

Frequency of caseous lymphadenitis (CLA) in sheep slaughtered in an abattoir in Tabriz: comparison of bacterial culture and pathological study

Fereydon Rezazadeh Zavoshti ·
Amir Babak Sioofy Khoojine · Javad Ashrafi Helan ·
Belal Hassanzadeh · Ali Akbar Heydari

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Abstract From January to February 2008, 468 sheep carcasses (335 male and 133 female) in a Khosroshahr (suburb of Tabriz, East Azerbaijan province, Iran) abattoir were randomly selected for inspection. The aim of the study was to estimate the frequency of caseous lymphadenitis (CLA) in sheep and to compare the results of bacterial cultures and histopathology of suspected cases. The mean age of the population was 2.5 years. One hundred ninety-seven cases containing 153 (77.7%) males and 44 (22.3%) females had prominent enlargement of one of the lymph nodes (i.e., prescapular, prefemoral, inguinal, supramammary, or mediastinal); these were removed with the surrounding tissue for further evaluation. For confirmed diagnosis of CLA, samples were sent for microbiology and pathology analysis. Standard bacteriological culture methods for isolation of *Corynebacterium pseudotuberculosis* and tissue preparations

for histopathological sections were performed. To evaluate the effect of age on the frequency of CLA, animals were categorized in four groups: under 1, 1–2, 2–3, and over 3 years of age. Based on the results, in 59 (12.60%) carcasses *C. pseudotuberculosis* was isolated, and in 94 (20.08%) of the cases histopathological studies revealed pathognomonic signs (lamellated exudates or onion ring) of CLA. The frequency of CLA based on bacteriological culture was 12.60% and on histopathological study 20.08%. In 37 (18.8%) of the carcasses, both bacteriological and histopathological studies confirmed CLA. The frequency of CLA following microscopic examination (20.08%) presented a more precise diagnosis compared to bacteriological culture (12.60%) and macroscopic evaluation of the lymph nodes ($P<0.05$). Furthermore, there was a positive correlation rate between the bacteriological culture and histopathological study ($r=0.196$, $P=0.006$). The prescapular lymph node had the highest infection rate with 54 (1.70 ± 0.97) and supramammary lymph node had the lowest with two (0.07 ± 0.41) ($P<0.05$). There was an increase in CLA detection with increasing age ($P<0.05$), the mean age of animals with a positive CLA test were 2.92 years and in the oldest age group 31 (47%) cases had the highest infection.

F. R. Zavoshti (✉)
Department of Clinical Sciences, Faculty of Veterinary Medicine,
University of Tabriz,
Tabriz, Iran
e-mail: f_rezazadeh@tabrizu.ac.ir

A. B. S. Khoojine · J. A. Helan
Department of Pathobiology, Faculty of veterinary Medicine,
University of Tabriz,
Tabriz, Iran

B. Hassanzadeh
Department of Basic Science, Faculty of Veterinary Medicine,
Ferdowsi University of Mashhad,
Mashhad, Iran

A. A. Heydari
Department of Statistics, Faculty of Mathematical Sciences,
University of Tabriz,
Tabriz, Iran

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Introduction

Caseous lymphadenitis (CLA) was reported for the first time in the world by Preisz and Guinard in 1891 and Nocard in 1896 and in Iran by Afnan and his colleagues in

