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# Caseous Lymphadenitis Caused by *Corynebacterium pseudotuberculosis* in Alpine Chamois (*Rupicapra r. rupicapra*): a Review of 98 Cases

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## Summary

*Corynebacterium pseudotuberculosis* is the causative agent of caseous lymphadenitis (CLA) in domestic and wild ruminants. Here we describe CLA in alpine chamois (*Rupicapra r. rupicapra*) based on a series of 98 cases of *C. pseudotuberculosis* infection confirmed by bacteriology and gene sequence analysis. The population included 53 males and 45 females distributed within three age groups: up to 18 months ($n = 14$), 18 months to 4 years ($n = 11$) and over 4 years ($n = 73$). Four different gross lesion distribution patterns, observed individually or variably combined in the same animal, were defined: (1) cutaneous/external (i.e. subcutaneous lymph nodes with or without muscle involvement, $n = 34$); (2) abdominal visceral (i.e. only abdominal organs involved: liver and/or spleen and/or kidney and/or lymph nodes, $n = 35$); (3) thoracic visceral (i.e. only thoracic organs involved: lung and/or heart and/or lymph nodes, $n = 26$); and (4) generalized visceral (i.e. abdominal and thoracic organs involved, $n = 26$). In six particularly severe cases, mammary gland, testis, vertebral bone and the central nervous system were also affected. Macroscopically, most abscesses were characterized by fluid pus, confirmed by microscopy that showed the absence of distinct concentric layers and coagulative necrosis, which are typically seen in sheep and goats raised in areas where the infection is endemic. In three cases amyloid deposits were observed in the liver and kidney. The *C. pseudotuberculosis* strains isolated were highly homologous to the reference strain ATCC 19410, except for some variability in their ability to ferment maltose and mannitol. Based on the production of nitrate reductase, 95 strains were attributed to the *ovis* biovar (nitrate reduction negative) and three to the *equi* biovar (nitrate reduction positive). All strains were sensitive to antibiotics, except to ampicillin (62.3% resistant strains) and gentamicin (83.7% resistant strains). Using an indirect enzyme-linked immunosorbent assay designed for CLA in sheep and goats, seven (58.3%) of 12 serum samples tested positive for antibodies.



*Keywords:* caseous lymphadenitis; chamois; *Corynebacterium pseudotuberculosis*; *Rupicapra r. rupicapra*

## Introduction

Caseous lymphadenitis (CLA), also termed pseudotuberculosis owing to its similarity with tuberculosis lesions, is an infectious disease affecting domestic and wild ruminants worldwide. Considered a minor zoonosis in people exposed occupationally to infected animals (Romero-Pérez *et al.*, 2004; Join-Lambert *et al.*, 2006; Heggelund *et al.*, 2015), CLA causes considerable economic losses to the sheep meat industry due to condemnation and downgrading of carcasses, decreased meat yield and reduced wool production (Paton *et al.*, 1988). The aetiological agent, isolated for the first time in 1888 by the French

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bacteriologist Nocard from a case of bovine lymphangitis and some 3 years later by the Bulgarian bacteriologist von Preïsz from a ewe (Preïsz and Guinard, 1891; Nocard, 1896), is *Corynebacterium pseudotuberculosis*, a gram-positive and catalase-positive coccobacillus of the order Actinomycetales, which also contains the genera *Mycobacterium*, *Rhodococcus* and *Nocardia*. Two subtypes are distinguished according to nitrate reductase production: *equi* biovar (isolated from cattle and horses; nitrate reduction positive) and *ovis* biovar (isolated from goats and sheep; nitrate reduction negative) (Biberstein *et al.*, 1971; Songer *et al.*, 1988). The main virulence factor of *C. pseudotuberculosis* is phospholipase D, an enzyme that catalyzes the breakdown of proteins and lipids in the cell membrane. In small domestic ruminants, it causes the formation of purulent encapsulated abscesses with a caseous, lamellated ('onion ring') appearance, which can develop in peripheral lymph nodes (cutaneous or external form) or internal organs and lymph nodes (visceral or internal form).

