DOI: 10.1002/vms3.744

ORIGINAL ARTICLE

WILEY

Cytopathological and bacteriological studies on caseous lymphadenitis in cattle slaughtered at Bishoftu municipal abattoir, Ethiopia

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Abstract

Background: Caseous lymphadenitis (CLA) is a chronic bacterial infectious disease that affects cattle, shoats, and other domestic and wild ruminants.

Methods: A purposive cross-sectional study was conducted on 30 cattle with enlarged lymph nodes to investigate CLA using cytopathological and bacteriological techniques from cattle slaughtered at Bishoftu municipal abattoir.

Results: From a total of 30 cattle subjected to clinical and post-mortem examinations, only one bull was found to be infected with a rare case of CLA in Bishoftu municipal abattoir, Ethiopia. Enlargement of the pre-scapular lymph node was the only clinical finding during ante-mortem inspection of the bull. The gross pathological lesion showed a pre-scapular lymph node with a caseo-necrotic dystrophic calcification that was accompanied by a rough texture and white to grayish hue. Histopathologically, the lymph node was characterized by central liquefactive necrosis that was surrounded by coagulative necrosis containing multiple foci of mineralization, infiltration of polymorphonuclear neutrophils and mononuclear immature fibrosis containing inflammatory cells and also with some sort of a thick layer of mature fibrosis that defines the magnitude of the lesion. Cytologically, multi-lobulated (intact and degenerated) neutrophils, a few reactive lymphocytes, macrophages and some crenated histocytes have been recognized. The bacterial culture of the sample revealed small, white cream, dry, waxy colonies with a narrow area of β-haemolysis. The isolate of the sample was a Gram-positive cocci-bacilli that was arranged in a Chinese pattern on Gram staining, and catalase and urease were positive in the biochemical analysis of this organism, which was able to ferment glucose and maltose but not trehalose and xylose.

Conclusions: The present investigation indicated that CLA was prevalent as sporadic cases among cattle slaughtered in Bishoftu municipal abattoir. Thus, effective preventive and control measures, such as good sanitation and hygiene, should be followed during meat inspection.

KEYWORDS
Bishoftu, caseous lymphadenitis, cattle, Ethiopia

1 | INTRODUCTION

A lymph node is an oval-shaped organ of the lymphatic system that is distributed widely throughout the body and becomes inflamed or enlarged in various infections and diseases (Standring et al., 2005; Susan, 2015). Lymphadenitis is an inflammation of the lymph nodes (also referred to as the lymph glands), causing swelling of the lymph nodes, pain and tenderness that typically occurs when one or more lymph nodes are infected by bacteria, a virus, a parasite, a fungus or other non-infectious causes (Witte et al., 2006).

Caseous lymphadenitis (CLA) is a chronic highly contagious disease of sheep, goat and occasionally cattle, deer, horse, camelids, water buffalo, wild ruminants, primates, pigs and fowl (Fontaine & Baird, 2008; Kariuki & Poulton, 1982; Sood et al., 2012) that is caused by Gram-positive actinomycete called *Corynebacterium pseudotuberculosis*, which results for significant economic losses due to deterioration and condemnation carcass and organs, culling of the infected animals, loss of fertility and decreased meat and milk yield (de Sá Guimarães et al., 2011; Dorella et al., 2006; Mahmood et al., 2015; Williamson, 2001). The disease has also zoonotic importance as it may on rare occasions cause regional lymphadenitis in humans, particularly in farm workers and meat inspectors (Join-Lambert et al., 2006; Peel et al., 1997).

According to the World Organization for Animal Health (OIE), out of 201 countries that registered their health status between 1996 and 2004, 64 reported that within their borders, they had animals with CLA including Americas (19 out of 42 countries), Africa (18 out of 51), Asia (11 out of 43), Europe (14 out of 51) and Oceania (2 out of 14) (de Sá Guimarães et al., 2011). Ethiopia was one of the countries that announced its sanitary condition to the OIE in 1996. However, the number of countries that have issues with this disease may be under-notified. Since the OIE declaration is made only by the official health authorities of each country, some countries that have documented the disease in scientific articles have not made an official declaration (Abebe & Sisay Tessema, 2015).

