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Field validation of an antibiotic-free hoof spray to effectively treat ovine footrot by eliminating virulent *Dichelobacter nodosus*

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ABSTRACT

Ovine footrot caused by *Dichelobacter nodosus* is a highly contagious hoof disease negatively impacting animal welfare and causing major economic losses to the sheep industry. Bactericidal footbaths have shown to be an efficient treatment option and will be used in the national footrot control program in Switzerland. However, the application of footbaths is laborious and economically not sound for small flock holders. We therefore tested in a field study the Intra Repiderma spray for its applicability and efficacy to treat ovine footrot. Ten independent flocks fulfilling defined parameters (e.g. clinical signs, positive for *D. nodosus*, flock size) could be identified and were included in the study. Farms were visited weekly to fortnightly and clinical scores and swabs for *D. nodosus* real-time (rt)PCR were taken. Treatment with the Intra Repiderma spray was started after initial claw trimming at the very first visit and was carried out three times within a week. Clearly visible clinical improvement was evident after one week of treatment. Virulent *D. nodosus* amounts on feet declined constantly during treatment which was continued until all sheep of a flock tested rtPCR-negative (1–10 weeks). Results indicate that a highly effective improvement of clinical signs and complete elimination of virulent *D. nodosus* can be achieved with the spray treatment. Therefore, it is a valuable alternative to cumbersome footbaths especially for small flocks. A sustainable control of footrot and its pathogen in a successfully treated flock can be maintained by strict biosecurity measures and continued treatment as far as necessary.

1. Introduction

Ovine footrot caused by the gram-negative anaerobic bacterium *Dichelobacter nodosus* is a highly contagious disease generating great economic loss and suffering of the animals due to painful lesions (Bennett et al., 2009; Nieuwhof and Bishop, 2005; Zanolari et al., 2021; Zingg et al., 2017). Clinical symptoms range from mild interdigital dermatitis to detachment of the claw horn starting in the interdigital gap resulting in lameness, reduced growth, reduced wool production and lower birth rate (Marshall et al., 1991; Stewart et al., 1984). The main infection route is through contact between animals, the environment including pastures and bedding, or other vectors, such as boots, trimming knife and even through hands by the trimming personnel (Locher et al., 2018; Muzafar et al., 2015; Muzafar et al., 2016). The development of the disease depends on the virulence of the infecting *D. nodosus* strain as well as on the environmental conditions and animal husbandry (Kennan et al., 2011). High animal densities and poor claw hygiene in combination with moist environment and temperatures $>$ 10 °C promote the transmission of the pathogen.

While benign strains of *D. nodosus* may cause interdigital dermatitis, infection with virulent strains normally results in severe footrot with the typical clinical symptoms (Kennan et al., 2011). The key virulence factor of virulent *D. nodosus* is the protease AprV2, while the homologous protease AprB2 in benign *D. nodosus* differs in one amino acid as a result of a 2 bp difference in the corresponding genes *aprV2* and *aprB2* (Kennan et al., 2010; Riffkin et al., 1995). This minimal genetic difference allows to detect and differentiate virulent and benign *D. nodosus* by a competitive real-time (rt)PCR which tremendously improved diagnosis and control of the disease (Stauble et al., 2014). An additional role of *Fusobacterium necrophorum* in ovine footrot is often claimed but this might be limited to being a second invader rather than a driver of the disease (Zanolari et al., 2021).

Like in many countries ovine footrot is endemic in Switzerland with a prevalence of *aprV2*-positive *D. nodosus* of 16.9% in the sheep population (Arduser et al., 2020). The aim of the Swiss footrot control program planned to be started in 2024 is to reduce this prevalence to 1% within 5

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years. To achieve this goal claw trimming and regular footbaths with appropriate disinfectants will be applied, which has been shown to be effective (Greber et al., 2016; Schmid et al., 2022). By claw trimming loose or overgrown horn is removed carefully with a hoof knife while at the same time claws can be inspected and lesions scored. Finally, freshly cleaned and trimmed hoofs allow the disinfectant to better penetrate the lesions (Greber et al., 2016). Alternatives to footbaths are vaccination or antibiotic treatment. However, vaccination will not be allowed during the control program and the commercially available vaccine does not provide a long-term immunity. Moreover, efficiency of the vaccine depends on the serotype of *D. nodosus* present (Green and George, 2008). Although the vaccine covers multiple serotypes the vaccination does not protect against all ten serotypes known for *D. nodosus*. Severely affected animals might be treated individually with systemic antibiotics. The application of systemic antibiotics reduces the inflammation in the feet and promotes a fast recovery (Strobel et al., 2014). For farmers, the application of local antibiotic spray is a simple and quick practice to treat affected sheep individually. The local treatment kills *D. nodosus* on the hoof surface and prevents further pathogen contamination of the environment. To date, no antibiotic resistance of *D. nodosus* is known. However, the use of antibiotics should generally be avoided, since the development of antibiotic resistance became a serious global health problem (O'Neill, 2016).

