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Caseous lymphadenitis in small ruminants: An overview on reproductive implications

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Abstract
Caseous lymphadenitis (CLA) is a chronic infectious disease of sheep and goat caused by *Corynebacterium pseudotuberculosis* (*C. pseudotuberculosis*). This disease is characterised by the formation of abscesses in lymph nodes and visceral organs. However, it might be involved in reproductive disorders such as orchitic, abortion, and stillbirth. Current knowledge on the complications regarding reproductive system induced by CLA indicates that thees changes were observed in the gonads, seminal secretions, cytokines (IL-1β and IL-6) as well as hormonal concentration which overall impaired the reproductive performance of small ruminants. Despite the importance of CLA in small ruminants, reasonable information regarding reproductive pathology caused by this disease has not been provided. In this review, we present the pathogenicity characteristics of the bacterium, disease and its virulence effects on the histology, cytokine production, semen attributes and reproductive hormone profiles. Furthermore, we discuss an update on the isolation of the bacterium from different reproductive organs of bucks and does.

Keywords: Reproduction, caseous lymphadenitis, small ruminants

1. Introduction
In 1888, unusual organisms were isolated by the French bacteriologist Edward Nocard from the case of lymphadenitis in a cow (Nocard, 1896) [66]. Then after three years of this discovery, the Bulgarian bacteriologist Hugo von Preisz recognized the same kind of organism in cultures obtained from a renal abscess of an ewe (Preisz & Guinard, 1891) [82]. The *Corynebacterium* belongs to a suprageneric group of *Actinomycetes* that also include the genera *Mycobacterium*, *Rhodococcus* and *Nocardia* and group CMN. This heterogeneous CMN group shares some characteristics such as cell wall organization with the presence of a vast polymer complex of peptidoglycan, arabinogalactan and mycolic acid (Dorella *et al.*, 2006) [29]. Consequently the organism in question came to be known as "Preisz and Nocard" bacillus, a vernacular name with which it was linked for decades (Baird & Fontaine, 2007) [6]. Subsequently, Lehmann and Neumann (1896) [55] renamed the bacterium in their publication as "*Bacillus pseudo-tuberculosisca"* derived from Greek *pseudes-tuberculosisor* "false tuberculosis" and they further referenced it with the lesions to caseous nodules from mycobacterium tuberculosis. These organisms were placed under the category of *Corynebacterium* genus in the first Edition of Bergey's Manual of Determinative Bacteriology, published in 1923. In 1948, the bacterium was renamed as *C. pseudotuberculosis,* which has remained in its official designation even though some literature showed the evidence that it is known as *C. ovis* (Euzeby, 2005) [32].
This micro-organisms falls in the category of facultative intracellular pathogen which reveals the characteristic pleomorphic forms, such as coccoid and filamentous rods, measuring from 0.5µm to 0.6µm by 1.0µm to 3.0µm (Merchant & Packer, 1967; Hard, 1969; Buxton & Fraser, 1977; Connor *et al.*, 2000; Selim, 2001) [59, 35, 19, 23, 87]. It is a non-sporulating, non-capsulated and non-motile bacterium; however, it has fimbriae. This bacterium is a facultative anaerobe that grows well at 37 °C, at a pH of 7.0 to 7.2 (Merchant & Packer, 1967; Hard, 1969; Buxton & Fraser, 1977; Connor *et al.*, 2000; Selim, 2001) [59, 35, 19, 23, 87]. *C. pseudotuberculosis* is the causative agent of CLA in small ruminants associated with granulomatous, necrotizing type of