1964 (Hosseinzadeh et al. 1996; Baird and Fontaine 2007; Fontaine and Baird 2008). The disease was recognized in sheep in Britain in 1991 and in the Netherlands in 1978 (Dercksen et al. 2000). The agent was isolated in Venezuela in 1962 (Chirino-Zarraga et al. 2006). The agent of CLA is a gram-positive actinomyces called *Corynebacterium pseudotuberculosis* but other agents such as *Staphylococcus* spp., *Streptococcus* spp., *Bacillus* spp., *Micrococcus* spp., *Antrobacter* spp., *Aeromonas* spp., *Morexilla* spp., and *Pasturella* spp. have also been isolated from infected lymph nodes (Hosseinzadeh et al. 1996; Fontaine and Baird 2008; Radostits et al. 2007; Richard et al. 1979). The old name of the bacterium was *Corynebacterium ovis* (Ghadimi 1993; Smith 2002). The presence of CLA had been recognized for over 100 years (Fontaine and Baird 2008) and has been reported in infected sheep in Australia, New Zealand, South Africa, Middle east, North and South America, England, and in most parts of Northern and Southern Europe (Ghadimi 1993; Dercksen et al. 2000; Fontaine and Baird 2008; Fontaine et al. 2006; O'Reilly et al. 2008; Radostits et al. 2007; Smith 2002). The disease has been reported in other animals such as horse, lama, alpaca, buffalo, deer, and camels (Ghadimi 1993; Braga 2007; Fontaine and Baird 2008; Smith 2002). Occasionally, abortions have been reported due to *C. pseudotuberculosis* in sheep (Fontaine and Baird 2008). According to one study, the economic loss due to the disease in Australia was 17 million Australian dollars (Cetinkaya et al. 2002). Contamination of meat in slaughterhouses in the presence of an infectious agent in adult sheep is 3–5% and 0.02–0.03% in lambs (Hodgson et al. 1999; Radostits et al. 2007). The source of the infection is sheep that have lacerated superficial lymph node abscesses resulting in the agent being disseminated to other animals (O'Reilly et al. 2008; Sunil et al. 2008). Nasal and oral discharge is very important in the transmission of the disease in sheep with abscess in the lungs due to *C. pseudotuberculosis* (Ellis et al. 1987; Fontaine and Baird 2008; O'Reilly et al. 2008; Radostits et al. 2007; Sunil et al. 2008). The mediastinal lymph nodes and lungs are the most visceral organs that are infected with *C. pseudotuberculosis*, but other internal organs can present with the abscess (Ghadimi 1993; Binns et al. 2007; Fontaine and Baird 2008).

Phospholipidase D, a 31.5-kDa molecule, is the specific exotoxin of *C. pseudotuberculosis*; it causes the lysis of the sphingomyelin and the transfer of the agent which causes plasma to exit from the blood vessels into the lymph nodes (Radostits et al. 2007; Smith 2002). Abscesses may form in 60–80% of infected lymph nodes and the agent can persist against the phagocytic action of the immune system due to the high lipid content in their cell wall, hence making it an intracellular parasite (Ghadimi 1993; Fontaine and Baird 2008; Hodgson et al. 1999; Radostits

et al. 2007; Smith 2002). Capsule formation causes the organism to establish in the body (Fontaine and Baird 2008). Cellular immunity is more important than humoral immunity against CLA (Prescott et al. 2002). One of the characteristics of *C. pseudotuberculosis* is that it is reactive in old abscesses and can cause a relapse of the disease and clinical signs in the animal (Fontaine and Baird 2008). The clinical signs of CLA are prominent enlargement of superficial lymph nodes such as submandibular, prescapular, prefemoral, supramammary, popliteal, or in visceral organs such as lungs (Binns et al. 2007; Dercksen et al. 2000; Fontaine and Baird 2008; Radostits et al. 2007; Smith 2002). The postmortem signs are green yellowish exudates from infected superficial lymph nodes in animals (Radostits et al. 2007). In the primary stage, the pus in the lymph nodes is smooth and in the progressive stages is lamellate (onion skin or onion ring), a pathognomonic signs of CLA (Fontaine and Baird 2008; Radostits et al. 2007). The standard bacterial culture from intact lymph nodes and/or histopathological study from lymph nodes fixed in formalin is used to identify CLA (Radostits et al. 2007; Smith 2002).

