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Etiological Agent, Pathogenesis, Diagnosis, Treatment, Measures for Prevention and Control of Caseous Lymphadenitis Disease in the Small Ruminants with Special Reference to Sheep

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Abstract

Caseous lymphadenitis (CL) is a chronic infectious disease caused by *Corynebacterium pseudotuberculosis* (*C. pseudotuberculosis*) that is transmitted mainly by contact with contaminated exudate wounds. Caseous lymphadenitis affects different species of animals and is considered an occupational zoonotic disease. CL is responsible for important economic losses in the small ruminant industry, which include decreased production, damaged quality of milk and wool, reproductive disorders, total or partial confiscation of carcasses, and depreciation of the skin. Caseous lymphadenitis disease can present in two clinical pictures: a skin or superficial picture and a visceral or deep picture. The presumptive diagnosis of CL in the skin is based on the exploration of superficial lymph nodes. These have little value in diagnosing visceral CL, where the main sign is emaciation. The prevention and control of CL involve identifying the skin condition and debriding the abscesses. In addition to the treatment of superficial lesions, the animal facilities must be repaired and adapted. To avoid exposure of negative herds to CL, new animals must be subjected to observation to identify any of the clinical manifestations. Another form of control is vaccination, though only some countries have commercial vaccines. General information on the etiological agent and its characteristics can be used to improve the understanding of this disease.

Keywords

Abscess, CMNR, *Corynebacterium pseudotuberculosis*, Goat, CL, Lymph Nodes, Pseudotuberculosis, Sheep

1. Introduction

Caseous lymphadenitis (CL), also known as pseudotuberculosis, is a chronic infectious disease caused by *C. pseudotuberculosis*, characterized by the formation of abscesses and is caused by *C. pseudotuberculosis*. This disease has been reported in different geographical areas and affects various animal species, but mainly small ruminants. CL is widely disseminated in herds for different zootechnical purposes, so much so that in some countries, it is even commented that "if you have sheep/goats, you have CL." The prevalence in each productive unit is variable and typical. The diagnosis is mainly clinical when superficial abscesses are identified, and only in some cases is a laboratory diagnosis reached. The socioeconomic reasons are diverse and include not recognizing the importance of the disease. The importance of recognizing the disease depends on the quality-quantity approach; that is, if the depreciation of skins due to the lesions is appreciable at first glance, but not the quality of the wool or milk, even if there is a decrease in these qualities, many farms would not considered this a disease of high economic impact. Additionally, with the progress made by studies focused on reproduction, it has been found that CL damages spermatozoa, possibly even rendering the animal infertile, causing important productive losses. In turn, although CL is a zoonotic disease, the precautions that owners and workers take in production facilities or in slaughterhouses are minimal when it can be considered an occupational disease.

In Caseous lymphadenitis, the infection occurs mainly by direct contact with the caseous exudate of the abscess, but there are reports of transmission through milk consumption. The clinical picture, which is unpredictable and has almost zero mortality, makes the diagnosis and control of the disease difficult. In addition, this disease has not been given due importance since economic losses are small for some producers. In recent years, there have been several studies in molecular biology and bioinformatics to find virulence factors that would facilitate our understanding of the pathogenic mechanisms of this disease to support the development of immunogens that would reduce infection. Despite this growing focus, many aspects of the pathogenesis of CL are still unknown.

This review aims to provide a description of the knowledge required by zootechnical veterinarians and laboratories for diagnosing CL. Measures for prevention, control, and diagnosis should be studied and improved to reduce the prevalence of CL.

2. Search Strategy

This review was done through a search in ScienceDirect [https://www-sciencedirect-com] using the terms *C. pseudotuberculosis*, caseous lymphadenitis, and CL in combination with each of the following words: sheep, goats, small ruminants, pathogenesis, abscesses, and pseudotuberculosis.

