ADDIS ABABA UNIVERSITY

COLLEGE OF VETERINARY MEDICINE AND AGRICULTURE

ISOLATION AND IDENTIFICATION OF *Corynebacterium pseudotuberculosis* FROM SUPERFICIAL LYMPHNODES OF SHEEP AND GOAT AT ORGANIC EXPORT ABBATOIR, MODJO ETHIOPIA

Msc Thesis

By

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A thesis submitted to the College of Veterinary Medicine and Agriculture, Addis Ababa University in the partial fulfillment for the requirements for the Degree of Master of Science in Tropical Veterinary Microbiology

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At last I would like to dedicate this thesis in the memory of my mother Almaz Dubale and my little brother Zerihun.

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LIST OF ABBREVIATIONS

CAMP	Christie Atkins Munch-Petersen
CLA	Caseous lymphadenitis
CSA	Central Statistics Authority
DNA	Deoxy Ribonucliec Acid
EARO	Ethiopian Agricultural Research Organization
ELISA	Enzyme Linked Immunosorbent Assay
GDP	Gross Domestic Product
ILRI	International Livestock Research Institute
MHC	Major histocompatibility complex
MoARD	Ministry of Agriculture and Rural Development
NCCLS	National Committee for Clinical Laboratory Standard
OIE	Office International Des Epizooties
PCR	Polymerase chain reaction
PLD	Phospholipase D
RFLP	Restriction fragment length polymorphism
RNA	Ribonucliec Acid
CVMA-AAU	College of Veterinary Medicine and Agriculture of Addis Ababa University
TB	Tuberculosis

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ABSTRACT

Corynebacterium pseudotuberculosis is the causative agent of caseous lymphadenitis (CLA), a disease characterized by the formation of suppurative abscesses, particularly in superficial and internal lymph nodes, and in internal organs in small ruminants. The present study was conducted from November to April 2011/ 2012 only on the superficial lymph nodes and carcass of small ruminants. Out of 768 small ruminants slaughtered at Organic slaughterhouse during the study period, 82 (68 in goats and 14 in sheep) were found to have gross evidence of abscess or caseous lymphadenitis. The typical gross lesion is a discrete abscess distended by thick and often dry, greenish yellow or white, purulent exudates. The most frequent sites of abscesses in goats occurred in the prescapular, prefemoral and poplitial lymph node and carcass 34(5.54%), 24(3.91%), 2(0.33%) and 8(1.30%) respectively. In sheep 7(4.54%) in the prescapular , 5(3.25%) in prefemoral and 0% in poplitial lymph node and 2(1.23%) abscess on carcass were found. The study indicated that in both species young animals are more in number than adult, however, caseous lymphadenitis was found to be higher in adult than young animals in both species. There was statistically significant difference in the occurrence of CLA between age groups ($P<0.05$). Out of 68 bacterial cultures from goats 18 and 50, pure and mixed colonies, respectively, were obtained; while out of 14 bacterial cultures from sheep 4 and 10, pure and mixed colonies, respectively, were obtained. *Corynebacterium pseudotubrculosis* isolates were obtained from 59 out of 82 collected abscessed lymph nodes and carcass. Bacterial colonies were identified on the basis of morphological characteristics, primary and secondary biochemical tests, i.e. catalase, urease, trehalose, xylose, maltose, and glucose fermentation tests and reverse CAMP test (antagonistic haemolysis between *C. pseudotuberculosis* and *Staphylococcus aureus*). Isolates positive for catalase, urease, maltose, and glucose, but with inhibiting β- haemolysin of *S. aureus* by pospholipase D of *C. pseudotuberculosis* and negative for trehalose, and xylose were considered as *C. pseudotuberculosis.* The susceptibility pattern of *C. pseudotuberculosis* to antimicrobial agents varied among isolates. Out of 59 isolates of *C. pseudotuberculosis* isolated from lesions of caseous lymphadenitis in goats and sheep were susceptible to the antibiotics norfloxacin, tetracycline, doxycyline HCl and kanamycine.

Keywords: Antimicrobial agents; Caseous lymphadenitis; Corynebacterium pseudotuberculosis; Goat; Lymph nodes; Mixed colonies; Pure colonies; Sheep.

1. INTRODUCTION

The Ethiopian economy is heavily dependent on agriculture sector which contributes between 40-50 percent of the total GDP. Over the last three years the economy is exhibiting encouraging growth rate. With the rural sector accounting for about 90% of Ethiopia's population and providing about 85% of the country's agricultural production, the sector is the major source of employment as well as a major source of raw materials for local industry. It is also a major source of foreign exchange, livestock, livestock products and by-products being one of the major sources of export earning (MoARD, 2006). Ethiopia is believed to have the largest livestock population in Africa. This livestock sector has been contributing considerable portion to the economy of the country, and still promising to rally round the economic development of the country (CSA, 2009). The total export value from small ruminants in the form of meat and live animals during a 2 years period (1995-1996) is estimated to be about 4.6 million US$ (EARO, 2000).

According to CSA (2009) Ethiopia posses an estimated of 25.02 million sheep and 21.88 million goats, respectively. Sheep and goats are owned by smallholder farmers as an integral part of the livestock sub-sector (Tekelye and Kasali, 1992; Workneh, 2000), and contribute to both subsistence and cash income generation (EARO, 2000; Ehui *et al.*, 2000). Sheep and goats play a significant role in the nation's economy. Meat and milk are major sources of protein, and hides, live animals, and carcasses account for a significant proportion of exports. In Ethiopia, sheep and goats are affected by many infectious and parasitic diseases (Shiferaw *et al*., 2006).

