbetrieben mit Almbeweidung

Salmonella enterica subspezies *enterica serovar* Dublin (*S.* Dublin) ist ein Verursacher von Salmonelleninfektionen in Milchviehbetrieben und wird in Österreich regelmäßig nachgewiesen. Ziel dieser Studie war es, das Vorkommen und die Verbreitung von *S.* Dublin in Tiroler Milchviehbetrieben zu untersuchen. Ein weiterer Schwerpunkt dieser Studie lag auf der Untersuchung von Almen, um die Rolle von Oberflächenwasser als mögliche Übertragungsquelle zu ermitteln.

Im Zuge dieser Studie wurden zwölf Milchviehbetriebe in Tirol (440 Rinder) mit bekannt hoch positiven Tankmilchergebnissen (prozentuale Positivität PP ≥ 100 %) für *S.* Dublin auf Einzeltierebene (Serum, Milch und Kot) und Herdenebene (Tankmilch, Stiefeltupfer, Gülle, Wischschwämmchen und Futter) untersucht. Zusätzlich wurden sechs Almen, auf denen Rinder dieser zwölf Betriebe gehalten wurden, vor, in der Mitte und am Ende der Almsaison auf Herdenebene untersucht (Tankmilch, Stiefeltupfer, Wischschwämmchen und Oberflächenwasser).

Die Ergebnisse zeigen, dass auf Herdenebene Tankmilchuntersuchungen (80,0 % positiv) empfindlicher als Stiefeltupfer (75,0 % positiv) zu sein scheinen, um

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intermittent excretion of the pathogen, repeated bacteriological examinations should be carried out at the individual animal level in affected herds to identify and eliminate latent carriers. The results also confirm that alpine pasturing of dairy cows may be a source of infection due to the mixing of cattle from different farms and to the surface water used for drinking. Good hygienic practices and vaccination against *S.* Dublin should be considered to prevent spreading of salmonellosis in cattle.

Abbreviations: AGES = Austrian Agency for Health and Food Safety; BVD = Bovine Viral Diarrhea; IVET = Institute of Veterinary Disease Control; PP = percent positivity; T-TGD = Animal Health Service Tyrol

Abkürzungen: AGES = Österreichische Agentur für Gesundheit und Ernährungssicherheit; BVD = Bovine Virusdiarrhoe; IVET = Institut für veterinärmedizinische Untersuchungen; PP = prozentuale Positivität; T-TGD = Tiroler Tiergesundheitsdienst

den Infektionsstatus der Herde zu überwachen. Die Seroprävalenz bei Kälbern betrug 5,4 %, beim Jungvieh 4,1 % und bei Milchkühen 24,3 %. Im Gegensatz dazu hatten 50,0 % der Milchkühe Antikörper in der Einzelmilchprobe. Bakteriologisch positiv mittels Kotkultur waren 35,0 % der Milchkühe, 13,3 % davon waren chronische Ausscheider und von diesen hatten 60,0 % Antikörper in Serum und Milch. Auf den Almen wurden alle Stiefeltupferproben und Tankmilchproben positiv getestet. Von insgesamt 47 untersuchten Wasserproben wurden drei Proben (6,4 %) auf zwei verschiedenen Almen positiv für *S.* Dublin getestet.

Die Ergebnisse zeigen, dass die Tankmilchuntersuchung für die Untersuchung auf Herdenebene ein geeignetes Screeningverfahren ist. Aufgrund der intermittierenden Ausscheidung des Erregers sollten in betroffenen Herden wiederholte bakteriologische Untersuchungen auf Einzeltierebene durchgeführt werden, um auch latente Ausscheider zu identifizieren und auszuscheiden. Die Alpung der Rinder stellt eine Übertragungsquelle dar, da Tiere aus unterschiedlichen Herkunftsbetrieben aufgetrieben werden und kontaminiertes Oberflächenwasser als Tränkwasser benutzt wird. Eine gute hygienische Praxis und eine Impfung gegen *S.* Dublin sollten angestrebt werden.

Introduction

Salmonella enterica subsp. *enterica* serovar Dublin (*S.* Dublin) infection is a serious threat to dairy herds, causing both economic losses and public health concerns (Henderson & Mason 2017). The cattle-adapted serovar can cause enteric, septicaemic and reproductive diseases associated with abortions (Holschbach & Peek 2018). There has been an increased reported prevalence in several countries in recent years (Velasquez-Munoz et al. 2023). Animals infected with *S.* Dublin periodically shed bacteria in their faeces or milk and this may lead to a high intra-herd prevalence in affected herds (Nielsen et al. 2004). Persistently infected asymptomatic carriers make monitoring difficult (Holschbach & Peek 2018) and these latently infected animals are responsible for the spread within and between herds (Velasquez-Munoz et al. 2023). Control and eradication of the latent carriers is essential (Velasquez-Munoz et al. 2023).

The presence of *S.* Dublin infections in dairy herds may lead to considerable economic losses stemming from reduced milk yield, culled animals, treatment costs, abortions and reduced income from selling animals (Nielsen et al. 2013). *S.* Dublin is also a notable zoonotic pathogen and may lead to severe disease in humans (Harvey et al. 2017; Velasquez-Munoz et al. 2023). Infection can cause a range of illnesses, particularly through the consumption of contaminated dairy products or direct contact with infected animals (Henderson & Mason 2017). In Austria, the Austrian Agency for Health and Food Safety (AGES) Institute Graz (National Reference Center for Salmonellosis) detected 34 infections of humans with *S.* Dublin from 1993 to September 2023. The most affected federal state was Tyrol with 15 cases (unpublished data).

The epidemiology and dynamics of *S.* Dublin infections are complex and depend on factors such as herd size, husbandry and feeding management (Vaessen et al. 1998; Holschbach & Peek 2018). The host-adapted serovar in cattle seems to be endemic in some regions of Tyrol (Glawischnig et al. 2017), specifically in the districts of Kufstein and Kitzbühel. The traditional form of husbandry there includes alpine pastures in summer, where cattle from various farms are kept together, which may increase the risk of *S.* Dublin infection (Allerberger et al. 2003). Across Austria, there are about 8,706 mountain pastures with livestock farming, of which 2,151 pastures are in Tyrol. About 33,639 dairy cows are herded on these pastures during the summer months (Federal Institute of Agriculture Economics, Rural and Mountain Research, Austria).