inflammation which develops within one or more lymph nodes, leading to the formation of chronic abscesses, with hair loss and ultimately ruptured followed by discharge of pus (Baird & Fontaine, 2007) [6]. *CLA* is associated with the appearance of large abscesses externally that leads to death or abortions and loss of body weight. It affects young ovine population resulting in delayed sexually maturity and decreased wool production (Burrell, 1981) [18]. The external form of *CLA* is characterized by abscess formation in superficial lymph nodes of mandibular, parotid, cervical, sub-iliac, popliteal or mammary tissue and also in subcutaneous tissues. Characterizing visceral form of the *CLA*, these abscesses may also develop in internal organs, such as the lungs, liver, kidneys, and spleen (Merchant & Packer, 1967; Piontkowski & Shivvers, 1998) [59, 81]. Once *CLA* has been established, it avoids the immune system with ease, hence referred to as the perfect parasite (Baird and Fontaine, 2007) [6]. This review discusses the reproductive complications caused by CLA in sheep and goats, as well as the prominence of the disease in small ruminant populations.

2. Transmission

Despite the fact that various routes of inoculation (i.e. oral, intranasal, Intradermal, intra-tracheal, intra-venous or intra-vaginal) have been attempted successfully in experiments, it is accepted that the transmission of *CLA* happens fundamentally through contamination of superficial skin wounds inflicted during shearing, ear tagging, castration, fighting among herds, or rubbing against defiled surfaces (Brown & Olander, 1987; Williamson, 2001; Othman *et al.*, 2014b; Latif *et al.*, 2015) [17, 96, 71, 53].

Infected animals are considered as a possible source of contamination as they may shed immense numbers of wild organisms through purulent discharge from cracked abscesses or by coughing up discharge from lesions present in the lungs (Pepin *et al.*, 1994a; Baird & Fontaine, 2007) [78, 6]. Other animals might be then unprotected through direct contact with infected animals through contaminated equipment and environment. Certainly, *C. pseudotuberculosis* has been demonstrated to survive for a long time in fomites or different natural material and additionally in contaminated sheep dipping (Nairn & Robertson, 1974; Augustine & Renshaw, 1986) [65, 4].

Goats having traumatized buccal mucosa have more chances of taking the bacterium from contaminated feed (Brown & Olander, 1987) [17]. Because of the high prevalence of thoracic and pulmonary lesions from the visceral form of CLA, it is possible for airborne transmission. However, the dissemination of the pulmonary lesions demonstrates a hematogenous or lymphogeneous instead of erogenous spread (Augustine & Renshaw, 1986) [4]. Nonetheless, animals with lung lesions play a paramount part in transmission of the bacterium. Similarly, they could debride the wounds of skin in other animals, particularly when they are in close contact (Pepin *et al.*, 1994a) [78]. Investigations on the part of insects and arthropods serving as vectors for *C. pseudotuberculosis* is inconclusive (Brown & Olander, 1987) [17].

3. Virulent Factors and Pathogenesis

Currently, two main virulence factors have been identified by the researchers. These are identified as phospholipase D and mycolic acids (Baird & Fontaine, 2007) [6]. Phospholipase D is the glycol-phospho-lipid-hydrolyzing enzyme responsible for many biological processes such as dermo-necrosis (Muckle & Gyles, 1986; Brogden *et al.*, 1990; Songer, 1997) [63, 15, 90] lethal synergistic lyses of erythrocytes in the presence of an extracellular *Rhodococcus equi* factor (Fraser, 1961) [34], and blockage of staphylococcal lysin-induced lysis of erythrocytes (Zaki, 1976) [99]. Jolly (1965) [45] reported that PLD increased the permeability of the vascular wall, caused leakage of plasma from the blood vessels by sphingomyelin hydrolysis in membranes of endothelial. Pathogenesis may be assisted by this effect and it allows the bacterium to migrate from the infection site to the lymph nodes around the peripheral regions (Brown & Olander, 1987; Pepin *et al.*, 1994a) [17, 78].