Many important livestock diseases that inflict major socio-economic losses in Ethiopia occur every year. Annual disease losses amount to 8–10%, 14–16%, and 11–13% of the cattle, sheep and goat populations, respectively. It is estimated that some 700 million Birr (1US$ = 9.2 Birr) is lost annually due to helminth (internal parasite) infestation of domestic animals. Besides affecting the quantity and quality of livestock products, the prevalence of infectious and economically important animal diseases in Ethiopia excludes the country from profitable international markets, thereby greatly reducing the country's foreign exchange earnings. Poor husbandry practices and inadequate veterinary services are the major factors favoring the expansion of livestock diseases (Yami and Merkel, 2008).

Abscess disease, commonly known as Morel's disease and caseous lymphadenitis (pseudo-tuberculosis) deserves interest because of its contagious nature, worldwide distribution and lack of effective control measures. It is primarily a disease of sheep and goats, and once introduced into a flock, it is very difficult to control because of its poor response to treatment, its ability to persist in the environment and the limitations in detecting sub-clinically infected animals (Ivanovic *et al*., 2009; Williamson, 2001).

Caseous lymphadenitis (CLA) is an important cause of organ and carcass condemnation in goat and sheep in South Africa, Tanzania, Kenya, Ethiopia, Mali and Nigeria. The prevalence of CLA in Kenya has been estimated to be 7% in goats and 2% in sheep, while a 50% morbidity of caseous lymphadenitis in goats has been reported in Nigeria (Kusiluka and Kambarge, 1996). From 1996 - 2004, among the 201 countries that reported their sanitary situation to the World Animal Health Organization (OIE), 64 declared that they had animals with caseous lymphadenitis within their borders (OIE, 2009). 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). According to de Sá Guimarães *et al.* (2011) Ethiopia is one of the countries that had reported its sanitary situation to the OIE in 1996. 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 (de Sá Guimarães *et al.*, 2011).

The actual prevalence of CLA in small ruminants is usually underestimated, because it is not a notifiable disease in many countries and since animal owners are not aware of its economical impacts and do not usually apply for veterinary advice for superficial abscesses. However, relatively high figures ranging from 8 to 90% have been reported in some countries (Kuria and Holstad, 1989; Middleton *et al*., 1991; Stanford *et al*., 1998). CLA usually will not be diagnosed clinically. Therefore, it has higher prevalence rate and the majority of cases are diagnosed during postmortem inspection. When CLA is detected the carcass is trimmed and usually downgraded. Added to this, are the unknown costs of abattoir line interruptions when CLA affected carcasses fill the trimming rail. Meat inspectors on mutton chains spend 60% to 70% of their time detecting and trimming CLA. The economic effects will thus depend on the production system in which

CLA is occurring (Paton, 2001). Due to its importance in the contamination of meat and meat products, CLA has a great economic importance (Howard and Smith, 1999). The overall cost of CLA to the Australian sheep industry has been estimated to be $10 to 15 million (Australian dollars) from lost wool production and $10 million (Australian dollars) for the inspection and subsequent trimming of abscesses from carcasses, particularly in export abattoirs (Paton, 1993; Paton *et al*., 1988).

Recently, several export abattoirs like Luna, Helimex, Elfora, Metehara, Organic and Modern export abattoirs have been established to slaughter small ruminants and provide red meat and other edible organs to international markets. The export of meat from sheep and goats to Middle East countries has become profitable business. This has great potential to contribute to the national economic development through acquisition of foreign valutta. These abattoirs constitute important places for disease search and identification of health problems.

The importance and contribution of *Corynebacterium pseudotuberclosis* as a cause for caseous lymphadenitis and hence for the condemnation of organ and carcass in goats and sheep in Ethiopia is not well studied.

Therefore, the objectives of this paper are as follows:

General objective:

- ❖ To isolate and identify *Corynebacterium pseudotuberclosis* from superficial lymph nodes and carcass of sheep and goats from slaughterhouse.

Specific objectives:

- ❖ To isolate and identify *Corynebacterium pseudotuberclosis* from superficial lymph nodes and carcass of sheep and goats from slaughterhouse.
- ❖ To determine the prevalence of caseous lymphadenitis from sheep and goats at slaughterhouse.
- ❖ To evaluate the susceptibility of the *Corynebacterium pseudotuberclosis* for different antibacterial agents.

2. LITERATURE REVIEW

2.1. Caseous lymphadenitis

Corynebacterium pseudotuberculosis is the causative agent of caseous lymphadenitis (CLA), a disease characterized by the formation of suppurative abscesses, particularly in superficial and internal lymph nodes, and in internal organs in small ruminants. The disease occurs worldwide and causes significant economical losses particularly in the sheep industry due to wasting, poor wool growth, decreased milk production, reproductive disorders, premature culling, carcass condemnation and rarely death (Alonso *et al*., 1992; Paton *et al*., 1994; Williamson, 2001; Arsenault *et al*., 2003; Dorella *et al*., 2006; Baird, 2001). The disease is considered to be one of the most economically important diseases of sheep and goats in the USA, Canada and Australia (Burrell, 1980; Paton *et al*., 1994; Stanford *et al*., 1998). An annual loss of $ 17 million has been reported in wool production alone in Australia (Paton *et al*., 1994). C. *pseudotuberculosis* is also involved in occasional infections of cattle, horses and man (Mills *et al*., 1997; Peel *et al*., 1997; Yeruham *et al*., 1997; Doherr *et al*., 1999). Infection in humans, particularly in those occupationally exposed to farm animals (farmers, abattoir workers, etc.) has been reported to be frequent in Australia (Peel *et al*., 1997). *C. pseudotuberculosis* is found in milk and the ingestion of raw milk from CLA-affected animals may therefore pose a risk for humans (Goldberger *et al*., 1981).

