



Short communication

Short communication: Isolation frequency of bacteria causing lymphadenitis and abscesses in small ruminants in central Spain



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ABSTRACT

Infectious lymphadenitis in small ruminants caused by *Corynebacterium pseudotuberculosis* and *Staphylococcus aureus* subsp. *anaerobius* are widely distributed throughout the world, and result in significant economic losses. *Trueperella pyogenes* has also been associated with lymphadenitis in sheep and goats. In order to determinate the isolation frequency of the different agents associated with lymphadenitis and abscesses, 171 pus samples (135 from sheep and 36 from goats) from 46 flocks were investigated. Isolated bacteria were identified by MALDI-TOF method. *S. aureus* subsp. *anaerobius* was the most frequently detected agent. It was identified in 76 animals (59 sheep and 17 goats) from 24 of the surveyed flocks. Of these infected animal, 25 (32,9%) were over one year old, confirming that abscess disease may occur in a significant percentage of adult animals. *C. pseudotuberculosis* was identified in 45 of the sampled animals (36 sheep and 9 goats) from 24 flocks. Only 5 of animals suffering caseous lymphadenitis were under one year old. *T. pyogenes* was isolated from 17 animals (13 sheep and 4 goats) in 11 flocks. Seven of these samples were taken from subcutaneous abscesses located in not lymph nodes regions. A notable finding of this work is the isolation of *Actinomyces hyovaginalis* from 5 of the samples analyzed all of them taken from subcutaneous abscesses located in superficial lymph nodes regions. Thus, *T. pyogenes* and *A. hyovaginalis* should be included in the differential diagnosis of lymphadenitis in small ruminants. In 19 of the 46 surveyed flocks at least two of the four agents were detected, which underlines the need to analyze samples from several animals to reach an accurate diagnosis in flocks affected by lymphadenitis.

1. Introduction

Two specific lymphadenitis are currently recognized in small ruminants, caseous lymphadenitis and abscess disease. These diseases are widely distributed throughout the world and result in significant economic losses. Caseous lymphadenitis is a chronic, contagious disease of worldwide distribution caused by *Corynebacterium pseudotuberculosis*. The condition affects mainly sheep and goats and is characterized by abscess formation in superficial lymph nodes (external form) or within internal organs and lymph nodes (internal form) (Williamson, 2001). The disease is most frequently seen in adult animals. *C. pseudotuberculosis* infection may also occur in other animal species, and it is relatively common in horses, llamas and alpacas and buffalos (Fontaine and Baird, 2008). Human cases, although rare, have been reported especially in Australia and New Zealand, and it is considered an occupational zoonosis (Dorella et al., 2006).

Abscess disease produced by *Staphylococcus aureus* subsp. *anaerobius*

affects mainly young animals up to 6 months of age and is characterized by the presence of abscesses in superficial lymph nodes (De la Fuente and Suarez, 1985; De la Fuente et al., 1985, 2011). Subcutaneous abscesses not involving lymph node are occasionally reported. The geographic distribution of abscess disease is much more limited than caseous lymphadenitis. In Europe, the condition has been mainly diagnosed in France, where the disease was first described in 1911, and Spain. The condition has also been detected in Italy, Poland and Croatia, and a unique outbreak of the disease has been described in both Denmark and Hungary in animals imported from France. In Africa, abscess disease has been reported in Tunisia, Kenya, Nigeria, Egypt, Sudan and Somalia. The condition has also been diagnosed in occidental Asia (Saudi Arabia and Iran) (De la Fuente et al., 2011).

Trueperella pyogenes causes a variety of clinical manifestations in domestic animals, being bovine mastitis the most common (Quinn et al., 2011; Radostits et al., 2007). *T. pyogenes* infection has also recently been associated with lymphadenitis and abscesses in sheep and

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goats (Ribeiro et al., 2015).

In recent years, MALDI-TOF technique has been demonstrated as a rapid and reliable technique used for identification of microorganisms in the routine clinical laboratory both in human and veterinary medicine (Angeletti, 2017; Randall et al., 2015; Wattal et al., 2017).

This study reports the isolation frequency of agents causing lymphadenitis and abscesses in small ruminants in a convenience sample of 171 animals (135 sheep and 36 goats) from 46 flocks located in 5 provinces in central Spain.

2. Materials and methods

2.1. Sampling procedure

In April 2015, small ruminant practitioners working in 4 provinces of central Spain (Madrid, Ávila, Toledo and Palencia) were contacted, asking for their collaboration for sampling cases of lymphadenitis and abscesses in sheep and goats. Pus samples from subcutaneous abscesses located or not in lymph nodes regions were collected by veterinarians using a sterile syringe. Submitted samples were accompanied by a record sheet containing information on the host species of the flock (sheep, goat or both), breed, size of the flock, sex and age of each sampled animal and site of collection.

Significant differences in the frequencies of *S. aureus* subsp. *anaerobius* infection between sheep breeds were determined by the Fisher exact test. Statistical significance was set at $P \leq 0.05$.

2.2. Bacteria isolation and identification

Samples were inoculated in two plates of Columbia agar supplemented with 5% sheep blood (bioMérieux, Marcy l'Etoile, France), which were subsequently incubated for 48 h at 37 °C, one aerobically, and the other microaerophilically (candle jar system).

