Outbreak of severe foot rot associated with benign *Dichelobacter nodosus* in an Alpine ibex colony in the Swiss Prealps

J. Wimmershoff¹, M.-P. Ryser-Degiorgis¹, N. Marreros¹, J. Frey², P. Romanens³, K. Gendron⁴, F. C. Origgi¹

¹Centre for Fish and Wildlife Health and ²Institute of Veterinary Bacteriology, Vetsuisse-Faculty, University of Bern, ³Service des Forêts et de la Faune, Givisiez, ⁴Clinical Radiology Division, Vetsuisse-Faculty, University of Bern

Abstract

An outbreak of foot rot occurred in the ibex colony “Vanil Noir” in Switzerland from May to December 2014. This article describes field observations and the analyses carried out on the limbs of 3 animals submitted for postmortem examination. Disease signs observed in the field included lameness, poor body condition and overgrown hooves. Macroscopic examination of selected limbs revealed severe lesions in all of them, including interdigital inflammation with ulceration and malodorous exudation. Histological changes were consistent with chronic laminitis with bone resorption, which was not detected at radiographical examination. Grocott-positive organisms compatible with *Dichelobacter nodosus* were detected in the lesions. Samples collected from the lesions were positive by polymerase chain reaction for benign *D. nodosus*, which is typically associated with only mild lesions in domestic sheep. Whether *D. nodosus* is endemic in the colony or had previously been transmitted from sympatric domestic livestock is unclear. The unusual warm and humid weather conditions in 2014 may well have contributed to the outbreak.

Keywords: Capra ibex ibex, Dichelobacter nodosus, histology, PCR, radiography

Ausbruch von schwerer Moderhinke in Verbindung mit benignem *Dichelobacter nodosus* in einer Alpensteinbockkolonie in den Schweizer Voralpen


Schlüsselwörter: Capra ibex ibex, Dichelobacter nodosus, Histologie, PCR, Radiologie
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Introduction

The Alpine ibex (*Capra ibex*, Fig. 1) had disappeared from the Alpine massif between the 16th and 19th century, except in the region of the Gran Paradiso National Park in Italy. First reintroductions into Switzerland took place in 1911 and, although the species is still classified as protected, it has been subject to controlled hunting since 1977 (Hindenland and Nievergelt, 1995). Currently, the ibex population in Switzerland is estimated at 17,000 animals distributed among distinct colonies of various sizes (Swiss Federal Hunting Statistics, 2013). Interactions between ibex and domestic livestock as well as other wild ruminants regularly occur on Alpine pastures (Casaubon et al., 2012) and interspecific pathogen transmission has been documented in Switzerland and elsewhere (Belloy et al., 2007).

Wild ruminants can be affected by a range of foot diseases. Reports include foot and mouth disease in various species (Vosloo et al., 2009; Caron et al., 2013), necrobacillosis in Norwegian reindeer (*Rangifer tarandus tarandus*; Handeland et al., 2010), *Treponema* infection in north-American elks (*Cervus elaphus nelsoni*; Clegg et al., 2015) and foot rot in mullflon (*Ovis aries musimon*) and Alpine ibex (Belloy et al., 2007). Foot rot is an infectious bacterial disease affecting the hooves of both domestic and wild ungulates (Belloy et al., 2007; Ginn et al., 2007; Volmer et al., 2008). The disease occurs worldwide, particularly in high rainfall areas with temperate climate (Ginn et al., 2007). A pool of microorganisms, including *Dichelobacter nodosus*, *Fusobacterium necrophorum*, aerobic diphteroides, coliformes and other common bacteria found in the soil, on animal skin and in feces have been shown to contribute to the development of the disease. However, the Gram negative, obligate anaerobe bacterium *D. nodosus* is considered to be the main etiologic agent (Ginn et al., 2007; Witcomb et al., 2014).