Attention must also be paid to surface water as a possible transmission route (Vaessen et al. 1998), especially manure contaminated water, which can be a source of infection (Glawischnig et al. 2017). However, good hygiene management, especially of the water and feed troughs, of alpine pastures is often difficult because of limited infrastructure.

S. Dublin is regularly detected in Austrian dairy cattle herds (Allerberger et al. 2003; Glawischnig et al. 2017; Sodoma et al. 2019) and bulk milk seroprevalence is

14.8 % for Tyrol (Hofer et al. 2024). Individual cattle testing has shown a seroprevalence of 18.9 % and 11.3 % of the cattle were *S.* Dublin shedders (Hofer et al. 2024). These reports represent the only source of reliable data on the distribution and significance of the infection in Austrian dairy herds.

We have investigated the occurrence and distribution of *S.* Dublin in Tyrolean dairy farms, with a focus on alpine pastures and the potential role of surface water as a source of infection and mode of transmission. The summer grazing of animals may play a major part in the transmission of the disease, as it involves the mixing of animals from different farms on a single mountain pasture, with the animals also sharing drinking water sourced from surface water. We studied, if alpine pasturing plays a major role in the transmission of *S.* Dublin in dairy cattle herds in Tyrol and this should be considered in measures to control the infection.

Materials and methods

Study population, selection of herds and alpine pastures

The study was part of a project conducted by the Austrian Agency for Health and Food Safety (AGES), the Clinical Center for Ruminant and Camelid Medicine of the University of Veterinary Medicine Vienna (Vetmeduni) and the Animal Health Service Tyrol (T-TGD). In 2019, 2020 and 2022, bulk milk screenings for the detection of *S.* Dublin antibodies in dairy herds in Tyrol were performed by ELISA (Hofer et al. 2024). The surveys were carried out using the samples collected as part of the annual national Bovine Virus Diarrhea (BVD) monitoring programme. In 2019, 584 of 3,854 dairy farms (15.3 %), in 2020, 512 of 3,821 dairy farms (13.4 %) and in 2022, 579 of 3,670 dairy farms (15.8 %) tested positive for *S.* Dublin antibodies (percent positivity PP ≥ 35 %). Combining the results of the three years, 6.2 % (211/3,386) of the participating dairy farms were found positive in all three bulk milk screenings (Hofer et al. 2024). Farms with highly positive bulk milk antibody test results (percent positivity PP ≥ 100 %) for *S.* Dublin were invited to participate in faecal testing of all animals over 24 months of age. The value PP ≥ 100 % for highly positive test results was determined by an internal classification system as suggested by Hofer et al. (2024). Of the 213 (5.5 %) highly positive farms in 2019 and the 190 (5.0 %) in 2020, 63 dairy farms participated in this voluntary testing programme. Two additional farms with a negative bulk milk result were included due to clinical symptoms suggesting salmonellosis (Hofer et al. 2024). The individual faecal samples were taken by the local farm veterinarians from fall 2021 to spring 2022. Animals with a positive culture result for *S.* Dublin were resampled after approximately twelve weeks. Individuals with two positive results were rated as permanent shedders and the T-TGD recommended their culling.

We selected twelve farms to participate based on highly positive bulk milk antibody test results (PP ≥ 100 %) in 2019 and 2020 (12/12 farms) and the willingness of the farmer. All farms enrolled in the study were visited and sampled once during the period from January 2022 to March 2022 (Fig. 1).

The study was approved by the Ethics and Animal Welfare Committee of the University of Veterinary Medicine, Vienna, in accordance with the University's guidelines for Good Scientific Practice and authorized by the Austrian Federal Ministry of Education, Science and Research (ref BMBWF GZ2021-0.909.888), in accordance with current legislation.

A total of 440 cattle were enrolled and sampled. The herd size varied between twelve and 82 cattle with an average of 37 animals. Due to the lack of faecal culture test results, 16 animals from six different farms were excluded from the dataset. The individual animals were divided into three age categories: calves (aged

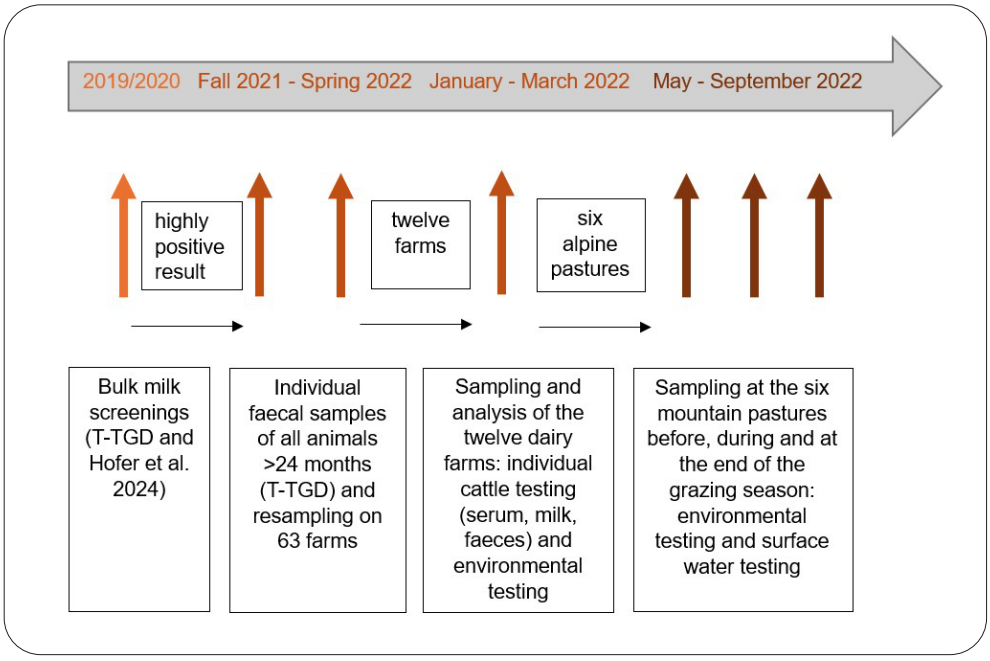


Fig. 1: Timetable of sampling and overview of the sampling material / Zeitplan der Probenahme und Überblick des Probenmaterials

0-6 months), youngstock (older than six months until first calving) and dairy cows (cattle from the time of first calving and first lactation) (Tab. 1).