Caseous lymphadenitis (CL) is a contagious disease with a chronic course and worldwide distribution. Caused by *C. pseudotuberculosis*, it affects mainly sheep

and goats, although it can also affect horses, cattle, llamas, and alpacas. It is a zoonotic disease that is mainly occupational in nature. The disease is characterized by the formation of caseous lesions in the lymph nodes and sometimes in other organs. Serious cases of toxemia have also been described, generating anemia with emaciation that can even lead to death in kids and lambs. In goats, the lymph nodes of the head (parotid, lateral retropharyngeal, and mandibular) and neck (superficial cervical) are affected. In sheep, the most affected lymph nodes are the parotid, lateral retropharyngeal, mandibular, superficial cervical, ischial, popliteal, and inguinal [1]-[10].

The economic importance of CL lies in losses for the producer, which include a decrease in the production and quality of milk and wool and reproductive disorders (mastitis and hormonal problems in females and males, leading to infertility due to morphological changes in sperm). Total or partial confiscation of the carcass and depreciation of the skin are also recorded. On free-standing farms, this pest lowers the economic value of these animals and is a risk factor for the spread of the disease to herds where these animals enter as breeders [1] [11] [12] [13] [14] [15].

3. Etiological Agent

Corynebacterium is a member of the phylogenetic branch of the order *Corynebacteriales*. According to studies of the mycolic acids present in the cell wall of these bacteria, they maintain a close relationship with the genera *Mycobacterium*, *Nocardia*, and *Rhodococcus* and are associated with the genera *Corynebacterium*, *Mycobacterium*, *Nocardia*, and *Rhodococcus* (CMNR).

Corynebacterium pseudotuberculosis was first isolated in 1888 by Nocard and fully identified in 1894 by Preisz [3]. *C. pseudotuberculosis* is a Gram-positive, nonsporulated, immobile, encapsulated bacterium with a short, thin, or pleomorphic bacillary shape (0.5 to 0.6 μm × 1.0 to 3.0 μm). This bacterium is isolated or grouped into pairs that form V or L angles, palisades, or even Chinese character-like forms. It is a facultative intracellular bacterium. In blood agar, it undergoes *β*-hemolysis. It can grow under aerobic or anaerobic conditions (5% - 10% CO_2) at 37˚C at a pH of 7 to 7.2. For the development of the bacteria, the culture media (solid or liquid) must be supplemented with serum or blood, although good growth occurs in unsupplemented PPLO medium [1].

On solid media, the colonies are dry and friable. After incubating at 37˚C for 24 h, small yellowish-white colonies are observed; these colonies grow and increase in diameter by 1 - 2 mm after 48 h [3] [14] [15]. In liquid media, the bacteria grow in the lower part, a film may form on the surface, and there is no turbidity. To obtain large amounts of *C. pseudotuberculosis*, the use of 0.1% or 1% Tween 20 favors bacterial growth [13] [14] [16].

Corynebacterium pseudotuberculosis strains are classified into two biotypes based on their ability to reduce nitrates: the *ovis* biotype, which does not reduce nitrates, and the *equi* biotype, which reduces nitrates. Most of the strains iso-

lated from small ruminants do not reduce nitrates, but nitrate-reducing strains have been reported from sheep [1] [16] [17]. For identification via biochemical tests, the carbohydrate reduction test, and some strains may or may not reduce arabinose, dextrin, or sucrose [3].

Corynebacterium pseudotuberculosis strains are viable at −15˚C for 480 days and for 170 days when stored with glycerin in the refrigerator. Ayers, 1977 [18] demonstrated the survival of the bacteria for 80 and 120 days in soils with moderate and high concentrations of organic matter, respectively. In conclusion, long-term bacterial survival is favored by the higher content of organic matter added to the loamy-silty texture, and pH and soil salinity do not seem to have an effect.

The antimicrobial spectrum of *in vitro* strains is generally susceptible to ampicillin, chloramphenicol, lincomycin, gentamicin, tetracycline, penicillin G, tetracyclines, sulfamethoxazole-trimethoprim, and neomycin. Resistance to streptomycin, penicillin, nitrofurantoin, and furozolidone has been reported in some studies [3] [19] [20] [21]. replicated the environment of a natural infection, showing that when this bacterium forms biofilms, it becomes resistant to all antibiotics.

