# Early Infection Dynamics of *Dichelobacter nodosus* During an Ovine Experimental Footrot In Contact Infection

P. Kuhnert[1], V. Cippà[1], M. C. Härdi-Landerer[2], M. Schmicke[3], S. E. Ulbrich[2], I. Locher[4], A. Steiner[4], J. Jores[1]

[1] Institute of Veterinary Bacteriology, Vetsuisse Faculty, University of Bern, Switzerland; [2] ETH Zurich, Animal Physiology, Institute of Agricultural Sciences, Switzerland; [3] Clinic for Cattle, University of Veterinary Medicine Hannover, Germany; [4] Clinic for Ruminants, Vetsuisse Faculty, University of Bern, Switzerland

## Summary

Ovine footrot caused by *Dichelobacter nodosus* is a highly contagious and painful disease representing an economic as well as an animal welfare problem. In order to get more information on the infection dynamics, 26 lambs and 4 ewes enrolled in an in-contact infection trial were monitored over two weeks for the presence of *D. nodosus*-specific DNA. Two *D. nodosus*-positive ewes were housed together with 13 confirmed negative lambs. The control group consisted of another 13 lamb siblings and two confirmed *D. nodosus*-negative ewes. Every foot of all sheep was sampled seven times over the two weeks experiment period and subsequently analyzed for the presence of *D. nodosus* by quantitative real-time PCR. The control group was negative at the beginning and the end of the experiment and showed no clinical symptoms of footrot. The two positive ewes showed a high, but hundred fold differing level of virulent *D. nodosus* that remained constant over time with one of the ewes being also weakly positive for benign *D. nodosus*. All lambs of the infection group were positive for virulent *D. nodosus* at 14 days post infection (dpi). The first positive animals were observed on 3 dpi. The *D. nodosus* load remained at a low level and only increased in a few lambs at the end of the trial. Five of the contact lambs showed suspicious clincal signs (score 1-2) at 14 dpi corroborating the PCR results and indicating that the disease starts as early as two weeks after contact with positive sheep.

**Keywords:** *aprV2*, lameness, molecular diagnostics, PCR, sheep, transmission

## Infektionsdynamik einer experimentellen Moderhinke durch eine Kontaktinfektion von *Dichelobacter nodosus* beim Schaf

Moderhinke verursacht durch *Dichelobacter nodosus* ist eine hoch ansteckende und schmerzhafte Krankheit, die sowohl ein wirtschaftliches als auch ein Tierschutzproblem darstellt. In einer Kontaktinfektionsstudie wurden 26 Lämmer und 4 Mutterschafe über einen Zeitraum von zwei Wochen auf das Vorhandensein von *D. nodosus*-spezifischer Desoxyribonukleinsäure (DNS) untersucht. Zwei *D. nodosus*-positive Mutterschafe wurden zusammen mit 13 bestätigten negativen Lämmern gehalten. Die Kontrollgruppe bestand aus weiteren 13 Lammgeschwistern und zwei bestätigten negativen Mutterschafen. Während des zweiwöchigen Versuchszeitraums wurden von jedem Schaffuss sieben Abstrichproben entnommen und mittels einer quantitativen Echtzeit-Polymerase Kettenreaktion (PCR) auf das Vorhandensein von *D. nodosus* analysiert. Die Kontrollgruppe war zu Beginn und am Ende des Experiments negativ und zeigte keine klinischen Symptome von Moderhinke. Im Versuchszeitraum wurde bei beiden positiven Mutterschafen ein konstant hoher, aber hundertfach unterschiedlicher Gehalt an virulentem *D. nodosus* nachgewiesen. Ein Mutterschaf war auch schwach positiv für benigne *D. nodosus*. Alle Lämmer der Infektionsgruppe waren 14 Tage nach dem Kontakt (TnK) positiv für virulente *D. nodosus*. Die ersten positiven Tiere wurden 3 TnK beobachtet. Der *D. nodosus* Nachweis blieb auf einem niedrigen Niveau und stieg am Ende des Versuchs nur bei einigen Lämmern an. Bei 14 TnK zeigten fünf der Kontaktlämmer verdächtige klinische Symptome (Score 1-2) was die PCR-Ergebnisse bestätigte und darauf

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hindeutete, dass die Krankheit bereits zwei Wochen nach dem Kontakt mit positiven Schafen begann.