Infection with *C. pseudotuberculosis* causes two main forms of disease: an external form (abscesses in superficial lymph nodes and subcutaneous tissues) and an internal form (abscesses located internally in organs and lymph nodes, especially the lungs, livers, kidneys and mediastinal, bronchial and lumbar lymph nodes) (Merchant and Packer, 1967; Piontkowski and Shivvers, 1998; Ivanović *et al*., 2009). In some cases, the infection produces few obvious clinical signs in the animal, remaining unrecognized until a post-mortem examination has been carried out, making it difficult to obtain definitive data about the prevalence of this disease (Buxton and Fraser, 1977; Paton *et al*., 1994; Arsenault *et al*., 2003). Both forms can exist concomitantly (Piontkowski and Shivvers, 1998).

2.2. The bacterium; *Corynebacterium pseudotuberculosis*

C. pseudotuberculosis was isolated from bovine farcy in 1888 by Nocard. Preisz, in 1894, was the first to completely describe this microorganism and to observe its resemblance to the diphtheria bacillus. Synonyms for *C. pseudotuberculosis* were *Bacillus pseudotuberculosis ovis*, *Bacillus pseudotuberculosis*, *Corynebacterium ovis* and Preisz-Nocard bacillus (Merchant and Packer, 1967; Jones and Collins, 1986). *Corynebacterium pseudotuberculosis* belongs to the genus *Corynebacterium*, family *Corynebacteriaceae*, suborder *Corynebacterineae*, order *Actinomycetales*, subclass *Actinobacteridae*, and class *Actinobacteria* (Stackebrandt *et al*., 1997). The genus *Corynebacterium* belongs to a suprageneric group of actinomycetes that also includes the genera *Mycobacterium*, *Nocardia* and *Rhodococcus* (Paule *et al*., 2004; Songer *et al*., 1988). These gram-positive bacteria (*Corynebacterium*, *Mycobacterium*, *Nocardia* and *Rhodococcus* species), termed the CMN group, constitute a very heterogeneous group; however, most of the species share particular characteristics, such as: (i) a specific cell wall organization, mainly characterized by the presence of a huge polymer complex composed of peptidoglycan, arabinogalactan and mycolic acids (Funke *et al*., 1995; Connor *et al*., 2000; Bayan *et al*., 2003; Hall *et al*.,2003) and (ii) high G+C content (47–74%) (Garg and Nain 1985; Goodfellow, 1989; Funke *et al*., 1995). Biotypes associated with ulcerative lymphangitis in cattle and horses are different from the biotype associated with caseous lymphadenitis in sheep and goats (Radostitis *et al.*, 2006). The bacterium *Corynebacterium pseudotuberculosis* is classified into two biovars (Biberstein *et al.*, 1971), 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 (Costa *et al*., 1998; Connor *et al*., 2000; Connor, *et al*., 2007). Cattle are infected by both reducing and non-reducing nitrate strains (Songer *et al*., 1988; Sutherland *et al*., 1996). A strain of *C pseudotuberculosis* from sheep, when inoculated into camels, produced only a local abscess (Afzal *et al*., 1996).

Corynebacterium pseudotuberculosis is a short (0.5 to 0.6 x 1.0 to 3.0 μm), irregular, ovoid, gram-positive rod almost resembling a coccus, and is facultative anaerobic. In smears made from lesions, the organism show marked pleomorphism, with coccoid, bacillary and filamentous forms invariably being present. They are often clumped together and are more numerous in early lesions. The filamentous forms may exhibit a barred or beaded appearance when stained. Pleomorphism is not as marked in cultured organisms. After 48 hours' incubation at 37°C on blood tryptose agar, slow-growing, light cream-colored colonies are produced, surrounded by a narrow zone of β-haemolysis. The colonies are granular, opaque, and flat with a matt surface, do not attach to the medium and can be moved around on the surface of the agar (Collins and Cummins, 1986). *C. 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 (Biberstein *et al.*, 1971).

2.3. Epidemiology

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 (OIE, 2009). CLA is prevalent worldwide but incidence is higher in areas where intensive husbandry is practiced (Brown and Olander, 1987; Lindsay and Lloyd, 1991; Literak *et al*., 1999). The epidemiology of CLA seems to vary between different sheep production systems and fall into two broad forms of disease. In one form, the disease is prevalent in adults but much less so in lambs (for example in Australia, South Africa, Newzealand and some USA wool producing flocks). This form of disease appears to occur more frequently in extensive production system. The other form of disease is characterized by a moderate prevalence in lambs and adult sheep with some adults showing chronic wasting disease (for example in Saudi Arabia, Canada, France, United kingdom (UK) and more intensive production system in USA). This form of CLA seems more common where sheep are raised intensively or where some housing is involved (Paton, 1997). In goats, abrasions on the head caused by head-butting, ear-biting, and browsing may predispose to infection (Batey *et al*., 1986; Prescott and Muckle, 1986; Timoney *et al*., 1988). Ingestion of infective material has been reported to be a cause of abscess development in the mandibular lymphnodes of goats (Ashfaq and Campbell, 1980).

Dissemination of this disease throughout the world probably occurred through importation of infected animals. 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. 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).