4. Genetics

Corynebacterium pseudotuberculosis has a chromosome of 2.28 to 2.34 Mb that contains roughly 2111 to 2195 genes and presents 52.2% to 52.88% G + C bases. The central genome comprises 1810 genes, which are considered highly conserved. The *16SrRNA*, *rpoB*, and *pld* genes have been used for the differentiation of *C. pseudotuberculosis* from other closely related species, such as *C. ulcerans* and *C. diphtheriae*. To identify the genus *Corynebacterium*, 16S rRNA gene analysis is performed. The *rpoB* gene, which encodes the *β* subunit of RNA polymerase and is approximately 3500 bp, has also been used for the identification of the genus and for the construction of the phylogenetic tree. Fragments of 434 to 452 bp of the *rpoB* gene are useful for the identification of related pathogenic species within the diphtheria group, and the *pld* gene, which encodes phospholipase D, is used for the identification of the species and is the main factor involved in the virulence of *C. pseudotuberculosis* [22] [23].

Li *et al.* (2018) [20] proposed the use of the *fusA* gene for differentiating between the *ovis* biotype and the *equi* biotype through phylogenetic analyses and epidemiological studies. The absence or presence of the *narG* gene has been proposed for use in classifying the biotype *ovis* and biotype *equi,* respectively [24]. The strains of the *ovis* biotype present genetic similarities to each other, as with the strains of the *equi* biotype, but when comparing the strains of the *ovis* biotype with those of the *equi* biotype, there are genetic differences between them. This difference can be explained by the difference between the signs and clinical pictures of the disease.

Corynebacterium pseudotuberculosis has characteristics such as loss of genes,

low G + C content, and a reduced genome that differentiates it from those of nonpathogenic species of corynebacteria. The presence of 50 - 53 pseudogenes has also been reported. All *ovis* biotype strains present four copies of *rDNA* operons and 95% similarity of amino acids in terms of G + C content, which may explain their slow replication [25] [26]. Advances in molecular biology have allowed the description of the genetic content of *C. pseudotuberculosis.* D'Alfonseca *et al.* (2010) [27] mentioned phospholipase D, dehydrogenase enzymes, proteins for obtaining iron, and the heat shock proteins as important parts of the identification of bacteria and pathogenicity factors. Among the virulence factors that have been recognized as the most important for the pathogenesis of *C. pseudotuberculosis* are the exotoxin (phospholipase D) and mycolic acid forms that are part of its wall. Other virulence factors and proteins relevant to survival during pathogenesis have also been proposed.

Hodgson *et al.* (1990) [28] reported the first virulence gene, *pld*, describing its function and importance in the pathogenesis and the characterization of *C. pseudotuberculosis.* Iron acquisition is a survival strategy during infection. In the case of *C. pseudotuberculosis*, the genes that code for this function are located within the pathogenicity islands described for this microorganism, which suggests that its acquisition was due to horizontal transfer. Billington *et al.* (2002) [29] described the presence of the *fagABCD* operon, which is located downstream of the *pld* gene; within this operon are the genes *fagA*, *fagB*, *fagC*, and *fagD*, which present 32% - 47% identity to proteins that acquire iron, such as *ABC* transporters. In the strains that can cause disease, the *aroB* and *aroQ* genes, which encode aromatic amino acid synthesis proteins, have been identified by removing genes and challenging animals [27] [30].

Pathogenicity islands contain virulence elements, including iron-producing genes, fimbrial subunits, adhesion factors, insertion elements, and secreted proteins, which are acquired by horizontal transfer. Ruiz *et al.* (2011) [26] reported pathogenicity islands for the first time for *C. pseudotuberculosis* using prediction software for two strains (sheep and goats) of biotype *ovis*. They found seven characteristic regions of codon usage different from those of the gene in tRNA-flanking and transposon genes. These regions were classified as islands of pathogenicity in *C. pseudotuberculosis* (PiCp), which encode proteins of the AB transport system, glycosyl transferase, two components of systems for obtaining iron, the operon *fagABC*, and phospholipase D. Soares *et al.* (2013) [25] sequenced the genome of a biotype *equi* strain and, with the use of the PIPS software, found 11 islands of pathogenicity in *C. pseudotuberculosis*. As reported by Ruiz *et al.* (2011) [26], seven additional strains (PiCp 1 - 7) and four additional strains (PiCp 8 - 11) exhibit great genetic plasticity (insertion, deletion, and substitution). D'Afonseca *et al.* (2010) [27] discussed the different methods used to determine the pathogenicity of *C. pseudotuberculosis* with reduced costs and sequencing time. These authors proposed technologies for sequencing, such as SOLiD, GS FLX, Ion Torrent PGM, and Illumina platforms. The knowledge ob-

tained with the study of pathogenicity islands will allow us to know what elements will be necessary to create an immunogen that prevents.