The disease is characterized by abscessing of the superficial and visceral lymph nodes, resulting in chronic granulomatous lesions, commonly known as 'cheesy gland', due to the accumulation of infected phagocytes, eosinophils and cellular debris, forming distinct abscesses with multi-centric layers (Cetinkaya et al., 2002; Gascoigne et al., 2020). The external form of CLA involves abscessation at the superficial lymph node. The most common superficial lymph nodes infected are the parotid, sub-mandibular, pre-scapular, pre-femoral, popliteal and supra-mammary lymph nodes. The internal form afflicts the lungs, liver and spleen (Abdullah et al., 2017).

The transmission of this disease occurs through the release of a large number of bacteria from a ruptured abscess, which spreads over the skin and fleece of infected animals and also the environment. Other animals may be exposed either via direct physical contact with the infected animal or indirectly through contaminated fomites (Anil, 2019; Fontaine & Baird, 2008; Gascoigne et al., 2020; Soares et al., 2013; Sood et al., 2012; Umer et al., 2017). The source of natural infection and the means of entry into cattle are not well documented (Sood et al., 2012). However, the possible role of arthropod vectors in the transmission of the bacterium has been studied (McGuire & Durant, 1957; Selim, 2001; Yeruham et al., 2003).

A cytological diagnostic technique like fine-needle aspiration is considered an appropriate procedure in terms of its potential complications, the time required, expense, sensitivity and specificity (de Gopegui et al., 2004). Additional tests applied to reach a final diagnosis included histopathology, haematology, serology, PCR, isolation and biochemical identification, mainly to differentiate other pathogens that also cause abscesses (Baird & Fontaine, 2007; Singh et al., 2017).

Several clinical forms of the disease caused by *C. pseudotuberculosis* have been described in cattle: pyogranulomatous reactions; abscess formation; ulcerative lymphangitis and mastitic and visceral forms (Yeruham et al., 1997). Recently, an additional form was described: necrotic and ulcerative dermatitis on the heel of the foot; accompanied by oedematous swelling and lameness (Yeruham et al., 2003). However, there is a paucity of information on CLA in cattle worldwide and there is no report of the disease in cattle in Ethiopia. Therefore, the objective of this study was to investigate CLA through cytopathological and bacteriological techniques in cattle slaughtered at Bishoftu municipal abattoir.

2 | MATERIALS AND METHODS

2.1 | Study area

The study was conducted from October 2017 to May 2018 to investigate CLA through cytopathological and bacteriological techniques in cattle slaughtered at Bishoftu municipal abattoir. Bishoftu is located 45 km southeast of Addis Ababa. The area is located at 9° N latitude and 40° E longitude at an altitude of 1850 meters above sea level with an annual rainfall of 866 mm, of which 84% is in the long rainy season (June to September). The dry season extends from October to February. The mean annual maximum and minimum temperatures are 26 and 4°C, respectively, with a mean relative humidity of 61.3% (Belay & Muktar, 2015). The domestic animals raised in the area are 91,040 cattle population, 39,055 goats, 39,048 sheep, 22,676 donkeys, 6136 horses and 2015 mules. However, the total number of pet animals is not yet known (Genzebu et al., 2016).

2.2 | Study animals

The study animals were those cattle that were brought to Bishoftu municipal abattoir for slaughter from the different production systems.

2.3 | Inclusion criteria

Cattle of all age groups, sex and breeds that were kept in different husbandry systems but had enlarged lymph nodes were included in the study period for assessing the lesions of the lymph nodes and collecting specimens for cytopathological and bacteriological examination as described by Gatenby (1991) and Sheferaw et al. (2014).

2.4 | Exclusion criteria

Those animals which are healthy and have normal lymph nodes upon palpation and inspection of the cattle during ante-mortem and post-mortem inspection were ruled out in the current study.

