

## REVIEW: VETERINARY MICROBIOLOGY

## CASEOUS LYMPHADENITIS: EPIDEMIOLOGY, DIAGNOSIS, AND CONTROL

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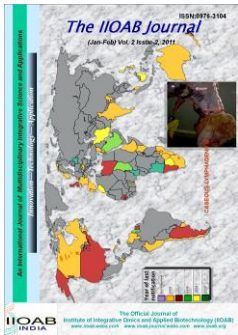
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## ABSTRACT



**Caseous lymphadenitis, caused by *Corynebacterium pseudotuberculosis*, is one of the most important diseases of sheep and goats, causing considerable losses for herd owners. Due to the chronic and generally subclinical nature of infection, control is difficult and prevalence in animals and herds is high. This review describes the principal characteristics of *C. pseudotuberculosis*, including pathogenesis, epidemiology and principal manifestations of caseous lymphadenitis, as well as management practices, diagnostic tests and vaccination as disease control tools.**

**Keywords:** Caseous lymphadenitis; *Corynebacterium pseudotuberculosis*; sheep; goat

## [1] INTRODUCTION

Caseous lymphadenitis is a chronic and subclinical disease of sheep and goat of worldwide distribution, presenting high animal and flock prevalences. *Corynebacterium pseudotuberculosis*, its causal agent, affects sheep and goats, though it can also infect cattle and horses, and rarely, humans; thus, it is considered an occupational zoonosis. The pathogen has been isolated from other species, including pigs, buffaloes, deers, porcupines, llamas, camels and laboratory animals [1, 2].

Distributed throughout much of the world, this disease is found in North and South America, Australia, New Zealand, Europe, Asia and Africa; it causes considerable economic losses, from condemnation of skins and carcasses because of abscesses, to expressive losses in reproductive efficiency, and in wool, meat and milk production. It is the main cause of condemnation of sheep carcasses in slaughterhouses in Australia, one of the world's largest producers of meat and wool [3, 4, 5].

This disease is characterized by abscessing of the lymph nodes; both superficial and visceral. In the superficial form, the peripheral lymph nodes swell and abscess, while in the visceral form there are systemic

complications that can lead to chronic thinning [6]. *C. pseudotuberculosis* is easily disseminated throughout the herd by normal management practices and by environmental contamination [7].

## [II] CLASSIFICATION OF CORYNEBACTERIUM PSEUDOTUBERCULOSIS

*Corynebacterium pseudotuberculosis* belongs to the genus *Corynebacterium*, family *Corynebacteriaceae*, suborder *Corynebacterineae*, order *Actinomycetales*, subclass *Actinobacteridae*, class *Actinobacteria* [8]. The genus

*Corynebacterium* belongs to the *Actinomycetes* group, which also includes the genera *Mycobacterium*, *Nocardia* and *Rhodococcus* [2]. Though the species of these genera, also denominated the CMN group, are quite diverse, they have some characteristics in common, such organization of the cell wall, composed principally of peptidoglycans, arabinogalactan, and mycolic acids, and a high proportion of guanine and cytosine in the genome (G + C = 47 - 74%). The CMN group includes many species of medical and veterinary importance, including *Mycobacterium tuberculosis*, *M. bovis* and *M. leprae*, etiological agents of human and bovine tuberculosis, and of leprosy, respectively, and *C. pseudotuberculosis*.

The bacterium *Corynebacterium pseudotuberculosis* is classified into two biovars [9], the biovar Ovis, which mainly affects sheep and goats, causing superficial and visceral abscesses, and the biovar Equi, which mainly affects horses, causing ulcerating lymphangitis of the distal extremities, ventral abscesses of the thorax and abdomen, and furunculosis. The existence of these two biovars has been confirmed by biomolecular techniques [10, 11, 12, 13].

*Corynebacterium pseudotuberculosis* is a Gram-positive, nonencapsulated, nonsporing, fimbriated bacterium. The cell wall is composed of mesodiaminopimelic, arabinogalactan and corinomycolic acids (lipids), similar to mycolic acid from *Mycobacterium tuberculosis*, but it is not acid-alcohol resistant [14]. The attenuation generated by successive passages is due to thinning of this lipid layer [15].

In stained smears, the rods appear isolated and have pleomorphic forms, from coccoids to filamentous rods, grouped in parallel cells or in a format similar to Chinese letters [16]. According to Collet [17], the microorganism, when removed from culture, does not appear pleomorphic; this was also found for 208 strains of *C. pseudotuberculosis* isolated and identified at the Escola de Veterinária da Universidade Federal de Minas Gerais, obtained from cultures of caseous material collected at a slaughterhouse. The cells are small (0.5-0.6  $\mu\text{m}$  x 1.0- 3.0  $\mu\text{m}$ ), facultative anaerobes and generally contain metachromatic granules [14, 17].