Footbaths envisaged for the Swiss footrot control program will be difficult to implement by all farmers especially those that only keep a few animals, which are rather common in Switzerland. At the same time such small flocks are often part of alpine farming during summer together with animals from various other sheep holders. Thus, they must be certified *D. nodosus* free as well which is an important part of the control program aiming to interfere with infection chains occurring at common grazing places. We therefore investigated an antibiotic-free spray-based protocol for individual treatment of sheep. Carefully selected flocks affected by footrot were included in the field-study and could be successfully treated resulting in complete clinical improvement and elimination of virulent *D. nodosus*.

2. Materials and methods

2.1. Selection of farms

To acquire suitable farms for the project an information letter explaining the study design and stating requirements for participation was drafted. Participation conditions for the study were: a maximum flock size of 25 animals, presence of animals with clinical signs of footrot, no treatment going on with another agent, strict implementation of the treatment protocol, and no animal transport nor purchase or integration of foreign sheep during the project. Different associations were asked to distribute the letter in forums and by email, like the Small Ruminant Advisory and Health Service (BGK), the Swiss Sheep Breeders Association, the Association of Swiss Professional Shepherds and various cantonal veterinary offices.

Interested animal owners contacted the veterinarian of the study (first author) and a questionnaire was sent to them with specific questions about the size of the herd, the number of affected animals at the time and the severity of lesions to make an initial selection of the farms.

2.2. Sampling of farms and treatment of animals

All farm visits were carried out by the same veterinarian from September 2022 to April 2023. On the first visit all sheep were individually examined. Each claw of the animal was cleaned from gross dirt, inspected and scored using the scoring system of the BGK ranging from 0 (healthy claw) to 5 (loss of the horn capsule) (Greber et al., 2018). If needed claws were then trimmed by the farmer and a Transwab® Amies Charcoal (MWE Medical Wire and Equipment, Corsham, UK) was used to take swabs from each animal for culture and rtPCR. Swabs were taken using a standardized protocol, where one clean quarter of the same swab was used for each foot to obtain a 4-feet sample (Locher et al., 2015). The swab samples were always processed as single animal samples. For every swab a new pair of gloves were used to avoid cross contamination of samples and transmission of the bacteria between sheep (Locher et al., 2018). In one farm no clinical signs were observed at the first visit, but the farm veterinarian had previously taken swab samples to test for footrot and confirmed an infection with virulent *D. nodosus* of the sheep flock. The status was first verified by rtPCR before treatment started one week later. In the other nine flocks the first treatment period was directly initiated during the first visit. The claws and interdigital space were washed with water and dried before being treated with the Intra Repiderma spray containing 6% chelated zinc and copper (Intracare, Veghel, the Netherlands). Its chelated elements, local application and strong adhesion is protective, environmental-friendly and allows a long lasting effect. Moreover, its micronized form allows optimal absorbtion into the skin where it promotes vasculation, cell growth and wound healing. Claws were spread, and the spray was applied 3 s from a distance of 15–20 cm. Treated animals, clearly identified by the green color of the spray, were then left for one hour on a clean solid floor. Two more treatments were done by the farmer himself on day 3 and day 7 post first treatment. Two to five days after the third treatment a follow-up farm visit was done, closing the first treatment cycle. All sheep feet were scored again, and claws were trimmed if needed by the farmer under surveillance of the veterinarian. A dry cotton swab in a tube with screw cap and without medium (101 ×16.5 mm; Sarstedt, Nümbrecht, Germany) was used to take a sample for rtPCR analysis. If clinical signs were still present in an animal, the treatment was directly repeated for the whole flock. Again, the 2nd and 3rd spray application was done by the farmer on day 3 and 7 followed by a farm visit of the veterinarian. These treatment cycles continued until all animals tested negative for virulent (*aprV2*-positive) *D. nodosus* in the rtPCR (Fig. 1).

One month after all sheep of a herd were tested negative for the *aprV2* gene, a final inspection and sampling was done by the veterinarian. All claws of the animals were again examined, and a dry cotton swap sample was taken for rtPCR like described before.

