Caseous Lymphadenitis in sheep and goats – "Cheese Glands"

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Abstract

Caseous lymphadenitis (CLA) is a chronic infectious disease that affects small ruminants and is caused by *Corynebacterium pseudotuberculosis*. This highly contagious pathogen leads to significant economic losses in the livestock industry due to loss of productivity, rejection of carcasses, and increased veterinary costs. CLA is characterised by the formation of abscesses in lymph nodes and internal organs. Rupture of these nodes can lead to additional contamination of the environment and further transmission. Diagnosis of CLA involves clinical examination, bacterial cultures, serological testing, and advanced molecular techniques for more accurate detection. Treatment options are limited and often ineffective as the pathogen can survive in abscesses and evade the host's immune response. Antibiotic therapy can provide temporary relief but does not eliminate infection, emphasising the importance of prevention measures. Control strategies focus on biosecurity, culling infected animals, and vaccination. While currently available vaccines reduce the incidence and severity of the disease, they do not provide complete immunity and need to be further improved. Understanding the virulence mechanisms of the pathogen and the interactions between the host and pathogen is crucial for the development of more effective vaccines and therapeutic approaches. Ongoing research and new ideas are crucial to reduce the impact of CLA on animal health and the farm economy. This emphasises the need for comprehensive management strategies, including strict hygiene measures, regular checks, and targeted vaccination plans. In addition, due to its zoonotic potential, *C. pseudotuberculosis* can contaminate meat and milk from infected animals, posing a risk to consumers. The ability of the pathogen to infect both animals and humans emphasises the importance of research into its prevention and diagnosis.

Key words: *small ruminants; caseous lymphadenitis; Corynebacterium pseudotuberculosis; abscess; zoonotic potential*

Introduction

Caseous lymphadenitis (CLA), commonly called 'cheese gland', is a chronic bacterial disease of small ruminants that causes pyogranulomatous lesions (Bettini et al., 2022). CLA is a worldwide contagious disease for which there are no effective control measures. Once it infects a flock of sheep or goats, it is difficult to

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control due to its resistance to treatment, its persistence in the environment, and the difficulty of identifying animals with subclinical infections (Yitagesu et al., 2020).

The causative agent of the disease, *Corynebacterium (C.) pseudotuberculosis* biovar *ovis*, is a facultative, anaerobic, Gram-positive bacterium that does not form spores, has no capsule, and is non-motile. It has a pleomorphic form and can live both inside and outside cells (Oreiby, 2015). The bacterium is characterised by its virulence due to its strong phospholipase D (PLD) exotoxin and mycolic acid-rich cell wall, which play a crucial role in disease pathogenesis (Schlicher et al., 2021). Due to differences in the results of the nitrate reduction test, *C. pseudotuberculosis* is divided into two biovars—biovar *ovis* and biovar *equi*—with biovar *ovis* being of greater importance for sheep and goats (Markova et al., 2024).

Clinically, CLA can present either as palpable superficial abscesses or with symptoms of internal organ involvement. These manifestations can occur separately or simultaneously (Oreiby, 2015). When these abscesses rupture, transmission between animals occurs through direct contact as well as ingestion, inhalation, or contact with contaminated objects such as ear tags, shearing equipment, castration instruments, and feed, leading to rapid spread within the herd (Osman et al., 2018).

The potential consequences of CLA include reduced fertility, progressive weight loss, rejection of carcasses at slaughterhouses, and reduced milk and wool production, all leading to significant economic losses (Arsenault et al., 2003; Al-Gaabary et al., 2009; Abebe and Sisay, 2015; Thongkwow et al., 2019; Ruiz et al., 2020). For this reason, the detection of infected animals is crucial for the success of control measures. The diagnosis of CLA in small ruminants primarily involves the identification of the characteristic clinical signs of the disease and the isolation of *C. pseudotuberculosis* from the abscesses of affected animals (Baird and Fontaine, 2007). Subclinical carriers that are undetectable during clinical examinations represent a significant source of infection for healthy animals (Kaba et al., 2024). Identification of these cases requires alternative diagnostic methods, such as enzyme-linked immunosorbent assays (ELISAs) or molecular techniques (Selim et al., 2021). Developing a diagnostic plan that includes one or more diagnostic methods, preferably repeated, is crucial as no single test can detect all CLA cases. This plan should take into account the prevalence of the disease, symptoms, vaccination strategy, presence of other infections, economic capacity, and available diagnostic facilities (Oreiby, 2015).