The majority of the cattle were Simmental with 79.5 % (350), with the remainder comprised of 6.8 % (30) Simmental crossed with Red Friesian or Holstein Friesian, 3.6 % (16) Tyrolean Grey, 3.0 % (13) Simmental crossed with white-blue Belgian, 1.6 % (7) Brown Swiss, 1.4 % (6) Holstein and Red Friesian, 1.1 % (5) Pinzgauer and 3.0 % (13) crossbreeds of these breeds with beef cattle such as white-blue Belgian, Wagyu, Angus and Limousin (Tab. 1).

The twelve dairy farms included eight farms with tethered stalls and four free stall farms. One third of the farms fed hay and silage and two thirds fed solely hay as forage. All of them used alpine pasture in summer for their cattle, of which nine farms kept their animals on community alpine pastures with contact to livestock from other farms and three utilized a closed system with animals from one farm only. Six alpine pastures accommodating animals from the twelve participating farms that had been tested for *S.* Dublin were included. Three of them were communal pastures, with cattle from a minimum of two and a maximum of eight herds. The other three alpine pastures were grazed by individuals from one farm only. Four alpine pastures and premises were sampled for *S.* Dublin before the start (May 2022), in the middle (July 2022) and at the end of the alpine pasture season (August 2022 and September 2022). For logistical reasons, the remaining two alpine pastures were only sampled once, in the middle (July 2022 and August 2022) of the alpine season (Fig. 1).

Tab. 1: Herd size and age distribution of the twelve dairy farms enrolled in the study / Herdengröße und Altersverteilung der Rinder der zwölf Milchviehbetriebe, die an der Studie teilgenommen haben

dairy farm no.	no. of calves[1]	no. of young-stock[2]	no. of dairy cows[3]	stock size	Simmental cattle (%)
1	16	22	25	63	82.5 (52)
2	10	15	18	43	90.7 (39)
3	2	4	9	15	100.0 (15)
4	9	9	18	36	69.4 (25)
5	5	8	13	26	30.8 (8)
6	4	7	16	27	100.0 (27)
7	10	11	11	32	100.0 (32)
8	9	10	12	31	45.2 (14)
9	14	24	44	82	87.8 (72)
10	3	0	9	12	75.0 (9)
11	6	3	27	36	58.3 (21)
12	5	8	24	37	97.3 (36)
Total	**93**	**121**	**226**	**440**	**79.5 (350)**

[1]calves < six months of age; [2]cattle older than six months until first calving; [3]cattle after first calving

Herd-level and environmental sampling

A questionnaire, to collect management data and information on the health status of the herds, was conducted and bulk milk, boot swabs and slurry samples were collected from the participating herds. For bulk milk samples, 8 ml of milk was collected and stored in sterile tubes (ProClin™, Kabe Labortechnik GmbH; Germany) from the bulk tank at the end of milking. Boot swab (Stérisox®, Sodibox; France) samples were collected by walking through the barns of the dairy cows and the youngstock areas as described (Eisenberg et al. 2013). Where feasible, slurry samples were collected from the slurry pit by immersing 100 ml sterile, sealable plastic universal cups (Universalbecher PP/PE 120 ml Deckel grün, Semadeni AG; Austria). Sponge-sticks (Surface™ Sponge-Stick, Romer Labs Division Holding GmbH; Austria) were used to collect samples from visibly dirty areas in the calf pens by swiping over surfaces. Feed samples (at least 300 g) were collected from the feeding area of the cows, the feeding alley or the feed trough and filled into sterile universal plastic bags. If present, faecal samples from the environment from other animals living on the farm were also collected in sterile sealable plastic universal cups (Universalbecher PP/PE 120 ml Deckel grün, Semadeni AG; Austria).

Individual animal sampling

For the testing of individual animals, faecal samples were collected for microbiological testing and milk and blood (serum) samples for serological analysis. Faecal samples of all animals ≤ 24 months of age were collected rectally, according to general clinical examination standards (Baumgartner & Wittek 2017), and were transferred into sterile sealable plastic universal cups (Universalbecher PP/PE 120 ml Deckel grün, Semadeni AG; Austria). One milk sample per lactating cow, combining milk of all four quarters (8 ml), was taken aseptically during the evening milking process and filled into sterile milk tubes (ProClin™, Kabe Labortechnik GmbH; Germany) as described (Baumgartner & Wittek 2017). Blood samples were collected from the vena coccygea media or vena jugularis via serum tubes (Vacuette®, Greiner Bio-One International GmbH; Austria) using a 20 Gx1 needle (Vacuette®), according to general clinical examination standards (Baumgartner & Wittek 2017).

Alpine pasture and water testing

During the alpine pasture visits, sponge-sticks (Surface Sponge-Stick, Romer Labs Division Holding GmbH; Austria) were collected from visibly contaminated areas from the feeding barn and the manure trail. Boot swabs (Stérisox®, Sodibox; France) were collected by walking through the manure channels of the barns as described (Eisenberg et al. 2013). Bulk milk samples were collected in 8 ml sterile milk tubes (ProClin™, Kabe Labortechnik GmbH; Germany). As there were no cattle on the pastures during the first sampling, neither boot swabs nor bulk milk tests were sampled. Surface water samples from two to five different water points were collected into sterile 500 ml plastic bottles (PET 500 ml steril, Semadeni AG; Austria). Depending on which water points were available, the surface water samples were taken from troughs, wells, running streams or small ponds. The samples were collected via direct extraction of the water into the plastic bottles or using the scooping method with a rectal glove, whereby an immersion depth of approximately 40 cm was maintained. Where possible, the same water points were sampled and analysed during all three visits.