*Corynebacterium pseudotuberculosis* is identified by its morphology, colony characteristics, and biochemical features, mainly carbohydrate fermentation. It produces catalase, sulfidric

acid, phospholipase D (PLD) and hydrolyzes urea. Nitrate reduction varies; it differentiates biovar Ovis, which is nitrate reductase negative, from biovar Equi, nitrate reductase positive [9]. In sheep blood agar, incubated at 37°C, cream-colored colonies, with a  $\beta$ -hemolysis zone, are observed after 48 h. It presents a reverse CAMP test, because there is inhibition of  $\beta$ -hemolysis by *Staphylococcus aureus* and synergy with *Rhodococcus equi* [14, 16]. In liquid culture, it forms a surface film, though the culture remains clear; this film is broken by agitation, forming flakes [14]. The principal characteristics of *C. pseudotuberculosis* that are important for its identification are shown in Table-1 [14, 16].

**Table 1.** Principal phenotypic characteristics of *Corynebacterium pseudotuberculosis* used for identification

| Tests                  | Carbohydrate fermentation |           |   |
|------------------------|---------------------------|-----------|---|
|                        |                           |           |   |
| Metachromatic granules | +                         | Starch    | - |
| $\beta$ -hemolysis     | +                         | Arabinose | V |
| CAMP                   | Reverse                   | Fructose  | + |
| <i>S. aureus</i>       | Inhibition                | Galactose | + |
| <i>R. equi</i>         | Increase                  | Glucose   | + |
| Motility               | -                         | Lactose   | - |
| Oxidase                | -                         | Maltose   | + |
| Catalase               | +                         | Mannitol  | V |
| Nitrate Reduction      | V                         | Mannose   | + |
| Methylene Red          | +                         | Ribose    | + |
| Hydrolysis of:         |                           | Sucrose   | V |
| Casein                 | -                         | Trehalose | - |
| Esculin                | -                         | Xylose    | - |
| Gelatin                | V                         |           |   |
| Hippurate              | -                         |           |   |
| Pyrazinamide           | -                         |           |   |
| Urea                   | +                         |           |   |

+: more than 90% positive; v: 21–89% positive; -: more than 90% negative. Adapted from Jones and Collins (1986)<sup>14</sup> and Quinn et al. (2005).<sup>16</sup>

## [III] EPIDEMIOLOGY AND ECONOMIC IMPACT

Caseous lymphadenitis is distributed worldwide and generally follows the distribution of sheep and goat herds, though in some regions its prevalence may be under-notified. Dissemination of this disease throughout the world probably occurred through importation of infected animals [18]. From 1996 - 2004, among the 201 countries that reported their sanitary situation to the World Animal Health Organization (OIE) [Figure-1], 64 declared that they had animals with caseous lymphadenitis within their borders [19]. These countries are distributed in the Americas (19 of 42 countries), Africa (18 of 51), Asia (11 of 43), Europe (14 of 51) and Oceania (2 of 14) (OIE, 2009). However, the number of countries that have problems with this disease is probably under-notified, because the declaration to OIE is only done by the official sanitary authorities of each country; some countries that have had this disease reported in scientific papers have not made an official declaration, including Brazil.

Prevalences of caseous lymphadenitis as high as 61% were found in Australia [20]; however, more recent studies indicate a prevalence of 20 - 30%, after vaccination began 5. In the USA, prevalences of up to 43% have been estimated [21], similar to the range of 21 - 36% found among sheep in Quebec province in

Canada [4]. In Alberta, also in Canada, vaccination was effective in the reduction of the prevalence of infection.<sup>3</sup> In the United Kingdom, 45% of the producers that were interviewed reported abscesses in their sheep [22].

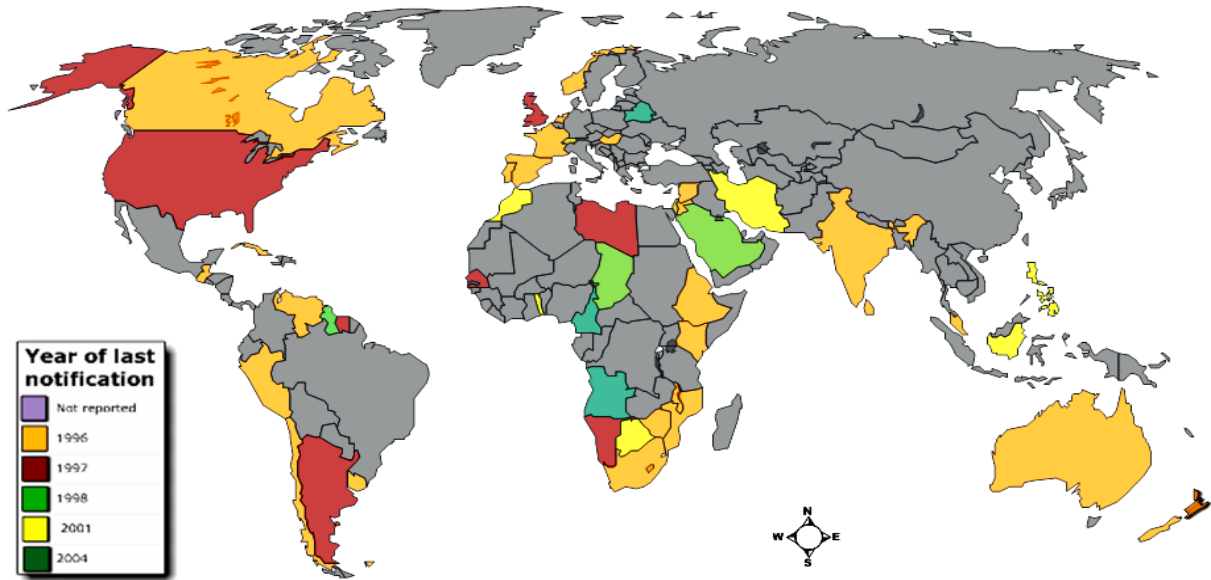


Fig: 1. Map with countries that reported their sanitary situation as a caseous lymphadenitis to the World Animal Health Organization (OIE), from 1996 - 2004