**Schlüsselwörter:** *aprV2*, Lahmheit, molekulare Diagnostik, PCR, Schaf, Übertragung

## Introduction

*Dichelobacter nodosus* is the etiological agent of ovine footrot affecting sheep worldwide.[7] This infectious and debilitating disease is the major cause of lameness in sheep and a significant animal welfare issue.[19,22] It also presents an economic burden through an enhanced fattening duration, weight loss and reduced wool production.[5,11] The disease starts with interdigital dermatitis, an inflammation of the interdigital skin that can progress to complete separation of the horn from the underlying tissue. Severity of the disease is determined by the virulence of the infecting *D. nodosus* strain as well as environmental conditions and breed.[1,7] In Switzerland, the disease is endemic and 57% of sheep farmers reported problems with footrot in a representative recent study.[4] The stage of the disease is routinely assessed based on the clinical presentation according to a scoring system adapted from Stewart et al.[18] This phenotypic diagnostic has recently been improved by a molecular test able to detect *D. nodosus* even prior to development of clinical signs.[10,16] The real-time PCR allows differentiating between benign and virulent *D. nodosus* strains and provides also information on the amount of bacteria present. Virulence of *D. nodosus* is primarily determined by the presence of the acidic protease AprV2 encoded by *aprV2*, while benign strains possess AprB2 encoded by *aprB2*. The real-time PCR allows the discrimination of *aprV2* from *aprB2* by detecting a 2 base pair difference, which accounts for two discriminative amino acids in the respective proteases AprV2 and AprB2.[8,17] As shown by whole genome analyses, benign and virulent *D. nodosus* represent two well separated lineages of this species that can reliably be differentiated by the real-time PCR.[6] Currently, a nationwide coordinated progressive control strategy against footrot in Switzerland is being developed and implemented.[15] In this context, more information is needed to fill knowledge gaps on transmission dynamics of *D. nodosus*. While we recently investigated the potential risk associated with shows, markets and claw trimming,[9] little is known on the infection dynamics of *D. nodosus*. Previous studies indicate that the bacterial load with *D. nodosus* is highest before clinical symptoms are evident.[16,20] However, to the best of our knowledge no systematic investigation was undertaken to analyze the presence and the bacterial load of *D. nodosus* during the initial course of an infection. We therefore examined foot swab samples of sheep from an experimental in contact infection over a two week period by real-time PCR for the presence of *D. nodosus*.

## Animals, Material and Methods

### Animals and contact infection set up

The animal study was undertaken according to Schmicke et al.[13] and was approved by the Cantonal Animal Protection Authorities (no. 28770).

In brief, a total of 26 lambs from *D. nodosus* negative herds (11 twin pairs and four singletons) were included. The lambs were between 2.5 and 5 months of age. Five twin pairs belonged to the breed Swiss White Alpine sheep (SWA) and the remaining lambs were Swiss Black-Brown Mountain sheep (SBS).

On day 0, the eleven twin lambs were split randomly across two groups, so that one sibling of each pair was allocated to the infection group, while the other was allocated to the control group. In addition, the four unrelated lambs were randomly split across the two groups. Subsequently, two adult ewes previously tested positive for virulent *D. nodosus* served as a source of infection and were grouped with the 13 lambs of the positive infection group. Two *D. nodosus*-negative ewes were grouped with the 13 lambs of the negative control group. The two groups were held in neighboring pens in the same stable. The footrot-positive ewes were SWA (no. 6987) and SBS (no. 9223), while the footrot-negative ewes both were SBS.

All feet of lambs and ewes were daily assessed and scored for footrot using scores between 0 (healthy foot) and 5 (progressed destruction of the epidermis of the whole pododerma with complete hoof separation).

### Sampling and DNA extraction

Samples for the detection of *D. nodosus* were collected from sheep at the following days post infection (dpi) 0, 3, 5, 7, 9, 11 and 14. Each foot of each sheep was individually sampled with a sterile cotton swab (2 mm × 15 cm, Applimed SA, Châtel-Saint-Denis) according to a standardized protocol.[16,17] In short, swabs were taken from the interdigital skin region of the hoof and placed for about one minute into 1 ml of lysis buffer (4 M guanindinethiocyanate, 0.01 M Tris–HCl, 1% β-mercaptoethanol) contained in a KingFisher™ 96-deep well

plate (Thermo Fisher Scientific, Reinach, Switzerland) and then discarded. Only non-neighboring wells in sets of plates devoted to an individual animal group were used for lysate preparation and storage in order to minimize the risk of contamination. Plates were transported to the laboratory and kept at 4 °C for maximal one week until processing.

DNA extraction from 500 µl of each sample was achieved with a KingFisher™ Flex Magnetic Particle Processor (Thermo Fisher Scientific) allowing 96 simultaneous automated DNA purifications and the DNA was eluted using 60 µl of sterile $H_2O$. The VetMAX™ Xeno™ Internal Positive Control (IPC) DNA (20,000 copies; Thermo Fisher Scientific) was added to each extraction sample prior to purification as a control. Purified DNA was stored at -20 °C until further analysis.