This enzyme also interrupts the normal function of ovine neutrophil chemotaxis and it may be dangerous to cells (Yozwiak & Songer, 1993) [98]. This may be associated with the pathogenesis by lymphatic drainage to tissues (Batey, 1986b) [10]. It can assist the organisms at the onset of infections and is also responsible for the creation of pathway for immune system. The enzyme depletes the bacterial infections which protect the organisms from opsonization (Yozwiak & Songer, 1993) [98]. Chemo-taxis in Neutrophils are hampered by PLD, thus reducing the chances of phagocytosis during the early stage of infection (Tashjian & Campbell, 1983; Yozwiak & Songer, 1993; Pepin *et al.*, 1994b) [91, 98, 79]. *Corybacterium pseudotuberculosis* has the ability to replicate within macrophages and to be released, some other workers recommended that PLD may play an important role in discharge from phagosome and cause death of macrophages (Pepin *et al.*, 1994a; McKean *et al.*, 2007) [78, 58].

The cell wall of *C. pseudotuberculosis* is coated by a waxy mycolic acid that plays an important role in pathogenesis. This protective coating has cytotoxin properties as well as mechanical strength that plays a vital role in the pathogenicity of the organism (tashjian and capbell 1983, Baird & Fontaine, 2007) [91, 6]. According to some researchers, the mycolic acid coat has the capability to allow *C. pseudotuberculosis* to survive even in extreme conditions for a long period of time in the environment; similar features were also seen in the *Actinomycetes* and *Mycobacterium* (Paton *et al.*, 2002; Baird & Fontaine, 2007) [75, 6].

Typically, the infection of *C. pseudotuberculosis* arise through wounds of skin or mucous-membrane, resulting in the spreading of the bacteria to superficial lymph nodes, where the caseous abscesses develop and necrosis take place. Some other sites of the contagion, particularly the visceral organs, might also be infected. Both free and phagocyte borne microorganisms migrate into the local drainage of lymph nodes. Lesions are the consequences of bacterial multiplication within cells (intracellular), which is preceded by the death of host's cells (Jolly, 1965; Hard, 1969) [45, 35]. The lesion size perhaps fluctuates with the initial concentration of pathogen, multiplication rate of organism and the availability of the defence cells to the lesion of host (Batey, 1986a) [9].

4. Clinical signs in Different Species and Zoonotic Importance

CLA in sheep and goats is a chronic type of disease, characterised by formation of abscesses in the lymph nodes, particularly in the parotid, retropharyngeal and in visceral organs (Baird & Fontaine, 2007; Michael, 2010) [6, 60]. Some infected sheep might have abscesses internally, which repeatedly develop in the lungs or lymph nodes of mediastinal without any external clinical signs of contagion (Binns *et al.*, 2007) [11]. *C. pseudotuberculosis* has a wide range of hosts

and induces clinical infection in cattle, pigs, horses, deer, camels and many laboratory animals (Moore *et al.*, 2010) [62]. Similarly, it causes reproductive disorders such as abortion, stillbirth and neonatal infection in ewes (Dennis & Bamford, 1966; Addo, 1979; Alonso *et al.*, 1992) [25, 2, 3] and in rams, suppurative orchitis (Ladds, 1993) [51]. (Williamson & Nairn, 1980) [97] also reported arthritis, bursitis and mastitis in ewes (Radostits *et al.*, 2007) [83] and finally toxemia which, leads to rapid death in experimentally challenged neonatal lambs and kids. In zoonotic view, humans are rarely affected, some cases of infections has been documented regarding the possible risks of infection in veterinary doctors and assistant as well as farm experts (Peel *et al.*, 1997) [77].

5. Histopathology of CLA Lesion

Histologically, the earliest inflammatory alteration in lymph nodes of sheep and goats contain numerous micro-abscesses and an enormous invasion of neutrophils and on a few eosinophils, thus conferring a greenish tinge to the pus (Valli, 1993) [92]. As abscesses augment, they might coalesce with pus constantly encompassed perusing an inflammatory response of neutrophils, epithelioid cells, lymphocytes and macrophages (Pepin *et al.*, 1994a) [78]. The abscesses quickly become encapsulated with connective tissue; however, it increases in size as necrosis of peripheral tissue and capsule reformation progresses. The progressive layers of necrotic tissue which develops as lesion expands and undergoes mineralisation which is responsible for the lamellate appearance of few lesions. Finally, the whole lymph node might be enlarged greatly and comprises of necrotic debris bounded by a connective fibrous tissue capsule subverted by epithelioid cells, lymphocytes, macrophages, neutrophils and occasionally plasma cells (Brogden *et al.*, 1984; Ellis, 1988; Holstad & Teige, 1988; Holstad *et al.*, 1989) [16, 31, 36, 37].