5. Virulence Factors

The secretion of exotoxins and mycolic acids has long been recognized as a major virulence factor. Currently, the mechanisms for obtaining iron (operon *fagABC*) and the formation of heat shock proteins for survival under various temperatures are beginning to be recognized as important factors for survival during infection. Phospholipase D, a powerful exotoxin produced by bacteria, can hydrolyze sphingomyelin, which is responsible for hemolysis in blood agar and for its pathogenic action on the membranes of phagocytes. It also promotes dissemination by increasing permeability in the vascular endothelial membrane and allowing invasion of *C. pseudotuberculosis* through lymphatic drainage and necrosis during infection [1] [3] [7] [9] [31] [32]. Mycolic acids (corynemycolic acids) are chemotactic elements for phagocytes, especially for neutrophils, and they have leukotoxic effects that cause degeneration and lysis of macrophages and polymorphonuclear cells. They contribute to the formation of abscesses and induces resistance to enzymes in the phagolysosome, as well as to the environment [1] [4] [12].

6. Pathogenesis

Corynebacterium pseudotuberculosis is transmitted mainly through skin wounds but can also be spread through mucosal lesions. Chronic infection in small ruminants results in the formation of abscesses, which can present in two different forms: 1) external abscesses, known as cutaneous or superficial abscesses, characteristic of abscess formation in superficial lymph nodes or subcutaneous tissue; 2) visceral or deep abscesses, characterized by unobservable lesions [1] [9], commonly affecting the internal lymph nodes, mainly the mediastinal nodes, frequently spreading to parenchymal organs such as the lung, liver, kidney, mammary gland or testes and less frequently to the heart, brain, spinal cord, uterus, and joints [33] [34].

When infection occurs, the bacteria spread within the host phagocytes to regional lymph nodes, where a short period of inflammation occurs. In the cortical region of the lymph node, microabscesses develop within 24 hours of infection. Approximately 6 days later, they fuse and elongate to form significant lesions containing a cluster of bacteria, cellular dendrites, and purulent exudate. The lesions continue to expand, repeating cycles of necrosis and encapsulation. Initially, the content is soft and semifluid, until it solidifies and acquires concentric capsular layers (onion ring injury). Dissemination via blood or lymphatics can lead to lesions in parenchymal organs or internal lymph nodes [35] [36].

7. Immunity

Corynebacterium pseudotuberculosis induces a complex humoral and cellular

immune response that relies on components of the bacterial cell wall and on phospholipase D [7] [33] [37] [38]. Normally, the antibodies produced against *C. pseudotuberculosis* and its exotoxin are of the IgG and IgM types [39].

The macrophage response tends to be nonspecific, so that once activated, macrophages can destroy a wide variety of bacteria. Macrophages are activated through stimulation with interferon (IFN)-γ and interleukin 2 (IL)-2. Upon phagocytosis, many nonactivated macrophages undergo degeneration (first 6 h); on the other hand, activated macrophages resist degradation due to the increase in lysosomal granules, and polymorphonuclear cells undergo degradation (up to 20 h) [18].

IFN-γ is produced by $CD4^+$ lymphocytes after stimulation by an antigen and activates macrophages. After being stimulated, tumor necrosis factor (TNF)-α is produced by macrophages and causes the activation of neutrophils and macrophages, an increase in the expression of adhesion molecules on leukocytes, and an increase in the expression of the major histocompatibility complex. The gene expression levels for the generation of TNF-α and INF-γ are highest on day 7 postinoculation, a time that corresponds to an amplification phase in the formation of pyogranulomas, highlighting macrophages and lymphocytes. The initiation phase is characterized by the arrival of neutrophils at the site of inoculation and at the lymph nodes, and the stabilization phase is characterized by the maturation and persistence of the pyogranuloma [35] [36] [40] [41].