Although caseous lymphadenitis is not considered zoonosis, it is important to note that *C. pseudotuberculosis* has zoonotic potential (Bastos et al., 2012). Since the first case of human infection in 1966, around 30 cases have been reported, highlighting the potential risks for veterinarians and farmers (Heggelung et al., 2015). In humans, the infection can occur through direct contact with CLA pus or by consuming contaminated products such as unpasteurised milk or undercooked meat (Thongkwow et al., 2019; Bettini et al., 2022). In addition, bacteria can be transmitted via air in laboratories, posing a risk of pneumonia in laboratory workers (Heggelung et al., 2015).

This disease has been present in Croatia for decades, but its prevalence and its potential economic impact remain largely unknown. Even after diagnosis, control programmes are not implemented, po-

tentially leading to a rise in disease incidence (Baćan, 2021).

Brief history

Caseous lymphadenitis (CLA) was isolated for the first time in 1888 by French veterinarian Edward Nocard, from a bovine suffering from lymphangitis. Three years later, Bulgarian bacteriologist Hugo von Preisz discovered a similar bacterium in an ewe with a renal abscess. This organism was formerly known as the "Preisz-Nocard" bacillus (Bastos, 2012).

Due to its resemblance to mycobacterial tuberculosis lesions, German bacteriologists Lehmann and Neumann termed it *Bacillus pseudotuberculosis* at the end of the 19th century. This name is derived from the Greek term *pseudes tuberculosis*, meaning "false tuberculosis" (Baird and Fontaine, 2007; Bastos, 2012).

The first edition of Bergey's Manual of Determinative Bacteriology, published in 1923, reclassified the organism as *Corynebacterium ovis* due to its resemblance to *Corynebacterium diphtheriae*. However, additional isolations from a variety of mammal species, including humans, led to a 1948 reclassification as *Corynebacterium pseudotuberculosis* in Bergey's Manual; this classification has remained in place ever since (Baird and Fontaine, 2007).

Etiology

Corynebacterium pseudotuberculosis is a Gram-positive bacterium in the genus *Corynebacterium* belonging to the class *Actinobacteria* . It is closely related to other genera like *Mycobacterium*, *Nocardia*, and *Rhodococcus*, collectively known as the CMNR group. Due to their common traits, including a high guanine-cytosine (G + C) content of the genome (47–74%) and a unique cell wall structure, these species are important in both veterinary and human medicine. They also cause pyogenic to granulomatous clinical infections (Bastos et al., 2012; de Oliveira Zamprogna et al., 2021; Markova et al., 2024).

Based on host preferences and nitrate-reducing activity, which is determined by the presence or lack of the *narG* gene in a PCR Multiplex test, *C. pseudotuberculosis* is divided into two biovars: biovar *ovis* and biovar *equi* (Dorneles et al., 2014; Parise et al., 2018; Schlichter et al., 2021). These two biovars have been confirmed by biomolecular techniques, and sequencing data was deposited at the National Center for Biotechnology Information (NCBI). Biovar *ovis* is mainly isolated from infections in sheep and goats resulting in superficial and visceral abscesses, while the biovar *equi*, from horses and cattle, causes ulcerating lymphangitis of the distal extremities and ventral abscesses of the thorax and abdomen (Munoz et al., 2016; Markova et al., 2024). However, according to Schlichter et al. (2021), the biovars do not exhibit specificity for a single species host, as there have also been reports of the illness in llamas, alpacas, pigs, deer, camels, and buffalos (Ruiz et al., 2020).

Corynebacterium pseudotuberculosis is a nonencapsulated, non-sporing, non-motile, and fimbriated bacterium (Baazizi et al., 2024). This pleomorphic strain of the facultative intracellular pathogen coccobacillus frequently displays a characteristic palisade or "Chinese letter" pattern. The tiny cells range in size from 0.5–0.6 μm to 1.0–3.0 μm (Ivanović et al., 2009). The bacterial cell wall is made up of lipids, mesodiaminopimelic acid, arabinogalactan, and mycolic acid. The waxy covering of mycolic acid shields the bacteria from the phagocyte lysosomes' en-

zymatic activity and aids in the formation of abscesses (Fontaine and Baird, 2008; Guimaraes et al., 2011; McVey et al., 2013). In terms of biological activity, it hydrolyses urea and generates catalase, sulfuric acid, and phospholipase D, while nitrate reduction varies: biovar *ovis* is nitrate reductase negative, whereas the biovar *equi* is positive (Dominiguez et al., 2021).

