



Research Paper

Clinical and Microbiological Insights into Caseous
Lymphadenitis in Sheep and Goats in Khorasan Razavi, IranAnoosh Firozeh¹, Gholamreza Mohammadi^{1*}, Mehrnaz Rad²

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ABSTRACT

Introduction: Caseous lymphadenitis (CLA), a chronic bacterial disease caused by *Corynebacterium pseudotuberculosis*, significantly impacts small-ruminant health and productivity worldwide, causing economic losses through reduced wool and milk yields, reproductive issues, and carcass condemnation. Despite its importance, CLA prevalence and microbial dynamics remain under explored in Iran, where small ruminants are vital to rural economies. This study assessed the prevalence, clinical manifestations, and bacteriological profile of CLA in Khorasan Razavi Province, northeast Iran, to inform regional control strategies and address potential zoonotic risks.**Materials & Methods:** We examined 15 flocks totaling 4,733 animals (4,640 sheep, 93 goats) through clinical inspections and microbiological analysis of pus samples from affected lymph nodes.**Results:** The results revealed a lymphadenitis prevalence of 11.59% (95% CI, 10.58%, 12.66%), with 8.62% of sheep (400/4640) and 8.60% of goats (8/93) affected, varying across flocks from 0% to 28.57%. Submandibular lymph nodes were most commonly affected (51.35%), followed by retropharyngeal (18.02%) and parotid (15.32%) nodes, with peak incidence in the 2–3-year age group (38.24%), likely linked to shearing practices. Bacteriological analysis of 102 pus samples identified *C. pseudotuberculosis* in 19.6% (20/102) of cases, characterized by small, dry, white colonies with β -hemolysis on Columbia blood agar. A diverse microbial profile included *Actinobacillus* spp. (7.8%), *Trueperella pyogenes* (3.9%), and novel isolates like *Acinetobacter* spp. and *Yersinia* spp. (1.0% each), with 43.14% of samples sterile, suggesting chronicity or sampling challenges.

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Conclusion: These findings indicate CLA etiology is complex, extending beyond a single pathogen and influenced by local husbandry practices. The study underscores CLA's economic burden and zoonotic potential, given rare but documented human cases. Integrated control measures—enhanced molecular diagnostics, recombinant phospholipase D (PLD) vaccine trials, and improved biosecurity—are urgently needed. Future research should prioritize genomic strain typing and environmental reservoir analysis to refine CLA management in Northeast Iran, offering insights applicable to similar agroecosystems globally.

1. Introduction

Caseous lymphadenitis (CLA), caused by *Corynebacterium pseudotuberculosis*, is a major bacterial disease affecting small ruminants globally, leading to significant economic losses through reduced wool and milk production, reproductive challenges, premature culling, carcass condemnation, and occasional mortality. This gram-positive, facultative intracellular, non-spore-forming, non-capsulated, non-motile pleomorphic bacterium uses a potent phospholipase D (PLD) exotoxin and a mycolic acid-rich cell wall to evade host defenses and cause tissue necrosis [1-5]. In Iran, where small ruminants are critical to rural livelihoods, CLA impact is substantial yet poorly documented [6, 7].

CLA typically presents as enlarged superficial lymph nodes (e.g. submandibular, parotid, prescapular, prefemoral, popliteal, supramammary) and visceral lesions in organs such as the liver, lungs, and kidneys [8]. Lesions are characterized by necrotizing, purulent inflammation with caseous cores [9]. Diagnosis relies on bacterial culture, though chronic lesions often yield few viable bacteria, complicating detection [10]. Biochemical tests and molecular methods, such as polymerase chain reaction (PCR), improve confirmation, despite variability in results [11, 12].

Recent studies have expanded CLA's epidemiological scope. Research by de Sá et al. (2023) and Almeida et al. (2024) highlights co-infections with pathogens like *Staphylococcus* spp. and *Trueperella pyogenes*, alongside environmental triggers such as shearing and overcrowding [13, 14]. Genomic analyses reveal strain diversity, influencing virulence and vaccine response [5, 15]. Emerging evidence also suggests zoonotic potential, with human cases linked to occupational exposure [16]. This study investigates CLA prevalence, clinical features, and bacteriological profile in Khorasan Razavi Province, Northeast Iran, to inform regional control strategies and contribute to global understanding.