Colostral transfer of antibodies is important in naturally acquired immunity. Maternal antibody levels are maintained in lambs up to 2.5 months of age, explaining why lambs under 3 months of age are rarely infected [13] [42] [43] [44].

8. Diagnosis

The clinical diagnosis is based on the signs, symptoms, epidemiological parameters, and morphopathology of the abscess, which is suspected to be CL, so a confirmatory laboratory diagnosis (bacteriological and/or immunological) is necessary in all patients. Ayers (1977) [18] established the phases of CL diagnosis in the field and on the trail [1] [5] [18] [45]. In the cutaneous form, the isolation of *C. pseudotuberculosis* from the contents of abscesses is easy. Isolation is more difficult in animals that have the visceral form, in which postmortem findings are generally obtained [1] [45] [46].

9. Clinical Diagnosis

The clinical manifestations of CL can vary widely from the superficial form (cutaneous lymphadenitis) to the visceral form (internal lymph nodes and/or foci of caseous necrosis in parenchymal organs). Superficial CL is characterized by an increase in the size of superficial lymph nodes due to the purulent focus, which is hard and consistent, without local heat or pain. The consistency changes until it fistulates, and the purulent exudate drains. In visceral CL, CL can occur subclinically since if a vital organ is not affected and clinically shows emaciation or

cachexia, chronic respiratory problems or signs depend on the affected organs [5] [45] [46].

For the morphopathological diagnosis, a lesion represented by a concentric lamination of the caseating necrosis ("onion lesion") should be considered highly suggestive of CL. The abscess may be a creamy, greenish-yellowish, purulent exudate [6] [47] reported that epidemic spread begins with the presentation of superficial abscesses and later with an increase in the frequency of abscesses in the lung, mediastinal and bronchial lymph nodes, eventually becoming endemic. Some of the epidemiological characteristics of the temporal evolution of an outbreak of CL include the following: 1) period from the primary infection to the appearance of the first cases of up to 4 - 6 months; 2) period of increase in prevalence from 3 to 5 years; 3) outbreaks after shearing, collective surgical interventions, or the introduction of new animals; 4) sickness mainly in adults [1] [5] [45].

Caseous lymphadenitis is associated with three syndromes: pyogenic, posterior respiratory, and cachectic lymphadenitis. For subcutaneous abscess syndrome, CL, pyobacillosis (*Arcanobacterium pyogenes*), and abscess disease (*Staphylococcus aureus* subsp. *anaerobicus*) are considered. For posterior respiratory syndrome, especially cachexia syndrome, it is necessary to differentiate between tuberculosis and CL; an anatomopathological diagnosis must be made, but this approach is not conclusive [1] [5] [45] [48].

10. Microbiological Diagnosis

The definitive diagnosis of CL is made by the isolation and identification of the causative agent. In the foci of infection, the exudate contains many corynebacteria; in more advanced lesions, it is possible that the causative agent is nearly absent, with the pyogenic exudative reaction dominating over the proliferative cell [1] [6] [13]. Blood agar is the most widely used isolation medium. On it, colonies are opaque and slippery, with a faint bulge (buttoned) and a smooth edge, surrounded by a halo of beta hemolysis. Selective media such as *Corynebacterium* selective agar (Merck), Muller tellurite agar (Difco), and chocolate tellurite agar can also be used. These media are enriched with bovine or equine serum and blood and supplemented with potassium tellurite. On it, the colonies are black [1] [3] [45] [49]. The miniaturized API bacterial identification system, API Coryne, is effective at identifying and differentiating corynebacteria [3]. According to the results of the CAMP bacterial characterization, a synergistic effect between the hemolysin (equifactor) from *Rhodococcus equi* and *C. pseudotuberculosis* was observed. In contrast, hemolysis (inhibition of CAMP) occurs when the PLD of *C. pseudotuberculosis* is neutralized by the *β*-hemolysin of *Staphylococcus aureus* [1] [3].

11. Immunological, Laboratory, and Molecular Biology Diagnostics

Clinical signs are not present in those animals because they are carriers of inter-

nal lesions or are in the incubation period. Therefore, there are a great variety of immunological tests that use different samples, such as serum, milk, or blood [3] [13] [50] [51] [52] [53].