C. pseudotuberculosis can grow in both aerobic and anaerobic conditions. Following 48–72 hours of incubation on sheep blood agar at 37°C, tiny, white, dry-in-consistency colonies with a thin zone of β-haemolysis around them are observed. On the other hand, in Brain-Heart Infusion (BHI) broth, abundant growth with yellowish-white sediment is seen (Pepin and Paton, 2009; Markey et al., 2013).

According to *in vitro* studies, various strains typically respond to ampicillin, chloramphenicol, lincomycin, gentamicin, tetracycline, penicillin G, tetracyclines, sulfamethoxazole-trimethoprim, and neomycin. However, some researchers have identified resistance to penicillin, nitrofurantoin, furazolidone, and streptomycin. It has been observed that the bacterium forms biofilms when simulating a natural infection environment, leading to resistance against every antibiotic that has been tested (Dorella et al., 2006; Stefanska et al., 2010).

Pathogenesis

The most common entry route is through skin lesions or mucosal membranes of the eyes, nose, and mouth, although some sources suggest it can also enter through intact skin (Williamson, 2001; Pepin and Paton, 2009; Habuš et al., 2015). The moment when the bacteria enters the host, lysosomes begin to phagocytose them due to the triggered immunological response. Because of its waxy mycolic acid coat, the bacterium is protected from the hydrolytic enzymes of lysosomes and survives phagocytosis, persisting within the host as a facultative intracellular parasite. The bacteria disseminates by lymphatic drainage to regional lymph nodes, where they continue to multiply and cause the lysis of lysosomes. Mycolic acid leads to degenerative changes and death in phagocytizing leucocytes, contributing to abscess formation (Baird, 2007; Guimaraes et al., 2011; Osman et al., 2018).

The bacteria lyses, breaks loose, and is phagocytosed once more. This recurring process of bacterial multiplication within lysosomes and host cell necrosis leads to a classic CLA lymph node abscess, which soon develops a fibrous capsule (Williamson, 2001; Baird, 2007). The body's inflammatory response can often stop an infection from spreading beyond the skin, though this is not always the case. Usually, the infection leads to inflammation of proximal lymph nodes, eventually causing them to break down. If these lesions do not create an external opening, the infection often progresses to a chronic state, characterised by the formation of 'cheesy gland' lesions (Windsor and Bush, 2016). In sheep, these abscesses have an onion-like structure with concentric fibrous layers and caseous material, whereas in goats, they form a dry, uniform, purulent paste due to differences in phagocytic enzyme activity between the two species (Ruiz et al., 2020; Habte et al., 2023).

The infection may occasionally spread to other areas of the body, affecting visceral organs like the brain, liver, kidneys, and lungs, where secondary abscesses may form, as a result of ongoing inflammatory cell infiltration and increased blood vessel permeability (Williamson, 2001;

Baird, 2003). This is caused by another major virulence factor, the potent exotoxin phospholipase D. It breaks down sphingomyelin in blood vessel walls, which leads to increased permeability and contributes to the spread of bacteria through tissues (Williamson, 2001; Constable et al., 2017; Oliviera et al., 2017).

Moreover, *C. pseudotuberculosis* belongs to the group of bacteria known as the "*Corynebacterium diphtheriae* complex," which also contains species with veterinary and medical importance that can produce the diphtheria toxin (DT). It can induce DT using a beta-corynebacteriophage that encodes the diphtheria toxin gene for the deadly human disease diphtheria. However, there are not many strains of this species that are capable of producing the toxin (Schlicher et al., 2021; do Nascimento Sousa et al., 2024).

Clinical signs

Depending on where pyogranulomatous lesions are located, CLA manifests in small ruminants in two primary forms: external (superficial or cutaneous) and internal (visceral) form. These forms can also coexist within a single animal (Dorella et al., 2006).

