

Foot health and prevalence of *Dichelobacter nodosus* in 11 ungulate species at Berne Animal Park

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Klauengesundheit und Prävalenz von *Dichelobacter nodosus* bei 11 Huftierarten im Tierpark Bern

Dichelobacter nodosus (*D. nodosus*) ist der ätiologische Erreger der Moderhinke bei Wiederkäuern, der weltweit hauptsächlich Schafe, aber auch freilebende wilde Huftiere wie Alpensteinbock (*Capra ibex ibex*) und Mufflon (*Ovis orientalis orientalis*) befällt. Für die kommenden Jahre ist ein landesweites Programm zur Tilgung der Moderhinke geplant, das auf PCR-Test (Polymerase Ketten Reaktion) von Abstrichen aus dem Interdigitalspalt und regelmässigen Fussbädern basiert. In dieser Querschnittsstudie haben wir die Klauengesundheit klinisch bewertet und das Vorhandensein von *D. nodosus* bei 11 verschiedenen Paarhufern (hauptsächlich europäische Arten) während eines Zeitraums von 13 Monaten (2018–2019) im Tierpark Bern analysiert. Basierend auf den Beschreibungen von Rindern und Schafen und unter Verwendung einer Skala von 0 bis 5 (während 0 klinisch gesunde Füße und 5 Verlust der Hornkapsel beschreibt) wurden die Klauenläsionen auf pathologische Veränderungen und Anzeichen von Klauenfäule bewertet. Von insgesamt 53 Tieren wurden jeweils vier Tupferproben aus den Interdigitalspalten entnommen und mittels Echtzeit-PCR auf *D. nodosus* untersucht. Klauenläsionen wurden bei fünf verschiedenen Arten festgestellt. Bei 3/5 Moschusochsen (*Ovibos moschatus wardi*), 7/12 Bezoarziegen (*Capra hircus cretica*) und 2/3 Zwergziegen (*Capra hircus aegagrus*) bestanden sie hauptsächlich aus der Weisslinienkrankheit, während bei 9/10 Wisenten (*Bison bonasus*) eine Dermatitis interdigitalis diagnostiziert wurde. Bei 1/3 Alpakas (*Vicugna pacos*) wurde eine Chorioptes Räude im Fesselbereich festgestellt. Keines der untersuchten Tiere zeigte klinische Anzeichen von Moderhinke (Punktzahl 0), und in keiner der Proben wurden benigne (*aprB2*-positive) oder virulente (*aprV2*-positive) *D. nodosus* nachgewiesen. Diese Studie liefert zusätzliche Informationen für ein effizientes Bekämpfungsprogramm der Moderhinke bei Schafen und zeigt, dass

Summary

Dichelobacter nodosus (*D. nodosus*) is the etiological agent of ovine footrot affecting mainly sheep worldwide, but also free-ranging wild ungulates such as Alpine ibex (*Capra ibex ibex*) and mufﬂon (*Ovis orientalis orientalis*). A nationwide ovine footrot eradication program is planned for the years to come, based on polymerase chain reaction (PCR)-testing of interdigital swab samples and regular footbathing. In this cross-sectional study, we clinically evaluated the foot health and analysed presence of *D. nodosus* in 11 different even-toed ungulate species (mainly European species) during a 13 months (2018–2019) period in Berne Animal Park. The foot lesions were scored for any clinical signs of pathologies as described in cattle and simultaneously for clinical signs of footrot as described for sheep, using a scale from 0 to 5 (while 0 describes clinically healthy feet and 5 loss of the horn capsule). From a total of 53 animals, 4-feet swab samples were taken from the interdigital cleft and subjected to real-time PCR assays to detect *D. nodosus* at animal level. Foot lesions were detected in five different species. In 3/5 muskoxen (*Ovibos moschatus wardi*), 7/12 Cretan wild goats (*Capra hircus cretica*) and 2/3 dwarf goats (*Capra hircus aegagrus*), they mainly consisted of white line disease, whereas in 9/10 European bison, dermatitis of the interdigital cleft was diagnosed. 1/3 alpaca was diagnosed with chorioptic mange of the heel area. None of the examined animals showed clinical signs of footrot (score 0), and neither benign (*aprB2*-positive) nor virulent (*aprV2*-positive) *D. nodosus* were detected in any of the samples. This study provides additional information to facilitate an efficient ovine footrot control program in Switzerland and suggests that captive wild even-toed ungulates do not pose a risk to the planned footrot control program.