1) Skin test: Methods used to verify infections caused by intracellular microorganisms and cell-mediated immune status [54]. The formation of a population of mononuclear cells specialized in phagocytizing and destroying microorganisms is induced [55]. The first to try this was Cesari [56], who inoculated and cultivated a filtrate of corynebacteria in guinea pigs, proposing this test for the diagnosis of the disease in rams. Carne y Onon (1978) [57] performed a toxin test, which revealed irregular reactions, and concluded that the test was not adequate for the diagnosis of CL. In 1999, Alves y Olander [58] concluded that purification of the antigen is essential for the intradermal CL test and that the use of a specific antigen can favor diagnosis at the field level and thus be able to detect subclinical infection in herds.

2) Agglutination test: The mechanism is based on the agglutinins that develop after infection. Cameron (1973) [59] used the test, but the results were less than encouraging. In contrast, using a strain of corynebacteria with a lower degree of agglutination, Award (1960) [60] achieved good results.

3) Complement fixation: After agglutination, infected animals seroconvert in the 3rd week of infection, but after 2 months, they are negative, so it is not recommended for the diagnosis of chronic CL. This test is easy to use because it detects antibodies quickly after a week after infection [13].

4) Inhibition of *Staphylococcus aureus* β-hemolysin: The test serum is incubated with PLD, bovine erythrocytes, and staphylococcal hemolysin. The corynebacterium toxin-specific antibodies present in the serum neutralize the exotoxin staphylococcal hemolysin in the absence of PLD, which lyses erythrocytes. This test detects infections beginning at the 3rd week of infection but fails after 5 months postinfection [13].

5) Inhibition of hemolysis via the detection of antibodies against the exotoxin of *C. pseudotuberculosis*: The mechanism consists of the inhibition of the hemolytic activity of the exotoxin by the immune serum of sheep, which can detect subclinically affected animals and antitoxin in the serum of lambs suckled by females that present positive titers at the time of the test [42].

6) Synergistic hemolysis inhibition test: It detects antibodies against the exotoxin of *C. pseudotuberculosis*. Originally developed for horses, it uses PLD in erythrocytes previously treated with a sterile filtrate of phospholipase C produced by *Rhodococcus equi*. This technique is a reliable indicator of active infection and could also be useful for use in disease control schemes in goats [44].

7) Indirect hemagglutination test and tube agglutination: This technique is based on the ability of formalin-sensitized lamb erythrocytes and bis-diazobenzidine to agglutinate in the presence of antibodies against corynebacteria. Being able to do so allows early and chronic infections to be diagnosed [45].

8) Double immunodiffusion technique: It is performed on an agarose gel, where

wells are made, one central (antigen) and six around the central one (sera). As antigens, exotoxin (a nonconcentrated supernatant) and test serum are used as reagents. Those sera where there is a precipitation line are considered positive. An economical and practical method, this approach can yield results within 24 hours [38] [42].

9) Enzyme-linked immunosorbent assay (ELISA): This is based on the principles of antigen-antibody interactions on plastic plates [1]. The following solid-phase antigens have been used: cell wall, whole cells, and sonicated cells [61] [62]. The liquid-phase antigen has also been used as a toxin [1] [63] [64] [65]. ELISAs have been used on goats and sheep, showing promising results, so they are recommended as good alternatives for the diagnosis of infection caused by *C. pseudotuberculosis*. On several occasions, ELISA has even been used to obtain prevalence data and to establish CL eradication programs in herds [3] [12] [45] [65] [66].

10) Detection of the IFN-γ response: This assay is based on the detection of response to whole-cell antigens. Tests are performed for both sheep and goats. Notably, immunization with the vaccine does not interfere with the prognosis, suggesting that the test has value for detecting infection and can be used in CL control programs. Currently, it is performed not only at the serum level but also in milk [53] [67] [68] [69] [70].

11) Surface plasmon resonance: Surface plasmons interact with light, producing a color for the detection of antibodies against different bacteria. In *C. pseudotuberculosis*, specific antibodies against phospholipase D are detected, allowing real-time detection, which is fast and fully automated [71].