2. Materials and Methods

The study covered 15 small ruminant flocks in Khorasan Razavi Province, Northeast Iran, comprising 4,733 animals (4,640 sheep, 93 goats). Clinical examinations identified lymphadenitis cases, documenting age, sex, affected lymph nodes, lesion size, and consistency (e.g. firm, caseous, liquefied). Pus samples were collected from 10–25% of affected animals per flock (102 total), using manual restraint, 70% alcohol disinfection, and a 16-gauge sterile syringe. Samples were stored near ice packs and transported to Ferdowsi University of Mashhad's microbiological laboratory within 6 hours.

Samples were inoculated onto Columbia blood agar (with 5% sheep blood) and MacConkey agar, incubated at 37 °C for 48–72 hours under aerobic conditions, and inspected for colony morphology. Subcultures purified isolates as needed. Smears underwent Gram staining and microscopic analysis (1000×magnification), followed by biochemical tests: Catalase, oxidase, urease, motility, and fermentation (glucose, maltose, sucrose). Suspected *C. pseudotuberculosis* isolates were confirmed via synergistic hemolysis with *Rhodococcus equi* [2, 11].

Descriptive statistics calculated prevalence by flock, species, sex, and age group. Confidence intervals (95% CI) were computed for prevalence estimates using the Wilson score method. Pearson correlation coefficients assessed the relationship between flock size and prevalence. Chi-square tests evaluated associations between lymphadenitis prevalence and categorical variables (sex, age group, lymph node site). All statistical analyses were performed using SPSS (version 27.0; IBM Corp., Armonk, NY, USA), with the significance level set at $P < 0.05$.

3. Results

3.1. Descriptive outcome

Lymphadenitis prevalence across the study area was 11.59% (95% CI, 10.58%, 12.66%), with flock-specific rates ranging from 0% to 28.57% (Table 1). No signifi-



Figure 1. Ewe showing evidence of CLA in the submandibular lymph node (red arrow)

cant linear relationship was found between flock size and prevalence ($r=-0.018$, $P=0.23$).

Affected lymph nodes included submandibular (51.35%), retropharyngeal (18.02%), parotid (15.32%), prescapular (9.01%), superficial cervical (3.60%), and others (inguinal, facial, etc., 2.70%) (Figures 1 and 2, Table 2). A chi-square test showed significant variation in lymph node site distribution ($P<0.001$).

Lesions averaged 2–5 cm in diameter, with 80% exhibiting caseous consistency and 15% showing liquefaction, indicative of chronicity. Females were more affected (66.67%) than males (33.33%) ($P<0.001$), possibly due to management practices like milking or shearing exposure. Age distribution peaked at 2–3 years (38.24%), followed by <1 year (33.33%), 1–2 years (24.51%), and >3 years (3.92%) ($P<0.001$) (Table 3, Figure 3).