Material and methods

From January to February 2008, 468 sheep carcasses (335 male and 133 female) in a Khosroshahr (suburb of Tabriz, East Azerbaijan province, Iran) abattoir were randomly selected for inspection. The aim of the study was to estimate the frequency of CLA in sheep and to compare the results of bacterial cultures and histopathology of suspected cases. The samples were taken from the carcasses that had prominent enlargement of at least one of the submandibular, prescapular, prefemoral, supramammary, popliteal, or visceral organs such as lungs; the lymph capsule with the surrounding tissue was excised (care was taken not to cut any of the infected surfaces) and sent in an ice container at 4°C to the microbiology laboratory. The surrounding tissue was cut and removed and the capsules of lymph nodes were burned and culture of pus performed in brain heart infusion broth for isolation of the *C. pseudotuberculosis*. One hundred microlitres of culture were transferred to blood agar enriched with sheep blood and incubated at 37°C. The resulting single colonies were purified and studied for their morphology. The suspected *Coccobacillus* to *C. pseudotuberculosis* was examined using biochemical tests. Single colonies with the same biochemical test profile as *C. pseudotuberculosis* were identified and sampled (Ghadimi 1993). In all suspected cases, for histopathological study the lymph nodes were divided into two slices in the sagittal plane and transferred for fixation in 10% formalin. After 24–48 h the formalin solution was changed and the lymph nodes were inspected for size, color, numbers, consistency of pus, and macroscopic

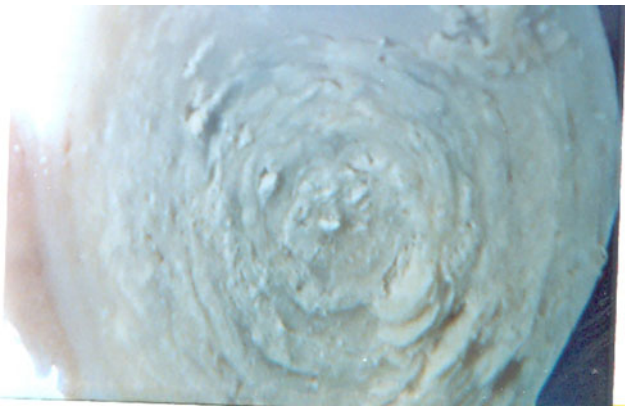


Fig. 1 Cross-sectional view of infected lymph node (mediastinal) to CLA in the present study shows the calcium granules that deposited and had rule to formation of lamellate in abscess

view of exudates (lamellate or onion rings). Tissue processing was undertaken using a tissue processor, (Leica, Jung Histokinette 200, Germany) and sectioning of the tissue using a sliding microtome (Leica, Jung Histo cuts, Germany). Sections were stained manually with hematoxylin and eosin (H & E). Microscopic evaluation of tissue was performed using an Olympus microscope (model CH36 RF200, Japan). Photomicrographs were taken with a digital camera (DP12, U-TVO.5XC-2, and Japan) (Luna 1968). Pathognomonic lesion (lamellate, onion, or onion ring) were used for diagnosis. The onion ring signs (centripetal circles) viewed under macroscopic or microscopic examination (Figs. 1, 2, and 3) or from isolation of *C. pseudotuberculosis* in the bacteriological study was the basis of CLA determination in sheep. If the exudate circles were not seen in histopathological examination, it was not recognized as CLA in our study. Age determination was based on dental eruption and change between milk and permanent teeth.



Fig. 2 Prescapular lymph node of a sheep infected with *Corynebacterium pseudotuberculosis* shows the centripetal circles of exudates. Note the connective tissue capsules that surrounded the exudates