Foot rot occurs following maceration and devitalization of the interdigital skin of the ruminant foot with subsequent invasion of bacteria. Interdigital skin damage may be favored by continuous exposure to wet pastures and the occurrence of minor wounds or abrasions of traumatic origin (Ginn et al., 2007). Three forms of clinical foot rot have been described, i.e. benign, intermediate and virulent (Green et al., 2008). The benign and virulent forms refer to the severity of lesions at individual level. They are indistinguishable in their early stages, which are characterized by pale discoloration, edema and erosions normally affecting only the caudal portion of the interdigital cleft, but in case of virulent foot rot, progression of the inflammation leads to separation of the horn (under-running) in association with a typical mild exudation of a gray, malodorous material (Abbott et al., 2005; Ginn et al., 2007). In addition, some authors mention an intermediate form of foot rot at herd level in sheep, which refers to outbreaks with only a moderate number of animals developing the severe under-running lesions and a relatively minor impact on the overall health and productivity of the affected sheep herds as compared to outbreaks in which virulent foot rot predominates (Abbott and Egerton, 2003). The benign and virulent forms of foot rot have been shown to correlate with the presence of strains of *D. nodosus* carrying either the *aprB2* or *aprV2* genes (respectively) which encode for proteases playing a pivotal role in strain virulence (Kennan et al., 2014; Stäuble et al., 2014a). Foot rot is a frequent disease in domestic sheep but cattle, domestic goats and even pigs can also be affected or at least act as *D. nodosus* carriers (Depiazzi et al., 1998; Ginn et al., 2007). The disease is endemic in sheep flocks worldwide, including in Switzerland. As there is appar-

![Figure 1: Group of adult male Alpine ibex of the colony Vanil Noir, canton of Fribourg.](image-url)
ently an absence of long term immunity, reinfections and recurrences are frequent (Egerton and Roberts, 1971). However, although sheep are considered to be the main reservoir of *D. nodosus*, virulent isolates may persist for up to 10 months on bovine feet after co-grazing with sheep. Furthermore, one grazing season without contact with sheep is insufficient to eliminate all of the pathogenic isolates from the cattle feet (Knappe-Poindecker et al., 2014).

In wild ruminants, foot rot has to date been poorly described. Epidemics of pododermatitis have been reported to affect free-ranging Alpine ibex and mouflon populations in different European Alpine countries including France, Austria, Germany and Switzerland (Deletraz, 2002; Belloy et al., 2007; Volmer et al., 2008) but the presence of *D. nodosus* has so far only been demonstrated in affected feet of mouflon in Germany and ibex in Switzerland (Belloy et al., 2007) and post-mortem investigations have been limited to a macroscopic assessment of the lesions. The epidemiology of the disease has not yet been thoroughly investigated but domestic livestock is thought to be the main source of infection, and unusually mild and wet weather conditions are suspected to contribute to the occurrence of outbreaks (Deletraz, 2002; Belloy et al. 2007). Here we report an outbreak of foot rot in Alpine ibex in Switzerland and the analyses conducted on the limbs of 3 dead animals, including radiologic, histopathological and bacteriological investigations. We then discuss the potential origin of the outbreak.

Field observations

The outbreak occurred in the ibex colony “Vanil Noir” in the Swiss Prealps. This is a relatively small colony under a low hunting pressure. It consists of approximately 200 animals (direct counts performed by cantonal game warden). The region of Vanil Noir is located in a protected natural area of about 15 km², which is a popular hiking site. The region corresponds to the mountain formation around the central peak called Vanil Noir (2,389 m above sea level). From this peak, a single mountain range of about 4 km extends to the south west and two parallel ranges separated by the valley of Morteyes extend to the north-east. The range north of this valley (about 4.5 km) stretches across the Selle des Morteyes (2,129) and Dent de Brenleire (2,352 m) and the southern range (about 2.5 km) stretches across the Dent des Bimis (2,161 m). Ibex share their habitat with an estimated number of 250 Alpine chamois (*Rupicapra r. rupicapra*) and small-sized wildlife. In summer male ibex are mainly found on the northern mountain range to the north-east of Vanil Noir and in the fall they migrate to the southern mountain range for the rut.

Domestic livestock is taken to the pastures each summer for the grazing season. Three large flocks of domestic sheep occupy the alpine pastures to the west and south of the peak of Vanil Noir. Furthermore, a cattle herd and a few domestic goats graze on the pastures close to the chalet des Morteyes at the south-western end of the valley of Morteyes for 3 to 6 weeks approximately from the end of July/beginning of August. In August and September a herd of heifers with a history of recurrent foot disorders go to the higher pastures directly below the Dent de Brenleire in the valley of Morteyes. Although direct interactions between ibex and sheep do not occur (no habitat overlap in summer) and contacts with cattle and goats (which remain at the bottom of the valley) are unlikely, ibex have been observed intermixed with heifers.

Ibex with signs of foot rot were observed by the local game warden from May to October 2014 between Selle des Morteyes and Dent de Brenleire, mainly on the slope of the valley of Morteyes. Between the end of October and the beginning of December, two affected males with similar clinical signs were observed south of the Vanil Noir peak (in the area of the sheep grazing pastures).