Laboratory analysis

Detection of antibodies in bulk milk, individual milk and blood samples by ELISA

The analysis for antibodies against *S.* Dublin was performed at the AGES Institute of Veterinary Disease Control (IVET), Linz. Blood and milk samples (bulk milk and individual milk samples) were tested using a commercial *S.* Dublin ELISA kit (PrioCheck *Salmonella* Antibody ELISA Dublin, Thermo Scientific; USA) and photometric analysis was performed using the Sunrise™ absorbance reader and Magellan™ software (Tecan Trading AG; Switzerland). According to the manufacturer's instructions, test results were interpreted as positive for *S.* Dublin if the PP was ≥ 35 % and, according to an internal classification system as highly positive if the PP was ≥ 100 % as suggested by Hofer et al. (2024).

Bacteriological culture of *Salmonella* in faecal, boot swabs and environmental samples

Faecal and environmental samples were examined microbiologically at the AGES IVET, Linz for the presence of *Salmonella* spp., according to the ISO 6579-1:2017/Amd 1:2020 standard. Pre-enrichment faecal and environmental samples were mixed with buffered peptone water (BPW, VWR International, LLC; USA) at a dilution of 1:10 and boot swabs were mixed with 250 ml BPW and then incubated at 37°C for 18 ± 2 hours. Three drops of the test material were added to modified semi-solid Rappaport Vassiliadis agar plates (MSRV-agar, VWR International, LLC; USA) and incubated at 41.5°C ± 1°C for 24—48 hours. Bacterial growth from migration zones on MSRV were inoculated onto Rambach, MacConkey and Xylose Lysine Deoxycholate (XLD) agar plates (VWR International, LLC; USA). All culture media were prepared at AGES IVET, Linz. Colonies with a characteristic appearance were analysed by matrix-assisted laser desorption-ionization (MALDI-TOF) using the direct transfer (DT) method (Bruker Corporation; USA). For more accurate identification of *Salmonella* spp., a rapid slide agglutination test using Anti-*Salmonella* Omnivalent (Sifin Diagnostics GmbH; Germany) and Agglutinating Sera, *Salmonella* D (Oxoid™; UK) was performed, according to the Kauffmann-White Le Minor classification serotyping with O and H antigens. Confirmation of positive *Salmonella* spp. isolates was conducted at the AGES Institute for Microbiology and Hygiene, Graz, the Austrian National Reference Laboratory for the Analysis and Testing of Zoonoses (*Salmonella*) (Hofer et al. 2024).

Bacteriological culture of *Salmonella* in feed samples

Feed samples were examined at the AGES Institute for Animal Nutrition and Feed, Linz. The samples were diluted 1:10 in BPW (Sigma Aldrich; USA) and incubated at 37°C for 16—20 hours. MSRV-agar plates (Biokar Diagnostics, Solabia Group; France) were inoculated as previously mentioned and incubated at 41.5°C for 21—27 hours. Bacterial growth from migration zones on MSRV-agar were inoculated onto XLD- (Oxoid™; UK) and Brilliant-green phenol-red lactose sucrose (BPLS)-agars (Millipore® Merck; Germany). The sample matrices were analyzed in two different departments of the AGES, Linz, as the Institute of Animal Nutrition and Feed is the accredited testing laboratory responsible for feed samples. *Salmonella* was detected in accordance with ISO 6579-1:2017/Amd 1:2020, which allows the use of different selective nutrient media. As a result, the selective culture media differs depending on the sample matrix, as the laboratory-specific standard operating procedures vary. Further identification, serotyping and confirmation of positive *Salmonella* spp. isolates were conducted as described (Hofer et al. 2024).

Bacteriological culture of *Salmonella* in water samples

Water samples were processed microbiologically at AGES IVET, Linz, for the presence of *Salmonella* spp., according to the ÖNORM EN ISO 19250 standard. 250 ml samples were diluted 1:2 with double concentrated BPW (VWR International, LLC; USA) and incubated at 37 °C for 18 ± 2 hours. After incubation, 0.1 ml of the pre-enrichment was added to a 10 ml MKTTn-tube (Müller-Kauffmann-Tetrathionate-Novobiocin-Bouillon,

VWR International, LLC; USA) and incubated at 37°C for 18 ± 2 hours. MSRV-agar plates (VWR International, LLC; USA) were inoculated with a further 0.1 ml of the pre-enrichment and incubated at 41.5°C ± 1°C for 24—48 hours. Positive test results from MSRV-agar were subcultured using Rambach, MacConkey and Xylose Lysine Deoxycholate (XLD) agar plates (VWR International, LLC; USA) and incubated at 37 °C for 18 ± 2 hours. Further identification, serotyping and confirmation of positive *Salmonella* spp. isolates were conducted as described above.

Statistical analysis

Microsoft Excel 365 (Microsoft Cooperation, Redmond Washington, USA) was used for data management, descriptive analysis and statistical analysis. Within-herd prevalence of antibody-positive (serum and milk) and bacteriologically positive cattle was calculated for all age groups.

Results

Analysis of the questionnaire and outcomes at herd level

The questionnaire was completed together with the farmers on the day of sampling and all responses referred to the two years before sampling. In none of the twelve farms, cattle were vaccinated against *S.* Dublin and other diseases. Ten farms (83.3 %) had purchased animals from other farms within the previous two years. The farms had an average calving interval of 381.67 days, all twelve (100.0 %) fed the newborn calves with colostrum from their own mother within two hours of birth and the calves were reared with raw milk. Six (50.0 %) of the farms stated that they had culled animals from the farm due to various health issues, mainly milk fever, claw diseases and poor fertility. Almost all farms (11 [91.7 %]) stated that the diseases occurred in dairy cows, with 50.0 % (6) reporting mastitis, 25.0 % (3) diarrhoea, 58.3 % (7) abortions and 33.3 % (4) impaired fertility (no oestrous cycle, poor conception rate, ovarian cysts). Calf illnesses were reported on 33.3 % (4) of the farms, with 25.0 % (3) reporting calf diarrhoea and 16.7 % (2) pneumonia as the main problem. Raw milk was regularly consumed by humans on all twelve farms. In retrospect, none of the farmers were aware of any health problems suggestive of *Salmonella* infection among animals or people living on the farm.