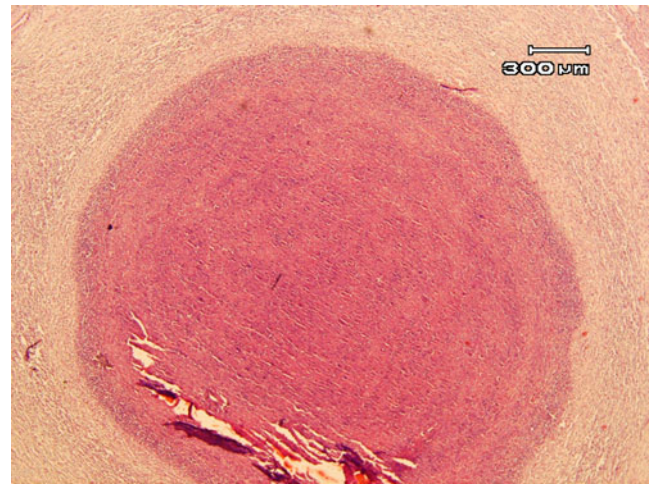


Fig. 3 Microscopic view of prescapular lymph node infected with *C. pseudotuberculosis* (CLA) H & E $\times 40$

Animals were grouped into four categories (under 1, 1–2, 2–3, and >3 years) (Hosseinzadeh et al. 1996; Dyce et al. 1995). Statistical analysis of the data was done using SPSS (version 16) software.

Results

From the 468 sheep carcasses randomly selected, 197 cases were suspected to have CLA with signs of prominent enlargement in one of the lymph nodes. In 59 cases (12.60%) pure *C. pseudotuberculosis* was isolated. In 94 (20.8%) cases histopathological study represented pathognomonic signs of CLA (onion ring signs). The average age of the population was 2.92 years, and there was an increase in the frequency of CLA with age ($P < 0.05$). From 59 cases that isolated *C. pseudotuberculosis*, 40 were male and 19 (4.05%) were female, this did not represent a statistical difference ($P > 0.05$). Tables 1, 2 and 3 and Figs. 1, 2, and 3 show details of results in the present study. One hundred twelve (56.9%) cases did not result in any isolated microorganisms. In 12 (2.56%) cases *Staphylococcus*

Table 1 Frequency of CLA based on microbiology isolation with sex in present study

Sex	Total number of inspected carcasses	Percentage and number of cases with isolation of <i>Corynebacterium pseudotuberculosis</i>
Male*	335	40 (8.54%)
Female	133	19 (4.05%)
Summation	468	59

*In 95% standard level (significance, 5%) did not have statistical differences between both sexes ($P > 0.05$)

Table 2 Results of isolation of *Corynebacterium pseudotuberculosis* in four groups in both sexes

Age group	Under 1 year	1–2 years	2–3 years	Above 3 years ^a	Summation	Total
The carcasses inspected	Male 80 Female 3	M. 179 F. 11	M. 21 F. 18	M. 55 F. 101	Male 338 Female 133	468
Number and percentage of positive	4 (6.7%) 0 (0%)	17 (28.8%) 2 (3.38%)	4 (6.7%) 1 (1.69%)	14 (23.7%) 17 (28.8%)	41 (69.4%) 19 (32.2%)	59 (100%)

M. male, F. female

^a Statistical differences with other groups ($P < 0.05$)

aureus, in four (0.85%) cases *Bacillus*, and in three (0.64%) cases both pure *C. pseudotuberculosis* and *Staphylococcus aureus* were isolated in microbiology. Macroscopic examination of prescapular lymph nodes represented 36 cases with lamellate exudates, but the microscopic study showed that 66 (61.68%) of the samples had CLA signs, resulting in the highest frequency of CLA; a statistical difference with infection compared with other lymph nodes ($P < 0.05$). In all cases studied the microscopic examination gave a more precise diagnosis compared with the macroscopic examination ($P < 0.05$). Thirty-seven cases out of 197 had both microbiology and histopathology results that identified CLA.