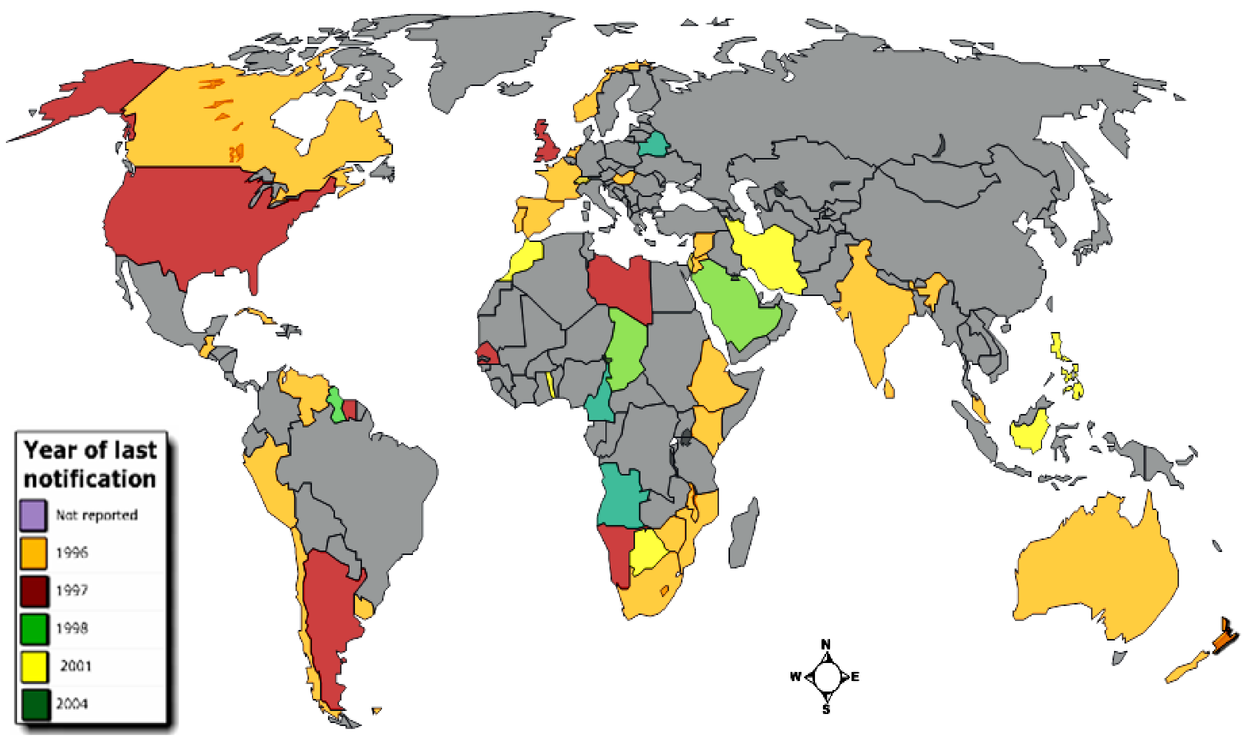

Figure 1. Map with countries that reported their sanitary situation as a caseous lymphadenitis to the World Animal Health Organization (OIE), from 1996 – 2004.

(Source: de Sá Guimarães *et al.* 2011)

2.3.1. Risk Factors

The following factors can have an effect on the occurrence of abscesses: Shearing wounds, advanced age, vaccination or treatments using contaminated needles, wounds and injuries, tail docking, castration, ear tagging, crutching, direct contact, sheep dips, arthropod vectors, environment, contaminated needles (Herenda, 1994; Homouda, 2009;Paton, 2001).

2.3.2. Modes of transmission

Corynebacterium pseudotuberculosis (*C. ovis*) survives for up to 8 months in soil contaminated with pus (Augustine and Renshaw, 1986; Amini *et al*., 2008). Infection typically occurs via skin or mucous-membrane wounds, followed by dissemination of the bacteria to the superficial lymph nodes, in which caseous abscesses develop and necrosis occurs. In some cases, other sites, in particular within the visceral organs, may also be infected (Batey, 1986). Possible way for entry of bacteria in the body is through the injured skin and mucous membrane as well traumatic skin lesions after fight, the ear lesions after marking or tattooing and skin lesions during shearing and browsing; lesions of mouth and gums during the falling out of teeth also, cause can entry in the organism *per os* when exist the primary contamination of feeding stuffs, water and troughs (Brown and Olander, 1987; Baird, 2001). Contaminated shearing, handling, and feeding equipment are responsible for spread of the organism, along with confinement housing at high stocking densities (Schreuder *et al*., 1994). Flies and other insects may act as mechanical vectors for transmission of the disease **(**Doherr *et al*., 1999).

2.4. Virulence factors and pathogenesis

The pathogenic process employed by C. *pseudotuberculosis* in causing CLA in sheep and goat is not well understood; at least two major virulence determinants have been identified (Dorella *et al*., 2006). Two major virulence factors enhance the organism’s ability to survive phagocytosis and spreading to secondary sites. Firstly, an external lipid coat provides mechanical and possibly biochemical protection from hydrolytic enzymes that enable the bacterium to resist being killed by phagocytes and to maintain a chronic infection despite a good immune response. The cell wall lipid is very pyogenic and similar to that of *Mycobacterium* species. Consequently, small ruminants with CLA can be tested false positive on a tuberculin skin test (Brown and Olander, 1987). The other identified virulence determinant is sphingomyelin-degraded phospholipase D (PLD) exotoxin. PLD has for some years been implicated as the major virulence factor of *C. pseudotuberculosis* (Hodgson *et al*., 1999). PLD is thought to mediate dissemination of the pathogen within the host by increasing local vascular permeability (Batey, 1986). This exotoxin is a permeability factor that promotes the hydrolysis of ester bonds in sphingomyelin in mammalian cell membranes, possibly contributing to the spread of the bacteria from the initial site of infection to secondary sites within the host (Carne and Onon, 1978; Coyle and Lipsky, 1990; McNamara *et al.,* 1995, Peel *et al*., 1997). Moreover, it provokes dermonecrotic lesions, and at higher doses it is lethal to a number of different species of laboratory and domestic animals (Egen *et al*., 1989; Songer, 1997). Damage and destruction of caprine macrophages have been observed during infection with *C. pseudotuberculosis.* This lethal effect is due to action of PLD (Tashjian and Campbell, 1983).