### Competitive real-time PCR for detection of virulent and benign *D. nodosus*

The real-time PCR described by Stäuble et al.[16] complemented by the IPC was used to analyze the purified DNA from extracted samples. The assay is able to simultaneously detect the *aprV2* gene indicative of virulent and the *aprB2* gene representing benign *D. nodosus* strains. In brief, the reaction mix contained 1x TaqMan™ Fast Advanced Master mix (Thermo Fisher Scientific), 300 nM of each primer DnAprTM-L and DnAprTM-R, 100 nM FAM-labelled probe DnAprTM-vMGB, 250 nM VIC-labelled probe DnAprTM-bMGB and 1 µl Xeno LIZ Primer Probe Mix (Thermo Fisher Scientific). Samples were analyzed in duplicates using 22.5 µl reaction mix and 2.5 µl DNA and run on a 7500 Real-Time PCR-System instrument (Thermo Fisher Scientific), using standard conditions, i.e. 2 min at 50 °C and 10 min at 95 °C followed by 40 cycles with 15 s at 95 °C and 1 min at 60 °C. Results were analyzed using the Sequence Detector 7500 software (v 2.3). A positive control including a mix of genomic DNA ($10^2$ genome equivalents per reaction) of the virulent *D. nodosus* strain ATCC25549$^T$ and the benign strain JF5922 was included in every run together with a negative control. The genomic DNA was also used to generate standard curves with 1:10 dilutions in $H_2O$. Samples lacking a fluorescent signal for *D. nodosus* ≥40 amplification cycles were considered negative in case that the IPC was positive. A total of 840 samples were analyzed.

### Statistical analysis

Significance was calculated using the Fisher's exact test with a level of significance set at $P < 0.01$ and $P < 0.05$.

## Results

### Clinical monitoring

*General condition:* At 3 dpi one animal (no. 2921) from the infection group was dyspneic and at 5 dpi aggravated such that it was treated systemically with 8 mg/kg body weight oxytetracyclin (Engemycin 10%, MSD Animal Health GmbH, Luzern). At 6 dpi, all lambs younger than three months old (5 from each group) were treated once with oxytetracyclin, to stop the recurrent reduced general condition in these animals.

*Claw scores:* as presented elsewhere[13] at 2 dpi, three animals (no. 2613, 2764 and 3927) of the infection group showed a score 1 on one foot each, which normalized at the following day. From 7 dpi, at least one of the SWA lambs exhibited at least one foot with score 1. From 9 dpi at least four out of five SWA lambs exhibited score 1 at more than one foot and at 13 dpi and 14 dpi, 3 out of the five SWA lambs showed score 2 at one or two feet (no. 2666, 2700 and 2767).

One of the footrot-positive ewes (SWA, no. 6987) scored between 1 and 2 at all feet throughout the experiment, whereas the other (SBS, no. 9223) showed score 1 at one to three feet at dpi 8, 10 and 11. However, she was lame at her right hind leg because of a purulent coronary band infection 1-7 dpi. This was treated locally with iodine daily. At the end of the experiment (14 dpi), all four claws of the two positive ewes were trimmed and treated with chlortetracycline (Cyclospray, Dr. E. Gräub AG, Bern).

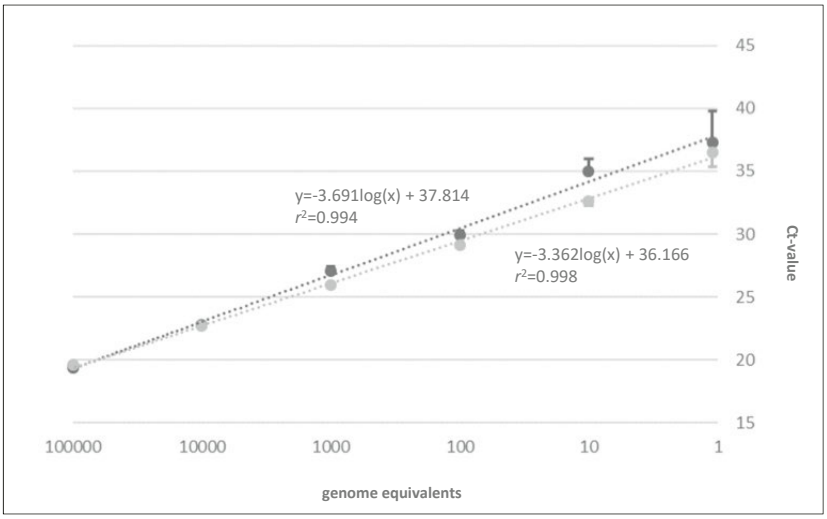


**Figure 1:** Standard curves for the real-time PCR of *aprV2* and *aprB2*. A series of 1:10 dilutions of genomic DNA of virulent *D. nodosus* strain ATCC25549T (*aprV2*; dark circles) and the benign strain JF5922 (*aprB2*; bright circles) with defined genome equivalents was analyzed with the competitive real-time PCR. Bars indicate standard deviation from triplicates of two independent runs. The equation including the slope as well as the correlation coefficient ($r^2$) are indicated beneath the dotted trend lines.