The external form is characterised by abscess of lymph nodes that may be palpated externally (mandibular, parotid, pre-scapular, superficial cervical, subiliac, popliteal, and supramammary) and, less frequently, swelling of the subcutaneous tissues (Baird, 2007; Ruiz et al., 2020). The internal form of pyogranulomatous lesions is characterised by its ability to develop internally in organs such as the lungs, liver, spleen, kidneys, uterus, and internal lymph nodes like the mediastinal, bronchial, and lumbar (Williamson, 2001; Baird, 2007; Guimaraes et al., 2011; Tongkwow et al., 2019). According to Guimaraes et al., (2009), goats are more prone than sheep to develop the external form of CLA, whereas the internal form is more prevalent in sheep as fewer than 20% of cases are recorded in goats (Habuš et al., 2015).

External abscesses are discrete, solid, painless swellings under the skin that eventually grow into encapsulated, visible masses. They typically occur in or near a peripheral lymph node. These lesions may last for several months or even years. After reaching maturity, they quickly burst through a fistula, releasing a thick, odorless, greenish-white, purulent material containing bacteria into the surrounding environment, where the microbe can survive for weeks to months. As the wound gradually heals, scar tissue forms. Mature abscesses often recur in the same animal months or years later due to incomplete infection elimination (Williamson, 2001; Baird, 2007; Guimaraes et al., 2011; Habte et al., 2023).

Unless the abscesses interfere with breathing or swallowing, these animals do not exhibit symptoms of disease (Piotr et al., 2016). Conversely, the internal form of the CLA shows few clinical signs (purulent nasal secretion, cough, fever, chronic weight loss) and remains undetected until a post-mortem examination, which makes gathering prevalence statistics more difficult (Arsenault et al., 2003). Unless the animal shows signs of chronic emaciation (weight loss and weakness), also known in sheep as thin-ewe syndrome, it is not possible to identify this form of the disease based solely on general appearance (Dorella et al., 2006).

Epizootiology

Whether or not they show clinical symptoms, infected animals are the main source of infection. These animals release

a significant amount of viable bacteria into the soil, water, feed, pastures, handling equipment, and facilities where the causative agent can remain for several months. This occurs through pus from spontaneously draining abscesses, nasal secretions, and faeces. Therefore, transmission can occur either by direct physical contact with the affected animal or indirectly via contaminated fomites, and also via ingestion or inhalation (Williamson, 2001; Guimaraes et al., 2011; Burmayan and Brundage, 2021). According to Spier et al. (2004), flies and other insects may act as possible disease vectors. Based on available data, it appears that *C. pseudotuberculosis* has been found on the exterior surfaces of domestic flies, acting as mechanical vectors, and in fly intestines and faeces, acting as biological vectors. This has considerable epidemiological importance for horses and cattle (Guimaraes et al., 2011). Transmission primarily occurs through contamination of skin injuries, including cuts and scratches, which frequently happen during procedures like shearing, docking, ear-tagging, and castration (de Oliveira Zamprogna et al., 2021; Baazizi et al., 2024). Additionally, fighting among herd or flock mates and other traumatic events, as well as environmental factors such as metal waves, nails, wire fences, and barbed wire, can significantly contribute to lesions in the skin of the animals, opening passage for the entry of bacteria (Guimaraes et al., 2011; Constable et al., 2017).

CLA primarily enters a flock or herd by introducing a clinically or subclinically infected carrier animal (O'Reilly et al., 2008). Baird (2003) stated that the most important source of infection in other flocks is an animal with lung lesions. They can produce an aerosol containing *C. pseudotuberculosis* organisms, releasing the pathogen through exhaled air, thereby transferring the infection to free animals within the flock (Ruiz et al., 2020). These flocks exhibit a rapid increase in CLA seroprevalence. Conditions such as close contact and poor ventilation, often found in small paddocks, could facilitate rapid spread of the infection through aerosols, potentially affecting many animals. This assertion was supported by El Khalfaoui et al. (2024), who indicated a strong correlation between the highest CLA risk and inadequate barn conditions.

Also, the risk of infection increases with shearers and their equipment, which are inevitably exposed to the purulent discharges of superficial CLA lesions. Shearing tools that are not cleansed before and after use, such as ear tagging or tattooing equipment, also raise the possibility of infection (Baird, 2003; Dorella et al., 2006; Fontaine and Baird, 2008). Baths for controlling ectoparasites also pose a risk as bacteria can persist within them. Sheep sheared just days before receiving ectoparasite therapy are more susceptible. Due to repeated exposure to infection during shearing, older individuals are more susceptible than younger ones (Baćan, 2021).