Between May 11 and October 16, 2014, a total of 8 ibex with signs of foot rot were found dead (n=2) or were culled (n=6) in the valley of Morteyes due to severe deterioration of body condition and abnormal behavior suggestive of an advanced disease stage with a probable fatal outcome. All these animals were adult males between 3.5 and 11 years old (median: 8.5 years) showing deformation of the hoof walls. If not dead, they were unable to walk normally and were found either on mountain ridges or at the bottom of the valley of Morteyes. They were lame or standing still, avoided touching the ground with one or the other foot, or else they were grazing on their carpal joints.

Eight limbs from 3 animals (ibex A: 10 years old, found dead on August 29, 2014; ibex B: 8 years old, culled on August 29, 2014; and ibex C: 9 years old, culled on September 1, 2014) were submitted for analysis to the Centre for Fish and Wildlife Health (FIWI) of the University of Bern. More specifically, they consisted of both hind limbs of ibex A, both front limbs of ibex B and all four limbs of ibex C.

Post-mortem investigations

Macroscopic examination

All examined limbs showed moderately moistened and ulcerated skin in the interdigital cleft with grey discoloration (Fig. 2A, B) associated with a strong unpleasant odor. In both hind limbs of ibex A, with the left one...
most severely affected, the subcutaneous tissue showed multiple, well defined foci (approximately 5 mm in diameter), containing light yellow to light tan, pasty material on longitudinal section. All limbs showed hoof walls moderately worn out and extending beyond the sole (Fig. 2C, D, E), with the most severe changes in ibex C, where in the front limbs they extended up to 12 cm and were multifocally fissurated, clefted and separated from the corium (Fig. 2F).

**Radiography**
A radiographical examination (Fuji CR System, Fujifilm Medical Systems, Düsseldorf, Germany) was carried out in two planes on the four limbs of ibex C without further preparation or paring of the overgrown hooves. The distal aspect of the medial P3 of the right front limb showed reduced sharpness of its profile and the interdigital tissues of the hind limbs were mildly swollen. No obvious changes of the bone structures were observed.

**Histopathology**
Histopathological examination was performed on the left hind limb of ibex A and on the right hind limb of ibex C. Findings in ibex A comprised vacuolization and degeneration of keratinocytes with spongiosis and neutrophilic infiltration at the level of the coronary band, where a deep clefting was also present. Multifocally, there was mild thickening of the arterioles of the dermal lamina with minimal associated perivascular mononuclear infiltrate. Deeper in the corion there was multifocal moderate perivascular edema associated with the main vessels, which were also surrounded by numerous others of smaller caliber embedded in abundant fibrous tissue. Frequently, only small caliber vessels were seen in these fibrous aggregates. Dorsally to P3 there was severe mixed inflammatory infiltrate, rimming locally the bone surface where multiple osteoclasts in Howship’s lacunae were seen, consistent with bone resorption (Fig. 3A). Grocott stain revealed the presence of few intralobular bacilli ranging from 1 to 5 µm in length and less than 1 µm in width morphologically compatible with *D. nodosus* (Fig. 3B). Similar changes were seen in ibex C. More specifically there was severe infiltration mainly of neutrophils and mixed mononuclear cells in the space defined by the distal portion of P2, the extensor tendon and the proximal aspect of P3, with only mild involvement of the joint space. Accumulation of osteoclasts with mild osteolysis was present in the bone of the affected portion of P3. The vascular changes observed in the dermal lamina were more severe than those seen in ibex A with also moderate vacuolization of the tunica media and adventitia of few arterioles. Grocott stain revealed few intralobular bacilli similar to those observed in ibex A. *Treponema*-like organisms were not detected with Warthin-Starry staining.

**Bacteriology**
Specimens for bacteriological examination were taken using dry sterile cotton swabs which were rubbed on the humid altered interdigital skin (one swab per foot) and immediately soaked for approximately one minute in 1 ml SV-lysis buffer (4 M guanidinethiocyanate, 0.01 M Tris–HCl, 1% b-mercaptoethanol), and finally discarded (Stäuble et al., 2014b). A total of 8 swabs (one per foot) were analysed by competitive real-time polymerase chain reaction (PCR) based on allelic discrimination of the protease genes *aprV2* and *aprB2*, which permits direct detection and discrimination of virulent and benign *D. nodosus* in a single test. Amplification was carried out in a 7500 Real-Time PCR-System thermocycler (Applied biosystems, Foster City, CA) using cycles...
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This was confirmed by pathological and bacteriological examination. All of the animals examined either in the field or in the laboratory showed the severe form of the disease, including interdigital inflammation, under-running lesions and typical malodorous exudation. The observed behavioral changes and macroscopic lesions were consistent with those described in domestic animals and previously reported in free-ranging ibex and mouflon (Deletraz, 2002; Belloy et al., 2007; Volmer et al., 2008). Histologically, we observed the presence of chronic lesions affecting the dermal lamina and the deep corion which were consistent with the gross lesions, and there were intralesional bacteria morphologically compatible with *D. nodosus*. Interestingly, we observed the presence of mild bone lesions and vascular changes similar to those reported in elks with *Treponema* infection (*Treponema medium*/*Treponema vincentii*-like and *Treponema phagedenis*-like digital dermatitis spirochetes; Han and Mansfield, 2014; Clegg et al., 2015). The absence of corresponding lesions on radiographs suggested that bone alterations were relatively recent, i.e. that these lesions only appeared in an advanced disease stage. However, differently from the elks affected with *Treponema*, in the ibex there were also clear spaces in the perivascular areas of the deep corion along with increased fibrous tissue. We interpreted these changes as chronic edema consistent with impaired blood flow in the affected tissues. Such lesions are expected to be very painful, explaining the impaired mobility of the affected ibex as well as the resulting reduced ware of the claw horn, reduced food intake and associated weight loss.