None of the control ewes and none of the SBS lambs ever scored above 0.

## Real-time PCR performance

Standard curves using ten-fold dilution series of virulent (*aprV2*-positive) and benign (*aprB2*-positive) *D. nodosus* DNA with defined genome equivalents was performed with triplicates in two independent runs (Fig. 1). Results indicate linearity within the range tested with correlation coefficients of $r^2$=0.994 and $r^2$=0.998 for the *aprV2* and *aprB2* target, respectively. Based on the slope the efficiency for the two real-time PCRs was calculated to be 87% and 98%, respectively. There was a higher standard deviation with the two triplicates of the single genome equivalent sample indicating that the limit of detection is between 1-10 genome equivalents. Thus, results with Ct-values >35 are at the limit of detection restricting reproducible results in this range. Positive and negative controls performed accordingly in each run. The IPC included as an extraction and PCR inhibition control was positive in all duplicates of 830 out of 840 samples (98.8%). In the remaining 10 samples, the IPC was negative in both duplicates in three samples and negative in only one of the duplicates in seven samples. The Ct-values for the IPC of most samples were in the same range, on average with a Ct-value of 30.5 (±1.9).

## Infected ewes

The two ewes included in the infection group were positive for virulent *D. nodosus* (*aprV2*-positive) as determined by real-time PCR. The time course of *D. nodosus* detection over the 14 days is given in Figure 2. Clinically, one ewe (no. 6987) showed acute type lesions (score 2) while the other ewe (no. 9223) showed a chronic type of footrot. The acute lesions correlated with a higher load of *D. nodosus* as indicated by a Ct-value difference of on average 7.4, representing an estimated more than 100-fold higher load of virulent *D. nodosus* in the acute lesion compared to the chronic form. In general, all four feet were tested positive except the ewe with chronic form showing only three positive feet on two of the seven sampling days (3 and 5 dpi). In the acute lesions, the benign *D. nodosus* was also specifically detected, only appearing on the left forefoot in the beginning of the trial. The foot was positive for *aprB2* with a Ct-value of 25.1 at 0 dpi followed by a constant reduction of bacterial load as indicated by Ct-value 27.3 at 3 and 5 dpi, Ct-value 29.7 at 7 dpi and then negative (Ct-value ≥ 40) at the following days.

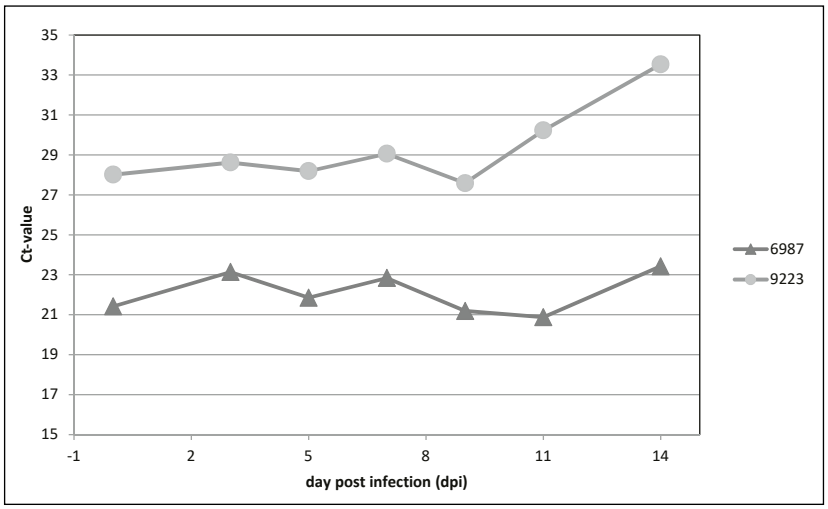


**Figure 2:** Time course of *aprV2*-positive, virulent *D. nodosus* load in positive ewes indicated by real-time PCR Ct-values obtained at each sampling day. Triangles represent the Swiss White Alpine (SWA) ewe with acute lesions (score 2) while dots show the Swiss Black-Brown Mountain (SBS) ewe with the chronic form of disease.