Key words: Captive wild and domestic ungulates, *Dichelobacter nodosus*, Foot health, PCR, Lameness

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Paarhufer in Zoologischen Einrichtungen kein Risiko für das geplante Eradikationsprogramm der Moderhinke in der Schweiz darstellen.

Schlüsselwörter: wilde und domestizierte Huftiere in Menschenobhut, *Dichelobacter nodosus*, Klauengesundheit, PCR, Lahmheit

Introduction

Despite its clinical importance, epidemiologic information on foot health and diseases is scarce in the nondomestic animal literature.²⁶ There are many predisposing factors that potentially contribute to foot disorders in captive nondomestic even-toed ungulates. Enclosure design, floor substrate, hygiene conditions and nutrition must meet with the natural history and welfare of the species to prevent health problems, including those of the feet.^{8,22} Spillover of foot pathogens between closely related species must be minimized by means of practical hygiene measures between enclosures and adequate quarantine investigations for new acquisitions. The inability to perform routine foot evaluations and trims in noncompliant species may further jeopardize foot health.

Dichelobacter nodosus (*D. nodosus*) is a gram-negative fastidious anaerobic bacterium and an essential pathogen involved in the multifactorial etiology of ovine footrot.^{4,15} The disease is reported worldwide as endemic, causing painful inflammation and lameness.^{11,12} The disease affects both domestic (mainly sheep) and to a much lesser extent free-ranging wild ungulates.^{3,21} A recent study in Switzerland estimated the true prevalence of virulent *D. nodosus* in sheep at 16.9% and 16.2% at animal and farm level, respectively.² On the other hand, another recent study revealed a low prevalence of both benign (1.97%) and virulent *D. nodosus* strains (0.05%) in all four indigenous wild ruminants in Switzerland.¹⁶ Footrot is an important welfare issue and causes large economic losses throughout sheep flocks in many countries. In Switzerland, costs for disease management without control measures were estimated at CHF 172.3 million for the years 2014–2030.²⁵

D. nodosus isolates can be classified into benign and virulent types by their presence of the *aprB2* or *aprV2* gene which code for thermolabile or thermostable acidic proteases, respectively. Benign strains (*aprB2*-positive) tend to cause non-progressive inflammation of the interdigital skin (mild interdigital dermatitis), while virulent strains (*aprV2*-positive) are associated mainly with clinical footrot, interdigital ulceration and may progress to separation of the horn shoe from the underlying dermal tissue.^{17,18,24}

In recent years, footrot diagnostics have been remarkably improved by establishing a competitive real-time polymerase chain reaction (PCR) method allowing detection and differentiation of the virulent and benign strains of *D. nodosus* directly from clinical samples.¹⁷ This, in turn, enabled the non-invasive swabbing of feet and early detection of animals harbouring *D. nodosus* with high sensitivity and specificity, and additionally to quantify the amount of bacteria present.

In Switzerland, the first footrot control program started in the 1990s in the canton of Grison (GR), more recently in the canton of Glarus (GL) and is currently still voluntarily in the other 24 cantons. The present footrot control program in sheep includes clinical examination and swab sampling from the interdigital cleft, followed by prudent removal of undermined horn without causing bleeding, and regular footbathing in disinfectant solutions of herds positive for virulent *D. nodosus*.⁹ However, information on footrot in animal parks and zoos is scarce, and there is a lack of knowledge concerning PCR diagnostics in diverse exotic ungulate species.²⁶ For these reasons, we aimed to clinically evaluate the distal limbs of the diverse even-toed ungulate stock housed at Berne Animal Park and to estimate the prevalence of *D. nodosus* using the established real-time PCR. This study provides relevant additional information for the planned footrot control program in Switzerland in order to ameliorate the prospects of success.