2.3. Culture and identification of D. nodosus

The Transwab® Amies Charcoal sampling done at the first farm visit allows for both, culture and subsequent rtPCR analysis (Zanolari et al., 2021). Culture was applied when an animal had a scoring of 3 or more. It was done according to Locher et al. (2018) by streaking the swab on one third of a 4% hoof agar (HA) plate, as soon as arriving at the laboratory. With a sterile toothpick, a grid pattern was made into the agar and the plate was incubated at 37 °C under anaerobic conditions. After 5 days, the primary culture plates were checked and colonies that looked like *D. nodosus* were subcultured on a new HA plate as described before. On the second HA plate suspicious colonies were subcultured on a brucellosis-blood-agar plate (BD Brucella Blood Agar with Hemin and Vitamin K1; Becton Dickinson GmbH, Heidelberg, Germany) and anaerobically incubated at 37 °C for a week. Colonies grown on brucellosis-blood-agar plate were then collected and identified by MALDI-TOF MS (Bruker Daltonik GmbH, Bremen, Germany). Primary HA plates that showed no suspicious colonies after growth for one week were discarded. Isolates were stored at − 70 °C.

2.4. DNA extraction and real-time PCR analysis

All samples were transported at room-temperature (RT) to the laboratory (Institute of Veterinary Bacteriology, University of Bern) and were immediately processed. The swabs were placed into a 1.5 mL screw cap microtube with 1 mL SV-lysis buffer (4 M guanidine thiocyanate, 0.01 M Tris- HCl, 1% beta-mercaptoethanol) for at least 1 min when the swab was squeezed and removed. After an incubation time of one hour at RT, DNA extraction was performed using the KingFisher Duo Prime

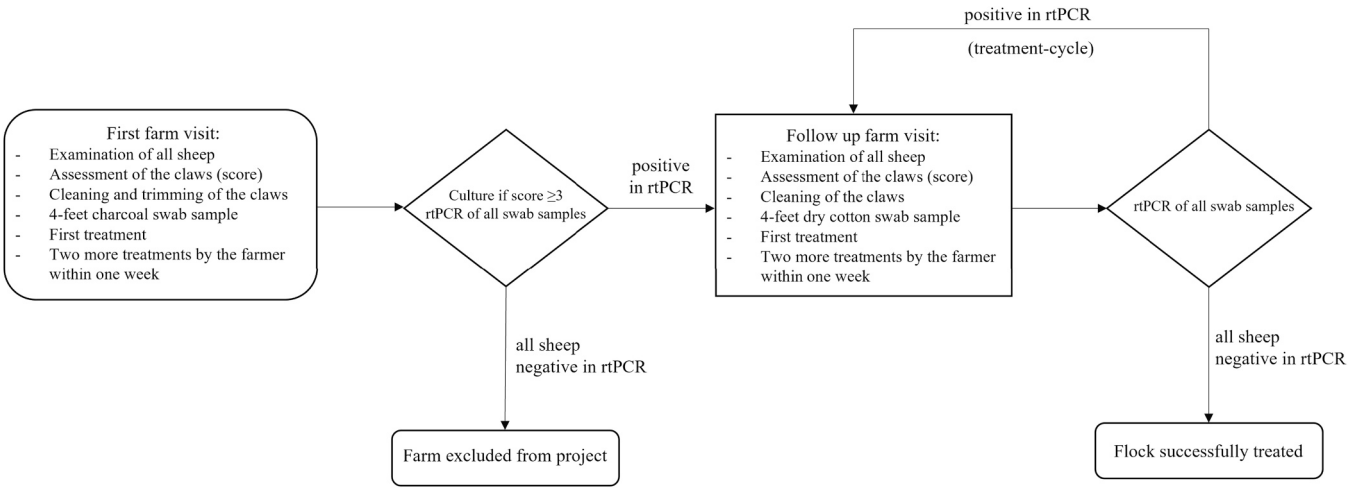

Fig. 1. Flow chart of the treatment process of farms initially selected for the project. The time from one week treatment to the other (in case the rtPCR was positive) is referred to as "treatment cycle".

(Thermo Fisher Scientific, Reinach, Switzerland). 500 µl of each sample was pipetted in a KingFisher 96 deep-well plate and VetMAX™ Xeno™ Internal Positive Control (IPC) DNA (20,000 copies; Thermo Fisher Scientific) was added to every extraction sample as a control (Kuhnert et al., 2019). The DNA was eluted using 80 µl of pyrogen-free water (Dr. Bichsel AG, Interlaken, Switzerland). All samples from a farm were accompanied by a negative extraction control containing 500 µl SV-lysis buffer.