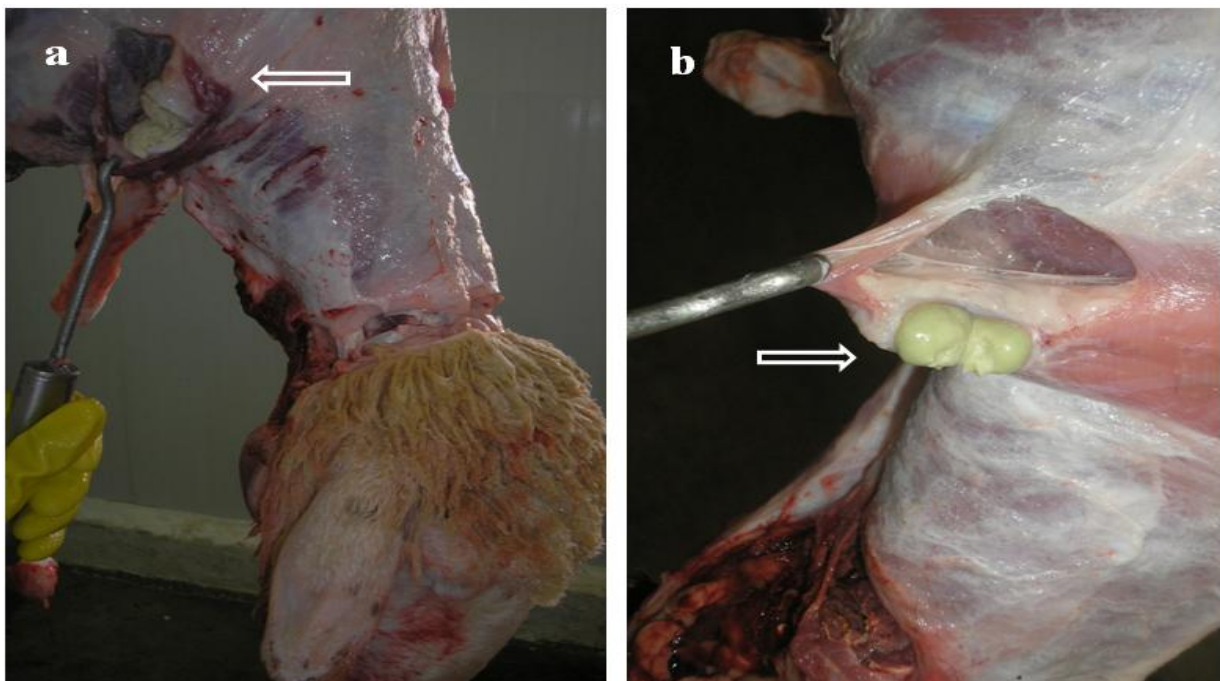


Fig: 2. Condemnation of sheep carcass at slaughterhouse inspection. (a) Pre-scapular lymph node. (b) Superficial lymph node. Arrows indicate pre-scapular lymph nodes with caseous material, characteristic of caseous lymphadenitis in federally inspected slaughterhouse

In Brazil, the first report of caseous lymphadenitis was made by Duport in 1918 [23]. Epidemiological studies have estimated that most Brazilian herds are infected and that clinical prevalence exceeds 30%. In goats, Pinheiro [24] reported 66.9% of animals to have clinical signs of caseous lymphadenitis in Ceará. In Rio de Janeiro, prevalence was reported to vary from 3.6-100% [25] and in a seroepidemiological ELISA study made by our group, in the State of Minas Gerais, we found prevalence figures of 75.8% for sheep [26] and 78.9% for goats [27]. In an ELISA analysis for *C. pseudotuberculosis* in 805 serum samples from sheep from a federally-inspected slaughterhouse in Minas Gerais; we found 377 positive animals, and a high frequency of alterations in the lymph nodes and internal organs [Figure-2]. This confirms the great economical importance of *C. pseudotuberculosis* infection for the sheep industry due to the high rate of carcass condemnation. Various molecular techniques have been used to type *C. pseudotuberculosis*, including RFLP of chromosomal DNA [11], RFLP of ribosomal 16S DNA [10, 11], ribotyping [10, 11], PFGE (pulsed-field gel electrophoresis) [12, 13] and RAPD (randomly amplified DNA polymorphisms) [28, 29]. Though these various techniques have been useful for separating the biovars Ovis and Equi, the species *C. pseudotuberculosis* has been found to be genetically very homogeneous. The two techniques that have given promising results for typing *C. pseudotuberculosis* strains are PFGE and RAPD.

