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FREQUENCY AND ANTIMICROBIAL SUSCEPTIBILITY OF BACTERIAL ISOLATES FROM CASEOUS LYMPHADENITIS IN SHEEP IN KOSOVO

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Abstract

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The aim of the study was to estimate the frequency of caseous lymphadenitis (CLA) in sheep in Kosovo and to determine the susceptibility of *Corynebacterium pseudotuberculosis* isolates to some antimicrobial agents. Live animals (284 sheep) were examined clinically for the presence of abscesses and enlarged superficial lymph nodes. 38 samples (pyogenic aspirates) from enlarged lymph nodes were collected using sterile needles and syringes according to the techniques recommended by Quinn et al. (1994). Samples were subjected to culture on heart infusion agar containing 5% defibrinated sheep blood, while isolates were differentiated by using biochemical API tests. *Corynebacterium pseudotuberculosis* was isolated from 32 (84.2%) of the 38 samples, followed by *Staphylococcus aureus* 4/38 (10.5%), and *Streptococcus pyogenes* 2/38 (5.3%). The frequency of CLA in sheep was 11.3%. A total of 56 bacterial isolates were recovered and most predominant were *Corynebacterium pseudotuberculosis* (*n* = 48), followed by *Staphylococcus aureus* (*n* = 5), and *Streptococcus pyogenes* (*n* = 3). These bacterial isolates were subjected to antimicrobial susceptibility test using disc diffusion method and performed according to CLSI guidelines in Mueller-Hinton agar. *In vitro* antibiogram studies of *Corynebacterium pseudotuberculosis* isolates were found to be complete susceptible to oxytetracycline (100%), amoxicillin/clavulanic acid (100%), and highly susceptible to cloxacillin (95.8%), gentamicin (91.7%), followed by trimethoprim (83.3%). However these isolates were highly resistant to streptomycin (83.3%), and penicillin G (62.5%).

Key words: *Caseous lymphadenitis*; sheep; *Corynebacterium pseudotuberculosis*; antimicrobial susceptibility

Introduction

Caseous lymphadenitis (CLA) is chronic bacterial infectious disease of sheep and goat caused by *Corynebacterium pseudotuberculosis*. It is characterized by abscesses in superficial and visceral lymph nodes. In the superficial form, the peripheral lymph nodes swell and abscess, while in the visceral form there are systemic complications that can lead to chronic thinning (Radostits et al., 2002). The clinical signs of CLA are prominent enlargement of superficial lymph nodes such as prescapular, submandibular, prefemoral, parotid, or in visceral organs such as lungs (Dercksen et al., 2000; Fontaine and Baird, 2008). The disease is present in many countries throughout the world. Dissemination of this disease throughout the world probably occurred through importation of infected animals (Baird GJ et al., 2007). Studies in Australian sheep flocks have indicated that most spread of CLA occurs shortly after shearing (Paton et al., 1988). The source of the infection is sheep that have lacerated superficial lymph node abscesses resulting in the agent being dissemination to other animals (Sunil et al., 2008). Caseous lymphadenitis is responsible for substantial economic losses in sheep flocks and goat herds (Williamson, 2001). Economic losses result from reduced weight gain, reproductive efficiency and wool and milk production, as well as from condemnation of carcass and devaluation of hides/skins and also from culling of

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infected animals (Paton et al., 1994; Merck, 2005; Michael, 2015). *CLA* is not only economically important disease but has also zoonotic importance. The disease in human is rare but well documented and it causes a suppurative granulomatous lymphadenitis (Nicastro et al., 2004). Treatment for CLA is not curative. Antibiotic therapy is generally not effective as antimicrobials are unable to penetrate the heavily encapsulated lesions. The best way to control this infection in a flock is to cull those showing clinical signs with confirmed diagnosis (East, 1998).

There is no data available in Kosovo about CLA disease and its etiological agents. The aim of this study was to estimate the frequency of caseous lymphadenitis (CLA) in sheep flocks in south-eastern of Kosovo and to determine the susceptibility of *Corynebacterium pseudotuberculosis* isolates to some antimicrobial agents.

Material and Methods

Flock history

The study was performed on two sheep flocks of about 284 sheeps, which were affected of CLA infection in south-eastern of Kosovo. Flock owners have informed local veterinarians about these changes in the animal body (enlarged lymph node and abscesses in different regions). CLA lesions were detected on sheep flocks from April to July 2016 during a clinical examination.