Characteristically, the abscesses are surrounded by fibrous and firm capsules comprised of thick, greenish or white, odourless substance. The abscessed lymph nodes frequently develop in sheep that have characteristic like "onion ring" formation with concentric processes due to recurrent stages of capsule formation and necrosis. The lesion progresses chronically through draining and healing of old lymph node abscesses and establishment of new lesions. Such lesion may take months to a year to be expressed later in different lymph nodes. Depending on the location and size of the abscesses, clinical indications such as mastitis may be seen. More frequently the lesion progresses sub-clinically as it does not affect the comfort of infected animals, and might not be distinguished until abscesses are discovered at slaughter (Batey, 1986b; Brown & Olander, 1987; Williamson, 2001) [10, 17, 96].

6. Caseous Lesion Distribution

The lesions caused by *C. pseudotuberculosis* in sheep are characteristically associated with the pyo-granuloma formation (Valli *et al.*, 1993; Khuder *et al.*, 2012; Khuder, 2015) [92, 49, 48]. Generally the lesion is characterized into external and visceral forms. The external form of CLA is related with the formation of abscesses in the superficial lymph nodes of the body, which can be easily palpated depending on the original point of pathogen entry (Radostits *et al.*, 2000). Visceral form of *CLA* is associated with abscess in internal lymph nodes and other organs. In sheep, the main sites of internal lesions are mediastinal lymph nodes and parenchyma of lungs. These lesions may be established in the liver, kidneys or udder, and in the heart. The *C. pseudotuberculosis* may also affect the male and female reproductive organs such as testes, scrotum, and uterus. Brain or spinal cord and joints are rarely affected (Valli *et al.*, 1993; Binns *et al.*, 2007) [92, 11]. Visceral lesions arise from hematogenous spread from local lesions and frequently join with an extreme form of infection. Consequent to the location of the lesion, pneumonia, pleurisy or general ill-thrift ("thin ewe syndrome") that is associated with loss of body weight has a severe effect on the productivity of herds (Batey, 1986b; Pepin *et al.*, 1994a; Williamson, 2001) [78, 96].

Infection of *C. pseudotuberculosis* intermittently causes other diverse conditions in sheep, including formation of abscesses in the heart, tongue, liver, kidneys, spleen, eyes, diaphragm, mammary gland, testes, bones, joints, vertebral bodies, skeletal muscles, brain, and spinal cord (Maddy, 1953; Renshaw *et al.*, 1979; Davis, 1990; Hulland, 1993; Kennedy & Miller, 1993; Maxie & Newman, 1993; Palmer, 1993; Valli, 1993; Radostits *et al.*, 2007) [56, 85, 57, 73, 83, 92].

7. Reproductive Histopathology

Bacterial infections can lead to abortion in doe and orchitis and/or epididymitis in bucks (Robert & Walter, 2007) [86]. Bacterial epididymitis and orchitis are more common in ram than the buck. It was found that clinically healthy rams harbor *C. pseudotuberculosis* in their accessory sex organs, epididymis and pre-putial cavity. Nevertheless, *Pseudomonas* and Coliform bacteria were also recovered from young buck semen; hence coliform bacteria can cause epididymitis and orchitis upon inoculation in testis (Robert & Walter, 2007) [86]. In mature and sexually exposed rams, epididymitis is most commonly caused by *Brucella ovis* and *Actinobacillus spp* or *Histophilus spp* in young virgin rams. *Brucella ovis* causes genital lesions in the rams that can lead to fertility diminution. The clinical manifestations of these lesions are usually unilateral or at times bilateral epididymitis (OIE-Terrestrial manual, 2009). Epididymitis in bucks and bulls has a deleterious effect on semen quality, testicular degeneration and/or cause testicular atrophy (Robert & Walter, 2007) [86].