Pulsed-field electrophoresis was used to characterize 50 strains of *C. pseudotuberculosis* isolated from goats, sheep and horses in the United Kingdom [12]; six “pulsetypes” were observed, which allowed the researchers to determine the origin of an outbreak of caseous lymphadenitis. However, in a study of 36 sheep samples and six goat samples from Australia, Canada, Eire, Holland and Northern Ireland, the same research team reported four different “pulsetypes”, with the conclusion that these *C. pseudotuberculosis* strains, both those from sheep and goats, were quite homogeneous [13]. RAPDs were useful in a study of 54 strains of *C. pseudotuberculosis* isolated from horses in four different states of the USA, identifying 10 different genotypes [28]. Also, RAPDs made with other initiators made it possible to define eight genotypes among 61 strains of *C. pseudotuberculosis* isolated from goats in Poland, with a diversity index of 0.539 [29].

The importance of caseous lymphadenitis in Brazil can be estimated by the increase in the participation of goats and sheep in national animal husbandry and its relationship with the economic impact of this disease. Brazil has 16,628,571 sheep and 9,355,220 goats, totaling 25,983,791 animals [30]. The economic losses include decreased milk production, decreased weight gain, reduced value of skins due to scarring, and the cost of the drugs and labor needed to treat superficial abscesses. Losses are increased when the affected lymph nodes are in critical areas (jaw, crural region, udder) negatively affecting chewing, locomotion and milk and meat production; however, economic losses due to this disease have not yet been computed. In industry, losses are due to the lower percent utility of carcasses from affected animals, damage to skins, along with the need for

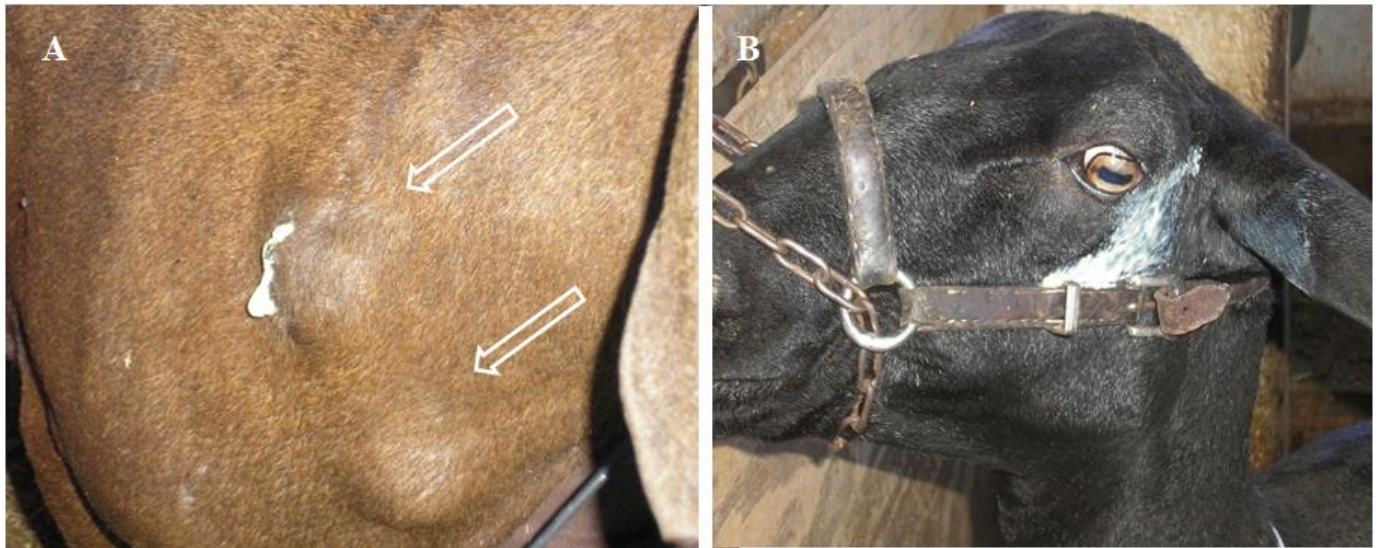
detailed inspection of carcasses. In the Brazilian Northeast, where goat and sheep husbandry are important sources of food and income, the situation is even more critical because of the type of vegetation (spiny) and the low level of schooling of the farmers [31, 24]. It is also becoming more of a problem in the Southeastern, Northern and Midwestern regions, in which this activity is increasing rapidly, negatively affecting the meat-processing industries [26, 32].

#### [IV] SOURCES OF INFECTION AND FORM OF TRANSMISSION

The main source of infection is infected animals, with or without clinical symptoms; these animals contaminate the soil, water, feed, pastures and facilities with nasal secretions, feces and pus from abscesses that drain spontaneously [Figure-3]. Infected animals that do not present clinical symptoms can eliminate the bacteria through their respiratory tract. Evaluation of the coefficients of transmission of *C. pseudotuberculosis* by respiratory tract infection and by pus from spontaneously-draining abscesses, using a mathematical model of transmission, showed that pulmonary abscesses have a small coefficient of transmission, but they are more important for maintaining the infection in the herd (endemic phase) [33].