Competitive rtPCR detecting and discriminating virulent (*aprV2*-positive) and benign (*aprB2*-positive) *D. nodosus* was done according to the protocol of Kuhnert et al. (2019). Every rtPCR assay included a negative (H_2O) and a positive (*aprV2/aprB2*) control as well as a negative extraction control sample, to ensure that no contamination occurred during neither the extraction nor the rtPCR. To check for correct rtPCR performance the Xeno LIZ Primer Probe Mix (Thermo Fisher Scientific) was added to the reaction mix to amplify the IPC DNA. All samples were analyzed in duplicate, and samples were defined positive if at least one of the duplicates showed a Ct-value < 40 in the *aprV2*-specific reaction. Results from rtPCR were promptly reported to the farmer after each farm visit by phone or email to immediately start another treatment cycle in case of any positive animal still present in the flock.

Detection of *Fusobacterium necrophorum* discriminating between the two subspecies *F. necrophorum* subsp. *necrophorum* and *F. necrophorum* subsp. *fundiliformis* was done by rtPCR according to Jensen et al. (2007).

2.5. Data analysis

R version 4.2.2 was used for the statistical analysis of the data and generation of plots. To determine the correlation between rtPCR Ct-values and the clinical score Spearman's rank correlation was calculated with the function cor.test(). Plots were generated using the function ggplot().

3. Results

3.1. Selection of farms

A total of 53 farmers were interested in participation in the project and were asked to fill out the questionnaire. Based on the feedback of the farmers, 32 farms did not fulfill the project criteria due to either the number of animals exceeding the maximum number for participation or, more frequently, the absence of footrot. Therefore, 21 farms were initially enrolled in the study. From these, 8 farms did not present any clinical symptoms of which 7 tested negative for virulent *D. nodosus* and were therefore excluded. Two farms were excluded from the project since they did not comply with the conditions of participation (non-compliance with treatment protocol and animal purchase). One farm decided to withdraw from the project after clinical improvement under treatment, however animals were still positive for virulent *D. nodosus*. On one farm an animal suddenly showed deterioration unrelated to the treatment (diarrhea and respiratory problems), causing the farmer to treat all sheep with antibiotics prescribed by the herd veterinarian and therefore the farm was excluded from the project. Finally, the project included 10 farms where the treatment could be fully implemented, samples be collected and statistically analyzed.

3.2. Clinical scores and rtPCR results during treatment

A total of 141 sheep from 10 farms were tested by rtPCR for virulent *D. nodosus* at a first farm visit (Supplementary data). After the first week of treatment an improvement of clinical symptoms (scores) was observed and Ct-values also increased, i.e. bacterial load decreased (Fig. 2 and 3). The Spearman correlation test showed a significant negative correlation between the scores and Ct-values before treatment start and after the first two treatment cycles ($p < 0.05$) (Table 1). The score distribution varied greatly from farm to farm. The higher the score, the longer it normally took for the lesions to heal during treatment (Fig. 2). In comparison, after one treatment cycle a strong reduction of pathogen presence was already evident (Fig. 3). Farms that also observed biosecurity measures, such as pasture management and regular mucking out of barns, turned more quickly negative, both clinically and in the rtPCR (farm B, C, D, E and F), than farms which only focused on treatment.

For example, on farm D many sheep showed severe clinical symptoms (score 3–5) on the first visit. The farmer implemented a good process of the treatment protocol for his farm. The sheep were brought from one side of the pasture into an anteroom of the barn. They were then treated one by one and left on the concrete floor on the other part of the barn for one hour allowing the sprayed agent to take effect. Afterwards, the animals left the barn through the front door to a new part of the pasture. The barn and the anteroom were then washed each time by the animal owner with a high- pressure cleaner. With this spatial separation, reinfection was prevented and the farm was negative for the virulent strain of *D. nodosus* after 3 treatment cycles. Similarly, farm E followed an ideal treatment process allowing the therapy being completed after three treatment cycles. The animals were in a shelter with solid ground. After each treatment cycle, the stable was mucked out and cleaned and freshly bedded.