The progression of CLA in sheep and goats starts as primary wound infection, with lymphatic and hematogenous dissemination, followed by secondary infection of the lymph nodes and various visceral organs. This is followed by the elimination or containment of infection, the latter presenting as characteristic caseous lesions. The steps of infection have been separated into the following phases: an initial phase (day 1–4 p.i.), characterized by the recruitment of neutrophils to the inoculation site and the draining of the lymph nodes; an amplification phase (day 5–10 p.i.), characterized by the development of pyogranuloma; and a stabilization phase, characterized by the maturation and persistence of the pyogranuloma (Pepin *et al*., 1997). Bacterial factors, including PLD and cytotoxic lipids, contribute to pathogenesis at a local level but have little effect on the systemic disease. After *C. pseudotuberculosis* is captured by phagocytic cells such as neutrophils and macrophages, the phagosome fuses with the lysosome, forming the phagolysosome (Batey, 1986). However, *C. pseudotuberculosis* is a facultative intracellular pathogen that is capable of surviving within macrophages for more than 48 hours. During that time, bacteria are released as a result of a process that leads to phagocyte death, although this property varies among different strains. The specific mechanisms of cell death caused by *C. pseudotuberculosis* are still unclear, as it does not induce the autophagy or apoptosis of macrophages. This has been demonstrated in murine macrophage cell lines, as evidenced by stable levels of microtubule-associated protein I light chain 3 (MAP-I LC3) activity and caspase-3 activity and an absence of nuclear fragmentation in infected macrophages (Stefańska *et al*., 2010). The bacteria survive within macrophages because some macrophages cannot produce nitric oxide in response to *C. pseudotuberculosis in vivo*, which results in ineffective clearing of the organism (Bogdan *et al*., 1997).

As a result of the uncontrolled bacterial growth within macrophages, the host attempts to restrain and limit the infection through the formation of pyogranulomas, which are characterized by the encapsulation of the *C. pseudotuberculosis* infected cells. The formation of pyogranulomas is dependent on adaptive immunity, which is a complex process in the case of infection by *C. pseudotuberculosis* that involves both humoral and cell-mediated immunity (Batey, 1986; Paule *et al*., 2003). Immunohistochemical studies on the cellular composition of the pulmonary lesions in sheep infected by *C. pseudotuberculosis* have revealed a predominance of large macrophages that express major histocompatibility complex (MHC) class II molecules on their surfaces in the abscess walls and surrounding lung parenchyma. T lymphocytes were prominent in the same areas within the naturally occurring lesions, with a CD4+ T cell to CD8+ T cell ratio of 3.5:1. B lymphocytes and granulocytes comprised a minor portion of the infiltrating cells. These data revealed the participation of macrophages and MHC class II-restricted T lymphocytes in the pathogenesis of CLA (Ellis, 1988).

2.5. Clinical signs and pathology

The clinical signs of caseous lymphadenitis are prominent enlargement of superficial lymph nodes such as submandibular, prescapular, prefemoral, supramammary, popliteal, or in visceral organs such as lungs (Dercksen *et al*., 2000; Smith, 2002; Binns *et al*., 2007; Radostits *et al*., 2007; Fontaine and Baird, 2008;). The postmortem signs are greenish yellow exudates from infected superficial lymph nodes in animals (Radostits *et al.*, 2007). In the primary stage, the pus in the lymph nodes is smooth and in the progressive stages is lamellate (onion skin or onion ring), a pathognomonic signs of caseous lymphadenitis (Radostits *et al*., 2007; Fontaine and Baird, 2008). Abscesses in goats and sheep are very suggestive of caseous lymphadenitis, especially if animals of the same lot have similar clinical signs (Pekelder, 2003).

2.6. Diagnosis

Accurate CLA diagnosis is based primarily upon clinical observations (external abscesses) and the identification of *C. pseudotuberculosis* by phenotypic and biochemical tests; this is important to differentiate this bacterium from other abscess inducing pathogenic agents, such as *Arcanobacterium pyogenes* or *Pasteurella multocida* (Dercksen *et al*., 2000; Williamson, 2001; Dorella *et al*., 2006).Various diagnostic techniques have been developed for caseous lymphadenitis in goats and sheep (Williamson, 2001; Baird and Fontaine, 2007). Serological diagnosis is also of importance since subclinically animals represent a potential source of infection for apparently healthy animals (Binns *et al*., 2007). Serological tests, such as ELISA (Schreuder *et al*., 1994; Dercksen *et al*., 2000), complement fixation test (Shigidi, 1979), immunodiffusion test (Burrell, 1980) and the haemolysis inhibition test (Kuria and Holstad, 1989) have been applied for diagnosis of CLA, and though most of these tests lack either sensitivity or specificity, the ELISA is particularly proved to be a versatile tool in control and eradication programmes (Dercksen *et al*., 2000).

Several molecular typing methods have been used to determine the degree of relatedness between many different Corynebacterial species, including nucleic acid hybridization, 16S rRNA gene sequence analysis and 16S rRNA gene restriction fragment length polymorphism (RFLP) (Pascual *et al*., 1995; Riegel *et al*., 1995; Ruimy *et al*., 1995; Hou *et al*., 1997; Björkroth *et al*., 1999). 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 (Cetinkaya *et al*., 2002). Sequence comparison of a hypervariable region within the rpoB gene, encoding the RNA polymerase b-subunit, has been proposed to replace or complement the 16S rRNA gene analysis for phylogenetic studies or accurate identification of Corynebacterium species, as rpoB is significantly more polymorphic than the 16S rRNA gene for members of the Corynebacterium genus (Khamis *et al*., 2004). However, DNA sequencing involves high costs, technical complexity and a considerable amount of time, making it difficult to apply for routine diagnostic testing. Recently a multiplex PCR assay for the identification of *C. pseudotuberculosis* that uses the 16S rRNA gene, rpoB and pld (encoding the phospholipase D in *C. pseudotuberculosis*, *Corynebacterium ulcerans* and *Arcanobacterium haemolyticum*) has been described. It facilitates the diagnosis by differentiating *C. pseudotuberculosis* from other pathogens present in abscesses, chiefly *C. ulcerans* (Pacheco *et al*., 2007).