## Infection group

All lambs kept with the positive ewes were negative for *D. nodosus* as determined by real-time PCR at the beginning of the experiment. First positive animals (10/13) were observed at 3 dpi. At 5 dpi all lambs were positive and stayed positive throughout the experiment, except on 8 dpi when one lamb tested negative in all four feet. The mean Ct-value from positive samples at a given dpi was constantly high with 34.3 (+/- 0.5). When looking at the number of positive feet, a significant bimodal increase was observed, one at the beginning of the infection trial and the other at the end, when almost all feet of the 13 lambs were positive (Fig. 3). A clearer picture can be gained when looking at the dynamics in individual animals as shown in Fig. 4. Results from individual feet are very inconsistent at a certain time point as well as over the entire experiment as shown in Fig. 4A. This was the case with most of the lambs and their feet over time. However, a few lambs showed a clear drop of Ct-values in all four feet at 14 dpi indicating an increase in virulent *D. nodosus* load (Fig. 4B-D).

## Control group

All animals, namely lambs and ewes, of the control group were tested negative for *D. nodosus* at the beginning and at the end of the experiment. Both ewes were tested positive on a single occasion at 5 dpi on four and three feet, respectively, together with all four feet of a single lamb (no. 2765). Single feet of individual animals were once positive at 3 dpi (no. 2922), at 5 dpi (no. 3975), at 9 dpi (no. 3925) and at 11 dpi (no. 2667 and 3935). The time course of number of positive lamb feet is shown in Fig. 3. There was no significant increase in number of positive feet ($P > 0.05$) in the control group.

## Discussion

Knowledge on the dynamics of *D. nodosus* infections is lacking to date. However, such information is useful in order to assess risk of transmission when planning a footrot control program, since otherwise recurrent infection will impede efforts to combat the disease. This is especially of concern since infectious *D. nodosus* seems to be present in high amounts prior to the observation of clinical signs.[16,21] While a control program solely based on clinical diagnosis carried out in one part of Switzerland for several years has succeeded to reduce the prevalence of footrot drastically, reinfections occur especially during seasonal alpine pasture when sheep originating from different herds are kept together during the summer period. Subclinical carriers can thereby easily be introduced into sanitized footrot-free herds. An early diagnosis based on the molecular detection of virulent *D. nodosus* can identify such carriers and would allow for a successful control program as shown in a proof of concept study.[3] Knowledge on the infection dynamics would help to define quarantine time in order to be sure that the herds are free of footrot. For this purpose, we investigated the feet of lambs enrolled in

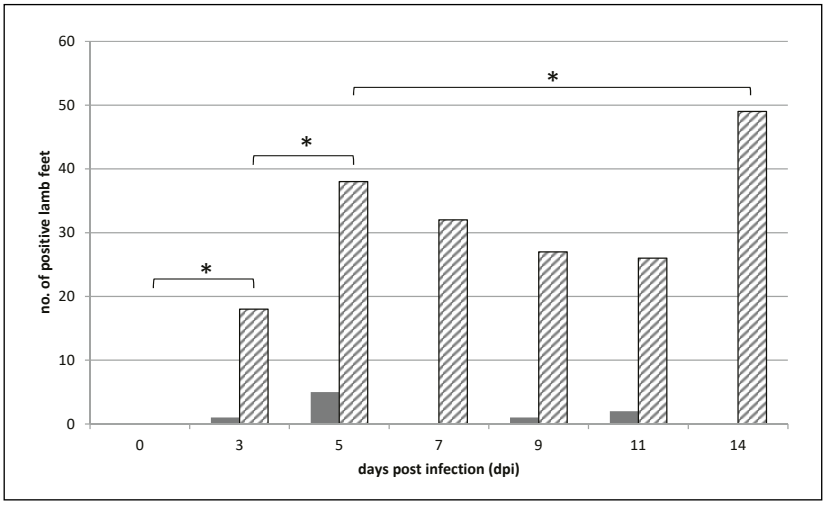


**Figure 3:** Number of *aprV2*-positive lamb feet from both experiment groups (maximum 52) at each sampling day. Filled boxes represent the number of positive feet in the control group and the shaded bars show the number of positive feet in the infection group. The asterisk indicates a significant difference in number between time points within the infection group at the level of $P < 0.01$. No significant difference in number within groups was observed with all the other time points ($P > 0.05$).