Material and Methods

Animal preparation

Random sampling of 53 even-toed ungulates from a total of 71 animals was performed during diagnostic, curative or prophylactic medical treatments as part of routine zoo veterinary practice, as well as during captures related to transports from the whole even-toed ungulate stock of Berne Animal Park (n=11 different species and n=71 animals totally kept). General anaesthesia for all species except Cretan wild goats (*Capra hircus cretica*) and dwarf goats (*Capra hircus aegagrus*) was necessary to perform clinical examination and sampling in order to reduce the risk of injuries to animals and staff. The day before sampling, individual animals were separated in a box and fasted overnight. Except for Javan

mouse-deer (*Tragulus javanicus*), a mixed syringe of medetomidine (Medetomidin HCL, Christoffel Apotheke, Bern, Switzerland or Dorbene, Graeb, Switzerland (0.04–0.08 mg/kg) and ketamine (Ketamin HCL, Christoffel Apotheke, Bern, Switzerland or Ketasol-100, Graeb, Switzerland, 1.5–3.0 mg/kg) was applied per estimated body weight (bw) via blow pipe or dart gun (Daninject, Gelsenkirchen, Germany) in the shoulder or thigh musculature. The animals were placed in right lateral recumbency, received 0.1 l/min oxygen per 10 kg bw via nasal probe and vitamin E and selenium. For reversal, atipamezole (Alzane, Graeb, Switzerland, 0.2–0.4 mg/kg) was administered at the end of the procedure. In total, 32 medetomidine-ketamine anaesthetics were performed. Minor complications occurred in a single muskox (superficial plane of anaesthesia with spontaneous movements at the end of the procedure) and two alpacas (transient respiratory depression and apnoea, treated with intubation and assisted ventilation), and recovery was uneventful in all the animals. Javan mouse-deer were trapped in an airtight box and anaesthesia was induced by 5.0 vol.% of isoflurane (Isoflo, Zoetis, Switzerland) in 2.0 liter oxygen, followed by maintenance with 2.0 vol.% of isoflurane in 0.5 l/min oxygen administered per face mask.

Additional samples were collected from freshly slaughtered (n=5) or dead (n=1) individuals.

Collection of swab samples and foot lesion description

Samples were collected between November 2018 and December 2019. All four feet were examined for clinical signs of footrot and scored according to the BGK (Swiss Health Service for Small Ruminants) and adapted from Egerton and Roberts⁶ using a scale from 0 to 5 (while 0 describes clinically healthy feet and 5 loss of the horn capsule). After removing the dirt with a dry paper towel, the feet were sampled with sterile, dry cotton swabs (2 mm × 15 cm, Stericlin, Germany) by rubbing the swab in the interdigital cleft of all four feet of the respective animal using one clean quarter of the same swab for each foot according to a standardized protocol.¹⁴ Attention was paid that the swabs had direct contact to the skin. Thereafter, standardized photographs were taken of all feet with dorsal and plantar views, digitally stored and blinded at animal level. Using these photographs, foot lesions were finally scored by the two trained and experienced first and last study authors according to the International Committee for Animal Recording (ICAR) claw health atlas (ICAR, 2015) for cattle (https://www.icar.org/Documents/ICAR_Claw_Health_Atlas.pdf, accessed 25.09.2020) and the guide for diagnosis and treatment of lameness in sheep (https://www.qm Scotland.co.uk/sites/default/files/diagnosis_treatment_of_lameness_guide.pdf, accessed 25.09.2020).

DNA extraction and real-time PCR

Individual swabs were placed immediately into an Eppendorf tube filled with 1ml of SV lysis buffer (4M guanidine thiocyanate, 0.01 M Tris-HCl, 1% β-mercaptoethanol) for DNA extraction according to an adapted protocol.¹⁷ The lysed samples were stored at +4 °C and transferred to the laboratory for DNA extraction within one week of sampling. DNA extraction was performed from a 500 µl lysate using a semi-automated extraction robot (KingFisher™ DuoPrime, Thermo Fisher Scientific, Reinach, Switzerland), and purified DNA was stored at –20 °C until further laboratory processing. Detection and typing of *D. nodosus* was performed using the competitive real-time PCR according to Stäuble et al.¹⁷ including an internal positive extraction and PCR inhibition control.¹³ This real-time PCR distinguishes between the presence of the gene *aprV2* encoding the thermostable protease AprV2, and the gene *aprB2* coding for the thermosensitive protease AprB2 of *D. nodosus* in sheep. All real-time PCR reactions were analyzed in duplicate, with a mean cycle (Ct) threshold of <40 rated positive.