Transmission can occur through direct or indirect contact or through wounds that come into contact with pus from the abscesses of sick animals [34]. Materials that are used in the management of the animals, such as during castration, identification with ear tags or by tattooing, contact with an uncauterized umbilical stump, and drainage of abscesses, can transmit the agent [Figure-3]. Vectors such as insects (especially flies) should be considered in the transmission of the disease, since *C. pseudotuberculosis* has been isolated from the bodies of domestic flies (mechanical vector) and from fly intestines and feces (biological vector). This bacterium has also been isolated from flies contaminated with milk from cows with mastitis in Israel [35, 36, 37]. In horses, flies have considerable epidemiological importance in the dissemination of *C. pseudotuberculosis*, because the higher frequency of infection in this species occurred during periods when there are large populations of flies [11].

*Corynebacterium pseudotuberculosis* survives long periods in the soil. Through experimental contaminations of soil and of sheep and goat facilities, it was found that *C. pseudotuberculosis* can survive up to eight months at various temperatures [7]. In bedding straw, it can remain viable for three weeks, during two months in hay, four months in shearing stalls and for more than eight months in the soil. This bacterium has been isolated after five months in places where there has been contamination with pus [34] and the concentration of viable microorganisms in the purulent material is estimated to be from 106 to 107 bacteria per gram of pus; consequently, environmental contamination due to a leaking abscess is very high and persistent [38].



**Fig: 3. Environmental and animal contamination with abscess suppurated in sheep.** (A) Two caeous abscesses, one spontaneous opened and other closed. (B) Ocular and cutaneous infection of sheep with caseous material

The use of barbed-wire fences or troughs and posts with sharp, cutting edges can cause lesions in the skin of the animals, opening passage for the entry of bacteria [26]. On farms that rear sheep for wool, the equipment and facilities used for shearing can transmit *C. pseudotuberculosis* among animals. Immersion baths immediately after shearing can disseminate the infectious agent, because these solutions can harbor bacteria for up to 24 h [39]. In the Brazilian Northeast, where non-wool sheep predominate almost completely, shearing and tail removal are not common and the sheep are rarely ear tagged [24]; however, the bacteria can penetrate through the respiratory system, transcutaneously or through skin wounds caused by the caatinga vegetation of this region [31].

Goat and sheep meat producers tend to make few periodic inspections of their herds because of the extensive type of rearing system, in which they do not identify individual animals, arguing that these animals are slaughtered within a short time interval. Conversely, goat milk producers tend to identify animals individually and are more likely to detect abscesses during daily contact, favoring the control of caseous lymphadenitis in these herds; this is proved by the fact that 103 (36.3%) of the 284 goat farmer interviewed in Minas Gerais, Brazil, have reported this disease in their herds, while only 13 (6.1%) of the 213 sheep farmers state the same [40]. In this State most goat herds are for milk production, while most sheep flocks are for meat production [40].

*Corynebacterium pseudotuberculosis* is sensitive to common disinfectants, such as hypochlorite, formalin and cresol; however, the surfaces should be cleaned before disinfection, because organic matter interferes with the action of these agents [41]. Iodine is recommended for chemical disinfection of wounds in

order to reduce bacterial transmission after surgical draining of the abscesses [42].

## [V] PATHOGENICITY AND VIRULENCE FACTORS

*Corynebacterium pseudotuberculosis* is a facultative intracellular bacterium, multiplying within macrophages and surviving the action of phagolysosomal enzymes, because of the external lipid layer of the cell wall [2, 18]. After penetrating into the host, which generally occurs through the oral, nasal and ocular mucosa, or through skin wounds, the agent disseminates freely or within macrophages, mainly through the afferent lymphatic system, to local lymph nodes and internal organs. This process depends on the ability of the agent to infect macrophages, resist phagolysosomes and kill cells, liberating new bacteria and causing necrosis [43]. Three minutes after intraperitoneal inoculation in mice, phagocytic vacuoles are observed; after an hour, 60-80% of the goat macrophages contain bacteria, and two hours after inoculation, acid phosphatase is present in the vesicles containing the bacteria [44]. A strong local reaction occurs four hours after challenge in sheep [45], and a few hours later macrophages are degenerated and polymorphonuclear cell infiltrates containing bacteria are seen [46, 44, 47]. A day after experimental cutaneous infection, microabscesses develop in draining lymph nodes, and pyogranulomas are formed three to 10 days post-infection [48, 49, 6].

The lipid cell layer of the bacteria is pyogenic, but not immunogenic. This same layer makes phagocytosis of the bacteria difficult, increasing its virulence (cytotoxicity), and survival inside macrophages; abscesses form through the release of lysosomal enzymes. Besides participating in pathogenicity,

mycolic acid appears to be important for the survival of this bacterium in the environment [50].

Phospholipase D (PLD) increases vascular permeability and bacterial survival in the host. It is important for the dissemination of the bacteria from the location of the primary infection (local lymph node) to other organs (lungs, regional lymph nodes, mesenteric lymph nodes, etc.), because it lyses mammal cell membranes, rich in phospholipids, causing microhemorrhages and vascular lesions, with increased vascular permeability [2].