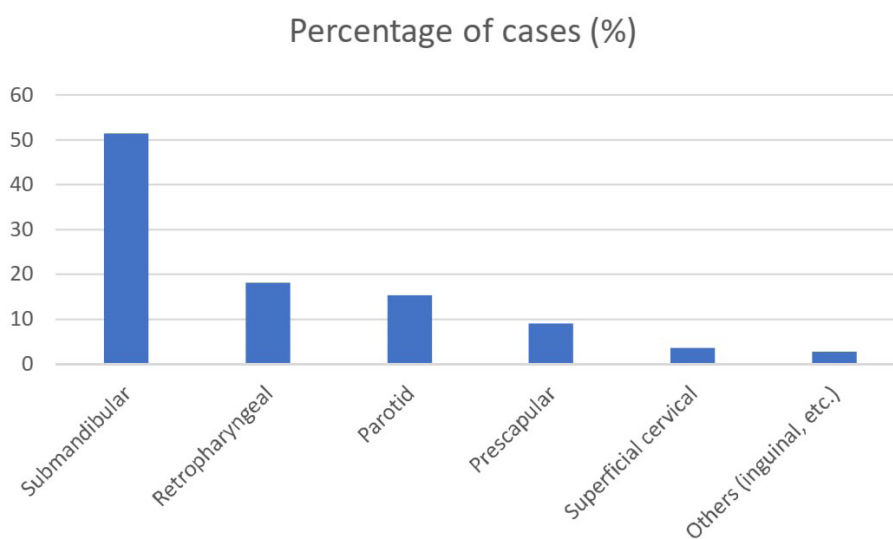


Figure 2. Distribution of CLA by lymph node site

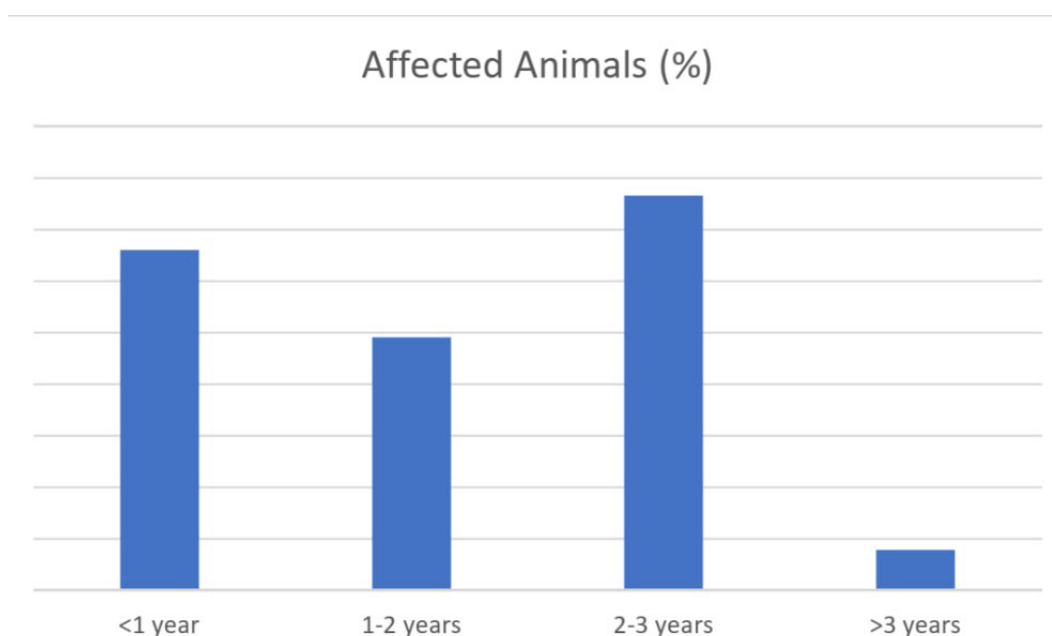


Figure 3. Prevalence of CLA by age group in Khorasan Razavi flocks

Table 1. Correlation between flock size and prevalence of CLA in Khorasan Razavi Province flocks

Flock ID	Flock Size (n)	Number Affected (n)	Prevalence (%)
1	200	0	0
2	250	5	2
3	300	10	3.33
4	350	15	4.29
5	400	25	6.25
6	450	35	7.78
7	500	45	9
8	550	60	10.91
9	600	70	11.67
10	350	50	14.29
11	300	45	15
12	250	40	16
13	200	35	17.5
14	150	30	20
15	128	43	28.57
Total	4733	408	11.59

Note: There is no significant linear relationship between herd size and the prevalence of caseous lymphadenitis in the studied population ($r=-0.018$, $P=0.23$).

Table 2. Distribution of affected lymph nodes

Lymph Node	No. (%)
Submandibular	209(51.35)
Retropharyngeal	74(18.02)
Parotid	63(15.32)
Prescapular	37(9.01)
Superficial cervical	15(3.6)
Others (inguinal, etc.)	11(2.7)

Note: Based on 408 affected animals; "Others" includes inguinal, facial, etc.

Table 3. Prevalence of lymphadenitis by age and sex

Age Group (y)	No. (%)		
	Male	Female	Total
<1	48(11.76)	88(21.57)	136(33.33)
1-2	36(8.82)	64(15.69)	100(24.51)
2-3	48(11.76)	108(26.47)	156(38.24)
>3	4(0.98)	12(2.94)	16(3.92)
Total	136(33.33)	272(66.67)	(100)

Note: Data derived from clinical examinations of 4,733 animals (4,640 sheep, 93 goats).

Table 4. Bacterial isolates from lymphadenitis samples (n=102)

No.	Isolate	No. (%)
1	<i>C. pseudotuberculosis</i>	20(19.6)
2	<i>Coryneform bacteria</i>	15(14.7)
3	<i>Actinobacillus</i> spp.	8(7.8)
4	<i>Actinomyces</i> spp.	5(4.9)
5	<i>Trueperella pyogenes</i>	4(3.9)
6	Mixed bacteria	3(2.9)
7	<i>A. lignieresii</i>	2(2)
8	<i>Micrococcus</i> spp	2(2)
9	<i>Acinetobacter</i> spp.	1(1)
10	<i>S. saprophyticus</i>	1(1)
11	<i>E. coli</i>	1(1)
12	<i>Yersinia</i> spp	1(1)
13	No growth	44(43.14)