Ethical statement

The study protocol was approved by the animal experimentation committee of the canton of Bern, Switzerland (permission # BE89/18, 30672).

Results

Overall, 53 animals of a total of 71 individuals from 11 different even-toed ungulate species kept at the Berne Animal Park were sampled between November 2018 and December 2019. Involved species, demographic data and the results of the clinical findings are presented in Table 1. In 3/5 muskoxen (*Ovibos moschatus wardi*), 7/12 Cretan wild goats and 2/3 dwarf goats, foot lesions were observed, mainly exhibiting signs of white line disease (WLD), apart from single cases of horn fissures and scissor claws. Feet of both fore and hind limbs were affected, and lesions were observed in the abaxial as well as the axial aspect of the claws. In 9/10 European bison (*Bison bonasus*), signs of active dermatitis of the interdigital cleft were diagnosed, which affected both the fore and hind limbs. one of three alpacas (*Vicugna pacos*) presented with scaly lesions of the heel area of all four feet and skin scrapings revealed *Chorioptes* sp. as the etiologic ectoparasite. None of the examined animals showed any clinical signs of footrot (score 0). The results of the competitive real-time PCR revealed the complete absence of both benign and virulent strains of *D. nodosus* (Table 1).

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Table 1: Clinical foot health findings and the prevalence of *Dichelobacter nodosus* (*D. nodosus*) in 53 even-toed ungulates from Berne Animal Park, sampled between November 2018 and December 2019.

Species	number of animals ¹ (m, f; mean age; bw range)	Clinical findings (number of animals)	² Footrot score	³ <i>D. nodosus</i>
Alpaca (<i>Vicugna pacos</i>)	3 (1, 2; 109; 53–74)	Dermatitis of the heel area (chorioptic mange) (1)	0	negative
Alpine chamois (<i>Rupicapra rupicapra rupicapra</i>)	5 (2, 3; 117; 30–37)	No lesion	0	negative
Alpine ibex (<i>Capra ibex ibex</i>)	3 (3, 0; 81; 82–97)	No lesion	0	negative
Cretan wild goat (<i>Capra hircus cretica</i>)	12 (4, 6; 37; 2–35)	⁴ WLD (7) Axial horn fissure (1)	0	negative
Dwarf goat (<i>Capra hircus aegagrus</i>)	3 (3, 0; 28; 10–41)	WLD (2)	0	negative
European Bison (<i>Bison bonasus</i>)	10 (4, 6; 91; 210–850)	Chronic dermatitis of the interdigital cleft (9)	0	negative
European elk (<i>Alces alces alces</i>)	2 (1, 1; 20; 205–400)	No lesion	0	negative
European forest reindeer (<i>Rangifer tarandus fennicus</i>)	2 (1, 1; 69; 8–143)	No lesion	0	negative
Javan mouse deer (<i>Tragulus javanicus</i>)	3 (2, 1; 29; 1.2–1.8)	No lesion	0	negative
Muskox (<i>Ovibos moschatus wardi</i>)	5 (1, 4; 66; 79–250)	WLD (3) ⁵ SC (1) Abaxial horn fissure (1)	0	negative
Red deer (<i>Cervus elaphus</i>)	5 (2, 3; 19; 70–123)	No lesion	0	negative

¹ m=male, f=female; mean age in months; body weight range in kg

² footrot score according to BGK (Swiss Health Service for Small Ruminants), adapted from Egerton and Roberts,⁶ using a scale from 0 to 5 (while 0 describes clinically healthy feet and 5 loss of the horn capsule)

³ The presence of *D. nodosus*-specific DNA encoded by *aprV2* and *aprB2* as evaluated by a quantitative real-time PCR according to Stäubli et al.¹⁷

⁴ WLD=white line disease

⁵ SC=scissor claws

Discussion

We assessed for the first time the foot health and the prevalence of *D. nodosus* in 11 different even-toed ungulate species from Berne Animal Park. The sample size allowed a reliable estimate of the foot health status of the stock, since more than two thirds of the individuals were sampled. The comparison of our findings with the currently reported data in other wild and domestic ungulate species from Switzerland is important to understand the dynamics of footrot and its interaction between domestic and wildlife species.¹⁶ Further investigations are required to evaluate the true prevalence of *D. nodosus* in Swiss animal parks and zoos including many other exotic even-toed ungulate species.