Boot swabs and environmental testing

The boot swabs for the dairy cows tested positive for *S.* Dublin in 75.0 % (9/12) and those for the youngstock in 57.1 % (4/7) of the farms (Tab. 2).

Bulk milk samples were positive in 80.0 % (8/10) of the farms, whereby 50.0 % rated as highly positive (PP ≥ 100 %) (Tab. 2).

Of 28 sponge-sticks taken from the calf pens, two (7.7 %) tested positive for *S.* Dublin. Of the 13 faecal samples from other animals on the farm that were examined, including chickens (4), pigs (2), goats (2), sheep (2), horses (2) and red deer (1), one combined from two horses tested positive for *S.* Dublin. At the follow-up sampling and examination 50 days later, the results were negative. Four of the 21 (19.0 %) feedstuff samples were positive, with three testing positive for *S.* Dublin and one testing positive for *Salmonella enterica* subsp. *salamae* (*Salmonella salamae*). For various reasons (e.g. harsh winter weather conditions, very low manure levels), slurry samples could only be taken from four farms; three of four tested positive for *S.* Dublin (Tab. 3).

Tab. 2: Results of boot swabs and bulk milk tested for *Salmonella* Dublin in previously positive farms (n=12) / Untersuchung von Stiefeltupfern und Tankmilch auf *Salmonella* Dublin in bekannt positiven Betrieben (n=12)

dairy farm no.	boot swab dairy cows	boot swab youngstock	*Salmonella* Dublin bulk milk PP (%)[2]
1	positive	positive	-[3]
2	positive	-[1]	163.5
3	positive	positive	173.1
4	positive	negative	102.9
5	positive	negative	208.5
6	positive	positive	79.4
7	negative	-[1]	13.0
8	positive	negative	144.2
9	negative	positive	25.9
10	negative	-[1]	75.9
11	positive	-[1]	-[3]
12	positive	-[1]	76.4
Total positive %	**75.0 %**	**57.1 %**	**80.0 %**

[1] no separate youngstock or no youngstock kept on farm at sampling date; [2] *Salmonella* Dublin antibody ELISA percentage positive (PP) values ≥ 35 % rated as positive, according to the manufacturer's specifications, values ≥ 100 % rated as highly positive; [3] no sample available

Tab. 3: Results of the environmental samples tested for *Salmonella* Dublin in previously positive cattle farms (n=12) / Ergebnisse der Umweltproben auf *Salmonella* Dublin in bekannt positiven Rinderbetrieben (n=12)

dairy farm no.	sponge-sticks calf pens (positive/negative)	faecal samples of other animals (positive/negative)	feed (positive/negative)	slurry positive/negative)
1	0/2	0/1 (goats)	**1**/0	-[1]
2	0/3	0/3 (sheep, pigs, chicken)	0/2	-[1]
3	**2**/0	-[1]	0/1	-[1]
4	0/3	0/2 (pigs, red deer)	**1**[2]/1	1/0
5	0/2	**1**/2 (**horses**, goats, chicken)	0/1	-[1]
6	0/2	-[1]	0/1	-[1]
7	0/3	-[1]	0/1	0/1
8	0/2	-[1]	0/3	-[1]
9	0/1	-[1]	0/2	-[1]
10	0/1	0/2 (sheep, chicken)	0/1	-[1]
11	0/3	-[1]	**1**/4	**1**/0
12	0/4	0/2 (horses, chicken)	**1**/0	**1**/0
Total positive %	**(2/26) 7.7 %**	**(1/13) 7.7 %**	**(4/21) 19.0 %**	**(3/4) 75.0 %**

[1]no sample available, [2]*Salmonella salamae*

Results of single animal testing

Calves

Of the 93 calves tested, five (5.4 %) from four different farms were seropositive for antibodies against *S.* Dublin by serum ELISA using the manufacturer's cut-off. Faecal samples of two (2.2 %) calves, from two different farms, tested positive for *S.* Dublin. The two calves, one day and the other 49 days old at the time of sampling, were also seropositive.

Youngstock

Five animals of 121 (4.1 %) in this age group, from four different farms, tested seropositive for *S.* Dublin. There were no positive faecal cultures.

Dairy cows

A total of 226 dairy cows were examined in the study. Of these, 24.3 % (55/226) were seropositive for *S.* Dublin and 35.0 % (79/226) had *S.* Dublin in their faeces. When the cows were re-examined by bacteriological faecal culture after approximately twelve weeks, 30 of the 226 (13.3 %) were still shedding *S.* Dublin and were rated as chronic carriers. For individual milking samples, 50.0 % (103/206) of the cows tested positive for *S.* Dublin antibodies. No individual milk sample could be taken from 20 animals, as they were dry on the sampling date (Tab. 4).

Figure 2 shows the distribution of dairy cows that tested positive for *S.* Dublin on the twelve farms. At least one cow tested positive for *S.* Dublin antibodies in milk on all the farms. Seropositive cows and *S.* Dublin shedders were present on eleven of the farms and chronic shedders were detected on eight.

Comparing the faecal culture test results of the dairy cows in relation to antibody testing for *S.* Dublin showed that 24 (34.3 %) of the 70 dairy cows that tested positive by faecal culture also tested positive for *S.* Dublin antibodies in serum and milk. No antibodies were found in serum or milk in 26 (37.1 %) of the dairy cows. Seronegative results together with antibodies for *S.* Dublin in the milk were found in 20 (28.6 %) of the 70 dairy cows. Of the 136 cows that tested negative for *S.* Dublin by faecal culture, 23 (16.9 %) showed antibodies in serum and milk. Negative antibody results for serum and milk were given by 56.6 % (77) of the cows with a negative faecal culture result. Positive antibodies in the milk and seronegative results were found in 36 (26.5 %) of the 136 cows. None of the cows were seropositive but negative for *S.* Dublin antibodies in the milk (Tab. 5).