2.7. Differential diagnosis

CLA in sheep and goats should be differentiated from pyo- or necrogranulomatous lesions found in diseases such as actinobacilosis, tuberculosis and melioidosis (Ellis, 1983; Collett *et al*., 1994; Yosefbaigy *et al*., 2004). The differential diagnosis should also include abscesses and lymphangitis caused by other bacteria such as *Staphylococcus aureus*, *Rhodococcus equi*, or *Streptococcus* or *Dermatophilus* species (Dorella *et al*., 2006).

2.8. Treatment and control

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 (Nozaki *et al*., 2000). 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 (Brown and Olander, 1987; Olson *et al*., 2002). The organism is susceptible to antibiotics other than the aminoglycoside group but treatment is not usually attempted because the abscess is encapsulated, the organism is intracellular and response is poor (Radostitis *et al*., 2006).

Once established in a herd or flock, CLA eradication is problematic due to the inefficacy of antimicrobial therapy (Piontkowski and Shivvers, 1998; Stanford *et al*., 1998; Williamson, 2001). 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* (Campbell *et al*., 1982). The most reliable control strategy for this disease involves vaccinating livestock and identifying and removing infected animals (Brown *et al.*, 1986; Paton *et al*., 2003). However, this approach is hindered by limitations in current diagnostic techniques (Williamson, 2001; Menzies *et al*., 2004; Dorella *et al*., 2006).

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 (Brown and Olander, 1987; Collett *et al*., 1994).

3. MATERIALS AND METHODS

3.1. Study area

The study was conducted from November to April 2011/ 2012 at Organic export abattoir which is situated at Modjo town, Ethiopia. Modjo town is the center of Lume district in Eastern Shewa Administrative Zone of Oromia Regional State. It is located 70 kilometers south east of Addis Ababa $8^0$35'N and 39^0 10' E at an altitude of 1777 masl. The average minimum and maximum temperature are 18^0c and 28^0c respectively it experience bimodal rain fall pattern with main rainy season occurring between June and September and short rainy season from March to May .The average annual rain fall is of 800mm (ILRI, 2005).

3.2. Study design

A cross-sectional type of study was conducted in between November 2011 to April 2012 on apparently healthy sheep and goats that are ready for slaughter at Organic Export abattoir.

3.3. Study population

At Organic abattoir 500-1500 sheep and goats are slaughtered every day depending on the demand from customers, availability of supply of animals and Air cargo space. This export abattoir produces safe, sound full and quality carcass and offal in a hygienically clean environment from veterinary inspected apparently healthy sheep and goats. The sheep and goats slaughtered at the abattoir were all male young and adult animals. These animals were considered to have been maintained under traditional management condition i.e., normal feeding and watering regime. Most of the animals slaughtered were originated from pastoral (low land areas) areas of the country mainly from Oromia region (Borena, Jimma, Metehara, Shashemene and its surroundings, Sodere and Dhera surroundings, and Babile), Southern Nations and Nationalities People (Arba minch, Konso, Wolayeta, Maji), Somali and Afar regional states.

3.4. Data collection

Data were collected from ante mortem and post mortem inspection, bacteriological culture and antimicrobial susceptibility tests.

3.5. Study animals

A total of 768 sheep and goats (614 goats and 154 sheep) that were ready for slaughter were included in this study. Sheep and goats were grouped into different categories of age. The age grouping was based on dentition. Those animals which have non erupted incisor teeth were classified as young, while those with one or more pair of erupted incisor teeth were classified as adult (Gatenby, 1991; Steele, 1998). Ante mortem inspection was conducted for the presence of abscesses in superficial lymph nodes at the lairage during movement and at rest.

3.6. Sample size

The sample size was determined at 95% confidence interval, 5% precision and with an expected prevalence of 50%. Thus, the sample size value was read from Thrusfeild (1995) sample size table to be 384 animals. To increase the precision the sample size was multiplied by two. Therefore, a total of 768 (614 goats and 154 sheep) have been sampled randomly. Large numbers of goat were included in the study because the numbers of goats slaughtered per day exceed the number sheep in the abattoir due to customer preferences.

$$n = \frac{1.96^2 \, Pexp\,(1-exp)}{d^2}$$

n = required sample size

Pexp= expected prevalence

d= desired absolute precision

3.7. Sample collection and transportation

Lymph nodes were examined grossly for the presence of abnormalities from sheep and goats at the slaughter line during post mortem inspection. Lymph nodes with abnormalities (enlargement, inflammation, swelling) that was suspected of having caseous, abscess or related lesions were collected and packed in sterile universal bottles and transported in ice box to the microbiology laboratory of College of Veterinary Medicine and Agriculture of Addis Ababa University (CVMA-AAU).

3.8. Bacterial isolation and identification

Bacterial isolation and identification have been conducted at the microbiology laboratory of CVMA-AAU. The collected lymph nodes were cut by sterile scissors. One swab was taken from each enlarged superficial lymph nodes after evacuation the contents under complete aseptic conditions. The swab was cultured on 7% sheep blood agar for 48 h at 37°C and bacterial colonies were identified on the basis of morphological characteristics, primary and secondary biochemical tests, i.e. catalase, urease, trehalose, xylose, maltose, and glucose fermentation tests and reverse CAMP test (antagonistic haemolysis between *Corynebacterium pseudotuberculosis* and *Staphylococcus aureus*). Smears were prepared from each colony type and stained with Gram's staining for microscopic examination. Isolates positive for catalase, urease, maltose, and glucose, but with inhibiting beta haemolysin of *S. aureus* by pospholipase D of *Corynebacterium pseudotuberculosis* and negative for trehalose, and xylose were considered as *Corynebacterium pseudotuberculosis* (Cowan and Steel, 1974; Quinn *et al*., 1994).