Firstly, identification of the isolates was performed by MALDI-TOF technique. Isolates were subjected to a protein-peptide extraction protocol according to the manufacture's instructions (Bruker Daltonik GmbH, Germany). Bacterial extracted proteins were deposited on a polished steel target plate, let dry at room temperature and overlaid HCCA matrix solution (α -cyano-4-hydroxycinnamic acid in 50% acetonitrile and 2.5% trifluoroacetic acid). After air-dried at room temperature, spectra acquisition was performed using an UltrafleXtreme equipment (Bruker Daltonik) in the linear and positive mode using the FlexControl software (version 3.4) in an automatic mode. Isolates were identified by comparing their MALDI profiles with those included in the BDAL Bruker database (5989 entries) using MALDI Biotyper software (Bruker Daltonik) according to the manufacture's instructions. Identification results was interpreted as follows: log-scores below 1.699 was considered not reliable while score values between 1.700–1.999 were considered probable identification at genus level. Finally, score values greater than 2.000 was interpreted as identification at species level being highly probable identification when scores were higher than 2.300.

Those isolates which showed score values lower than 1.700 were identified by partial sequencing of the 16S rRNA gene using the universal primers 20F (5'-AGAGTTTGATCATGGCTCAG-3') and 1500 (5'-GGTTACCTTGTTACGACTT-3'). (Weisburg et al., 1991). These analyses were performed using a C1000 ThermalCycler (Bio-Rad, Hercules, CA, USA) and the PCR products were checked in a 2% agarose gel (Biotools, Queensland, Australia). Molecular identification was performed by comparison of individual 16S rRNA gene sequences with those included in the Nucleotide BLAST NCBI (National Center for Biotechnology Information, www.ncbi.nlm.nih.gov).

3. Results and discussion

From May 2015 to June 2016, 171 pus samples (135 from sheep and

Table 1

Isolation frequency of the identified bacteria in the 171 clinical samples studied.

Identified bacteria	Number of isolates	% Referred to the samples studied
<i>Staphylococcus aureus</i> subsp. <i>anaerobius</i>	76 ^a	44.4
<i>Corynebacterium pseudotuberculosis</i>	45 ^b	26.3
<i>Treuerella pyogenes</i>	17 ^c	9.9
<i>Actinomyces hyovaginalis</i>	5 ^d	2.9
<i>Staphylococcus lentus</i>	5 ^e	2.9
<i>Streptococcus ovis</i>	4 ^f	2.3
<i>Staphylococcus simulans</i>	3 ^g	1.7
<i>Staphylococcus warneri</i> , <i>Staphylococcus caprae</i> ^h , <i>Staphylococcus equorum</i> , <i>Corynebacterium camporealensis</i> , <i>Pseudomonas aeruginosa</i> , <i>Pseudomonas hydrophila</i> , <i>Actinobacillus pleuropneumoniae</i> , <i>Vagococcus lutrae</i> , <i>Acinetobacter</i> spp.	1	0.6
Negative or non-significant growth	15	8.8

^a One in mixed infection with *T. pyogenes*.

^b Two in mixed infection with *S. lentus* and one with *S. simulans*.

^c One in mixed infection with *A. hyovaginalis*.

^d One in mixed infection with *S. ovis*.

^e One in mixed infection with *C. camporealensis*.

^f One in mixed infection with *Acinetobacter* spp.

^g One in mixed infection with *V. lutrae*.

^h In mixed infection with *P. hydrophila*.

36 from goats) from 46 flocks (28 sheep flocks, 9 goat flocks and 9 mixed sheep and goat flocks) were submitted. Flocks size ranged from 142 to 1700 (median 460). Sampled animals were from one month to 10 years of age (median 2 years). Fifty-five of the animals were less than one year old. The number of submitted samples per flock ranged from one to 9 (median 3). Only 17 of the samples were from subcutaneous abscesses located in not lymph nodes regions. Forty-eight of the sheep samples were taken from Assaf breed, 46 from Laucañe breed, 9 from Spanish Castellana breed, 6 from Spanish Colmenareña breed and the rest 21 from crosses.

The isolation frequencies of the different agents identified in this study are shown in Table 1. *S. aureus* subsp. *anaerobius* was the most commonly detected agent. Thus, abscess disease was diagnosed in 76 animals (59 sheep and 17 goats) from 24 of the surveyed flocks (18 sheep flocks, 4 goat flocks and 2 mixed), which shows the widespread of this condition in the analyzed herds. Twenty-five of these infected animals (32.9%) were over one year old. It has traditionally been considered that abscess disease affects mainly young animals up to 5–6 months of age (De la Fuente and Suárez, 1985; De la Fuente et al., 2011). However, in an epidemiological study performed in an enzootic area (Segovia, Spain) Tejedor et al. (1991) found that between 0.5 and 2.5% of 1–2 year old sheep were affected by the disease, and suggested that these animals can act as reservoir. The results of this study confirm that abscess disease may occur in a significant percentage of adult animals. Only 7 of the *S. aureus* subsp. *anaerobius* positive samples (4 from sheep and 3 from goats) were taken from abscesses located in not lymph nodes regions such as flanks, chest, back or croup. The most frequently affected lymph nodes were mandibular, parotid, lateral retropharyngeal and superficial cervical. The infection frequency of sheep from Assaf breed with *S. aureus* subsp. *anaerobius* (33/48, 68.7%) was significantly higher than those from Laucañe breed (19/46, 41.3%) and crosses (7/26, 26.9%). None of the sheep from Spanish breeds were infected with this bacterium. This results support field observations of the collaborating practitioners that Assaf sheep breed is more susceptible to abscess disease than other breeds.

Caseous lymphadenitis was also widespread in the surveyed flocks. *C. pseudotuberculosis* was identified in 45 of the sampled animals (36 sheep and 9 goats) from 24 flocks (15 sheep flocks, 6 goat flocks and 3 mixed). Only 5 of animals suffering caseous lymphadenitis were under one year old, corroborating that the disease affects mainly adult

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