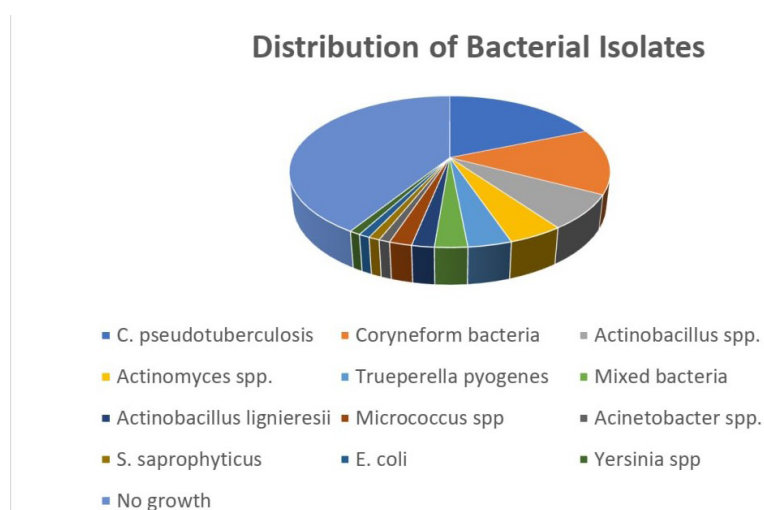


Figure 4. Bacterial isolates from lymphadenitis samples in Khorasan Razavi Province

3.2. Microbiological results

Bacteria were isolated in 56.86% of samples, with 43.14% sterile. *C. pseudotuberculosis* was isolated in 19.6% (20/102) of samples, forming small, dry, white colonies with β -hemolysis on Columbia blood agar. Other isolates included *Coryneform bacteria* (14.7%), *Actinobacillus spp.* (7.8%), *Actinomyces spp.* (4.9%), *Trueperella pyogenes* (3.9%), mixed bacteria (2.9%), *Actinobacillus lignieresii* (2.0%), *Micrococcus spp.* (2.0%), *Acinetobacter spp.* (1.0%), *Staphylococcus saprophyticus* (1.0%), *Escherichia coli* (1.0%), and *Yersinia spp.* (1.0%) (Table 4, Figure 4).

3.3. Epidemiological insights

Sheep showed a slightly higher prevalence (8.62%, 95% CI, 7.84%, 9.46%) than goats (8.60%, 95% CI, 4.43%, 15.99%), though the small goat sample (n=93)

limits robust comparison ($P=0.99$). Flock size showed no significant correlation with prevalence ($r=-0.018$, $P=0.23$), suggesting transmission dynamics beyond density (Figure 5). The submandibular focus (51.35%) may reflect regional feeding practices (e.g. prickly forage) or shearing injuries, differing from prescapular dominance reported elsewhere [12].

4. Discussion

This study confirms CLA as a significant concern in Khorasan Razavi's small ruminant populations, with an overall prevalence of 11.59% (95% CI, 10.58%, 12.66%), affecting 8.62% of sheep and 8.60% of goats. The flock-specific prevalence range (0–28.57%) aligns with global patterns but varies from other Iranian studies. For instance, Zavoshti et al. (2015) reported a higher abattoir-based prevalence of 12.60–20.08% in Iranian

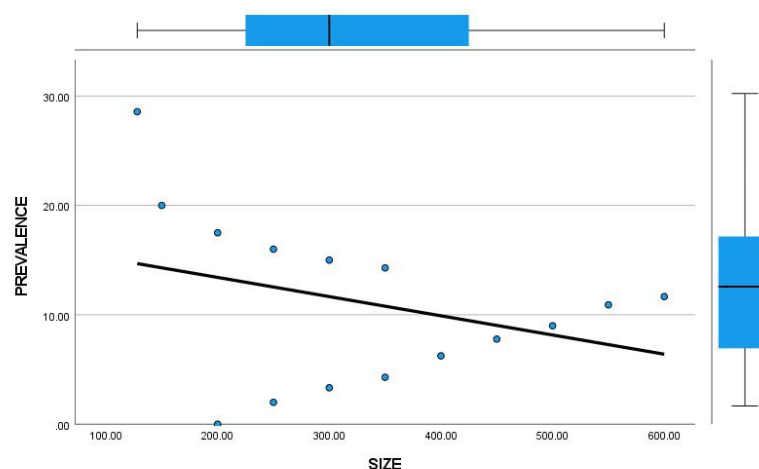


Figure 5. The relationship between the size of sheep and goat herds and the prevalence of lymphadenitis

sheep [17], likely capturing subclinical cases missed in our clinical inspections. Globally, Said et al. (2015) reported 5.1% in North African sheep [18], Nuttall et al. (2018) found 0.2–7.14% in New Zealand [19], and Guimarães et al. (2015) noted a serological prevalence of 70.9% in Brazil [20], highlighting diagnostic method influences. Our clinical prevalence is moderate compared to these, possibly due to regional differences in husbandry or detection methods.