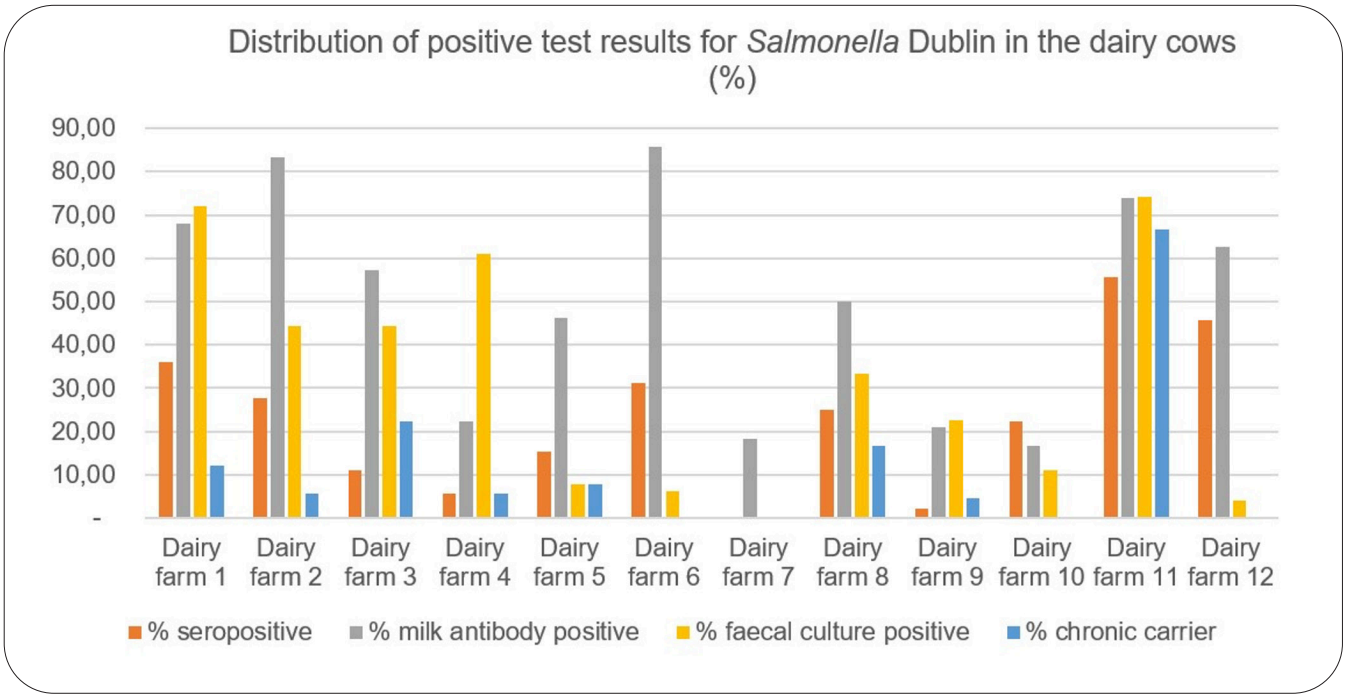
Of the 25 dairy cow chronic shedders, 60.0 % (15) exhibited antibodies in serum and milk. Five (20.0 %) of them were antibody negative. Five (20.0 %) cows had seronegative results but antibodies in the milk. As five chronic shedders were dry at the time of sampling, they were excluded from the calculation. Of the five, four tested seronegative and one tested seropositive for specific antibodies against *S.* Dublin.

Tab. 4: Results of serological (ELISA), microbiological faecal (n=226) and individual milk (ELISA) examinations (n=206) for *Salmonella* Dublin of the dairy cows on the twelve farms / Ergebnisse der serologischen Untersuchung (ELISA), der mikrobiologischen Kotuntersuchung (n=226) und der individuellen (ELISA) Milchuntersuchung (n=206) auf *Salmonella* Dublin der Milchkühe in den zwölf teilnehmenden Betrieben

dairy farm no.	no. of dairy cows[1] tested	% seropositive	% faecal culture positive	% chronic carrier[2]	no. of dairy cows[1] tested	% milk antibody positive
1	25	36.0 (9)	72.0 (18)	12.0 (3)	25	68.0 (17)
2	18	27.8 (5)	44.4 (8)	5.6 (1)	18	83.3 (15)
3	9	11.1 (1)	44.4 (4)	22.2 (2)	7	57.1 (4)
4	18	5.6 (1)	61.1 (11)	5.6 (1)	18	22.2 (4)
5	13	15.4 (2)	7.7 (1)	7.7 (1)	13	46.2 (6)
6	16	31.3 (5)	6.3 (1)	0.0 (0)	14	85.7 (12)
7	11	0.0 (0)	0.0 (0)	0.0 (0)	11	18.2 (2)
8	12	25.0 (3)	33.3 (4)	16.7 (2)	12	50.0 (6)
9	44	2.3 (1)	22.7 (10)	4.5 (2)	43	21.0 (9)
10	9	22.2 (2)	11.1 (1)	0.0 (0)	6	16.7 (1)
11	27	55.6 (15)	74.1 (20)	66.7 (18)	23	74.0 (17)
12	24	45.8 (11)	4.2 (1)	0.0 (0)	16	62.5 (10)
Total	**226**	**24.3 (55)**	**35.0 (79)**	**13.3 (30)**	**206**	**50.0 (103)**

[1]> first calving and first lactation; [2]tested positive twice at an interval of approximately twelve weeks

Fig. 2: Distribution of positive antibody and microbiological test results for *Salmonella* Dublin of dairy cows (n=226) on the twelve farms (%) / Verteilung der positiven Antikörper- und mikrobiologischen Testergebnisse für *Salmonella* Dublin der Milchkühe (n=226) auf den zwölf teilnehmenden Betrieben (%)



Tab. 5: Positive/negative[1] test results (%) for *Salmonella* Dublin for serum and milk in dairy cows compared to positive (n=70) and negative (n=136) results of bacteriological faecal examination / Antikörpertestergebnisse (%) für *Salmonella* Dublin aus Serum und Milch der Milchkühe im Vergleich zu positiven (n=70) und negativen (n=136) bakteriologischen Kotuntersuchungsergebnissen

dairy cows (n=206)	*Salmonella* Dublin positive antibodies serum and milk	*Salmonella* Dublin negative antibodies serum and milk	*Salmonella* Dublin positive antibodies milk and seronegative
***Salmonella* Dublin faecal culture positive (n=70)**	34.3 % (24)	37.1 % (26)	28.6 % (20)
***Salmonella* Dublin faecal culture negative (n=136)**	16.9 % (23)	56.6 % (77)	26.5 % (36)

[1] *Salmonella* Dublin antibody ELISA percentage positive (PP) values ≥ 35 % rated as positive

Findings from the alpine pastures

The environmental samples and the water samples from four alpine pastures before the start of the alpine grazing season were all negative for *S.* Dublin by bacterial culture (Tab. 6).