3.9. Antimicrobial susceptibility testing

Antimicrobial sensitivity test patterns of *Corynebacterium pseudotuberclosis* isolates were determined using Kirby-Bauer-disk diffusion method (Quinn *et al*., 1994). Pure colonies were inoculated into 5ml of the nutrient broth and incubated at 37 ºc for 24 hours until turbidity is seen and compared to the 0.5 McFarland standards. The isolates were streaked using sterile swab onto nutrient agar (Asghar *et al*., 2009) and then the antibiotic impregnated disks were applied on the surface of inoculated plates with sterile forceps. All the disks have been gently pressed down onto the agar with sterile forceps and waited for about 15 minutes to ensure complete contact with the agar surface. The plates were inverted and then incubated aerobically for 24 hours at 37 ºc. The diameters of growth-inhibition were measured in millimeters and reported as: Susceptible, Intermediate or Resistant. Presently, it is not clear which medium should be used for growing the inoculums for susceptibility testing, which medium to use for broth microdilution or agar dilution techniques, or which incubation conditions should be used (Funke *et al*., 1997). The selection of types of antimicrobial agents for use in the disk diffusion test was dependent on clinical considerations including the drugs that were available and in general use by veterinarian. Antimicrobials used in this study were Vancomycin, Kanamycin, Clindamycin, Ampicillin, Norfloxacin, Tetracycline and Doxycylin (Oxoid, England). The National Committee for Clinical Laboratory Standards (NCCLS) has not published specific guidelines for susceptibility testing of coryneform bacteria, in particular for fastidious organisms like lipophilic corynebacteria (Funke *et al*., 1997). Therefore, National Committee for Clinical Laboratory Standard (NCCLS) breakpoints adapted from Quinn *et al.* (1994) and the manufacturers' manual were used to interpret the inhibition zone.

3.10. Data analysis

All the research findings were stored in Microsoft Excel and descriptive statistics, such as percentage was used to determine the prevalence of caseous lymphadenitis, was performed using SPSS (V. 16, 2010). The association between caseous lymphadenitis, age and species was assessed by chi-squared (χ^2) test and differences were regarded statistically significant at P-value less than 0.05.

4. RESULT

4.1. Ante mortem inspection

Ante mortem inspection was done at the lairage as a part of daily routine work. Nevertheless, there were no signs detected for the presence abscess (swelling) of superficial lymph nodes in the animals in the abattoir. This is because at the reception site animals, which have any prominent sign of illness, will be rejected form admission as slaughter animals.

4.2. Postmortem inspection

Table1. Relative prevalence of caseous lymphadenitis and abscessed carcass

Species	**Proportion of affected lymph nodes and carcass No (%)**					
Goats	No. examined	Prescapular	Prefemoral	Poplitial	Carcass	Subtotal
Young	414	6 (1.45)	4 (0.97)	0 (0.00)	3 (0.73)	13(3.14)
Adult	200	28 (14.00)	20(10.00)	2 (1.00)	5 (2.5)	55(27.5)
Total	**616**	**34(5.54)**	**24(3.91)**	**2(0.33)**	**8(1.30)**	**68(11.07)**
Sheep	No. examined	Prescapular	Prefemoral	Poplitial	Carcass	Subtotal
Young	**109**	2 (1.83)	1 (0.92)	0	0 (0.00)	3(2.75)
Adult	**45**	5 (11.11)	4 (8.89)	0	2 (4.44)	11(24.44)
Total	**154**	**7(4.54)**	**5(3.25)**	**0**	**2(1.23)**	**14(9.09)**
Grand total	768	**41(5.33)**	**29 (3.77)**	**2(0.26)**	**10(1.30)**	**82(10.68)**

Out of 768 small ruminants slaughtered at Organic slaughterhouse during the study period, 82 (68 in goats and 14 in sheep) were found to have gross evidence of abscess or caseous lymphadenitis. The typical gross lesion is a discrete abscess distended by thick and often dry, greenish yellow or white, purulent exudates. Cut section of some of the affected lymph nodes revealed characteristic onion like appearance. The results in Table 1 indicate that the most frequent sites of abscesses in goats occurred in the prescapular, prefemoral and poplitial lymph nodes and carcass 34(5.54%), 24(3.91%), 2(0.33%) and 8(1.30%) respectively. In sheep 7(4.54%) in the prescapular, 5(3.25%) in prefemoral and 0% in poplitial lymph nodes and 2(1.23%) abscess on carcass were found. The study indicated that in both species young animals are more in number than adult, however, caseous lymphadenitis was found to be higher in adult than young animals in both species (Table1). This study showed statistically significant difference in the occurrence of caseous lymphadenitis between age groups ($P<0.05$) (Table 2). However, there is no statistically significant difference in the occurrence of caseous lymphadenitis between species (Table 3).

Table 2. Association between age and caseous lymphadenitis occurrences using Pearson's chi-square (χ^2)

Age * CLA Cross tabulation			Caseous lymphadenitis		Total	Chi-square (P-value)
			Negative	Positive		
Age	Young	Count	507	16	523	
		% within age	96.9	3.1	100	
	Adult	Count	179	66	245	99.75 (0.00)
		% within age	73.1	26.9	100	
Total		Count	686	82	768	
		% within age	89.3	10.7	100	

Table 3. Association between species and caseous lymphadenitis occurrences using Pearson's chi-square (χ^2)

Species * CLA Cross tabulation			Caseous lymphadenitis Negative	Caseous lymphadenitis Positive	Total	Chi-square (P-value)
Species	Sheep	Count	140	14	154	0.508 (0.475)
		% within species	90.9	9.1	100	
	Goat	Count	546	68	614	
		% within species	88.9	11.1	100	
Total		Count	686	82	768	
		% within species	89.3	10.7	100	