2.5 | Study design and sampling techniques

A cross-sectional study design with a purposive sampling technique was conducted on cattle slaughtered at Bishoftu municipal abattoirs. During the study period, a total of 30 cattle with the presence of enlarged lymph nodes were selected purposively for identifying the causes. Unfortunately, out of these, a 15-year-old exotic bull with enlarged pre-scapular lymph nodes was identified during an ante-mortem clinical examination, and different types of samples were taken from this animal during a post-mortem examination for further laboratory investigation.

2.6 | Sample handling, transportation and precaution

Lymph nodes were examined grossly for the presence of abnormalities at the lairage and slaughter line during ante-mortem and post-mortem inspection by wearing personal protective equipment. Lymph nodes with enlargement and swelling were cut by sterile scissors and tissue specimens were collected using sterile universal bottles and transported in an icebox for bacterial isolation and cytological studies in Addis Ababa University, College of Veterinary Medicine, Veterinary Microbiology Laboratory. Moreover, for histopathological examination by routine paraffin embedding technique, a tissue sample was collected in the universal bottle containing a 10% neutral buffered formalin, and processed at Sebeta National Animal Health Diagnosis and Investigation Center.

2.7 | Ante-mortem inspection

On ante-mortem inspection, a detailed physical examination was carried out by taking vital signs, such as temperature, pulse rate, respiratory rate, capillary refill time, physical body condition, normal demeanour and abnormal demeanour at lairage, as described by Jackson and Cockcroft (2008). During visual inspections, a total of 30 cattle suspected of having enlarged lymph nodes were selected. Unpredictably, all the cattle were asymptomatic.

2.8 | Post-mortem inspection

Standard necropsy procedure was followed and all organs were screened for gross visible abnormality, texture and attachments from the selected animals. On opening the pre-scapular region, pre-scapular lymph nodes were highly enlarged.

2.9 | Histopathology techniques

The formalin-fixed tissue specimens were trimmed and put into plastic tissue cassettes and then processed using an automatic tissue processer. Within the automatic tissue processer, tissues were dehydrated in graded concentrations of absolute alcohol (70, 95 and 100%), cleared by xylene and impregnated or infiltrated by molten paraffin. Impregnated tissues were made into a mould and solidified and then they were embedded. Tissues were sectioned to 4–5 mm thickness using a semi-automatic microtome machine, tissue ribbons were floated on a warm water bath at 45°C and they adhered to albumenized glass slides. The sectioned tissues were de-waxed (by heat and xylene), hydrated (by descending grades of alcohols (100, 95, 70%) and then stained with haematoxylin and eosin. The stained slides were dehydrated in increasing alcohol concenterations (70, 95 and 100%) and cleared with xylene, and then mounted by Canada balsam, and the slides were finally examined under a compound microscope at a different objective lens magnification (4–100×) (Talukder, 2007).

2.10 | Diagnostic cytology

Impression smears were made from the affected pre-scapular lymph nodes, and were fixed with methanol and stained with Giemsa stain. Then, the stained specimen was examined from low magnification to oil immersion (4-100× objective lens) to determine cellularity and cell morphology. The smears were also subjected to Gram stain to determine the morphological features and the presence of bacteria (Bancroft & Gamble, 2008).

2.11 | Bacterial isolation and identification

Lymph node contents were inoculated into cystine tellurite blood agar. After the incubation of plates aerobically for 48 h at 37°C, small, white, dry and crumbly colonies were searched for the characterization of *C. pseudotuberculosis*. Pure cultures were prepared from the colonies that were Gram-positive and small curved rod shaped in the microscopic examination. Then, routine biochemical tests, that is, catalase,