In bulls, bacterial infection can cause epididymitis which is usually associated with orchitis or vesiculitis. The most common bacteria that are involved in epididymitis in the bull are *Actinobacillus pyogenes*, *Brucella abortus* and *Mycoplasma bovigenitalium,* which have been reported to cause infertility subsequent to epididymal luminal obstruction (Robert & Walter, 2007) [86].

Abscesses are developed in goat's scrotum infected with casous lymphadenitis; the diameter of the lesion was 2-6 cm at the neck region. Generally these lesions are firm and smooth in texture (Murugaiyah *et al.*, 1990) [64]. In buck, orchitis and/or epididymitis are far less common compared to rams. However, orchitis can be acute in which case the buck develops fever, reduced appetite, lack of walking ability and loss of libido. The infected testes appear swollen, hot and painful to touch. In chronic cases of orchitis the testes appear smaller in size, firm with a loss of mobility due to testicular atrophy. *Brucella melitensis, Brucella ovis*, *C. pseudotuberculosis*, *Actinobacillus spp* or *Histophilus spp*, *Pseudomonas* spp. and *Actinobacillus seminis* have been incriminated as causes of infectious orchitis and/or epididymitis in bucks (Van Tonder, 1975; Robert & Walter, 2007) [95, 86].

Some studies have mentioned that *C. pseudotuberculosis* can infect the testes and cause orchitis in males and mastitis, abortion, perinatal abnormalities, neonatal infection and stillbirth in females in both sheep and goat. This pathogen is

also the cause of mastitis and abortion in mares and mastitis in cattle. In the United States, a survey conducted in a sheep abattoir showed that CLA lesions were found in many organs including the scrotum (8%) and mammary gland (6%) (Fontaine *et al*., 2006; Junior *et al.*, 2006; Radostits *et al.*, 2007; Paton, 2010) [33, 46, 83, 74]. *Corynebacterium pseudotuberculosis* is one of many organisms that can cause epididymitis (Robert & Walter, 2007) [86] in CLA infected sheep and goats, resulting in loss of body condition and subsequently leading to reproductive disturbances and infertility (Kuria *et al.*, 2001; Cetinkaya *et al.*, 2002; Connor *et al.*, 2007) [50, 20, 22]. Microscopic evaluation, the *C. pseudotuberculosis* shows changes in the shape of seminiferous tubules and presence of edema, degeneration, and necrosis in growing spermatogonia cells, necrosis of leydig cells and atrophy of testicular tissue. Besides that, the virulent factor (PLD) causes edema, sever congestion, irregular and shrinkage of the seminiferous tubules, lumen of seminiferous tubules showing less spermatids. The epididymis affected by *C. pseudotuberculosis* and PLD groups had degeneration, necrosis and oedema of the lining epithelium of epididymis (Khuder, 2015) [48].

Caseous lesions are normally present in the internal organs including udder and uterus (Valli *et al*., 1993) [92]. Placentitis is the common cause of infectious abortion in does which may develop into a uterine disease and subsequent infertility. The main common causes of infectious abortion in goats are *Brucella melitensis, Toxoplasma gondii, Clamydia psittaci, Mycoplasma* spp., *Campylobacter* spp. and *Coxiella burnetii* (Bretzlaff, 1994; Robert & Walter, 2007) [14, 86].