In wildlife species (free-ranging or in captivity), CLA has been reported in alpine ibex (*Capra ibex*) (Silinski and Walzer, 2004), Spanish ibex (*Capra pyrenaica*) (Cadena-Colom *et al.*, 2014), alpine chamois (*Rupicapra r. rupicapra*) (Bassano *et al.*, 1993), fallow deer (*Dama dama*) (Pérez *et al.*, 1996), red deer (*Cervus elaphus*) (Matos *et al.*, 2015), elk (*Cervus canadensis nelsoni*) (Jane Kelly *et al.*, 2012), white-tailed deer (*Odocoilecus virginianus*) (Stauber *et al.*, 1973), pronghorn antelope (*Antilocapra americana*) (Clark *et al.*, 1972), huemul (*Hippocamelus bisulcus*) (Morales *et al.*, 2017), white-tailed gnu (*Connochaetes gnou*) (Müller *et al.*, 2011), Arabian oryx (*Oryx leucorix*) (Tarello and Theneyan, 2008), cheetah (*Acinonyx jubatus*) (Boomker and Henton, 1980) and the aardvark (*Orycteropus afer*) (Roth and Vickers, 1966).

Sporadic case reports of CLA in chamois are found in the literature (Bassano *et al.*, 1993). Herein we describe CLA in alpine chamois based on a series of 98 confirmed cases of *C. pseudotuberculosis* infection, with a focus on gross and microscopical lesions, phenotypic and biochemical features, and antibiotic sensitivity of the strains isolated, as well as the serological response to *C. pseudotuberculosis* in some of the infected animals.

## Materials and Methods

### *Sampling*

We reviewed 98 cases of CLA in alpine chamois, collected over the past 16 years according to the availability of anamnestic data (i.e. sex, age and origin), description of gross lesions (i.e. type and distribution) and bacteriological test results (i.e. strain isolation and characterization). The animals came mainly from the Aosta Valley, a mountainous region of northwestern Italy, and to a lesser extent from bordering Piedmont (altitude 1,000–2,500 m). The provenance of the chamois included: (1) hunted with carcass fit for consumption ($n = 45$); (2) hunted, but with carcass condemnation due to widespread lesions and/or poor condition ($n = 32$); or (3) found dead or culled for health reasons ($n = 21$), generally due to starvation (often associated with hyporeactivity). Before necropsy examination, biometric data were recorded for age estimated from horn growth (range <1 year to 16 years) (Lovari, 1985). Three age groups were defined according to male reproductive physiology: group I, aged <18 months (age at which the male reaches maturity); group II, aged between 18 months and 4 years (age range when the sexually mature male generally does not reproduce due to competitiveness inside the herd); and group III, aged over 4 years (age when the male can mate and breed).

### *Necropsy Examination*

The suspected CLA lesions (typically the presence of yellow–green pus in lymph nodes and/or organs) were recorded according to the following groups: (1) cutaneous/external form (i.e. subcutaneous lymph nodes with or without muscle involvement); (2) abdominal visceral form (i.e. only abdominal organs involved: liver and/or spleen and/or kidney and/or lymph nodes); (3) thoracic visceral form (i.e. only thoracic organs involved: lung and/or heart and/or lymph nodes); and (4) generalized visceral form (i.e. abdominal and thoracic organs involved). More severe cases with bone and/or cerebral involvement or suppurative lesions in mammary gland and testis were also recorded.

### *Bacteriology and Antimicrobial Susceptibility*

The gross lesions of all animals were subjected to bacteriological examination for confirmation of macroscopical suspicion of CLA. Bacterial culture was performed using blood agar and MacConkey agar, with aerobic incubation at 37°C for up to 72 h. Typical colonies were confirmed initially by Gram staining and the catalase test, then subsequently identified with the API Coryne® system (Biomérieux, La Balme les Grottes, France). Once identified, the *C. pseudotuberculosis* strains were subjected to a Kirby–Bauer antibiogram based on measurement of the zone of inhibition of bacterial growth around the antimicrobial strips at known concentrations (according to CLSI guideline M45, 3rd