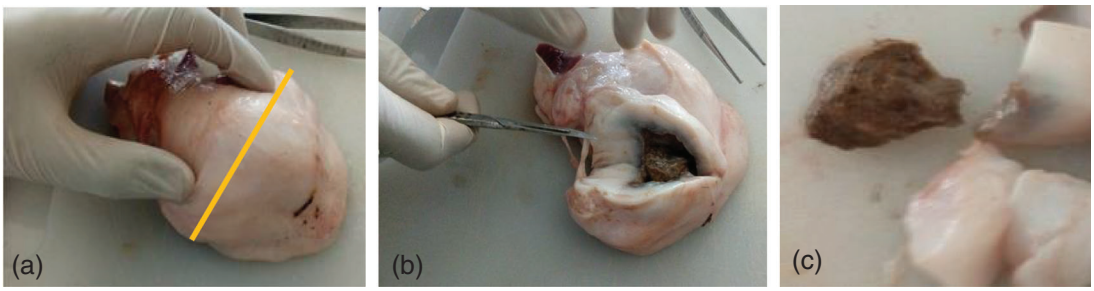

FIGURE 1 Gross lesion of caseous lymphadenitis in the pre-scapular lymph node of a bull

urease, trehalose, xylose, maltose and glucose fermentation and reverse CAMP (with *Staphylococcus aureus*) were carried out to identify the isolates. Isolates positive for catalase, urease, maltose and glucose but with *S. aureus* beta haemolysin inhibitor and trehalose and xylose negative are reported to be *C. pseudotuberculosis* (Cowan & Steel, 1965; Quinn, 1994).

2.12 | Data collection and interpretation

Data collected from the abattoir through ante-mortem and post-mortem inspection were recorded, screened and coded in the format developed for this study and entered into a Microsoft Excel 2016 spread sheet. Then, after cytopathological, histopathological and bacteriological findings were described using qualitative methods. Besides, detailed descriptions and interpretations of all figures were presented, respectively.

3 | RESULTS

3.1 | Gross findings of CLA

The present study revealed that a significant gross lesion of CLA in the pre-scapular lymph node of a bull was observed. A pre-scapular lymph node appeared, enlarged to a size of 13 cm (Figure 1a line arrow) in diameter and characterized by the presence of a distinct caseo-necrotic dystrophic calcified mass on a cut section, which was hard in texture and white to greyish in a colour (Figure 1c). The surface of a mass was smooth (Figure 1a) while a cut section revealed a rough surface. The central zone of an enlarged pre-scapular lymph node exhibited caseous necrosis (Figure 1b).

3.2 | Cytological findings of CLA

Cytological investigation of CLA in this study showed hyper-cellularity with basophilic cytoplasm, which contained an infiltration of several multi-lobulated neutrophils (intact and degenerated) across the slide area with a few bands of hyper-segmented neutrophils, reactive/hyperplastic/lymphocytes, macrophages (Figure 2a & b) and some form of crenated histocytes (Figure 2c). Some neutrophils had engulfed bacilli, suggesting phagocytosis of the infection (Figure 2b, double-headed arrow).

3.3 | Histopathological findings of CLA

Histopathologically, CLA from a pre-scapular lymph node revealed that numerous separate concentric layers within the lesion showed central liquefactive necrosis (D) which was surrounded by coagulative necrosis (C) and both of them contained multiple foci of mineralization (F) which were arranged by loose concentric layers. A thin layer of polymorphonuclear neutrophils surrounds the periphery of the coagulative necrosis, with a further outer layer of coagulative necrosis containing polymorphonuclear neutrophils migrating through it at adjacent densities, forming an apparent bi-layer (E). This was tightly bordered by a layer of immature fibrosis (B) contains mononuclear inflammatory cells and a thick layer of mature fibrosis (A) that outlines the extent of the lesion (Figure 3I). Note that the border between the layers of caseous necrosis (A) and active immature fibrosis containing mononuclear inflammatory cells (B); the presence of polymorphonuclear neutrophils migrating across the border into the caseous necrosis (C) and deeper at the higher magnification (Figure 3II).

3.4 | Bacterial isolation

The bacterial culture of the samples revealed the presence of bacterial growth, forming cream white, dry, waxy colonies with a narrow zone of β-haemolysis following 48 h of incubation and could be removed easily from the medium when scraped. Gram staining of smears showed Gram-positive cocci-bacilli arranged in Chinese pattern and presented in groups, pairs as well as singles (Figure 4a & b). The organisms were catalase and urease positive. They were able to ferment glucose and maltose but not trehalose and xylose.