Diagnostics

Numerous methods can be used to diagnose CLA. Lesions can be identified by clinical or postmortem exams, but the most reliable method is thought to be the isolation and identification of *C. pseudotuberculosis* bacteria. However, animals with internal abscesses pose a greater diagnostic challenge, as lesions may take up to six months to manifest (Gascoigne et al., 2020). These animals can be diagnosed through radiography and transtracheal aspiration (Williamson, 2001).

Furthermore, the bacterium can be cytologically identified by Giemsa and

Gramme staining. However, this may be limited, especially when sampling old and calcified lesions (Gascoigne et al., 2020). Further, the use of standardised and miniaturised test kits, such as the Analytical Profile Index Coryne kit from bioMérieux (UK), for the identification of coryneform bacteria, has simplified biochemical profiling. This kit includes 21 tests for enzymatic activity or carbohydrate fermentation (Baird and Fontaine, 2007).

The challenges of clinically identifying CLA have led to the development of several serodiagnostic tests. The Immunoenzymatic test (ELISA) is the most commonly used one in live animals. CLA stimulates both humoral and cellular immunity, allowing for the measurement of immunoglobulin G (IgG) and interferon-gamma (IFN-γ) as indicators of each, respectively (Gascoigne et al., 2020). Voigt et al. (2012) pointed out that the serology test against the exotoxin PLD (Elitest CLA; Hyphen Biomed) is most commonly used due to its cost efficacy and acceptable test performance, demonstrating a specificity of 98% and a sensitivity of 87%. Many other tests have been reported to have excellent specificity, though they all suffer from relatively poor sensitivity, which contributes to certain false negative test results (Baird, 2003). For that reason, ELISA has been developed to detect gamma interferon. The IFN-γ ELISA test appears to be more sensitive than the normal antibody ELISA, and it is unaffected by the vaccinal status of the sheep (Dorella et al., 2006; Gascoigne et al., 2020). The currently used commercially available serological test for CLA infection in Croatia is Elitest CLA Hyphen BioMed (France).

DNA-based techniques such as enzyme restriction of chromosomal DNA, ribotyping, multiplex polymerase chain reaction-restriction, polymerase chain reaction-restriction fragment length polymorphism (PCR - RFLP), Pulse-Field Gel Electrophoresis (PFGE) and Random Amplified Polymorphic DNA (RAPD) are used to classify *C. pseudotuberculosis* into two biovars. However, these methods do not provide further characterisation, particularly among biovar *ovis* isolates, because of the high genetic homogeneity within the species. Therefore, Enterobacterial Repetitive Intergenic Consensus PCR (ERIC-PCR) has proven to have good discriminatory power, typeability, and promising results for investigating the epidemiological relationships and sources of *C. pseudotuberculosis* infection in sheep and goats. (Dorneles et al., 2014; Schlicher et al., 2021; El Damaty et al., 2023). While ERIC-PCR is helpful, it has significant drawbacks, including limited repeatability, poor standardisation, and challenges in comparing typing patterns between laboratories. For better reproducibility, molecular typing methods based on the amplification of housekeeping genes, such as multilocus sequence typing (MLST) and multilocus sequence analysis (MLSA), are preferred (Schlicher et al., 2021). The most detailed and informative epidemiological tool available today is whole genome sequencing (WGS).

WGS of *C. pseudotuberculosis* strains offer high-quality comparative genomic studies. This helps identify genes related to virulence, antimicrobial resistance, and environmental adaptation, enabling targeted therapeutic and immunological interventions (Costa et al., 2017; Markova et al., 2024). Advancements in sequencing technology have led to the complete sequencing of 125 *C. pseudotuberculosis* strains from 19 countries and regions, providing valuable data for comparative genomics studies. The majority of isolates

were from goats (28%), sheep (24%), and horses (22%), with only one isolate from camel (biovar *ovis*) and one from llama (biovar *equi*) (Meng et al., 2023). Even though many genomes have been deciphered, virulence factors remain incompletely understood (Dias et al., 2016).

Differential diagnosis

Differentiating between CLA and other bacterial infections based solely on abscesses in the lymph node region is challenging. Therefore, for any purulent skin disorder, bacteriology should be used to obtain an early diagnosis as soon as possible. Due to the large amounts of bacteria in pus, it can be easily cultured on blood agar and identified after three to four days of incubation. All suspected CLA cases should be isolated from the flock until a definitive diagnosis is confirmed (Baird, 2003; Listos et al., 2016; Gascoigne et al., 2020).