Sample collection and microbiological examinations

Live animals (284 sheep) were examined clinically for the presence of abscesses and enlarged superficial lymph nodes. 38 samples (pyogenic aspirates) from enlarged lymph nodes of live sheep with CLA lesions were collected using sterile needles and syringes. The outer surrounding skin of the lymph nodes was shaved and disinfected with 70% alcohol. Sample collection was carried out according to the techniques recommended by Quinn et al. (1994). Direct smears were prepared from pus samples, heat fixed and stained with Gram stain. Samples were subjected to culture in blood agar. The isolated *C. pseudotuberculosis* grew clearly after 48h of incubation at 37°C on heart infusion agar containing 5% defibrinated sheep blood. *Staphylococcus aureus* isolates were differentiated from other staphylococci on basis of mannitol fermentation on Mannitol salt agar and by using biochemical test API Staph system (bioMėrieux SA), and streptococci isolates were identified using the API 20 Strep strips (bioMėrieux SA). Isolates were tested for antimicrobial susceptibility on Mueller Hinton agar (HIMEDIA) by disc diffusion method according to the Clinical Laboratory Standards Institute (CLSI, 2012). The following antibiotic disks were tested: amoxicillin/clavulanic acid AMC (30 µg, Oxoid), oxytetracycline T (30 µg, BD BBL™), streptomycin S (10 µg, Liofilchem), trimethoprim TM (2.5 µg, Liofilchem), cloxacillin CX (5 µg, Liofilchem), gentamicin CN (10 µg, Liofilchem) and penicillin P (10 IU, Oxoid). The plates were incubated at 35°C for 24 hours. The zone of inhibition around each disc was measured and the interpretation was made as per the zone size interpretation chart provided by the disc manufacturer.

Results

In this study, 284 live animals (sheep) were examined clinically for the presence of abscesses and enlarged superficial lymph nodes. *Corynebacterium pseudotuberculosis* was isolated from 32 (84.2%) of the 38 samples, followed by *Staphylococcus aureus* 4/38 (10.5%), and *Streptococcus pyogenes* 2/38 (5.3%), of pyogenic aspirates collected from enlarged lymph nodes of live sheep. The frequency of CLA in live sheep was 11.3%. (Table 1). Out of 38 samples bacteriologically examined, different bacterial isolates (N = 56) were recovered. Most predominant were *Corynebacterium pseudotuberculosis* (n = 48), followed by *Staphylococcus aureus isolates* (n = 5), and *Streptococcus pyogenes* (n = 3). Lesions of CLA in live sheep clinically examined were distributed as follows: 18 (42.8%) in parotid LN, 16 (38.1%) mandibular LN, 7 (16.7%) prescapular LN and 1 (2.4%) in precrural LN (Table 2). Bacteria isolated from affected lymph nodes of sheep with clinical abscesses were mostly *Corynebacterium pseudotuberculosis* with a frequency of 85.7%, followed by *Staphylococcus aureus* 8.9 %, and *Streptococcus pyogenes* 5.4% (Table 3).

Table 1
Frequency of CLA in live sheep examined clinically and bacterial positive samples

No of animals examined clinically	Animals with CLA lesions (external form)	No of positive samples (%)
284	38	*C. pseudotuberculosis* 32/38 (84.2) *Staphylococcus aureus* 4/38 (10.5) *Streptococcus pyogenes* 2/38 (5.3)

The antimicrobial susceptibility and resistance profile of bacterial isolates from CLA cases to antibiotics are shown in Table 4.

Table 2
Distribution of CLA lesions (lymph node abscesses) according to the localization in live sheep

Lymph nodes	No. of lesions/(%)
Parotid LN	18 (42.8%)
Mandibular LN	16 (38.1%)
Prescapular LN	7 (16.7%)
Precrural LN	1 (2.4%)
Total	42

Table 3
Bacterial isolates recovered from pyogenic aspirates (enlarged lymph node) of the CLA cases in sheep

Type of specimens	Isolated bacteria/Total sample (%)
Pyogenic aspirates (pus)	*C. pseudotuberculosis* 48/56 (85.7)
	Staphylococcus aureus 5/56 (8.9)
	Streptococcus pyogenes 3/56 (5.4)