4 | DISCUSSION

CLA is a chronic infectious disease of a wide range of mammalian species in the world, including Ethiopia, characterized by the

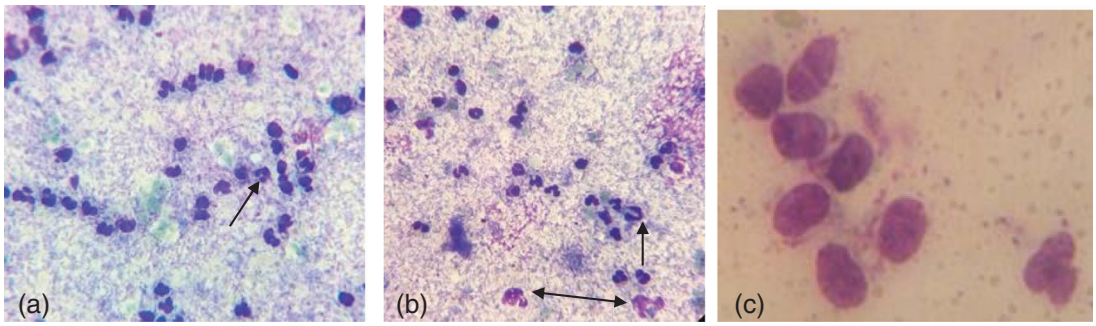

FIGURE 2 Cytological characterization of caseous lymphadenitis in a bull. Giemsa, original magnification 100×

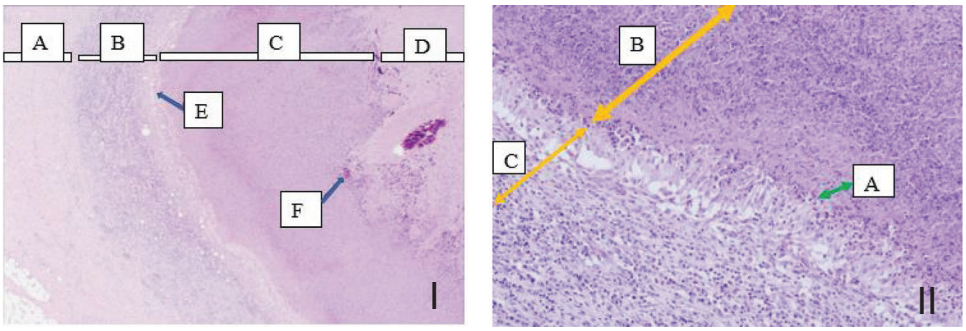

FIGURE 3 Histopathological characterization of caseous lymphadenitis in a bull, H&E, I (10×) and II (40×)

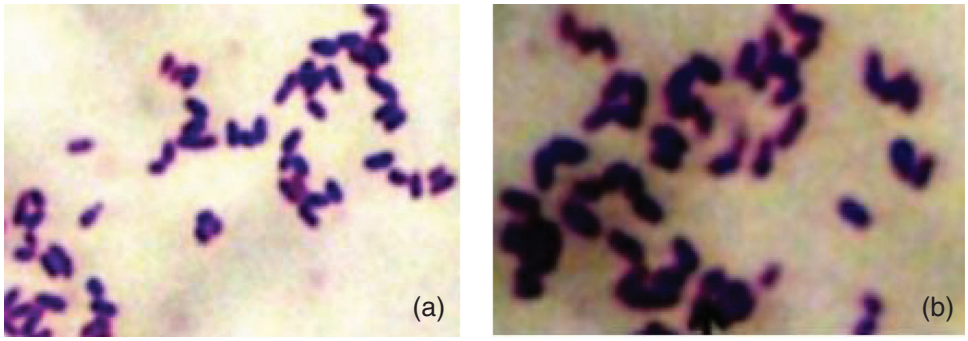

FIGURE 4 Gram stain of *Corynebacterium pseudotuberculosis* from caseous lymphadenitis in a bull. Gram stain, original magnification 100×