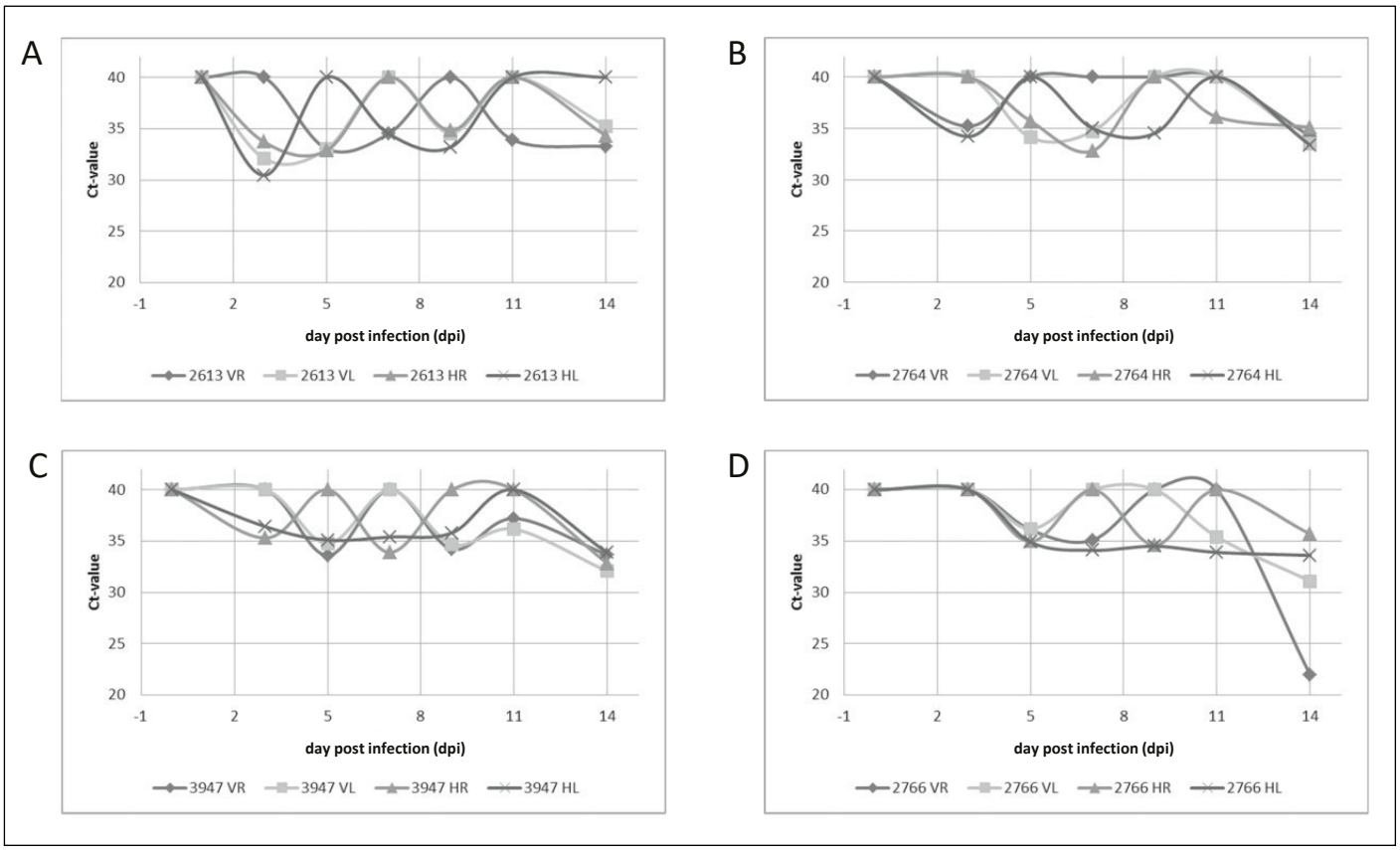


**Figure 4:** Time course of *aprV2*-positive, virulent *D. nodosus* load indicated by real-time PCR Ct-values determined for each foot at each sampling day. Results of 4 untreated sheep are given (Panel A-D). Sheep number together with the corresponding foot is given where VR, VL, HR, HL represent the right and left forefoot and right and left hind foot, respectively. Panel A is representative of most lambs in the infection group with varying Ct-values. This is also the case until day 11 in the three sheep presented in panels B-D. However, in these three individuals a clear tendency in all four feet towards higher bacterial load (lower Ct-values) can be seen at day 14.

an in contact infection trial. The study was part of a larger study to investigate the physiological stress response towards a chronic infection.[13]

By including an IPC extraction and PCR inhibition control, the original real-time PCR assay[16] was further improved to serve diagnostic purposes. The assay performed similar to the originally described one when looking at standard curves (Fig. 1). Moreover, only three out of the 840 samples were negative for the IPC in both duplicates tested (99.6% positive). Whether these three samples were inhibited or DNA extraction failed was not further investigated. In fact, some variation in the Ct-values of the IPC was observed which probably indicates various degrees of inhibition due to sampling material containing various degrees of fecal contamination. Feces is known to contain PCR inhibitors that cannot always be completely removed by DNA purification.[14] Given a success rate of nearly 100% though indicates that the automated DNA extraction protocol is reproducible and reliable.