In this study, *Corynebacterium pseudotuberculosis* isolates were found to be complete susceptible to oxytetracycline (100%), amoxicillin/clavulanic acid (100%), and highly susceptible to cloxacillin (95.8%), gentamicin (91.7%), followed by trimethoprim (83.3%). However these isolates were highly resistant to streptomycin (83.3%), and penicillin G (62.5%). The result *Staphylococcus aureus isolates* were also found more sensitive to amoxicillin/clavulanic acid (100%), trimethoprim (80%), oxytetracycline (80%), gentamicin (80%) and cloxacillin (60%), and resistance to streptomycin (80%) and penicillin G (60%). *Streptococcus pyogenes* that represented around 5.4% of the total isolates was found complete susceptible to trimethoprim (100%), amoxicillin/clavulanic acid (100%), and highly susceptible to oxytetracycline (66.7%), followed by gentamicin (66.7%), streptomycin (66.7%) and cloxacillin (66.7%), but not to penicillin G (33.7%).

Discussion

In the present study, 284 live animals (sheep) were examined clinically for the presence of abscesses and enlarged superficial lymph nodes. *Corynebacterium pseudotuberculosis* was isolated from 32 (84.2%) of the 38 samples, followed by *Staphylococcus aureus* 4/38 (10.5%), and *Streptococcus pyogenes* 2/38 (5.3%), of pyogenic aspirates collected from enlarged lymph nodes of live sheep. The frequency of CLA in sheep was 11.3% and is in agreement with previous studies (Smith PB, 2002). Bacteria isolated from samples of affected sheep with CLA lesions were mostly *Corynebacterium pseudotuberculosis* with a frequency of 85.7%, followed by *Staphylococcus aureus* 8.9%, and *Streptococcus pyogenes* 5.4%. The most frequently infected lymph node in sheep was the parotid lymph node, followed by mandibular lymph node, and this is not in agreement with the findings of many previous investigators (Williamson and Narin, 1980; O'Reilly et al., 2008). In this study, *Corynebacterium pseudotuberculosis* isolates were found to be complete susceptible to oxytetracycline (100%), amoxicillin/clavulanic acid (100%), and highly susceptible to cloxacillin (95.8%), gentamicin (91.7%), followed by trimethoprim (83.3%). However these isolates were highly resistant to streptomycin (83.3%), and penicillin G (62.5%), and these results are approximately in line with the findings of other authors (Garg et al., 1985; Pepin et al., 1989). Results of sensitivity of *Corynebacterium pseudotuberculosis* to antimicrobial agents were in agreement with that of Muckle and Gyles (1982), except that our isolates were resistant and to penicillin. Although *in vitro* antibiotic susceptibility test showed that *C. pseudotuberculosis* isolates were fully sensitive to most antibiotics used, caseous lymphadenitis may continue to be a serious problem for animal breeders because antibiotics in vivo are unable to penetrate the thick capsule of the abscesses.

Table 4
Antimicrobial susceptibility of bacterial isolates from clinical abscesses (CLA lesions) to antibiotics

Bacterial isolates (n)	Tested antimicrobial drug													
	Gentamicin		penicillin G		Streptomycin		amoxillin/clavulanic acid		trimethoprim		Oxytetracycline		Cloxacillin	
	S%	R%	S%	R%	S%	R%	S%	R%	S%	R%	S%	R%	S%	R%
C.pseudotuberc (48)	91.7	8.3	37.5	62.5	16.7	83.3	100	0	83.3	16.7	100	0	95.8	4.2
Staph. aureus (5)	80	20	40	60	20	80	100	0	80	20	80	20	60	40
S. pyogenes (3)	66.7	20	33.3	66.7	66.7	33.3	100	0	100	0	66.7	33.3	66.7	33.3

Legend: AMC: amoxycillin/clavulanic acid, T: oxytetracycline, CX: cloxacillin, CN: gentamicin, TM: trimethoprim, P: penicillin G and S: streptomycin

Conclusions

The results of this study, as well as other researchers, show that despite the frequency of disease and antibiotic susceptibility, ineffective antibiotic treatment due to the presence of the bacterial agent within the abscess surrounded by fibrous capsule poses a challenge for farmers and veterinarians to manage it and cause economic losses. Adequate control programs in small ruminants may contribute to reduction of disease. The best preventive measure would be to find effective vaccine against this disease in small ruminants.

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