Strikingly, *D. nodosus* detected in the lesions of the 3 animals examined were of the benign type. This is in contradiction with recent data on sheep showing that the detection of the virulent and benign *D. nodosus* strains fully correlates with the clinical status of the individual sheep or the foot rot history of the herd (Stäuble et al., 2014a, b). The lesions in the examined ibex were severe independently of the bacterial load. This has also been observed in domestic sheep: Although the amount of bacteria seems to be an important factor influencing the disease course, once the disease is clinically evident loads of *D. nodosus* are independent of clinical signs (Witcomb et al., 2014, Stäuble et al., 2014a). It is possible that wild species may be more prone than sheep to develop disease and that the reported association between the type of *D. nodosus* strains and the clinical form of foot rot might apply to sheep but not necessarily to other hosts. Similarly, infectious keratoconjunctivitis caused by *Mycoplasma conjunctivae* is known to be generally characterized by a less severe disease course in sheep than in wild *Caprinae* (Giacometti et al., 2002) and the occurrence and severity of clinical signs vary among species depending on the strain involved (Mavrot et al., 2012). Another potential explanation for

**Discussion**

Clinical field observations including behavioral changes and macroscopic foot lesions in several ibex from the colony “Vanil Noir” suggested an outbreak of foot rot.
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the severe foot lesions observed in ibex is a co-infection with other pathogens such as Treponema. Co-infections with D. nodosus and Treponema have been proposed to have a synergetic effect in the pathogenesis of digital dermatitis in cattle (Strub et al., 2007; Rasmussen et al., 2012). However, we found no histological evidence for Treponema in the examined ibex. Whether these animals were predisposed to developing severe lesions due to a preexisting or underlying disorder is unknown, as only their limbs were submitted for analysis.

Only adult male ibex were observed with foot rot in this outbreak. This is in accordance with previous observations in the Swiss and French Alps (Deletraz, 2002; Belloy et al. 2007). Adult male ibex live in groups separated from adult females and younger animals of both sexes and groups of adult males are more often observed on lower humid pastures where they come in contact with domestic sheep and other livestock (Bon et al., 2001). It has been proposed that this behavior may be the origin of a higher exposure of adult males to D. nodosus introduced on the pastures by domestic livestock and to humidity favoring foot skin damage, compared to other sex/age classes (Belloy et al., 2007). Dichelobacter nodosus is reported to survive on pastures for up to 10 days (Green et al., 2008), making transmission possible among sympatric species, and its optimal survival occurs in temperate climates (>10°C) with damp conditions (Whittington, 1995). Furthermore, both experimental trials and field studies have shown that D. nodosus is not host-specific (Belloy et al., 2007; Ghimire et al., 1999; Knapp-Poindecker et al., 2014), and the ibex is phylogenetically closely related to domestic goats and sheep (Hernández Fernández and Vrba, 2005). It is therefore reasonable to assume that a transmission between domestic ruminants and ibex could occur. In the present outbreak the sequence of events (observation of foot rot in ibex before the arrival of livestock on the alpine pastures used by ibex) does not support this hypothesis. Furthermore earlier observations in the ibex colony by the previous game warden in charge suggest the possible former occurrence of sporadic cases of foot rot. Cattle may have previously acted as a source of infection for ibex, as close interactions with heifers do occur in the valley of Mortey and cattle can be carriers of D. nodosus (Knapp-Poindecker, 2014). However, it is not possible to exclude an endemic occurrence of the bacteria in the ibex colony with only sporadic occurrence of clinical cases. The prevalence of D. nodosus infections in wild populations with and without disease occurrence has not yet been investigated and the dynamics of infection at the wildlife/livestock interface is largely unknown. The unusually humid weather conditions in Switzerland in 2014 might have contributed to a higher incidence of cases. The year 2014 was the warmest since the beginning of measurements in 1864, with a particularly mild winter followed by a summer season with extreme rainfall and cool temperatures and a warm fall (MeteoSchweiz, 2014). This may have favored D. nodosus transmission within ibex social groups by increasing the survival of the bacteria on the pastures and damaging the hooves of the animals.