enlargement of one or more lymph nodes (Ali et al., 2016; Belchior et al., 2009; Braga, 2007; Ruiz et al., 2020). This study reported a single case of CLA out of 30 cattle slaughtered in the Bishoftu municipal abattoir and exhibited an enlargement of the pre-scapular lymph node to the post-mortem carcass inspection, which appeared to be consistent with previous reports of Anil (2019) in cattle and Salgado et al. (2011) and Yitagesu et al. (2020) in shoats from different parts of the world. The low frequency of CLA in the present study was not in agreement with the reports of Al-Gaabary et al. (2010), Mira et al. (2014), Yitagesu et al. (2020) and Zeru and Kahsay (2014) that recorded a high occurrence of CLA in small ruminants. Perhaps, the likely explanation for this may be that the origins of most of the animals slaughtered originated in areas with robust husbandry systems. The occurrence of CLA is known to be higher in areas where intensive farming is practiced (Brown et al., 1987; Lindsay & Lloyd, 1991). On the other hand, the existence of a low frequency of CLA in the present study agreed with the report of Sood et al. (2012) from the mesenteric lymph nodes of a cow-calf and (Anil, 2019) from the superficial lymph nodes (sub-mandibular, pre-scapular and parotid) in non-descript cattle.

In this study, the clinical symptoms of CLA were not established. Eventually, only external lesions that are too large to affect the normal function of the limbs, organs or tissues were found during the clinical evaluation and these were consistent with the previous reports of Mahmood et al. (2015) and Paton (2010). External lesions, such as enlargement of the superficial lymph nodes, including sub-mandibular, parotid, pre-scapular, subiliary, popliteal and supra-mammary lymph

nodes, as defined by Baird and Fontaine (2007), Kumar et al. (2012) and Williamson (2001) in small ruminants, have not been observed in current studies. However, this study noted the enlargement of the second most affected superficial lymph node called pre-scapular lymph nodes which were in line with the previous reports described by Ali et al. (2016) and Anil (2019) in bovine and by Ayers (1977), Fontaine and Baird (2008), Mira et al. (2014), Stoops et al. (1984), Valli and Parry (1993), Williamson (2001), Yitagesu et al. (2020) and Zeru and Kahsay (2014) in shoats, who reported an enlarged superficial lymph node with a diameter of 13 cm. These results may be attributed to the habit of bulls that tend to scratch their shoulders and heads against walls and fences or any hard objects, resulting in a high percentage of superficial pre-scapular lymph node infections because they drain the shoulder region (Fontaine & Baird, 2008; Jubb et al., 2012; Yitagesu et al., 2020; Zeru & Kahsay, 2014).

Grossly, the pre-scapular lymph node was found to be calcified and to have a rough texture and a greyish hue. The surface of the mass was smooth at the inspection, while the section of the cut showed roughness. The appearance of hard to large calcified mass in the pre-scapular lymph node segment in the current study differed from the author's knowledge of CLA reported in ruminant animals in different countries around the globe by different authors.

Furthermore, reports by de Sá Guimarães et al. (2011), Radostitis et al. (2007) and Tripathi et al. (2016) in shoats and Sood et al. (2012) in the mesenteric lymph node of the cow-calf suggested a multi-focal caseo-calcified nodule with a brittle texture and convoyed by a small pea to the walnut size with multiple abscesses in both superficial and visceral lymph nodes, which did not comply with the present study that exhibited a hard calcified mass on cut section. The cross-sections of the lymph node with the spherical onion-skin appearance (pathognomonic signs of CLA) of abscess documented in the previous report of Abebe and Sisay Tessema (2015), Baird and Fontaine (2007), Crawshaw et al. (2008), Fubini and Campbell (1983) and Mahmood et al. (2015) in different lymph nodes of shoats were not observed in this study.