Key differential diagnoses include other potential infections such as actinobacillosis, also known as "cruels" or "king's evil," caused by *Actinobacillus lignieresii*. This infection occurs sporadically in sheep and leads to granulomatous lesions and suppurative adenitis in the lymph nodes of the head (Baird, 2003; Gascoigne et al., 2020). Although it is not commonly associated with lymph node lesions, *Actinomyces pyogenes* is another opportunistic pathogen that is occasionally isolated from subcutaneous abscesses in sheep and goats (Baird, 2003). Another disease that can be mistaken for CLA is Morel's disease, caused by *Staphylococcus aureus* subspecies *anaerobius*. According to Habuš et al. (2015), both diseases share a similar epizootiology and are characterised by the formation of abscesses in or close to major superficial lymph nodes. Unlike Morel's disease, which mainly affects young goats and has a shorter incubation period, the clinical picture of CLA usually involves a fewer and smaller abscesses per animal (Pepin and Paton, 2009; Saeed and Alharbi, 2014; Habuš et al., 2015).

Additional differential diagnoses to take into account include trauma, hematoma, healing fractures, salivary mucocele, granulomas, dermal cysts, submandibular oedema caused by parasites, *Fasciola hepatica* and *Haemonchus sp*. or lymphosarcoma (Gascoigne et al., 2020; Habte et al., 2023). It can be particularly challenging to diagnose the internal form of caseous lymphadenitis, as it can be mistaken for pneumonia caused by pathogens like *Mycobacterium bovis*, *Pasteurella haemolytica*, *Pasteurella multocida*, or ovine progressive pneumonia brought on by an infection with the Maedi-Visna virus infection (Guimaraes et al., 2011).

Treatment

Given CLA's highly contagious nature, capacity to impact many systems, and the ongoing struggle to eradicate infection, the limits of traditional therapeutic options become evident. This emphasises the critical need for alternative options in animal healthcare (Gascoigne et al., 2020).

Even though *C. pseudotuberculosis* is sensitive to almost all antibiotics tested *in vitro* (penicillin, tetracyclines, and cephalosporins), parenteral antibiotic treatment is ineffective against abscesses due to the presence of fibrosis, a thick, purulent exudate, and the bacteria's intracellular location (Pepin and Papon, 2009; Osman et al., 2018). Therefore, antibiotic treatment is an inviable option for herd-level disease management due to its inefficacy and high cost (Guimaraes et al., 2011). For external cutaneous lesions, palliative

care is administered locally, particularly for valuable animals, as an alternative to culling. This can involve debriding the abscess by extracting the purulent material or surgically excising the entire lesion with parenteral antibiotic treatment for 4-6 weeks to reduce the likelihood of recurrence (De la Fuente Mancera et al., 2024). This approach is still considered unreliable since it relies on the antibiotic to eliminate all infectious organisms from the treated lesions and assumes the absence of any internal lesions. As a result, reports of using such techniques remain discouraging (Baird and Fontaine, 2007).

However, Sellera et al. (2016) used antimicrobial photodynamic therapy (APDT) as an alternative treatment for localised infections. This was performed after surgically draining the lymph nodes. Within six months of the procedure, there were no recurrences in the treated lymph nodes.

Disease prevention and control

As previously mentioned, the introduction of infected or abscessed animals is the primary source of infection and, within two to three years, the prevalence of abscesses increases dramatically. For this reason, control programmes should involve regular clinical inspections and periodic serology testing of all animals in the flock. Animals showing clinical signs or testing serologically positive should be isolated from the healthy ones and culled because once infected, they rarely eliminate *C. pseudotuberculosis* (Guimaraes et al., 2009; Windsor, 2011; Baazizi et al., 2024). According to Voigt et al. (2012), by performing blood tests every three months and eliminating any animal that tested positive for culture or seropositivity, flock seropositivity was reported to have decreased from 10% to 0.4% in just two years. Nevertheless, this type of procedure has many drawbacks, including high expenses and difficulties like false positives and negatives, which can affect testing efficiency and economic outcomes (Gascoigne et al., 2020).