The presence of benign and virulent strains of *D. nodosus* was found to be associated with severe foot lesions in Alpine ibex (*Capra ibex ibex*).^{3,16,23} A recent study in Switzerland reported the presence of both benign and virulent strains of *D. nodosus* in free-ranging Alpine ibex associated with severe lesions consist-

ent with typical footrot in sheep.¹⁶ Despite the presence of both *D. nodosus* strain types in two clinically affected Alpine ibex, the authors reported that the infection with *D. nodosus* in Swiss free-ranging wild ungulates is only sporadic and thus maintenance and spread of this pathogen is unlikely. In accordance with these findings, in our study none of the examined species, involving three Swiss, five European, two domestic and a single exotic wild ungulate species, showed any clinical signs of footrot (all scored as 0). The absence of clinical footrot was further confirmed by the results of the competitive real-time PCR that showed the absence of both benign (*aprB2*-positive) and virulent (*aprV2*-positive) strains of *D. nodosus*. However, to prove a continuous footrot-free status, the biosecurity measures already in place and regular sampling and testing need to be continued. As a biosecurity measure, every even-toed ungulate that is introduced to Berne Animal Park passes through a standardized quarantine, and in this period, the feet are examined for possible foot pathogens.

Sheep are primary hosts for both virulent and benign *D. nodosus*, while cattle carry benign *D. nodosus*.² Previous studies showed a high prevalence (up to 88%) of benign *D. nodosus*, but absence of virulent *D. nodosus* in the Swiss cattle population,^{1,2} which seems epidemiologically irrelevant considering the fact that the national footrot eradication program of sheep focuses on the elimination of virulent strains (*aprV2*-positive) only. However, special attention is warranted when keeping sheep with other potentially susceptible ungulate species such as ibex and mouflon (*Ovis orientalis orientalis*).

Foot lesions were detected in 5/11 species and mainly consisted of WLD, apart from single cases of horn fissures and scissor claws. In cattle, these conditions are known as non-infectious foot lesions that affect the claw horn by multifactorial causes such as metabolic changes around calving associated with weakening of the connective tissue of the hoof suspensory structures, low body condition, poor claw conformation, poor flooring conditions, low digital cushion thickness and prolonged intervals of foot trimming.^{5,7,10,19,20} Based on the results of this study, regular foot trimming has been established for Cretan wild goats, dwarf goats and muskox.

In 9/10 European bison, dermatitis of the interdigital cleft of unknown origin was diagnosed, and research is currently in progress to determine the etiology of these lesions.

In conclusion, the absence of clinical signs of footrot and the negative results of the competitive real-time PCR for both, the benign (*aprB2*-positive) and virulent (*aprV2*-positive) strains of *D. nodosus* in 11 different even-toed ungulate species from Berne Animal Park provide useful information for the management of the upcoming nationwide footrot control program of domestic sheep in Switzerland. Our data suggest that captive wild even-toed ungulates do not pose a risk to the program.

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Santé des onglons et prévalence de *Dichelobacter nodosus* chez 11 espèces d'ongulés au parc animalier de Berne

Dichelobacter nodosus (*D. nodosus*) est l'agent étiologique du piétin chez les ruminants, qui affecte principalement les moutons dans le monde, mais aussi les ongulés sauvages en liberté tels que les bouquetins (*Capra ibex ibex*) et les mouflons (*Ovis orientalis orientalis*). Un programme d'éradication du piétin ovin à l'échelle Nationale, basé sur des tests PCR (réaction de polymérisation en chaîne) d'écouvillons de l'espace interdigité et des pédiluves réguliers, est prévu dans les années à venir en Suisse. Dans cette étude transversale, nous avons évalué cliniquement la santé des onglons et recherché la présence de *D. nodosus* chez 11 espèces différentes d'animaux biongulés (principalement des espèces européennes) pendant une période de 13 mois (2018–2019) au Parc animalier de Berne. Les lésions des onglons ont été notées pour tout signe clinique de pathologie et de présence de piétin, comme cela est décrit chez les bovins et les moutons et en utilisant une échelle de 0 à 5 (où 0 décrit des pieds cliniquement sains et 5 la perte de la boîte cornée). Des écouvillons ont été prélevés dans l'espace interdigité des 4 pieds sur un total de 53 animaux et soumis à des tests PCR en temps réel pour détecter *D. nodosus*. Des lésions aux onglons ont été détectées chez cinq espèces différentes. Chez 3 boeufs