12) Western blot: Western blot is normally used for the recognition of antigenic fractions of bacterial extracts, as described by Ellis *et al.* (1991) [37], who reported that sheep sera react with an antigen extracted from bacteria with ether, creating a three-band pattern (25.1, 68, and 31.6 kDa). In addition, immunedominant fractions (31.5, 68, and 120 kDa) and minor antigens (22, 40, 43, and 64 kDa) are detected in bacterial extracts, as is PLD (31 kDa) [19] [71]. Therefore, a reaction to recognized antigenic fractions of *C. pseudotuberculosis* could be diagnosed.

13) Molecular diagnosis using polymerase chain reaction (PCR): This approach allows direct amplification of specific DNA segments of *C. pseudotuberculosis* without the need for cloning [27]. An 815-base pair fragment of the *16S rRNA* gene was amplified [72]; Multiplex PCR (mPCR) has also been used with primers from three genetic regions (*16S rRNA*, *rpoB*, and *pld*) [1] [72] [73].

12. Treatment

Parenteral antibiotic treatment is not effective due to fibrosis in abscesses, the presence of purulent exudate and the intracellular nature of the bacteria. Therefore, palliative treatment is performed locally for superficial CL. This can be done by debriding the abscess by removing the purulent content or by dissecting

the entire lesion and removing it surgically [1] [5] [47].

13. Prevention

To avoid exposing the herd to infection, the animals to be introduced should be subjected to observation to identify possibly affected animals. For wool sheep, the injuries caused by shearing should be healed, and the instrument should be cleaned and disinfected each time it is used [1] [3] [5] [13].

There are different types of experimental vaccines, such as bacterins, toxoids, bacterin toxoids, attenuated toxins, and DNA vaccines. The first of these agents was described by Hodgson *et al.* (1994) [28], who administered a single dose of Toxminus (a mutant strain of *C. pseudotuberculosis*) in combination with PBS orally; however, these agents protected against the challenge and induced a humoral response, preventing good stimulation of Th1 lymphocytes. The researchers also sampled the feces of the vaccinated animals by isolating the Toxminus strain. Commercial vaccines include toxoids from different clostridial species (Glanvac-6) and mixtures of biotypes of *C. pseudotuberculosis*. All these vaccines have had different results and have not prevented infection of the herd [9] [10]. Furthermore, not all vaccines developed for sheep are functional for goats or *vice versa* [26]. In several countries, the main form of control of caseous lymphadenitis is through vaccination, as is the case in Australia, where the prevalence has decreased by up to 20%. Some of these countries have access to commercial vaccines such as Glanvac-6. In Mexico, no commercial vaccines are available [5] [16] [45] [74]. However, our group is investigating the PLD and the somatic antigens present in strain 129 biotype 1, in the design of an immunogen for the prevention of CL in sheep [75].

14. Control

Control becomes vital within the herd since the purulent exudate has a concentration of 1×10^6 to 5×10^7 colony-forming units per gram, and rupture of the abscess is sufficient for contamination of the environment. The ability of *C. pseudotuberculosis* to survive on land and in fomites allows for its continuous presence [5] [45]. The presence of organic matter in the soil gives the bacteria a longer time to survive, so its management should be considered to reduce the bacteria's survival.

Medication with antibacterial agents is ineffective because the bacteria cannot diffuse through the abscess between the purulent material, and the abscess is also an intracellular bacterium. Therefore, other strategies must be undertaken, such as those mentioned below [1] [3] [5] [47].

1) The main recommendation is to debride the abscess by removing the purulent content or by dissecting the entire lesion and removing it surgically [1].

2) Separate the young from their mothers after they are born and feed them colostrum and substitute milk. Manage the offspring in an independent herd to ensure that the animals were seronegative [16].

To supplement vaccination, a sanitation program is proposed: detect infected animals, eliminate all positive animals at diagnosis with or without signs, vaccinate those at risk, and administer general hygiene measures [3] [16] [45] [51] [75]. There are various methods for controlling CL, depending on the herd, which is why mathematical models have been designed to observe the impact of each strategy on the herd. Serological diagnosis is a strong weapon for combatting CL [5] [47].

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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