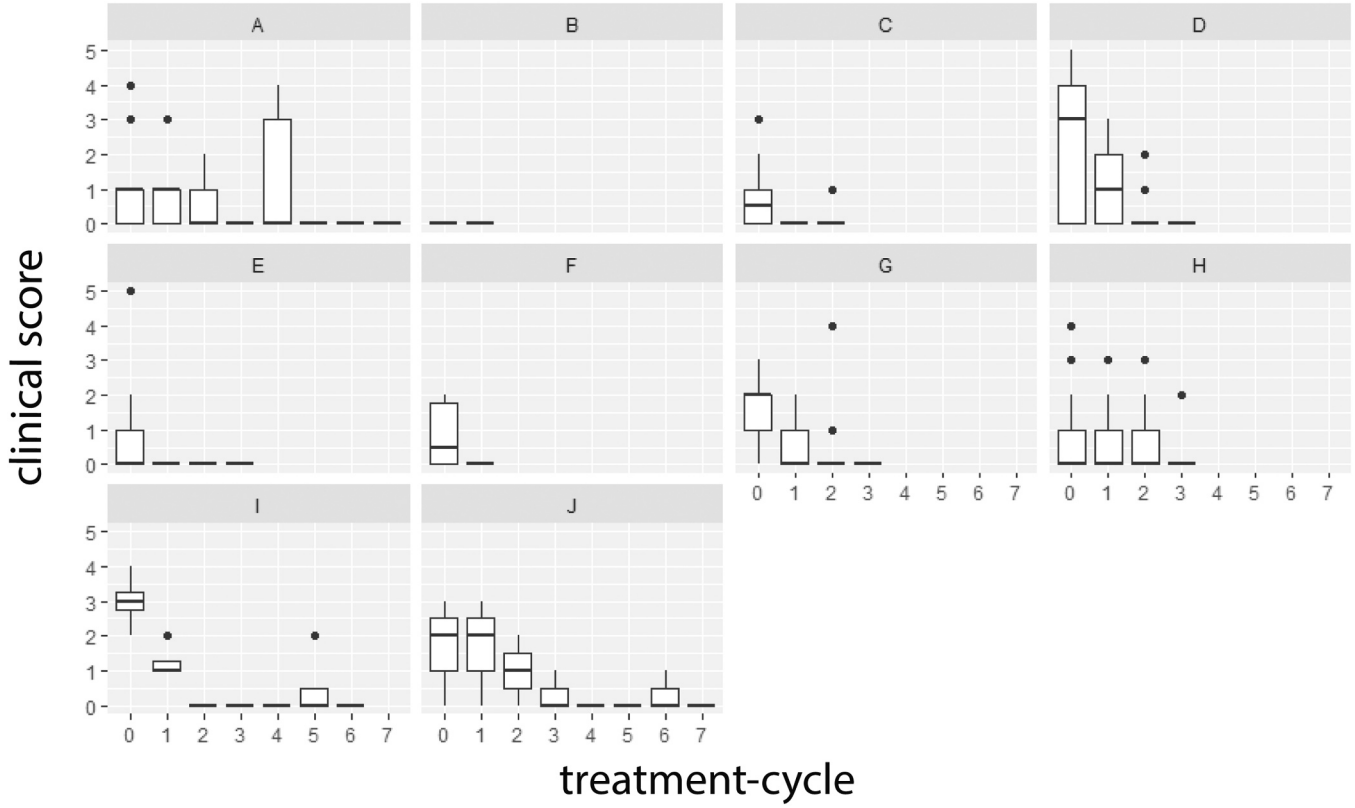

Fig. 2. Boxplots of clinical scores of Farms A – J over the treatment course. Each treatment-cycle included 3 spray treatments per week followed by rtPCR testing. Treatment-cycles were repeated until all animals of a farm tested rtPCR-negative. Treatment-cycle 0: time point before therapy started.

Table 1
Spearman correlation test between rtPCR and clinical scores over treatment cycle (TC).

TC	Correlation	p-value
0	-0.588	0
1	-0.316	0
2	-0.183	0.045
3	-0.113	0.29
4	-0.315	0.176
5	-0.313	0.179
6	-0.239	0.356

Overall, there was a strong improvement in both clinical symptoms (lower scores) and bacterial load (higher Ct-values) after the first two treatment cycles in all farms.

3.3. Treatment time until elimination of virulent D. nodosus

On all farms treatment and sampling continued until all sheep on the farm were negative in the rtPCR for virulent *D. nodosus*. The duration time until successful treatment (i.e. elimination of virulent *D. nodosus*) of a farm varied. The average treatment duration with three sprays per week was roughly 4 weeks ranging from 1 to 10 weeks (Fig. 2).

On farm B, none of the animals showed clinical signs and the farm tested free of *D. nodosus* after the first week of treatment (one treatment cycle).

On farm A, three out of 14 animals, had scores ≥ 3 and 5 animals had a score 1. The rtPCR results were positive in 12 out of 14 animals. After one week of treatment, only two animals were still rtPCR positive, with one animal being slaughtered due to other problems. The farmer repeated the treatment for another week, with the result that the two animals were still positive for the pathogen. After these two treatment cycles, the clinical signs had completely disappeared, and the animals were no longer lame. Another week of treatment was done, with the result that 11 of the 13 animals were rtPCR positive again. After consulting the owner, he mentioned that he had treated only the two positive animals instead of the entire flock. However, the remaining 11 animals that tested negative twice, were still in the same stable and on the same pasture as the animals that remained rtPCR positive. The lack of treatment and biosecurity measures led to reinfection of the animals. Animals that previously showed no clinical symptoms now showed symptoms with scores ≥ 3. After further 7 treatment cycles with the Intra Repiderma spray, clinical signs had disappeared and no more virulent *D. nodosus* could be detected. Farm I had a similar problem. The farmer reduced the treatment from 3 times a week to once a week. After one week the Ct-value decreased again and after two weeks one animal showed a score 2 again. As soon as the farmer treated again 3 times a week, the animals were negative for the footrot pathogen after 2 more treatment cycles.