The time course of the two positive ewes showed no difference within each ewe. Interestingly, the constant difference in *D. nodosus* load of more than 100-fold remained over the whole duration of the experiment. This could reflect the difference in clinical signs with the lower load associated with the more chronic and the higher load with the more acute form assessed by a clinical score of 2. Alternatively, it could also be associated with the different breed or color. While the higher load was found with the SBS sheep (brown-black color), the lower load was observed with the SWA sheep (white color). Skin color could also impair an objective clinical interpretation.

Benign *D. nodosus* was also detected in one of the ewes. However, the load of benign *D. nodosus* declined over time and it was no longer detectable at the end of the experiment. The presence of both virulent and benign strains has been previously reported, but seems to be rather an exception.[16] A displacement of benign by virulent *D. nodosus* during infection could be the reason for this. On the other side, benign *D. nodosus* could function as a placeholder eventually protecting from virulent strains, since sheep from the region with an ongoing control program more often carried benign than virulent strains in contrast to sheep from regions without control program.[9]

All animals of the control group tested negative at the beginning and at the end of the infection trial, indicating that no colonization occurred in these animals. However, single feet of a few lambs were positive at individual time points during the experiment. Comparing the numbers with the infection group, no significant increase was observed and the number of positive samples remained low and sporadic (Fig. 3). The pens for both the infection and the control group were in the same room and cats, mice or birds like swallows, which inhabit the farm, are likely vectors that might have caused the transfer of *D. nodosus*-containing material from one fenced area to the other. Theoretically, fast locomotion of the animals could have led to the transfer of minute amounts of beddings (that have not been changed throughout the entire experiment) from one area to the other. Iatrogenic transfer of material during the sampling process should be negligible, but cannot be ruled out. The results obtained clearly point towards the necessity of repeated contact with *D. nodosus* to cause infection, otherwise the negative herd would not have cleared the bacteria.

In a transmission study between ewes and their newborn lambs, Muzafar et al.[12] detected *D. nodosus* on feet of lambs 5-13 h after birth once they stood on naturally contaminated bedding. In our study, the first positive feet of lambs were observed in 10 of the 13 animals at 3 dpi in the infection group. The fact that even after 3 days standing in contaminated bedding not all lambs showed positive feet for *D. nodosus* could be due to a lower contamination level of bedding or the older age of lambs in our experimental setup (newborns vs 2.5-5 months old). The significant increase in positive feet in all 13 lambs of the infection group at 5 dpi might subsequently be the result of stable colonization. This then went on for more than a week before another significant increase in positive feet at 14 dpi may indicate the start of the infection and the spread of the disease (Fig. 3). This is corroborated by the time course seen with bacterial load in individual feet of animals (Fig. 4). By looking at individual feet, most lambs showed a rather heterogeneous course of bacterial load as indicated by the varying high Ct-values (Fig. 4A). Part of this is certainly due to the PCR assay which is at its detection limit not allowing reproducible detection at this range (Ct>35) as well as possible variation in the swab sampling procedure. However, a few lambs showed a trend towards higher loads, i.e. clearly lower Ct-values at 14 dpi (Fig. 4B-D). In one case, one foot of one lamb showed a dramatic increase of *D. nodosus* load (Fig. 4D). This, together with the bimodal increase of positive feet at 5 and 14 dpi (Fig. 3), reflects the infection dynamics and indicates start of the disease after 14 days. This is in agreement with a transmission study done in NSW, Australia.[2] In that study an experimental pen infection with defined *D. nodosus* strains was performed. After three weeks, clinically positive animals were put in paddocks together with negative sheep. Both were examined weekly for signs of footrot. After two weeks, half of the animals (10/20) developed clinical lesions. This Australian study was based solely on clinical signs and isolation, while our study looked at bacterial load by real-time

PCR, which is much more sensitive. In the infection group, only the SWA lambs showed mild clinical symptoms starting at 7 dpi and included progressively more animals and more feet per animal. At day 13, three SWA lambs started to show a score of 2 on one or two feet each. The clinical signs were also rather mild in the ewes and did not deteriorate during the experiment. In contrast to the Australian experiment, this trial took place inside a closed and more hygienic environment. This could account for the mild and delayed clinical development of the disease. The slightly different onsets of colonization reflect the in contact infection, which is close to a field situation and the outbred population is likely to correlate to slightly different individual infection dynamics. The experiment gives valuable information for future studies. While infected ewes were taken as a natural source of an infection, a defined experimental infection using virulent field isolates should further be considered. Such a new infection experiment should last longer than two weeks, since it takes at least one week or more before clinical signs can be expected.