The predominance of submandibular lymph node involvement (51.35%) contrasts with studies reporting prescapular or parotid dominance, such as Cetinkaya et al. (2016) in European flocks [12] or Kuria and Ngatia (1990) in Kenya [21]. This may stem from local practices, such as shearing injuries or thorny forage exposure, which facilitate bacterial entry at submandibular sites. The significant lymph node site variation ($P < 0.001$) underscores the need to consider regional management practices in CLA epidemiology.

The peak incidence in the 2–3-year age group (38.24%) aligns with shearing-related transmission, as noted by Paton et al. (1994) [22], with a significant age effect ($P < 0.001$). The decline in older animals (>3 years, 3.92%) likely reflects culling practices, consistent with Silva et al. (2018) [4]. The higher prevalence in females (66.67%, $P < 0.00$) may result from prolonged herd retention for milking or breeding, increasing exposure risks compared to males, a pattern also observed by Guimarães et al. (2015) [20].

Bacteriological analysis identified *C. pseudotuberculosis* in 19.6% of samples, consistent with its role as the primary CLA pathogen [23–25]. However, the diverse microbial profile, including *Actinobacillus* spp. (7.8%), *Trueperella pyogenes* (3.9%), and novel isolates like *Acinetobacter* spp. and *Yersinia* spp. (1.0% each), suggests a complex etiology. This mirrors findings by de Sá et al. (2023) and Almeida et al. (2024), who reported multi-pathogen dynamics in CLA lesions [13, 14]. The presence of *A. lignieresii* raises concerns about cross-species transmission, as noted by Rodriguez et al. (2025) [5]. The high sterility rate (43.14%) exceeds reports from acute cases (e.g. 20% in Martins et al., 2024 [15]), likely due to chronic lesion encapsulation or sampling limitations, as described by Costa et al. (2017) [10]. Compared to Magdy et al. (2017) in the Middle East, where *C. pseudotuberculosis* dominated (26.92%) [8], our lower isolation rate may reflect regional strain differences or diagnostic challenges.

The lack of correlation between flock size and prevalence ($r = -0.018$, $P = 0.23$) contrasts with studies like Hajtos et al. (2017), which linked larger flocks to higher CLA rates due to crowding [25]. This discrepancy suggests that transmission in Khorasan Razavi Province is driven more by husbandry practices (e.g. shearing, feeding) than flock density. The zoonotic potential, though rare, is concerning given reports of human cases [16], particularly for shepherds and shearers in this region.

These findings highlight CLA's economic and welfare impacts in northeast Iran, necessitating integrated control strategies. Compared to Iran's national data (e.g. Zavoshti et al., 2015 [17]), our prevalence is lower, possibly due to clinical versus abattoir-based detection. Globally, our microbial diversity aligns with emerging multi-pathogen models [13, 14], but the high sterility rate suggests a need for advanced diagnostics like real-time PCR or metagenomics, as recommended by Cetinkaya et al. (2016) [12]. Recombinant PLD vaccines, tested by Martins et al. (2024) [15], and CRISPR-based strain typing [27] offer promising solutions but are underutilized in Iran. Enhanced biosecurity, targeting shearing and environmental reservoirs, is critical to reducing CLA's burden, aligning with global trends toward precision epidemiology.

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Compliance with ethical guidelines

All applicable international, national, and institutional guidelines for the care and use of animals were followed.

Data availability

The data supporting this study's findings are available upon request from the corresponding author.

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Authors' contributions

Conceptualization, study design, statistical analysis, review and editing: Gholamreza Mohammadi; Data acquisition: Anoosh Firozeh; Project administration, technical, and material support, data analysis and inter-

pretation: Gholamreza Mohammadi and Mehrnaz Rad;
Writing the original draft: All authors.

Conflict of interest

The authors declared no conflict of interest.

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