The importance of regular removal of manure was demonstrated on farm J. The farmer kept three uncastrated rams in a barn over the winter until mid-spring. Due to the lack of space, it was not possible for him to muck out the barn regularly during this time. Thus, the bedding was accumulated, dirty and wet. The farmer always put a new layer of straw on top every week, but straw is not as absorbent as wood chips or other bedding material. After 4 treatment cycles, all clinical lesions were gone, but the animals were still slightly positive in the rtPCR. The Ct-values often fluctuated between 35 and 39 and sometimes only one of the duplicates was positive. For this reason, we hypothesize that the pathogen was repeatedly transferred from the bedding to the sheep claws. Farm F, on the other hand, did not have a stable at all for the animals. The sheep spent the whole year in a shelter protected from the weather in a grazing area that was very marshy. Due to the swampy landscape, the claws were constantly wet and dirty, and in addition the grazing area was very limited which favored the progression of footrot and made the

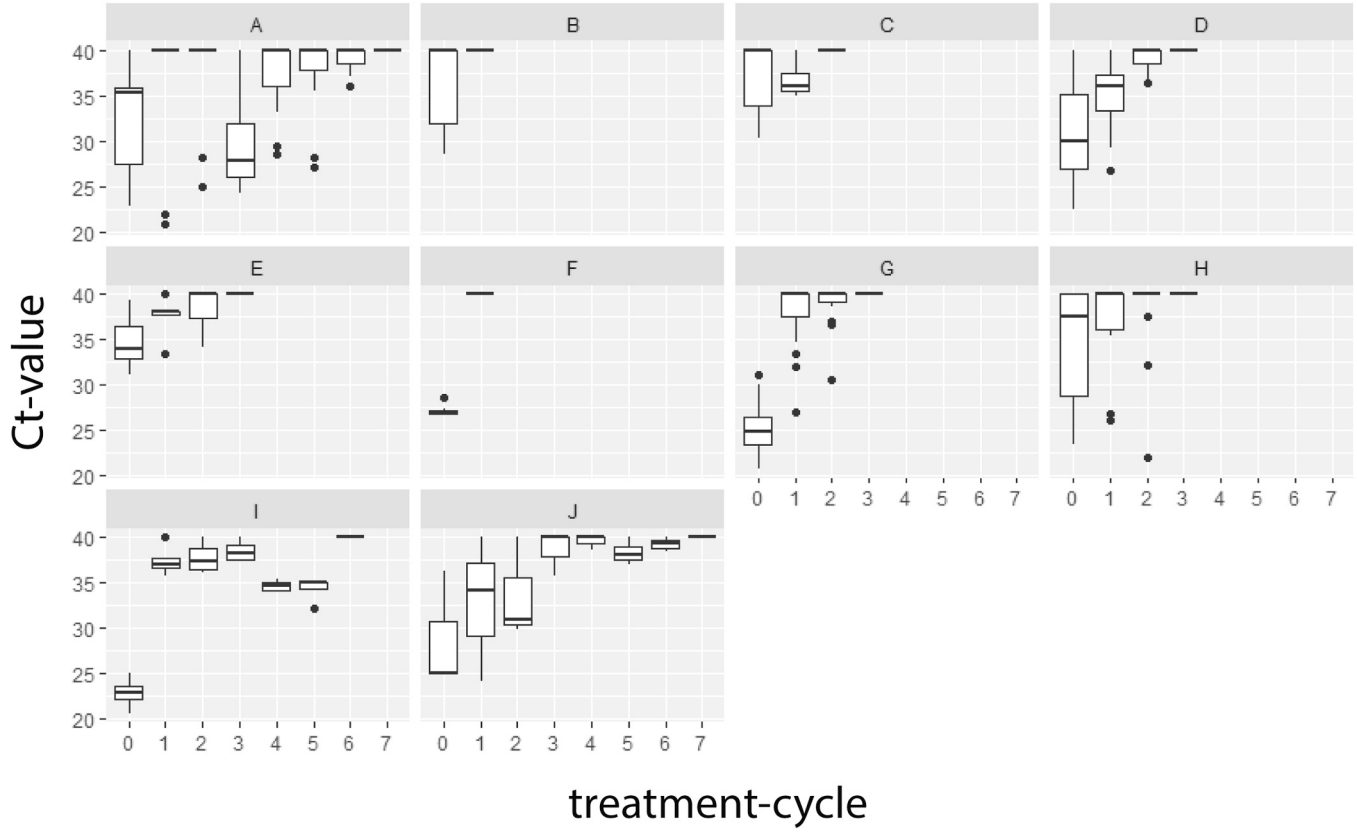

Fig. 3. Boxplots of Ct-values of Farms A- J over the treatment course. Each treatment-cycle included 3 spray treatments per week followed by rtPCR testing. Treatment-cycles were repeated until all animals of a farm tested rtPCR-negative. Treatment-Cycle (TC) 0: time point before therapy started.