The *C. pseudotuberculosis* has the ability to produced histological alteration regarding fibrous tissue formation in ovaries of infected does; however the ovaries of PLD infected does show congestion, degeneration, and necrosis of stromal cells infiltration. Similarly, infection with both *C. pseudotuberculosis* and its exotoxin (PLD) produced congestion, degeneration and necrosis post inoculation (Khuder, 2015) [48]. Moreover, histo-pathological changes such as congestion, degeneration and necrosis, infiltration of polymorph nuclear leukocytes, hemorrhages, edema and thrombus were seen in ovaries, uterus, testes and epididymis (Khuder *et al*., 2012) [49]. Histological alteration were observed in the reproductive organs and inguinal lymph nodes of non- pregnant does experimentally infected with *C. pseudotuberculosis* through intradermal, intranasal, and oral routes of inoculation. Only the intranasal route of infection had severe lesions as compared with other routes of inoculation. In the ovaries, leukocytes infiltration was seen and degeneration, necrosis, congestion as well as thrombosis were recorded due to *C. pseudotuberculosis* infection. Moreover the edema was the main lesion in the uterus of all infected does inoculated with intradermal, intranasal, and oral routes of inoculations; it might be due to the presence of the exotoxin (PLD). Histo-pathological lesions were also recorded in inguinal lymph nodes of intranasal inoculated non pregnant goats (Othman *et al.*, 2016) [68]. Similarly, infection with C. *pseudotuberculosis* was shown to increase systemic neutrophilia and mastitis development after inoculation *into* the mammary gland of goats (Junior *et al.*, 2006; Othman *et al.*, 2016) [46, 68].

8. Seminal Secretions

Semen assessment is the most critical instrument used to distinguish the regenerative wellbeing and execution of a creature (Zemjanis, 1969) [100]. Caseous lymphadenitis produces the caseous lesions in visceral organs and the testes (Murugaiyah *et al.*, 1990) [64]. As such, Khuder (2015) [48] reported that *C. pseudotuberculosis* significantly decreased the scrotal circumference of bucks experimentally inoculated with PLD. The volume of the semen showed a significant increase in both *C. pseudotuberculosis* and PLD. However, the other parameters including *pH,* wave pattern, Sperm motility, sperm concentration, dead sperms and abnormal sperm percentage were decreased in goats infected with *C. pseudotuberculosis* and its toxin PLD. In contrast, the semen parameters were not affected in CLA infected bucks. Although, if the lesions are present in the surface of scrotum it may be due to epididymis, spematocoeles, varicoeles; which might affects the semen attributes (Murugaiyah *et al.*, 1990) [64].

9. Reproductive Hormones Concentration

C. pseudotuberculosis causes hormonal imbalances through disrupting the normal function of hypo-pituitary-gonadal axis in goats and might be the cause of infertility (Othman *et al.*, 2014b; Khuder, 2015) [71, 48]. Moreover, chronic infection of CLA produces steroidogenesis as well as anti-steroidogenic effects, the serum level of progesterone hormone decrease throughout 90 days post infection period, however estrogen hormone was decreased after post infection (Abdullah *et al.*, 2015) [1]. Khuder *et al.* (2012) [49] stated that both *C. pseudotuberculosis* and its exotoxin PLD disturbed the normal serum progesterone and testosterone concentrations in mice used as an experimental model. Similarly, Khuder (2015) [48] also reported that experimentally infected goats with wild type of *C. pseudotuberculosis* and its PLD are responsible for decreasing the concentration of serum testosterone. Moreover, they further reported that the serum testosterone concentration of bucks infected with whole bacterium was 2.98 ± 3.70 pg/mL with a decrease of 7 folds as compared with control group (16.58 ± 3.67 pg/ml), however the exotoxin treated group had unchanged testosterone levels (11.84 ± 3.19 pg/ml). Approximately, two fold decrease in serum testosterone level was observed in CLA infected bucks (2.11±0.63 ng/ml) as compared to non-infected healthy rams 3.42±0.82 ng/ml (Ibtisam, 2008). Though steroidal hormones significantly decreases the serum level of estrogen and progesterone in does after infection with caseous lymphadenitis (Khuder, 2015), Othman (2014) [48, 68] illustrated that the serum concentration of progesterone as well as estrogen were higher in does experimentally infected with live bacterium of *C. pseudotuberculosis* via oral, intranasal and intradermal rout of infection.