Histopathologically, CLA of the pre-scapular lymph node of the bull revealed that there were numerous separate concentric layers within the lesion that showed central liquefactive necrosis encircled by coagulative necrosis, both of which contained multiple mineralization foci that were organized by loose concentric layers. Also, a thin layer of polymorphonuclear neutrophils covers the periphery of the coagulative necrosis, with a further outer layer of coagulative necrosis containing polymorphonuclear neutrophils migrating through it at adjacent densities, creating an obvious bi-layer. It was then closely bound by a layer of immature fibrosis containing mononuclear inflammatory cells and a thick layer of mature fibrosis that outlines the extent of the lesion. These histopathological findings were consistent with reports of Anderson et al. (2004), Baird (2000), Baird and Fontaine (2007), Domenis et al. (2018), Morales et al. (2017), Radostitis et al. (2007) and Saleh et al. (2019) that identified similar microscopic findings in domestic and wild ruminants abroad.

Cytological investigation of CLA in this study showed hypercellularity with basophilic cytoplasm, which contained an infiltration of several multi-lobulated neutrophils across the slide area with a few bands to hyper-segmented neutrophils (intact and degenerated), reactive/hyper-plastic/lymphocytes, macrophages and some form of crenated histocytes in line with the findings reported by Al-Rukibat et al. (2016), Elmore (2006), Sood et al. (2012), Thangapandiyan and Balachandran (2010) and Ward (1990) in bovine and canine species. Some neutrophils had engulfed bacilli, suggesting phagocytosis of the infection, which was consistent with reports of Sood et al. (2012) which recorded the same findings in a cow-calf.

The bacterial culture investigation showed the existence of bacterial growth that formed white cream, dry, waxy colonies with a narrow β-haemolysis zone after 48 h of incubation. On Gram staining, bacterial isolates revealed Gram-positive short cocci-bacilli, which were arranged in a Chinese pattern. These findings were consistent with previous studies by Abebe and Sisay Tessema (2015), de Sá Guimarães et al. (2011), Fontaine and Baird (2008), Quinn et al. (2011), Radostitis et al. (2007) and Singh et al. (2017) in flocks of caprine and ovine. When examined biochemically, the organisms were positive for catalase and urease and were able to ferment glucose and maltose but not trehalose and xylose. Similar findings were reported by Abebe and Sisay Tessema (2015), Chirino-Zárraga et al. (2006), Dorella et al. (2006), Singh et al. (2017) and Singh et al. (2018).

5 | CONCLUSIONS

CLA is prevalent as sporadic cases among cattle slaughtered in Bishoftu municipal abattoir in central Ethiopia that was confirmed using cytopathological and bacteriological investigation. Therefore, effective preventive and control measures, such as good sanitary and hygiene, should be followed during meat inspection. Moreover, public health education on the zoonotic importance of CLA through one health approach, and further molecular characterization of the lesions and epidemiological studies should be undertaken.

ACKNOWLEDGEMENTS

The author acknowledges Addis Ababa university college of veterinary medicine and NAHDIC.

FUNDING

This work was not supported by any funding source or institution.

CONFLICT OF INTEREST

All authors declared that there is no conflict of interest in this work.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This research was approved by the Animal Research Ethics and Review Committee of Addis Ababa University College of Veterinary Medicine and Agriculture and the study was done following the guidelines stated for animal use (VM/ERC/10/05/10/2018). Written consent for the use of samples and animals was obtained from the cattle owner before inclusion in the study.

AUTHORS CONTRIBUTIONS

All authors contributed to data gathering and manuscript write up, involved in data analysis and write up as well as editing of the manuscript. And also, all have approved the submission of the manuscript.

DATA AVAILABILITY STATEMENT

The datasets used and analysed during the current study are available from the corresponding author on reasonable request.

PEER REVIEW

The peer review history for this article is available at https://publons.com/publon/10.1002/vms3.744

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How to cite this article: Mathewos, M., & Fesseha, H. (2022). Cytopathological and bacteriological studies on caseous lymphadenitis in cattle slaughtered at Bishoftu municipal abattoir, Ethiopia. *Veterinary Medicine and Science*, *8*, 1211–1218. https://doi.org/10.1002/vms3.744