treatment process difficult but still surprisingly successful.

Farms were a last time examined one month after successful treatment. Of the 10 farms, 4 were still negative for the footrot pathogen both clinically and in the rtPCR. In five of the rtPCR positive farms no clinical changes were seen (score 0). On one farm (farm H), the rtPCR was positive in 3 out of 20 animals and some animals showed clinical lesions and inflammation signs again (score 2 and 3).

While generally benign *D. nodosus* was absent or only sporadically detected, two farms (A and G) showed an increased presence of benign *D. nodosus* at the final examination (Supplementary data).

3.4. Culture

From 4 out of the 10 farms (A, D, H and I) *D.nodosus* could be isolated from 11 out of 20 animals from which cultures were attempted. The colonies were identified using MALDI-TOF MS and the isolates were stored at -70 °C. All isolates were confirmed to be virulent *D. nodosus*. With cultures started from 7 animals of farms C, E, G and J isolation of *D. nodosus* was not successful. From farm C a massive growth of *Clostridium* sp. was observed on the agar from the two cultures, not seen in any of the other negative cultures from other farms. Since farms B and F had no or only few clinical signs, no culture was set up from these animals.

3.5. Detection of Fusobacterium necrophorum

Only two farms (A and D) were positive for *F. necrophorum* subsp. *necrophorum*. In farm D three animals (12.5%) were positive prior to treatment while during and after treatment none of the animals tested positive. In farm A three animals (21.4%) were positive prior to treatment all of them becoming negative during treatment. Three other animals tested positive each once during treatment but were negative at the final examination. Four animals of farm H (19.1%) were positive for *F. necrophorum* subsp. *funduliforme*. One of them tested positive prior, during and after treatment. The other three animals tested positive during treatment but were negative prior and after treatment (supplementary data).

4. Discussion

The application of a consequent Intra Repiderma spray-based treatment protocol without any antibiotics resulted in a rapid improvement of clinical signs of footrot and elimination of virulent *D. nodosus*. Despite this, several factors relevant for transmission and survival of *D. nodosus* can compromise an effective and successful treatment when not addressed (Table 2). Awareness for these factors followed by corresponding action and (biosecurity) measures are important to generally contain the risk and eventually eradicate the pathogen leading to a footrot negative status of a flock. This is exemplified with farms D and E showing clinical scores up to 5 but following an ideal treatment procedure well adapted to the available farm infrastructure: through consequent and proper treatment, spatial separation, subdividing the pasture and cleaning of the stable after treatment, a quite rapid cure of the disease was achieved, and reinfections prevented. Most of the other farms of this study did not properly follow the recommendations or had limited infrastructure available to do so beforehand. In that way the selection of farms included in the study well reflects the very heterogenous situations of small farms and flocks which the planned control program will have to face. Most important is that the animal owners consequently treat the entire flock at the given interval of three treatments a week. Farms not following this protocol (A and I) had serious problems to get rid of *D. nodosus* reflected by pathogen load and clinical scores going down and up until implementation of the proper protocol. Similarly, farms where a spatial separation during and after treatment

Table 2
Risk factors for transmission and measures for prevention of *D. nodosus* infection during and after treatment.

Factor	Risk	(biosecurity) measure
Treatment	only clin. affected animals	all animals if any rtPCR positive
Treatment processes	reduction of treatments, no herd treatment	consistent treatment, herd treatment until all animals rtPCR negative
Stable	wood and natural floor not good for cleaning	concrete better for cleaning
Paved area	missing	available
Pasture	always same	changing, compartmentalization
Bedding	litter	regular mucking out
Hoof hygiene	neglected	regular and proper claw trimming
Trimming knife	used for all animals	disinfected between animals
Hands	bare-handed	wearing gloves
Boots	always same	changing/desinfecting
Animal transport	direct/indirect animal contact	avoid, quarantine, rtPCR negative

was difficult to achieve (A and J) struggled to get their flock free of virulent *D. nodosus*. Likewise, farms with limited hygiene like same bedding (H and J) or wooden stables with a natural floor (G and C) impairing mucking out or cleaning, had more problems to cure the disease. Finally, farm B corroborated earlier findings that clinically healthy animals can still carry the pathogen which then, if not properly contained, could lead to recurrent footrot (Kraft et al., 2020; Stauble et al., 2014). In this case, however, treatment with the spray led to complete elimination of the etiologic agent within a very short time.