In the middle of the alpine pasture season, sponge-sticks from the feeding trough tested positive for *S.* Dublin on four of the six alpine pastures and all boot swabs tested positive for *S.* Dublin on pastures at that timepoint. Bulk milk also tested positive for *S.* Dublin on all alpine pastures in the middle of the alpine grazing season, whereby five of the pastures had a high positive ELISA result (PP ≥ 100 %). Of the 23 surface water samples collected during the middle of the grazing season, three tested positive for *Salmonella* spp. and one trough sample tested positive for *S.* Dublin. This trough, an old bathtub, was fed by a pipe from a small stream and was contaminated with faeces. The other two surface water samples, taken from two small ponds, tested positive for *S. enterica* subsp. *diarizonae*. Sampling at the end of the alpine pasture season revealed negative sponge-stick results for *S.* Dublin from the feeding trough on all pastures. Boot swab and bulk milk samples tested positive on all alpine premises (4/4) at the end of the grazing season. Three of the four alpine bulk milk samples had highly positive ELISA results. At the end of the alpine grazing season two of 16 (12.5 %) water samples tested positive for *S.* Dublin, both taken from troughs. One of the positive samples was taken from the same trough as in the middle of the alpine pasture season (alpine pasture no. 1), the second from a wooden trough fed by a pipe from a spring (alpine pasture no. 3). A water sample was taken from the same wooden trough on all three visits and the results on visits no. 1 and 2 were negative (Tab. 6).

Tab. 6: Results of the microbiological examination of sponge-sticks, boot swabs, water samples and bulk milk (ELISA) testing for *Salmonella* Dublin on alpine pastures before, during and at the end of the pasture season of known *Salmonella* positive dairy herds (n=6) / Ergebnisse der mikrobiologischen Untersuchung von Wischschwämmchen, Stiefeltupfern, Wasserproben und Tankmilchuntersuchungen (ELISA) auf den Almen, vor, während und am Ende der Weidesaison, von bekannt Salmonellen-positiven Milchviehherden (n=6)

alpine pasture no.	visit no.[1]	sponge-sticks from the feeding trough	boot swabs	water (positive/ negative)	bulk milk PP[2] %
1	1	negative	-[3]	0/2	-
	2	positive	positive	1/3	145.97
	3	negative	positive	1/3	149.32
2	1	negative	-[3]	0/4	-
	2	positive	positive	0/4	88.78
	3	negative	positive	0/4	70.87
3	1	negative	-[3]	0/5	-
	2	positive	positive	0/5	129.36
	3	negative	positive	1/4	119.16
4	1	negative	-[3]	0/2	-
	2	negative	positive	0/4	153.18
	3	negative	positive	0/3	158.35
5	2	negative	positive	0/2	128.82
6	2	positive	positive	2[4]/2	127.45
Total		**4/14 positive**	**10/10 positive**	**5/47**	**127.13**

[1] 1= before grazing season, 2= middle of grazing season, 3= end of grazing season; [2] percentage positive value; [3] no boot swabs and bulk milk samples taken on visit no. 1; [4] two water samples from this alpine pasture tested positive for *Salmonella enterica* subsp. *diarizonae*

Discussion

We have investigated the route of entry and the distribution of *S.* Dublin in smallholder alpine dairy farming structures. We have also investigated the role of the special form of husbandry associated with alpine pastures, where cattle from different farms are herded during the summer months, and especially the surface water in the alpine regions, as a source of infection. The study has its limitations, partly due to the small sizes of the herds but also because the twelve farms were located in only two Tyrolean districts, which limits the representativeness of the study.

At herd level, the results show that bulk milk antibody testing is more suitable than boot swab microbiological testing to survey the incidence of the infection in a herd, as 80.0 % of the participating farms tested positive in the bulk milk but 75.0 % in boot swabs only. Bacteriological faecal culture, by boot swabs, can be useful but has the limitation that latent carriers may remain undetected due to intermittent faecal shedding (Velasquez-Munoz et al. 2023; Hofer et al. 2024). Serological methods are thus more suitable for determining the herd level of *S.* Dublin infections (Veling et al. 2002). Nevertheless, the interpretation of *S.* Dublin-positive bulk milk ELISA findings can be complicated by vaccinations, as vaccinated cows have higher antibody titres in milk than unvaccinated cows (Smith et al. 2015).

The results show that attention should be paid to good management practices to prevent infection within the herd, as three feed samples and one faecal sample from horses also tested positive for *S.* Dublin. We can presume that the samples were contaminated with faeces from positive cattle due to poor hygiene on the farms, or from fomites such as rubber boots or equipment such as pitchforks but specific evidence is lacking.

Of the 93 calves examined, only two calves from different farms tested serologically and bacteriologically positive for *S.* Dublin. At the time of sampling, these calves were one and 49 days old. *S.* Dublin was also detected by sponge-sticks from an environmental sample in one barn of a positive calf. Three additional calves, 17, 102 and 109 days of age, on two different farms were seropositive for *S.* Dublin. Calves have been reported to be most susceptible to infection during the first 14 days of life up to three months in age (Henderson & Mason 2017), although this conclusion contrasts with our finding that more adult cattle were positive than calves. We should emphasize that all calves that tested positive in our study were clinically healthy and had no signs of illness. Calves can also be infected with *Salmonella* spp. by vertical transmission (Hanson et al. 2016), which helps to explain the positive finding in a one-day-old calf. The seroprevalence of the calves in our study was 4.3 %, which is much lower than reported seroprevalences of 19–37 % (Nielsen 2013) and 29.4 % (Veling et al. 2002) in this age group. One reason for this could be that the other studies sampled larger herds, specifically 38 to 154 (Nielsen 2013) lactating cows and 28 to 330 cattle (Veling et al. 2002). Other factors that can affect the interpretation of seropositivity in calves relate to the presence of maternally-derived antibodies and to the fact that antibody production in calves less than twelve weeks of age is poor (Henderson & Mason 2017). For the youngstock, a seroprevalence of 25–30 % in 14 endemically-infected herds has been reported (Nielsen 2013) but only five (4.1 %) of the 121 youngstock cattle in our study tested seropositive for antibodies against *S.* Dublin and none were positive for *S.* Dublin in the faeces. However, 57.1 % of the boot swab samples for this age group tested positive. The discrepancy might be explained by the lack of a physical boundary to separate the age groups due to the housing conditions on most of the participating farms, possibly leading to contamination of the area for youngstock by faeces from the dairy cows.