## [VI] IMMUNE RESPONSE

Immunity against *C. pseudotuberculosis* is complex and involves cellular and humoral immune responses [51]. Studies point to a greater cellular immune response, chiefly a Th1 response, because of the facultative intracellular nature of the microorganism, with production of gamma-interferon (IFN- $\gamma$ ) and other cytokines that are important for controlling infection [52, 53, 54]. The humoral immune response is observed to present, from 6 to 11 days post-infection, a low production of IFN- $\gamma$ , which significantly increases thereafter [55]. Inflammatory cytokines, such as TNF- $\alpha$  and IL-6, are mainly produced at the site of inoculation, while T cell-associated cytokines, such as IFN- $\gamma$ , are chiefly produced in drainage lymph nodes [47].

## [VII] CLINICAL SIGNS

Caseous lymphadenitis in its superficial form is characterized by infection of external lymph nodes, such as the submandibular, parotid, pre-scapular, subiliac, popliteal and supramammary lymph nodes, while the visceral form is characterized by abscessing of internal organs, such as lungs, liver, kidneys, uterus, spleen and internal lymph nodes, such as the mediastinal and bronchial lymph nodes. These two forms can coexist; however, other less common sites can be involved, such as mammary gland, scrotum, the central nervous system and joints. Internal abscesses are normally associated with weight loss and weakness, known in sheep as thin-ewe syndrome. The mature abscesses easily leak through fistulas, releasing purulent whitish-green discharges into the environment or into the affected organ. Abscesses usually recur, months or years later, in the same animal, due to the failure to eliminate the infection [1]. In some cases, infections produce few characteristic clinical signs, and a post-mortem examination becomes necessary for diagnosis; this makes it difficult to obtain objective data about disease prevalence [38].

Differences in the place of the abscesses between sheep and goats have been reported, the visceral form being more frequent among sheep and the superficial form among goats [7]. External abscesses in the lymph nodes of the head and neck are more common in goats, while the subiliac and pre-scapular lymph nodes are more commonly affected in sheep [7, 42]. Differences in the appearance of abscess content have also been reported

between sheep and goats; in sheep the contents have a laminar form when cut, similar to the layers of an onion, caused by the formation of layers of fibrous tissue and thick caseous material, while abscesses in goats have a thin and pasty exudate [7]. However, onion-like abscesses were not always present in sheep. Sheep carcass inspection at a federally inspected slaughterhouse in Minas Gerais, Brazil, showed that most of the abscesses in sheep were located in the head and neck lymph nodes and their content was essentially pasty. Isolation of *C. pseudotuberculosis* from these materials confirms the infection status of the animals. It is possible that older abscesses become more consistent, with a tendency towards fibrosis and calcification, progressing to an onion-like appearance, independent of animal species.

In horses, there have been reports of abortions and cases of mastitis associated with visceral abscesses. In Israel, this bacterium was isolated from subcutaneous abscesses in milking cows; which could occur in outbreaks and cases of mastitis, affecting the whole mammary gland, resulting in total loss of milk production [56].

## [VIII] CLINICAL AND LABORATORY DIAGNOSIS

Abscesses in goats and sheep are very suggestive of caseous lymphadenitis, especially if animals of the same lot have similar clinical signs, however bacterial isolation is necessary to identify the causative agent, since other bacteria such as *Arcanobacterium pyogenes*, *Staphylococcus aureus* subsp. *anaerobius*, *Actinobacillus licheniformis* and *Pasteurella multocida*, can be found in abscesses [57]. In animals with respiratory problems, a thoracic X-ray can reveal masses in the pulmonary parenchyma and lymph nodes; which also must be confirmed by culture of tracheal washes [58].

The use of aspirating puncture with a fine needle in the diagnosis of *C. pseudotuberculosis* was evaluated [59]. It proved to be easily performed, to have a low cost and to cause little damage to the tissues when compared to histopathology. It allows presumptive cytological diagnosis of the infection, before the affected lymph nodes abscessed, aiding in early adoption of prophylactic measures for the rest of the flock.

Gram and Giemsa staining can be used for cytological identification of the microorganism. Although Gram staining is not primarily indicated for staining tissues, the bluish color taken on by *C. pseudotuberculosis*, in contrast with the reddish color of the cellular and inflammatory material from the aspirated lymph nodes, helps in the identification of the infectious agent [6].

In order to make a definitive diagnosis of caseous lymphadenitis, the agent should be isolated from purulent material from abscessed lymph nodes samples from live animals. Besides aspirating puncture, the material can be obtained by excision after trichotomy and careful antiseptic cleaning of the skin [17, 42]. It can also be collected at necropsy or during slaughter, when internal abscesses, affecting the liver, lungs, intestine,

kidneys, internal lymph nodes and other tissues, become accessible [60].

In the laboratory, after isolation, the identification of *C. pseudotuberculosis* is done by its morphology, staining characteristics, profile and fermentation of various carbohydrates [14]. The main phenotypic characteristics of *C. pseudotuberculosis* used for identification are shown in Table-1.

Various diagnostic techniques have been developed for caseous lymphadenitis in goats and sheep, such as serological neutralization for antitoxins, immunodiffusion in agar gel, indirect hemagglutination, complement fixation and hypersensitivity tests [25, 1, 18].