## Conclusions

A constant bacterial load in previously infected animals with individual difference was observed. An increase of *D. nodosus* in the affected feet of lambs was only seen at the end of the trial, i.e. at 14 dpi. Therefore, in the framework of a control strategy, a quarantine of 2-3 weeks is recommended in case of contact with potentially infected sheep. Afterwards a PCR can verify if the animal is negative for virulent *D. nodosus*.

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## Dynamique de l'infection précoce de *Dichelobacter nodosus* au cours d'une infection expérimentale par contact sur les onglons des ovins

Le piétin causé par *Dichelobacter nodosus* est une maladie hautement contagieuse et douloureuse qui représente à la fois un problème économique et de bien-être animal. Pour avoir plus informations sur la dynamique de l'infection, 26 agneaux et 4 brebis appartenant à un groupe d'essai d'infection par contact ont été contrôlés pendant deux semaines quant à la présence d'ADN spécifique de *D. nodosus*.

Deux brebis positives pour *D. nodosus* ont été mises en contact avec 13 agneaux confirmés négatifs. Le groupe témoin était formé de 13 autres agneaux et deux brebis confirmées négatives.

Sept échantillons sur écouvillon ont été prélevés sur chaque pied de chaque mouton au cours des deux semaines de la période expérimentale et analysés quant à la présence de *D. nodosus* par PCR quantitative en temps réel. Le groupe témoin était négatif au début et à la fin de l'expérience et n'a montré aucun symptôme clinique de piétin. Les deux brebis positives ont montré une forte présence de *D. nodosus* virulent, mais de cent niveaux différents, qui est restée constant dans le temps, l'une des brebis étant aussi faiblement positive pour *D. nodosus* bénin. Tous les agneaux du groupe infecté étaient positifs pour *D. nodosus* virulent 14 jours après l'infection (dpi). Les premiers animaux positifs ont été observés à 3 dpi. La charge de *D. nodosus* est restée faible et

## Dinamica della propagazione della zoppina da *Dichelobacter nodosus* in uno studio a contatto dell'infezione nelle pecore

La zoppina negli ovini causata dal *Dichelobacter nodosus* è una malattia altamente contagiosa e dolorosa che rappresenta un problema sia economico che del benessere degli animali. Per capire maggiormente la dinamica della trasmissione, in uno studio a contatto dell'infezione, 26 agnelli e 4 pecore sono stati monitorati per due settimane per la presenza di ADN specifico a *D. nodosus*. Due pecore positive al *D. nodosus* sono state messe a contatto con 13 agnelli negativi al patogeno. Il gruppo di controllo era formato da altri 13 agnelli gemelli e da due pecore negative. Sette campioni sono stati prelevati dagli zoccoli di ogni animale durante le due settimane di sperimentazione e analizzati per la presenza di *D. nodosus* con un'analisi quantitativa di real-time PCR. Sia all'inizio che alla fine della sperimentazione, il gruppo di controllo è risultato negativo e non presentava nessun sintomo clinico alla zoppina. Durante il periodo dello studio, le due pecore positive hanno mostrato un livello alto ma costante nel tempo di *D. nodosus* virulento ma con un contenuto cento volte differente e una pecora è risultata pure debolmente positiva al *D. nodosus* benigno. Tutti gli agnelli del gruppo infettato sono risultati positivi al *D. nodosus* virulento dopo 14 giorni dal contatto (dpi). I primi animali positivi sono stati osservati a 3 dpi. La carica di *D. nodosus* è rimasta a livelli bassi ed è aumentata solo in pochi agnelli alla fine del-

n'a augmenté que chez quelques agneaux à la fin de l'expérience. Cinq des agneaux en contact ont présenté des symptômes suspects (score 1-2) à 14 dpi, corroborant les résultats de la PCR et indiquant que l'infection commence dès deux semaines après le contact avec des moutons positifs.

**Mots-clés:** *aprV2*, boiterie, diagnostic moléculaire, PCR, ovin, transmission

lo studio. Cinque degli agnelli del gruppo a contatto hanno mostrato dei sintomi sospetti (punteggio 1-2) a 14 dpi. Questo ha confermato i risultati dell'analisi PCR e ha indicato che l'infezione inizia al più presto due settimane dopo il contatto con delle pecore positive.

**Parole chiave:** *aprV2*, zoppia, diagnostica molecolare, PCR, pecore

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## Corresponding author:

Peter Kuhnert
Länggassstrasse 122
Postfach
3001 Bern
Tel.: 031 631 24 85
Fax: 031 631 26 34
E-Mail: peter.kuhnert@vetsuisse.unibe.ch