Salute dell'unghione e prevalenza del *Dichelobacter nodosus* in 11 specie di ungulati nel parco zoologico di Berna

Dichelobacter nodosus (*D. nodosus*) è l'agente eziologico della zoppina ovina, che colpisce un gran numero di pecore nel mondo, ma anche ungulati selvatici come lo stambecco (*Capra ibex ibex*) e il muflone (*Ovis orientalis orientalis*). In futuro, è previsto un piano di eradicazione per la zoppina ovina a livello nazionale, mediante lo screening molecolare (polymerase chain reaction-PCR) di tamponi interdigitali e il trattamento con bagni medicati. Nel presente studio trasversale (cross-sectional), realizzato allo Zoo di Berna e durato 13 mesi (2018–2019), abbiamo esaminato clinicamente le condizioni del piede di 11 diverse specie di ungulati (con prevalenza di specie Europee) e investigato la presenza di *D. nodosus*. Le lesioni del piede sono state classificate in base alla presenza di qualsiasi segno clinico, analogamente a quanto avviene per i bovini, e contemporaneamente per ogni lesione riconducibile a zoppina, analogamente a quanto avviene nelle pecore, usando una scala da 0 a 5 (dove 0 corrisponde un piede clinicamente sano e 5 la perdita del rivestimento corneo). In totale, sono stati raccolti ed analizzati per la presenza di *D. nodosus* tramite real-time PCR, 4 tamponi interdigitali, raccolti da ciascuno di 53 animali. Lesioni podali sono state rilevate in cinque specie differenti. In 3 su 5 buoi muschiati (*Ovis moschatatus wardi*), in 7 su 12 capre cretesi (*Capra hircus*

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musqués (*Ovibos moschatus wardi*) sur 5, 7 chèvres sauvages crétoises (*Capra hircus cretica*) sur 12 et 2 chèvres naines (*Capra hircus aegagrus*) sur 3, il s'agissait principalement de lésions de la ligne blanche, alors que dans 9 bisons sur 10, le diagnostic était une dermatite interdigitale. Un alpaga sur 3 a été diagnostiqué avec une gale chorioptique de la zone du paturon. Aucun des animaux examinés n'a montré de signes cliniques de piétin (score 0) et aucun *D. nodosus* bénin (aprB2-positif) ou virulent (aprV2-positif) n'a été mis en évidence dans les échantillons. Cette étude fournit des informations supplémentaires pour améliorer l'efficacité du programme d'éradication du piétin ovin et suggère que les biongulés sauvages détenus dans les parcs zoologiques ne présentent pas de risque pour le programme d'éradication prévu en Suisse.

Mots clés: Ongulés sauvages en captivité et ongulés domestiques, *Dichelobacter nodosus*, santé des onglons, PCR, boiterie

cretica) e in 2 su 3 capre domestiche (*Capra hircus aegagrus*), e' stata osservata principalmente la malattia della linea bianca, mentre in 9 su 10 bisonti Europei, e' stata diagnosticata la dermatite interdigitale. In 1 su 3 alpaca e' stata diagnosticata la rogna corioptica dell'area dello tallone. Nessuno degli animali esaminati ha mostrato segni sovrapponibili a zoppina (punteggio 0), e ne il ceppo benigno (aprB2-positivo), ne quello virulento (aprV2-positivo) di *D. nodosus* sono stati rilevati in alcun campione. Questo studio fornisce delle ulteriori informazioni rilevanti a facilitare il programma di controllo della zoppina in Svizzera e suggerisce che gli ungulate selvatici in cattività non rappresentano un rischio per il programma di controllo della zoppina.

Parole chiave: ungulati selvatici in cattività e domestici, *Dichelobacter nodosus*, salute dell'unghione, PCR, zoppia

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Foot health and prevalence of *Dichelobacter nodosus* in 11 ungulate species at Berne Animal Park

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