Immunoenzymatic tests (ELISA), using bacterial cells, toxins and secreted proteins of *C. pseudotuberculosis*, such as PLD [61, 62, 63, 64], have been reported to be effective in caseous lymphadenitis control and eradication programs. Indirect ELISA based on secreted proteins has shown a diagnostic sensitivity and specificity of 93.5% and 100%, respectively, in the diagnosis of caseous lymphadenitis in small ruminants [63].

Detection of INF- $\gamma$  by ELISA, an indicator of cell-mediated immunity, has been used for diagnosis of infection by *C. pseudotuberculosis*, with a sensitivity of 91% and a specificity of 98%, demonstrating its potential for use in caseous lymphadenitis eradication programs [51, 65].

Molecular techniques have also been used for the diagnosis of caseous lymphadenitis. Polymerase chain reaction (PCR), used to identify *C. pseudotuberculosis*, is an alternative to conventional diagnostic methods, with the advantage of being faster and more specific [66]. Multiplex PCR based on amplification of the genes 16S rDNA, rpoB and pld, presented 94.6% diagnostic sensitivity, for *C. pseudotuberculosis* isolates as well as for clinical material [67]. It facilitates the diagnosis by differentiating *C. pseudotuberculosis* from other pathogens present in abscesses, chiefly *C. ulcerans* [67].

Recently, the genome of two *C. pseudotuberculosis* strains isolated from goats and sheep has been sequenced by a Minas Gerais Genome Network and Pará Genomic and Proteomic Network. The genomic data will help to identify new specific targets, useful in the diagnosis as well as in the development of drugs and vaccines and in the understanding of *C. pseudotuberculosis* pathogenicity mechanisms.

## [IX] DIFFERENTIAL DIAGNOSIS

Pyogranulomatous lesions, such as found in actinobacillosis, tuberculosis and superficial abscesses caused by *Staphylococcus aureus* and *Actinomyces pyogenes*, must be differentiated from caseous lymphadenitis [17]. The superficial form of the disease should also be differentiated from submandibular edema caused by parasites, *Fasciola hepatica* and *Haemonchus sp.*, salivary cysts, lymphosarcoma and subcutaneous inoculation of vaccines.

The debilitating visceral form can be clinically similar to chronic parasitism, thinning due to abnormal waste of teeth, alveolar periodontitis, malnutrition and chronic diseases, such as pulmonary adenomatosis, neoplasias and scrapie [17].

Pneumonias caused by *Mycobacterium bovis*, *Pasteurella haemolytica*, *Pasteurella multocida* or ovine progressive pneumonia, due to Maedi-Visna virus infection, can make the diagnosis of caseous lymphadenitis even more difficult [58].

In sheep, orchitis and epididymitis caused by *C. pseudotuberculosis* needs to be differentiated from similar lesions caused by *Brucella ovis*, *Actinobacillus seminis*, *Histophilus ovis* and *Pasteurella spp* [17, 68].

## [X] TREATMENT

Treatment of affected animals consists of the drainage of abscesses, followed by cleansing and chemical cauterization, usually with 10% iodine, or even removal of the affected superficial lymph nodes [69]. Although it is an important control measure, this procedure might not be as effective as expected due to the presence of internal abscesses. Drainage of the abscess should be done in a way that avoids environmental contamination, with disinfection of the surgical material before and after the procedure, and all of the disposable materials should be incinerated and buried, including plastics and paper used to cover the area.

Another treatment option is antibiotic therapy, which is not very efficient, even though *C. pseudotuberculosis* is sensitive in vitro to almost all antibiotics that have been tested. The intracellular location of the bacteria and the formation of biofilm in natural infections reduces drug efficacy, making antimicrobials inefficient under these conditions [7, 70]. The inefficacy and high cost of antibiotic treatment make it an inviable option for herd-level disease management.

## [XI] CONTROL AND PROPHYLAXIS

An effective program for the control of caseous lymphadenitis should be based on clinical inspection and periodic serology of all animals in the flock, which includes recently-acquired animals and those that return to the herd, culling the ones that have clinical signs or that are serologically positive. Once infected, an animal hardly eliminates the *C. pseudotuberculosis* [71]. The main source of infection for a flock is introduction of infected or abscessed animals into a herd, which results in a high frequency of abscesses after two or three years. This stresses the importance of employing biosecurity procedures in all flocks, chiefly during the introduction of animals.

Measures designed to reduce the environmental risk of wounding should also be adopted, such as the use of smooth wire fences, troughs and facilities without sharp edges, disinfection of surgical, ear tagging and shearing instruments, systematic use of

individual disposable needles, effective control of insects, and disinfection of newborns' navels and any other wounds with 10% iodine. Although it is not recommended to be applied to swelled lymph nodes because of its irritating and caustic action on tissues (skin, mucosa and lungs), 10% formaldehydeshould be used for disinfection of herd facilities [7, 1, 18].

All control programs should be based on sanitary education of herd owners and technical personnel, otherwise success will be compromised. Information about losses throughout the production cycle, as well as concerning the zoonotic potential of *C. pseudotuberculosis*, should be supplied to the people who work with the herds directly or indirectly, reinforcing their importance in the success of the control program.