In the initial stage of the disease the bacterial load of *D. nodosus* in infected and clinically healthy sheep is generally high (Greber et al., 2018; Locher et al., 2018; Stauble et al., 2014; Witcomb et al., 2014). As the disease develops to severe footrot the load of *D. nodosus* gets less what supports the hypothesis that *D. nodosus* promotes the development of footrot but is displaced by other bacteria, such as *F. necrophorum* (Maboni et al., 2017; Witcomb et al., 2014). In our study only few sheep limited to two farms (A and D), were positive for *F. necrophorum* subsp. *necrophorum*. In the farm setting tested, *F. necrophorum* subsp. *necrophorum* was not related to clinical scores nor presence of *D. nodosus* and therefore does not seem to contribute to footrot and its detection resembled a chance finding. Interestingly, in a single farm (H) *F. necrophorum* subsp. *funduliforme* was continuously present in a number of animals.

Benign *D. nodosus* was only sporadically detected in six farms but prominently present at the final examination in two of them. This corroborates earlier observations that after successful sanitation benign *D. nodosus* could act as a placeholder (Allworth and Egerton, 2018; Kuhnert et al., 2019; Locher et al., 2015; Zanolari et al., 2021).

Virulent *D. nodosus* could be isolated from four farms confirming initial rtPCR results from swab samples. Since culture is much less sensitive than rtPCR a relatively high bacterial load is needed for successful isolation (Best et al., 2018; Locher et al., 2018).

The six farms testing rtPCR positive after one month were contacted again in June 2023. One farm stated that after re-treatment of the positive sheep they sold the animals and gave up sheep farming due to private matters. Two farms treated the positive animals again for a fortnight and so far, the sheep showed no signs of footrot. The one positive sheep of another farm was slaughtered, and the farm did not have any signs of footrot. Culling of sheep resistant to any treatment is in fact recommended in the framework of a control program since it increases treatment success of the flock (Greber et al., 2016; Schmid et al., 2022). The one farm having again sheep with clinical signs reported that besides the spray the animals were also treated with zinc sulfate foot baths. Nevertheless, some of the animals continued to have problems with footrot indicating a fundamental problem on that farm. Remarkably, this was the farm with *F. necrophorum* subsp. *funduliforme* but its role in that remains unclear.

The currently recommended treatment protocol for the Swiss eradication program includes claw trimming and foot bathing (BGK, 2023). Most treatments are carried out before sheep shows in spring and before the alpine farming season in summer. A further treatment period is done after alpine farming to ensure that returning animals are free of footrot and acceptable to attend autumn shows. Due to the small number of sheep, the workload and costs for small farms using weekly foot baths is too high and the lack of knowledge on the correct disposal of environmentally harmful foot bath products is also a cause for concern. Therefore, treatment of footrot with Intra Repiderma spray offers an alternative for small farms being effective and having very little impact on the environment due to the chelated formula, the targeted application and the adhesive effect. Moreover, farmers reported that they found the application of the hoof spray very practical and quick.

5. Conclusion

With the developed comprehensive treatment protocol using an antibiotic-free claw spray it is possible to effectively treat a sheep flock suffering from footrot and eliminate its etiologic agent. To this aim consequent adherence to the treatment protocol is paramount, including proper claw trimming. In addition, disease awareness of animal owners, knowledge of pathogen transmission routes and implementing the necessary biosecurity measures is important for a time efficient and successful treatment. Biosecurity measures include optimized farm infrastructure allowing spatial separation for the different treatment steps, regular pasture rotation and a generally high hygiene standard in the barn. Most important is to avoid animal traffic without quarantine and in general to prevent contact to possibly infected animals and vectors. Considering all factors, a successful treatment and lasting footrot-free status as given by negative rtPCR for virulent *D. nodosus* can be achieved by the Intra Repiderma spray with reasonable effort. The protocol is therefore a valuable alternative for more laborious foot baths in the framework of the planned Swiss footrot eradication program.

Declaration of Competing Interest

none.

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Ethics statement

The project was carried out under the permission of the Veterinary Office of the Canton of Bern, Switzerland (animal experimentation permit no. BE59/2022) and under the supervision of the Federal Food and Veterinary Office. Each sheep farmer enrolled in the project signed a written consent form to participate in study.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.vetmic.2023.109920.

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