To our knowledge, this is the first report of a foot rot outbreak in wildlife which includes a detailed histopathological description, radiological investigations and bacteriological examinations distinguishing between benign and virulent D. nodosus. Importantly, we document a severe disease form with fatal consequence in wildlife associated with benign D. nodosus. Our study underlines the lack of information on the spread of D. nodosus and occurrence of clinical disease in domestic and wild species, on the factors contributing to the severity of clinical disease, and on the potential role of other livestock species than sheep and other wild ruminants such as chamois (which are not known to be affected by foot rot) in the epidemiology of the disease.

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Epidémie de piétain grave associée avec *Dichelobacter nodosus* de type bénin dans une colonie de bouquetins dans les Préalpes suisses

Une épidémie de piétain s’est déclarée dans la colonie de bouquetins du "Vanil Noir" en Suisse de mai à décembre 2014. Cet article décrit les observations de terrain et les analyses effectuées sur les membres de 3 animaux soumis à un examen post-mortem. Les signes de maladie observés sur le terrain comprenaient boiterie, amaigrissement et croissance démesurée de la corne des ongulés. L’examen macroscopique des membres envoyés pour analyse a révélé des lésions graves sur chacun d’eux, y compris une inflammation interdigitale avec ulceration et une exudation malodorante. Les lésions histologiques concordaient avec une lamine chronique avec résorption osseuse, celle-ci n’ayant pas été détectée à l’examen radiographique. Des organismes positifs à la coloration de Grocott et compatibles avec *Dichelobacter nodosus* ont été détectés dans les lésions. Les échantillons prélevés dans les lésions et testés par réaction en chaîne par polymérase se sont avérés positifs pour *D. nodosus* de type bénin, qui est typiquement associé à des lésions mineures chez les moutons domestiques. Il n’a pas été possible de déterminer si *D. nodosus* est endémique dans la colonie de bouquetin ou s’il a été transmis dans le passé par du bétail domestique en estive sur le domaine de la colonie. Les conditions météorologiques anormales le long de l’été 2014 auraient pu contribuer à l’apparition de l’épidémie.

Un outbreak di forma grave di “zoppina” associata al tipo benigno di *Dichelobacter nodosus* in una colonia di stambecchi delle prealpi Svizzere

Tra il Maggio e il Dicembre 2014, si è manifestato un’outbreak di “zoppina” nella colonia di stambecchi “Vanil Noir” in Svizzera. Questo articolo descrive le osservazioni fatte sul campo e i risultati delle analisi condotte sugli arti di 3 animali che erano stati inviati per un’indagine anatomo-patologica. I segni della malattia osservati sul campo comprendevano, zoppia, dimagrimento e crescita eccessiva degli unghii. L’esame macroscopico ha rivelato la presenza di lesioni severe in tutti gli arti ispezionati come l’inflammazione nello spazio interdigitale con ulcerazione e odore pungente. L’indagine istologica ha rivelato lesioni compatibili con lamine cronica con riassorbimento osseo non evidente all’esame radiologico. La colorazione di Grocott ha rivelato la presenza di batteri morfologicamente compatibili con *Dichelobacter nodosus* nelle lesioni. Campioni raccolti dalle lesioni sono risultati positivi alla reazione di polimerizzazione a catena (PCR) per il tipo benigno di *D. nodosus*, che è tipicamente associato solo a lesioni di lieve entità nelle pecore domestiche. Se il *D. nodosus* sia endemico nella colonia o se sia stato trasmesso in precedenza da animali domestici simpatrici, non è chiaro. Il clima insolitamente caldo e umido del 2014 potrebbe aver contribuito all’insorgenza dell’outbreak.

References


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Corresponding author

PD Dr. med. vet. Marie-Pierre Ryser-Degiori, Dipl. ECZM (WPH)
Centre for Fish and Wildlife Health (FIWI)
Dept. Infectious Diseases and Pathobiology
Vetsuisse Faculty, University of Bern
Postfach 8466, Länggass-Str. 122
CH-3001 Bern, Switzerland
Tel. +41 31 631 24 43; Fax +41 31 631 24 43
E-Mail: marie-pierre.ryser@vetsuisse.unibe.ch