Control measures vary with the prevalence of infection. In countries free of this disease, importation should only be permitted from herds that have been certified free of caseous lymphadenitis for three years, all animals should be tested by ELISA before importing and they should initially be placed in quarantine. In countries with low disease prevalence, the clinically affected animals should be separated and submitted to ELISA testing, lambs and kids should be reared away from their mothers, and installations and equipment should be well disinfected. In countries with a high incidence, rigorous sanitary measures should be implemented, associated with vaccination [7, 17].

Disease eradication can be achieved in endemically-infected herds by initially discarding all animals that have clinical signs and those that are positive in serological tests [6]; however, this is difficult to accomplish because of the rapid dissemination of the agent within the herd and the difficulty in identifying animals that have a subclinical form of the disease [60, 66].

## [XII] VACCINATION

Given that caseous lymphadenitis treatment is ineffective and expensive, the best strategy for control and prevention of the disease is immunization, as it was observed in countries with high prevalence of infection [5]. The vaccines commercially available have different relevant features that should be considered on their use. Not all of the vaccines licensed for use in sheep have the same efficiency in goats, and normally it is necessary to adjust the vaccination program to the flock conditions. Also, the protection provided by vaccination is only partial, as external and internal abscess development can still occur [1].

The principal component of *C. pseudotuberculosis* used in the formulation of vaccines is PLD. The rationale for its use as a vaccination antigen is the good rates of protection obtained after immunization of goats and sheep with this toxin. Most of the commercial vaccines against *C. pseudotuberculosis* use inactivated PLD associated to antigens of other pathogens, such as *Clostridium tetani*, *Clostridium perfringens* type D, *Clostridium novyi*, *Clostridium chauvoei* and *Clostridium*

*septicum*, along with some vaccines that are associated with the endectocide moxidectin. Such a formulation is the basis of the Glanvac vaccine (Vetrepharm, Inc London), licensed for use in sheep and goats in Canada, Australia and New Zealand and the Biodectin vaccine (Fort Dodge Austrália PTY LTD), also licensed in Brazil for use in sheep.

The Glanvac vaccine has been evaluated in various countries [72]. Vaccination of sheep and goats with Glanvac resulted in protection against experimentally-induced infection with *C. pseudotuberculosis*, evidenced by a decrease in the number of lesions [73]. Another commercial vaccine that has been evaluated, Caseous D-T (PBS Animal Health, USA), has two formulations, one that only contains toxoids (clostridial and from *C. pseudotuberculosis*) and another that is a combination of clostridial toxoids and the bacterium *C. pseudotuberculosis*. Preliminary results indicate that this second formulation confers better protection against experimental infection than the first, reducing the number of internal and external lesions [74]. The use of PLD toxoid for the immunization of goats can have some negative consequences, including reduced milk production, fever, ventral edema, ataxia and convulsions; therefore, recommendations for its use in this species should be made with restrictions [1].

The partial protection provided by immunization of goats and sheep with commercial vaccines is associated with the type of immune response elicited. Protection against *C. pseudotuberculosis* is mainly dependent on immune response that involves INF- $\gamma$  production and cytotoxic T-cells. A humoral response alone is insufficient to protect the animal, and a good cellular response is not achieved with inactivated vaccines [75].

Hence, various attempts have been made to obtain an attenuated vaccine that is effective against caseous lymphadenitis [76, 52]. Attenuation can happen naturally or through manipulations using temperature, chemical and genetic (recombinant) agents. With this type of vaccine strategy, the microorganism maintains its capacity to replicate, mimicking natural infection and producing humoral and cellular responses. Also, this is the type of vaccine that confers the best and longest-lasting immune response, due to its similarity to natural infection [75]. Techniques such as deletion of multiple genes involved in virulence, and insertion of fragments that interrupt these genes in the pathogen, practically eliminate the risk that the pathogen can revert to its virulent form [77]. Live vaccines that have been attenuated in the laboratory (recombinants) usually have the PLD gene as a target for attenuation, because of its importance as a virulence factor [78].

In Brazil, the Bahia State Agency for Agricultural Development (EBDA) developed a vaccine based on strain 1002, a naturally attenuated strain, which is currently commercially available. It stimulates significant protection levels, 83%, in vaccine trials; however, immunization still presents collateral effects, such as local reactions, and field trials have not been as successful as the initial vaccine tests, presenting highly variable protection levels [79]. Another attenuated live vaccine, LinfoVac (Laboratórios Vencofarma do Brasil Ltda), licensed for use in sheep and goats, is also currently available in Brazil. The results obtained in the



field with these attenuated vaccines demonstrate the need to develop a more effective and safe vaccine [75].

## [XIII] CONCLUSIONS

Caseous lymphadenitis continues to be an important challenge for sheep and goat industries, limiting their profitability. The intense market and movement of small ruminants, without the necessary biosecurity measures, are important obstacles to the control of caseous lymphadenitis, maintaining its prevalence at high levels, which indicates that specific control measures must be adopted. There are various difficulties affecting the development and application of effective diagnosis and more effective immunogens need to be made available as vaccines. Thus, great efforts need to be made by all players in sheep and goat industries to control this awful disease.

## CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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