

Monitoring of *pseudotuberculosis* in an Italian Population of Alpine Chamois: Preliminary Results

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Abstract

Caseous lymphadenitis (CLA) of sheep and goats is a chronic and often sub-clinical disease, with high prevalence in different parts of the world, which can cause significant economic losses for farmers. The causative agent is *Corynebacterium pseudotuberculosis* that primarily infects domestic small ruminants, but it has been isolated also in wildlife such as pronghorns (*Antilocapra americana*) and elk (*Cervus elaphus canadensis*). Furthermore, a recent research has demonstrated a maintenance of the infection on an endemic level in a Spanish ibex (*Capra pyrenaica hispanica*) population after an outbreak with high morbidity and mortality. The typical clinical manifestation is lymph node abscess that may fistulize and discharge pus contaminating the environment where can survive for months in faeces, fomites, and soil. Infected subjects with the sub-clinical form can also shed bacteria through respiratory tract and mechanical vectors such as flies. Human infection is a rare event. Wildlife-livestock interfaces are dynamic and bidirectional and pathogens could be transmitted freely within and between the two species. The study area is the Alpine hunting district located in Vercelli province (formerly named C.A. VC1). The study was carried out during 2016 hunting season, between September and December and performed on hunted chamois. A macroscopic examination of each carcass has been conducted at the Control Centre, where each hunter had to bring the chamois after culling for the control by a veterinarian. Hunters collected post-mortem-blood samples via jugular or heart clot from their own bag and led up to the Control Centre where serum was obtained by centrifugation and stored at -20°C until further processing. A commercial enzyme-linked immunosorbent assay (ELISA) kit (ELITEST CLA, HYPHEN BioMed, France) has been used to detect antibodies (IgG) anti-PLD. Results have been expressed as OD after reading the plates at 450 nm in an ELISA microplate reader. The aim of this monitoring is to confirm the circulation of *C. pseudotuberculosis* in the chamois population as suspected on the basis of the finding of several chamois with characteristic lesions during previous hunting seasons and to identify risk factors for the infection and the development of clinical signs of disease. 196 chamois have been hunted in 2016 hunting season, a total of 34 sera have been processed (1 kid, 7 yearlings, 17 adult males, 9 adult females) showing a seroprevalence of 8.82% (c.i. 95% 0.0-18.26). One yearling female and two adult females resulted seropositive; at the macroscopic examination the two adults had showed typical lymph node abscesses in abdominal cavity and the oldest was underweight and presented typical dermatophilosis lesions. Furthermore, a 4-year-old male showed a grey zone OD. The results of this first year survey on the territory are to be considered as preliminary, the starting point for a data collection that can become a historical series that could give useful management guidance in the near future. In spite of this, it can be safely stated that the first results obtained confirm the circulation of *C. pseudotuberculosis*, together with the pathological and bacteriological diagnoses of previous years. The fact that a yearling seropositive has been found without any lesions found is evidence of the current circulation of the pathogen as this subject has come into contact with the infection during the previous year.

Keywords: *Corynebacterium pseudotuberculosis*, Caseous lymphadenitis; Wildlife; Alpine chamois

Spanish ibex (*Capra pyrenaica hispanica*) population after an outbreak with high morbidity and mortality [5,6].

Introduction

Caseous lymphadenitis (CLA) of sheep and goats is a chronic and often sub-clinical disease, with high prevalence in different parts of the world, which can cause significant economic losses for farmers [1]. The causative agent is *Corynebacterium pseudotuberculosis*, a gram-positive facultatively anaerobic rod resembling a coccus [2]; primarily infects domestic small ruminants [1], but it has been isolated also in wildlife such as pronghorns (*Antilocapra americana*) [3] and elk (*Cervus elaphus canadensis*) [4]. Furthermore, a recent research has demonstrated a maintenance of the infection on an endemic level in a

The typical clinical manifestation is lymph node abscess that may fistulise and discharge pus contaminating the environment where can survive for months in faeces, fomites, and soil; then the infection occurs through oral, nasal and ocular mucosa or skin wounds. Infected subjects with the sub-clinical form also can shed bacteria through respiratory tract and mechanical vectors such as flies [7,8].

Human infection is a rare event and most of the reported cases have been related to occupational exposure [9] and ingestion of raw meat and milk [10].

Wildlife-livestock interfaces are dynamic and bidirectional and pathogens could be transmitted freely within and between the two species [11] as they come into direct and, above all, indirect contact in

a communal environment, through use of shared pastures and water and via vectors [12]. Their spatial behavior may be variable according to the species considering environmental conditions such as natural barriers, and human management of wild and domestic herds; in mountain pastures, these conditions can be compared [13].