10. Responses of Cytokines (Interleukin 1β and IL- 6)

Pro-inflammatory cytokines (interleukins) are polypeptide in nature and produced by immune competent cells of the immune system during inflammation (Sirotkin, 2011) [89]. The ovary is the site of both reduction and action of Interleukins (ILs). The granulosa and theca cells have receptors that are responsible for ILs production, however, maximal production of ILs occur after gonadotropin action in the pre-ovulatory follicle (Brännström, 2004; Ingman & Jones, 2008) [13, 40].

Effects of IL-1 depends on the stage of ovarian follicle development; it prevents the steroidogenesis in the follicles that are undifferentiated, but stimulates the release of progesterone in ovaries (Bornstein *et al.*, 2004) [12]. Besides that, IL-1 might also be involved in several events associated with ovulation such as proteases synthesis, regulation activity of plasminogen activator, nitric and prostaglandin production

(Sirotkin, 2011) [89]. *Corynebacterium pseudotuberculosis* is responsible for steroidal hormones imbalances including estrogen and progesterone, which were seen to be elevated in non-pregnant does, which might be due to IL-1β and IL-6 secretion in does. Interleukin-1β represent a potent mediator in response to injury and infection (Dinarello, 1998) [27]. The increased plasma level of IL-1β and IL-6 were seen in non-pregnant goats after infection of *C. pseudotuberculosis* (Othman *et al.*, 2014a) [70].

Progesterone and estradiol are stimulated by IL-1 in small follicles, while antral gonadotropin dependent follicles and secretion are inhibited (Baratta *et al.*, 1996) [7]. Increases in interleukin-1, IL-6 and TNF influences the cross takes between the immune system and hypothalamic pituitary adrenocortical (HPA) axis. Finally, IL-1 could cause ovulation suppression, as well as the release of estradiol and progesterone and stimulates the prostaglandins E and F production and their receptors present in the corpus luteum of di-estrus phase of ovarian cycle (Bornstein *et al.*, 2004; Brännström, 2004) [12, 13]. Reported that the defence mechanism of host animals is stimulated by bacterial infection which enhances the secretion of pro-inflammatory (IL-1β). Similarly, the chronic infection of CLA elevates the concentration of IL-1β which stimulates the defence mechanism of the host (Jesse *et al.*, 2016) [69].

Interleukin-6 (IL-6) subfamily is a group of hematopoietic cytokines with a broad range of physiological functions including cell survival, immune and inflammatory responses (Jazayeri *et al.*, 2010) [43]. Secretion of IL-6 could be related with ovarian carcinogenesis and steroidogenesis (Dijsselbloem *et al.*, 2004) [26]. The peak level of IL-6 was observed in the 2nd month post infection and this indicated the severity of infection, however the level was decreased at the 3rd month post infection with *C. pseudotuberculosis* (Jesse *et al.*, 2016) [69]. In addition, ILs secreted through immune or reproductive systems can be molecules mediating known suppressive effect of inflammation on reproductive processes (Sirotkin, 2011) [89]. The serum concentration of IL-6 increased in chronic stage of disease may decline leading to activation of other cytokine concentration in the immune responses. The adverse effects of ILs of CLA disease in reproductive biomarker aspects have already been observed by many scientists (Othman *et al.*, 2014a; Jesse *et al.*, 2016) [70, 69]. This elevated level of ILs might be responsible for decreased ovulation rates in does and spermatogenesis as well as seminal secretions in bucks due to hormonal imbalances.

Cytokine may play a role in the development of pyogranulomas, which is very important in reducing the dissemination of bacteria. Cytokines have been known to be activated in a cascade and responsible for both pro-inflammatory and anti-inflammatory processes (Dinarello, 2010) [28]. *C. pseudotuberculosis* have the ability to produces pyogranulomas in visceral organs of goats (Khuder, 2015) [48]. Moreover, the inflammatory cytokines are the key functional parameters determining the outcome of immune response to infectious agent (Pepin *et al.*, 1997) [80]. Because of the facultative intracellular nature of the microorganism, production of gamma-interferon and other cytokines help in controlling infection (Simmons *et al.*, 1998; Lan *et al.*, 1999; El-Enbaawy *et al.*, 2005) [88, 52, 30].