Moreover, precautions against environmental wounding must be taken, such as the use of smooth wire fences, sterilizing tools and shearing equipment, disposable needles, insect control, and disinfecting wounds with 10% iodine. Herd facilities should be sanitised with 10% formaldehyde (Williamson, 2001; Baird and Fontaine, 2007). To reduce CLA's causal agent transmission risk, isolating young, newly sheared sheep, shearing young sheep first, minimizing post-shearing cover time, and reducing ectoparasite dips are recommended (Windsor, 2011).

The most suitable approach to disease control and prevention is continuous immunisation, which is primarily used in nations with high infection rates due to the inefficiency and high expense of treating caseous lymphadenitis. Although vaccination does not completely eradicate the disease, it does slow the spread of infection and cause a gradual decrease in disease prevalence (Windsor and Bush, 2016). Therefore, vaccinating animals regularly is necessary to lower the bacterial load and safeguard younger animals while older infected ones are culled. It also must be kept in mind that vaccines vary in efficacy between sheep and goats, requiring tailored vaccination programmes (Windsor, 2011; Burmayan and Brundage, 2021).

Both commercial and experimental vaccines exist. In many places, commercial CLA vaccines are licensed and accessible; these are primarily toxoid vaccines with several uses. They are made

using antigens from several *Clostridium* pathogens, including *Clostridium tetani*, *Clostridium perfringens*, *Clostridium novyi*, *Clostridium chauvoei*, and *Clostridium septicum*, along with inactivated PLD from *C. pseudotuberculosis*. Examples of these vaccine formulations include Glanvac® by Vetrepharm Inc. in England and Biodectin® by Fort Dodge LTD in Australia. Even though these commercial vaccines have been on the market for decades, none offer complete protection against CLA. The immunity they offer is frequently insufficient and only partially effective, and varies between goats and sheep. Additionally, their safety is debatable as there have been reports of side effects such as fever, lethargy, injection site infections or abscesses, and decreased milk production (Dorella et al., 2006; Ribeiro et al., 2014; De Pinho et al., 2021).

On the other hand, there are various types of experimental vaccines, such as bacterin, toxoid, combined, live, and DNA vaccines. Combined vaccines, including formalin-killed whole cells with PLD-rich supernate or clostridial toxoids, have demonstrated the most promising results by showing complete protection against experimental infections (Windsor, 2011; Gascoigne et al, 2020). Researchers continue to work toward creating vaccines that provide effective and long-lasting defense against CLA, though they also face difficulties in guaranteeing efficiency in a range of animal species and immune responses (Windsor, 2011; De Pinho et al., 2021).

In conclusion, controlling and eradicating CLA is challenging once introduced because of its rapid spread within a flock and the establishment of infected individuals as reservoirs. The consensus generally favours the vaccination of healthy animals combined with identifying and isolating or culling infected individuals as the most effective strategy for disease control (Habte, 2023).

Geographical distribution/ global prevalence

CLA is widely distributed in the world's major sheep-rearing areas, where its prevalence is often remarkably high. The disease is also prevalent within the smaller European small ruminant sector (Baird, 2003). However, only a few countries have conducted epidemiological studies to determine disease prevalence rates, and most of these studies were based on farm and abattoir research (Osman et al., 2018). Studies were performed in Australia, Canada, USA, Mexico, Argentina, Brazil, and Africa. These countries showed a high disease prevalence, ranging from 12.60% to 61.00%. In European countries, such as the Netherlands, Denmark, Norway, England, Italy, Germany, Island, Slovakia, Czech Republic, Poland, and Spain, studies have reported lower prevalences (0-6.4%), while Spain has the highest rates. Recent epidemiological investigations have also been conducted in Iran, Egypt, Algeria, the Falkland Islands, and Brazil. However, the number of countries affected by this disease is likely underreported (Zavoshti et al., 2011; Ruiz et al., 2020).

Dominguez et al. (2021) surveyed 264 veterinarians and 510 farmers in the UK. The survey revealed that only 18% of veterinarians had encountered at least one case of the disease, while 45% of farmers had observed abscesses in their sheep. Only a few farmers investigate the cause of these abscesses. However, laboratory diagnoses on 32 farms confirmed the disease in 24 cases, so the prevalence of CLA in different regions or countries largely depends on many factors.

Therefore, variations in disease frequency across studies can be attributed to differences in management systems, climatic conditions, and the viability of the causative organism in the contaminated environment, which is influenced by ambient temperature. Additionally, the endemic nature of the disease results in variation levels of animal immunity and the degree of animal susceptibility (Al-Gaabary et al., 2009).