Focusing attention on the Alps, the environment is probably one of the most valuable ecosystems, with a tricky balance [14].

Northern chamois (*Rupicapra rupicapra*) is widespread and has a large population of over 440 000 individuals. Although it is declining in some parts of its European and global range, the bulk of the population is found in the Alps and is relatively secure. Currently it is assessed as Least Concern by the Red List of Threatened Species of the International Union for Conservation of Nature (IUCN) [15]. Alpine chamois (*R. r. rupicapra*) inhabit steep, rocky areas in the mountains, utilizing a variety of habitats including alpine meadows, open rocky areas, mixed broadleaf woodland, and coniferous woodland [16]. This species feeds on grasses, herbs, leaves of trees, buds, shoots, and fungi [17]. Poaching and over-hunting may be a problem for the species in parts of its range, especially where it occurs outside protected areas and private hunting reserves [18,19]. Human disturbance, particularly as a result of increased tourism and leisure activities in mountain areas, may also be a problem [18,19]. Competition with domestic livestock and introduced species such as the mouflon (*Ovis musimon*) is a threat to the more vulnerable subspecies, although it is not considered to be a major problem for Alpine chamois. Alpine chamois does, however, suffer periodic outbreaks of sarcoptic mange, causing local population declines [18]. This species is widely hunted through its range. In the Alps, hunting is generally managed sustainably, but elsewhere it is a threat to the species.

The aim of this study is to confirm the circulation of *C. pseudotuberculosis* in a chamois population as suspected on the basis of the finding of several chamois with characteristic lesions during previous hunting seasons and to identify risk factors for the infection and the development of clinical signs of disease. This could give useful management guidance in the near future.

Materials and Methods

Study area and period

The study area is the Alpine hunting district located in Vercelli province (formerly named C.A. VC1), with an extent of 77.668 ha, of which 51.182 ha are used for hunting, and includes three geographically different district, "Alta Valsesia", "Media Valsesia" and "Prealpi Biellesi e Valsesiane". The agricultural, forestry and farming territory amounts to 46.315, 25 ha without considering unproductive surfaces, waters and urbanized areas [20].

Climatic, geological and anthropogenic factors, in particular high rainfall during summer season and soil acidity, have affected the development of a uniform vegetation of chestnut tree (*Castanea sativa*) than other Alpine Western valleys [21].

There are stable populations of chamois (species useful surface area of 48.792,11 ha), roe deer (*Capreolus capreolus*), red deer (*Cervus elaphus*), mouflon (*Ovis musimon*) and ibex (*Capra i. ibex*). Wild boar (*Sus scrofa*) is widespread, in particular in pre-alpine territories. Bovids and cervids hunting is based on the selective cull method which provides the assignment of determined species, age class and sex to each hunter based on previous census, hunters can shot their heads in

about two and half months. Each hunter can shoot one wild ruminant per species and twenty wild boars per year. Furthermore, several flocks are present in this territory, in particular the province is famous for wool production.

The study was carried out during 2016 hunting season, between September and December.

Macroscopic examination and blood sampling

The study was performed on hunted chamois during selective culling activities carried on by trained hunters. A macroscopic examination of each carcass has been conducted at the Control Centre, where each hunter had to bring the chamois after culling for the control of a veterinarian.

Soon after the shoot, hunters have to eviscerate the hunted animal removing at least the gastrointestinal tract. For this reason, at the Control Centre is not possible to check all the organs, in some cases there is no organ. Hunters collected post-mortem-blood samples via jugular or heart clot from their own bag and leaded up to the Control Centre where serum was obtained by centrifugation and stored at -20°C until further processing. Because of their quality, such as haemolysis or bacteriological contamination, not all sera could be used for further examination.

Serologic analyses

A commercial enzyme-linked immunosorbent assay (ELISA) kit (ELITEST CLA, HYPHEN BioMed®, France) has been used to detect antibodies (IgG) anti-PLD (*Corynebacterium pseudotuberculosis* phospholipase D). This direct ELISA utilises a recombinant form of the important, conserved *C. pseudotuberculosis* virulence factor, PLD to detect anti-PLD IgG antibodies in sera from sheep and goats with CLA. As PLD is not known to be produced by any other sheep pathogenic bacteria this makes it a very specific test. Infected subjects develop a specific immune response against PLD even in absence of clinical symptoms. Results were expressed as OD after reading the plates at 450 nm in an ELISA microplate reader (Bio-Rad® Model 680). Values <0.20 have been considered negative, values between 0.20 and 0.50 represent the grey zone, values >0.50 have been considered positive.

Results

For each head culled, the location of shot has been registered on the maps (scale 1:50000) with a precision of 1 km² and age and sex have been determined, subsequently morphobiometric measures have been taken and registered (Table 1).