Higher cytokine expression was measured in sheep with pyogranulomas in the draining lymph nodes as compared to those without, especially for interleukin-1 beta and interleukin-8 (Mikuni, 1995; Pepin *et al.*, 1997; Van der Hoek *et al.*, 1998) [61, 80, 94], it was also observed that interleukin-6 had no effect on progesterone levels but inhibited estradiol production. There is paucity of information on the relationship between inflammatory cytokine (interleukin-1β and interleukin-6) and reproductive hormones (progesterone and estrogen) in CLA infection.

11. Bacterium isolation from different organs through PCR

Caseous lympadenitis (CLA) is still a reason for alarm in small ruminant production areas throughout the world (Baird, 1997; Williamson, 2001; Paton *et al.*, 2003; Dorella *et al.*, 2006) [5, 96, 76, 6]. It is due to high rate of transmission of its pathogenic agent namely *C. pseudotuberculosis* (Baird, 1997) [5]. Microbiological and biochemical tests are not very sensitive tools in the identification of the bacteria; the improvement of a rapid and accurate diagnostic toolis important in the control of CLA within animal herds (Cetinkaya *et al.*, 2002) [20].

In 1984, an American biochemist named Kary Mullis developed PCR and he received Nobel Prize as well as Japan Prize on PCR innovation in 1993 (Bartlett & Stirling, 2003) [8]. The Multiplex PCR (mPCR) assay delivers an accurate, efficient, rapid identification method for *C. pseudotuberculosis* from cultures and its pus samples collected from CLA infected animals (Pacheco *et al.*, 2007) [72]. Similarly, the polymerase chain reaction (PCR) technique is a specific test that amplifies a single piece of DNA into thousands and millions of copies. The rRNA (16S) has been a reliable gene for identification as well as classification of a bacterium. The rRNA (16S) gene has revealed functional consistency with a relatively good positive behavior of expression (Chanama, 1999) [21] and approximately 1,500 bp of it is length is sufficient for the analysis of bio-informatics (Janda & Abbott, 2007) [41]. The rRNA gene (16S) PCR assay based has been used for identification of *C. pseudotuberculosis* (Cetinkaya *et al.*, 2002) [20]. Furthermore, this assay is very effective in assessing the prevalence rate of CLA in animals, however it has some limitations; firstly, it totally depends on the culture of bacterium; secondly, it has no ability to differentiate the *C. pseudotuberculosis* from *C. ulcerans* (Cetinkaya *et al.*, 2002) [20]. *C. pseudotuberculosis* has been isolated and identified from prenuptial cavity, accessory sex organs and epididymis of clinically normal rams (Jansen, 1983) [42]. Moreover, the bacterium was was successfully isolated from the ovary, uterine horns, uterus, cervix, vagina, and associated inguinal lymph nodes of the experimentally infected non-pregnant does through PCR applications (Latif *et al.*, 2015, Latif *et al.*, 2017) [53, 54].

12. Conclusion

Caseous lymphadenitis is a very destructive and highly prevalent bacterial disease in sheep and goat rearing areas all over the world. It causes economic losses due to condemnation of skin, carcasses and infertility in goats. It has been reported that the *C. pseudotuberculosis* has been isolated from the reproductive organs of buck and does and it caused changes in, histology, seminal characteristics, hormonal concentration and cytokines levels. It is believed that CLA may also be associated with infertility and abortion in small ruminants herds as a result of the alterations mentioned above.

13. Future Research

Caseous lymphadenitis in small ruminant remains a challenge for veterinary scientists. In fact, exact knowledge of CLA pathogenesis regarding its effect on the reproductive organs is

still scarce. Nevertheless, more in-depth studies need to be pursued in order to evaluate the association between CLA, infertility and cytokine production in small ruminants

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Conflict of Interest
The authors have no competing interest to declare.

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