Heifers and cows are most susceptible to *S.* Dublin infections around the period of calving (Henderson & Mason 2017). This fits with the largely negative results of the youngstock of this study, as heifers become *S.* Dublin carriers around the time of calving. One finding of the present study was that more of the dairy cows tested positive for specific antibodies in the milk (50.0 %) than in the serum (24.3 %). This is comparable with the finding of 26–34 % seroprevalence in dairy cows (Nielsen 2013). There are no results for seroprevalence of antibodies in conjunction with individual milk samples from dairy cows in other comparable studies. The prevalences of antibodies in the twelve farms varied widely from 0.0 % to 55.6 % in serum and from 16.7 % to 85.7 % in individual milk samples. About a third (34.3 %) of the cows with a positive bacteriological faecal result also had antibodies both in serum and milk. Cows with a negative bacteriological faecal test result had fewer positive antibody outcomes in serum and milk at 16.9 %. These findings suggest an association, as *S.* Dublin shedders tested positive for antibodies about twice as often as non-shedders. This would contradict the finding that there is no association between seroprevalence and faecal excretion of *S.* Dublin (Nielsen 2013). Of the chronic shedders, 60.0 % had antibodies in milk and serum. No dairy cows were seropositive yet negative for antibodies in their milk. Serological methods should thus be preferred for diagnosis at the herd level. A possibility is to combine bulk milk ELISA and serum ELISA from a specific group from the herd (Veling et al. 2002). However, boot swabs are also suitable to detect acute infections at the herd level (Hofer et al. 2024) and repeated bacteriological faecal culture examinations of individual animals are required to identify latent carriers.

There is a seasonal trend of *S.* Dublin infections (Nielsen 2013). This finding cannot be compared with the data from our study, as sampling only took place during a specific time of year. This may limit the gener-

alizability and our ability to observe temporal variations in the data. The use of common alpine pastures during summer, when cattle from different farms are herded, may play a major part in the epidemiology of *S.* Dublin in the alpine Tyrolean region. In neighboring Bavaria, Germany, 54 *S.* Dublin outbreaks in animals were investigated and analysed in more detail between 2017 and 2021 and the results show that at least two origins of infection can be traced back to alpine pastures and that summer grazing plays a source of infection, as animals from different farms are herded together (Klose et al. 2022). Similar surveys have been conducted in Tyrol between 1990 and 2001, as 64 non-human *S.* Dublin strains from five Austrian provinces were linked to outbreaks on cattle mountain pastures in Salzburg and Tyrol (Allerberger et al. 2003)

We identified surface water, used for drinking, as a potentially important source of infection. Three water samples collected on alpine pastures tested positive for *S.* Dublin. We assume that the surface water was soiled by faeces, as the water tested at the inflow was negative. A previous outbreak of *S.* Dublin in cattle in an alpine pasture area in the district of Kufstein, Tyrol was linked to deaths of chamois (*Rupicapra rupicapra*) and infected red foxes (*Vulpes vulpes*) as reservoirs. It was assumed that abortion material contaminated with *S.* Dublin or water contaminated with faeces containing *S.* Dublin was the source of the infection (Glawischnig et al. 2017).

Our results are consistent with our idea that alpine pasture farming and its surface water represent potential sources of *S.* Dublin infections, making water safety and hygiene crucial in preventing outbreaks. Boot swabs were positive on all alpine pastures from the mid-grazing season and four of the 14 sponge-stick samples from the feeding troughs tested positive for *S.* Dublin. The results also emphasize the high priority of good management practices for hygiene and biosecurity to prevent bacterial spread within and between herds. Particular precautions are essential around feed and water troughs (Henderson & Mason 2017) to prevent faecal contamination. Ideally, only cattle that have tested negative for *S.* Dublin should be kept on alpine pastures, as co-grazing, which is common in Tyrol, increases the risk of infection (Allerberger et al. 2003). Recommended biosecurity measures include maintaining closed herds, separating equipment for feed and manure management, separating calving and sick cow boxes and purchasing only cattle that have tested negative. Vaccinations against *S.* Dublin may also be implemented as a control and prevention measure (Holschbach & Peek 2018).

Conclusion for practice

Salmonella Dublin is an important cause of *Salmonella* disease in Tyrolean dairy farms. The special form of husbandry, with alpine pastures in summer, has a major role in transmission and the spread of infection. Contaminated surface water on alpine pastures, which is used as a source of drinking water for cattle, has tested positive for *S.* Dublin. As *S.* Dublin may cause substantial economic losses and poses a possible threat to producers and consumers, further measures to prevent infection are needed, including renewed consideration of the use of vaccinations against *S.* Dublin to improve the immunity of herds.

Fazit für die Praxis:
Das Serovar *Salmonella* Dublin ist ein bedeutender Verursacher der Salmonellose in Tiroler Milchviehbetrieben. Der besonderen Art der Haltung mit der Alpung im Sommer kommt eine größere Bedeutung bei der Übertragung und Ausbreitung der Infektion zu. Auf den Weiden konnte *S.* Dublin in Oberflächenwässern, die zur Tränkung verwendet wurden, nachgewiesen werden. Angesichts der wirtschaftlichen Einbußen durch Infektionen und der potenziellen Gesundheitsgefährdung für Landwirte und Konsumenten sind vorbeugende Maßnahmen nötig, insbesondere sollten Impfungen gegen *S.* Dublin zur Stärkung der Immunität der Herden angedacht werden.

Conflict of interest
The authors declare no conflicts of interest.

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