Discussion

CLA causes chronic emaciation, contamination of meat, loss of health, and reduction of wool production in sheep. Due to the slow progression of CLA and the little information and data on the disease, most countries do not pay attention to its eradication and prevention (Baird and Fontaine 2007; Fontaine and Baird 2008; O'Reilly et al. 2008; Ural et al. 2008). This study on CLA was undertaken in an abattoir because the visceral form of CLA can only be recognized after killing the animal (Hosseinzadeh et al. 1996; Zahraei and Shayeg 2007; Cetinkaya et al. 2002; Radostits et al. 2007). In a large-scale study in sheep in Australia, the prevalence of CLA was 26% (Paton et al. 2003, 1996; Radostits et al. 2007). Some other studies and references estimated the CLA rates between 5% and 10% (Smith 2002). In an abattoir study in Turkey, the prevalence of CLA was 3.5% (Cetinkaya et al. 2002). In the present study, the frequency of CLA based on bacteriological culture was 12.60% and on histopathology was 20.08%.

The defense mechanism of *C. pseudotuberculosis* prevents the organism from being isolated in all bacteriology cultures; therefore, we used both identification methods (culture and histopathology) to identify CLA (Radostits et al. 2007; Sunil et al. 2008). This may be the reason why the agent was not isolated from 112 carcasses. Furthermore, the abscess is formed in only 60–80% of infected animals, and it is important that we see the onion-skin or lamellate shape in the abscess in histopathology to identify CLA (Fontaine and Baird 2008; Jubb and Palmer 2007; Radostits et al. 2007). Furthermore, correlation rate between the bacteriological culture and histopathological study was positive ($r = 0.196$) and significant ($P = 0.006$). In 37 (18.8%) carcasses, both bacteriological and histopathological studies confirmed CLA. These results are in agreement with previous studies (Hosseinzadeh et al. 1996; Fontaine and Baird 2008; Hodgson et al. 1999; Radostits et al. 2007; Smith 2002). Reaction to other viruses can cause lymphadenitis in some animals (Ellis and DeMartini 1985; Ellis et al. 1987; Fulton et al. 2006; Yirell et al. 1991), which might explain why we did not obtain the bacteria in all cases in this study. The microbiology isolated 12 (2.56%) suspected *Staphylococcus aureus* cases, four (0.85%) *Bacillus* cases, and in three (0.64%) cases of both pure *C. pseudotuberculosis*, and *Staphylococcus aureus*, these results are in agreement with previous studies (Hosseinzadeh et al. 1996; Fontaine and Baird 2008; Radostits et al. 2007; Richard et al. 1979). The prescapular lymph node had the highest infection rate compared with other lymph nodes and this finding is the same with other studies (Hosseinzadeh et al. 1996; O'Reilly et al. 2008; Sunil et al. 2008). The mediastinal lymph node had the second highest rate of infection for *C. pseudotuberculosis*, illustrating the importance of blood and air in the transmission of CLA (Binns et al. 2007; Fontaine and Baird 2008).

Table 3 The results of macroscopic and microscopic examination in suspected animals with CLA (some cases were infected with more than one lymph node)

Lymph node	Prescapular	Prefemoral	Supramammary	Inguinal	Mediastinal	Summation
Macroscopic examination	36 (60%)	3 (5%)	2 (3.3%)	3 (5%)	16 (26.7%)	60 (100%)
Histopathology study ^a	66 (61.68%)	4 (3.70%)	3 (2.8%)	8 (7.4%)	26 (24.07%)	107 (100%)

^a In all groups the histopathology had precisely more than macroscopic examination of suspected lymph nodes to CLA ($P < 0.05$)

Our finding regarding the increase in CLA with increasing age is in agreement with Hosseinzadeh et al. and Radostits et al. (Hosseinzadeh et al. 1996; Arsenaault et al. 2003; Radostits et al. 2007). In the present study, the oldest group (>3 years of age) had the highest infection rate and was statistically different from the other groups. Based on these results and with this knowledge that we do not use vaccine against CLA in Iran, it seems that education of sheep owners, disinfection utilities in shearing sheep, and drug usage in the control of ectoparasites is very important in the prevention and control of CLA in this part of the country.

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