Only two studies have reported prevalence data of caseous lymphadenitis in Croatia based on clinical and bacteriological testing, reporting a prevalence of infected goat flocks of 12.5% (1/8) (Baćan, 2021) and 30% (15/50) (Habuš et al., 2015). The first study was conducted in Karlovačka County, while the location was not specified in the second study. There is no further information about the prevalence of CLA in sheep and goats in Croatia (Habuš et al., 2015; Baćan, 2021).

Future outlook

The worldwide small ruminant sector faces serious economic and welfare challenges due to CLA, which results in large losses for farmers in the form of decreased milk and wool production, as well as reproductive problems including mastitis and infertility brought on by hormone imbalances and sperm abnormalities. Also, CLA leads to skin depreciation and either total or partial confiscation of the carcasses (Osman et al., 2018; De la Fuente Mancera et al., 2024). Although vaccination has shown a great deal of effectiveness in reducing the prevalence of disease in infected flocks, it still requires ongoing immunisation, client cooperation, and good communication. Alternative strategies like serotesting and culling should be studied to achieve "CLA-free status" (Gascoigne et al., 2020). Therefore, to prevent the endemic situation that exists in most countries, small ruminant industries will inevitably need to implement control techniques (Baird, 2003).

To ensure success, hygiene education of herd owners and technical personnel must be prioritised in all control actions. For those working directly (shepherds, shearers, abattoir workers, butchers, and veterinarians) or indirectly (neighbors and farm visitors) with herds, it is essential to provide information on production cycle losses and the zoonotic potential of *C. pseudotuberculosis* (Guimaraes et al., 2011; Thongkwow et al., 2019). This knowledge is necessary for the successful implementation of control measures (Guimaraes et al., 2011).

Further research is needed to understand the economic impacts and prevalence of CLA in sheep and goats, since awareness of the disease is minimal. According to Pioquinto et al. (2023), the disease will eventually become endemic once it infects a flock and causes a rise in CLA prevalence. Therefore, greater efforts need to be invested into raising awareness of this disease.

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Kazeozni limfadenitis u ovaca i koza - 'Cheese Glands'

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Kazeozni limfadenitis je kronična zarazna bolest koja zahvaća male preživače, a prouzročena je bakterijom *Corynebacterium pseudotuberculosis.* Zbog svoje visoko kontagiozne prirode, uzrokuje znatne ekonomske gubitke u stočarstvu zbog smanjene produktivnosti, odbacivanja trupova i povećanih veterinarskih troškova. Bolest je karakterizirana stvaranjem apscesa u limfnim čvorovima i unutarnjim organima, koji nakon što puknu, prouzroče kontaminaciju okoliša i dovode do daljnjeg širenja bolesti. Dijagnoza bolesti uključuje: klinički pregled, bakterijsku kulturu, serološke testove i napredne molekularne metode za preciznije otkrivanje bolesti. Opcije liječenja su ograničene i često neučinkovite zbog sposobnosti patogena da preživi unutar apscesa i izbjegne imunološki odgovor domaćina. Terapija antibioticima može pružiti privremeno olakšanje, ali ne eliminira infekciju, naglašavajući važnost preventivnih mjera. Strategije kontrole bolesti usmjerene su na biološku sigurnost, klanje zaraženih životinja i cijepljenje. Trenutno dostupna cjepiva, iako smanjuju učestalost i težinu bolesti, ne pružaju potpunu zaštitu i zahtijevaju daljnja istraživanja. Razumijevanje virulentnih mehanizama patogena i interakcije domaćin-patogen ključno je za razvoj učinkovitijih cjepiva i liječenje. Kontinuirana istraživanja i nove ideje su ključne za smanjenje učinaka kazeoznog limfadenitisa na zdravlje životinja i stočarstva. To naglašava potrebu za sveobuhvatnim strategijama kontroliranja bolesti, uključujući stroge higijenske mjere, redovite preglede i ciljana cijepljenja. Nadalje, zbog zoonotskog potencijala, *C. pseudotuberculosis* može kontaminirati meso i mlijeko zaraženih životinja, što predstavlja rizik za potrošače. Sposobnost patogena da inficira životinje i ljude pridodaje važnosti za daljnja istraživanja metoda prevencije i dijagnoze.

Ključne riječi: *mali preživači, kazeozni limfadenitis, Corynebacterium pseudotuberculosis, apsces, potencijalna zoonoza*