Age	Sex	
Kids		05-Jul
Yearlings		55/95
Adults	Males	77/77
	Females	59/78

Total	196/257
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Table 1: Number of culled chamois classified by age and sex classes/ number of chamois provided by the shooting plan in the whole territory.

While the Table 2 shows the number of sampled chamois: 1 kid (20% of culled), 8 yearlings (14.5% of culled), 22 adult males (28.6% of culled) and 11 adult females (18.6% of culled).

Post mortem blood sampling of hunted chamois allows to obtain a large number of samples but sera could be contaminated or have low quality because of excessive haemolysis. For this reason, a preliminary selection of sera has been done before processing them.

Age	Sex	Sampled chamois	Processed sera
Kids		1	1
Yearlings		8	7
Adults	Males	22	17
	Females	11	9
Total		42	34

Table 2: Number of sampled chamois classified by age and sex classes or number of processed sera.

Three of 34 chamois were seropositive (Table 3), one adult male resulted in the grey zone with OD=0.498 (Table 4), and 30 chamois were seronegative. Seroprevalence has been calculated considering census data of the previous year and resulted 8.82% (c.i. 95% 0.0-18.26).

ID	Age	Sex	Weight	Date	Town	Loc.	Lesions
772	1	F	12.8 kg	02-Nov	Vocca	Avina	No one
119	11	F	Emaciation	11-Dec	Rassa	Vasnera	Abdominal abscess
863	3	F	17.5 kg	16-Nov	Alagna	Tagliaferro	Abdominal abscesses

Table 3: Identification mark, age, sex, weight of the carcass completely eviscerated, date and location of cull, presence of macroscopic lesions of the seropositive chamois.

ID	Age	Sex	Weight	Date	Town	Loc.	Lesions
68	4	M	20.6 kg	12-Nov	Rassa	Punta Gay	No one

Table 4: Identification mark, age, sex, weight of the carcass completely eviscerated, date and location of cull, presence of macroscopic lesions of the chamois with OD=0.498.

The three seropositive subjects are all females, two are adults and one is a 1-year-old chamois. During the inspection at the Control Centre, the two adult subjects presented abdominal abscesses in abdominal cavity related to caseous lymphadenitis, for this reason they

have been considered “sanitary heads” and the carcasses have been destroyed. Moreover, the oldest one presented evident cachexia and skin lesions on nasal bridge and distal limbs caused by *Dermatophylus congolensis*. Other sampled chamois did not show any lesions at the moment of the inspection, including 4-year-old chamois with OD=0.498.

Discussion and Conclusion

Little research had been done on caseous lymphadenitis in wildlife, few studies are found in Literature and no one about any population in Italy, even if the circulation of the infection is suspected in some areas. So this monitoring represents the first step to confirm the presence of *C. pseudotuberculosis* in an Alpine chamois population presenting typical lesions, and then to understand the trend of the infection, the possible effect on the population and the possible risk factors on which take action; moreover it could represent a useful model for other study areas.

Results obtained in this first year of investigation are to be considered absolutely preliminary, as a starting point of a data collection that will provide useful management suggestions in the near future. In fact, the sampling has not been enough, for sample size and duration, to draw any certain conclusion.

Despite this, the results give the serological confirmation of the circulation of *C. pseudotuberculosis* and, together with pathological and bacteriological diagnosis of previous years, confirm it to be the causative agent of the lesions observed in chamois.

In fact, the only two subjects tested with lesions also were seropositive; while the seropositivity of a yearling female without macroscopic lesions is evidence of the current circulation of the pathogen as this subject has come into contact with the infection during its one and a half year of life. Moreover, it is not excluded that in the future it could have presented the typical abscess injuries given the slowness they often develop in the infected subjects. The only grey-zone result is very close to positivity, so we might suspect a contact with the pathogen not enough to develop a higher antibody titre.

During years, the continued presence of dermatophilosis skin lesions in chamois represents a warning alarm. Dermatophilosis is a dermatitis caused by *Dermatophylus congolensis* not a highly pathogenic bacteria, it is an opportunistic bacteria living on host skin surface and causes clinical lesions in presence of several risk factors such as depression of the host immune system and high atmospheric humidity.

For the future, further investigations are needed, also considering wildlife/livestock interfaces and the role of flocks. The suggestion is to continue the monitoring in chamois and to plan a monitoring in sheep and goats sharing summer pastures. This may be useful to understand if the *C. pseudotuberculosis* is on endemic level in chamois population or there is a continuous spill-over from livestock.

The results of this first year survey on the territory are to be considered as preliminary, the starting point for a data collection that can become a historical series that could give useful management guidance in the near future.

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