4.3. Results of bacterial isolation and identification

On postmortem examination, 68 (11.07%) of 614 goats and 14(9.09%) of 154 sheep had lesions typical of caseous lymphadenitis; these were confirmed on culture as indicated in table 4 below. Out of 68 bacterial cultures from goats 18 and 50, pure and mixed colonies, respectively, were obtained; while out of 14 bacterial cultures from sheep 4 and 10, pure and mixed colonies, respectively, were obtained (Table 4) (Annex XI). *Corynebacterium pseudotubrculosis* isolates were obtained from 59 out of 82 collected abscessed lymph nodes and carcass. Bacterial isolates were identified and confirmed based on the basis of their morphological and biochemical characteristics. All bacterial isolates appear small, white, dry, opaque and concentrically ringed colonial characteristics. These colonies were surrounded by a narrow zone of hemolysis following 48h of incubation and can be removed easily from the medium when scraped. Gram staining of smears showed Gram positive short coccobacilli in groups, pairs or singly and also showed Chinese letter arrangements (Annex XI). The organisms were catalase and urease positive. They were able to ferment glucose and maltose but not trehalose and xylose. For presumptive identification reverse CAMP test were used and all the isolates were reverse CAMP test positive with *Staphylococcus aureus* (Annex XI).

Table 4. Frequency of pure colonies, mixed colonies and *C. pseudotuberculois* isolates

Animal species	**Number of animals No. (%)**	**Number of abscess found at post mortem No. (%)**	**Pure colonies No. (%)**	**Mixed colonies No. (%)**	***Corynebacterium pseudotuberculosis* No. (%)**
Goat	614 (80)	68(11.07)	18(26.5)	50(73.5)	48(81.35)
sheep	154 (20)	14(9.09)	4(28.57)	10(71.13)	11(18.64)
Total	**768 (100)**	* **82(10.67)**	**22(26.83)**	**60(73.17)**	****59(71.95)**

* Total number of abscess ** Total number of isolates

4.4. Results of antimicrobial susceptibility test

A total of 59 *C. pseudotuberculosis* isolates were tested for susceptibility to seven antibiotics. These antibiotics were ampicillin, clindamycin, doxycyline HCl, vancomycine, kanamycine, tetracycline and norfloxacin (Table 5). The susceptibility pattern of *C. pseudotuberculosis* to antimicrobial agents varied among isolates. In this study of 59 isolates of *C. pseudotuberculosis* isolated from lesions of caseous lymphadenitis in goats and sheep were susceptible to the antibiotics norfloxacin, tetracycline, doxycyline HCl and kanamycine. Resistance to ampicillin, clindamycin, and doxycyline HCl was observed while clindamycin, ampicillin, tetracycline and norfloxacin gave the intermediate sensitivity to some isolates of *C. pseudotuberculosis* (Annex XI).

Table 5. The susceptibility pattern of *C. pseudotuberculosis* isolates to antimicrobial agents

Antimicrobials	***Corynebacterium pseudotuberculosis***		
	Susceptible no. of isolates (%)	Intermediate no. of isolates (%)	Resistance no. of isolates (%)
Ampicillin (10µg)	35(59.32)	7(11.86)	17(28.81)
Clindamycin (2µg)	35(59.32)	9(15.25)	15(25.42)
Doxycyline Hcl (30µg)	43(72.88)	3(5.08)	13(22.04)
Kanamycine(30µg)	41(69.49)	6(10.17)	12(20.34)
Tetracycline (30 µg)	43(72.88)	7(11.86)	9(15.25)
Vancomycine(30µg)	41(69.49)	6(10.17)	12(20.34)
Norfloxacin (10µg)	46(77.97)	7(11.86)	6(10.17)

5. DISCUSSION

Caseous lymphadinitis is a chronic, recurring disease. A slowly enlarging , localized and non painful abscess may develop either at the point of entry into the skin or in the regional lymph node (superficial) or external form) from which it may spread via the blood or lymphatic system and cause abscessation of internal lymph nodes or organs (visceral or internal form). There is very scanty information about caseous lymphadenitis in the country. The present study was conducted only on the superficial lymph nodes and carcass of small ruminants thus it doesn't include the internal lymph nodes or organs due to difficulty of inspecting the carcass and internal organs at the same time. The external form of abscess disease, being visible, has attracted greater scientific research attention than the internal form of the disease. This is because of the difficulty of diagnosing internal abscesses, even though the greatest economic losses are attributed to it (Estevao *et al*., 2006). All the samples were collected from male animals because only males are allowed for slaughter. Ante mortem inspection was done at the lairage as a part of daily routine work. Nevertheless, there were no signs detected for the presence abscess (swelling) of superficial lymph nodes in the animals in the abattoir. The possible reason is at the reception site animals, which have any prominent sign of illness, will be rejected form admission as slaughter animals. All animals examined post mortem were those passed ante mortem inspection by veterinarians working for MoARD, in the abattoir.

The result of this study indicates that in both species young animals are more in number than adult, however, caseous lymphadenitis was found to be higher in adult than young animals in both species (Table 1). There was statistically significant association between age groups and occurrence of caseous lymphadenitis ($P < 0.05$) (Table 2). Caseous lymphadenitis increases in prevalence with age and reaches a peak incidence in adults. In one Australian population of unvaccinated sheep the frequency of infection at abattoir inspection was 3.4% for lambs and 54% for adult ewes and similar levels of prevalence are recorded in North and South America. In another large study of mature age slaughter sheep in Australia the overall prevalence of lesions was 26 %, with carcass lesions in 20.4% of sheep and offal lesions in 9.5 % (Radostitis *et al*., 2006) indicating that the findings are in agreement. Abscessation was the main causes of offal 33% of condemnation. All 49 abscesses were positive on culture for *C. pseudotuberculosis*, the causative agent of caseous lymphadenitis. The rather high prevalence of this condition is attributable to the old age of the sheep slaughtered. These were culled Merinos that are raised for wool production and exposed to repeated trauma at shearing (van Tonder, 1971).

Post mortem inspection of the carcass of animals with local swelling of the lymph nodes and abscess on the carcass revealed that caseous lymphadenitis was more prevalent in prescapular lymph node than the others mentioned lymph nodes in both species (Table 1). The reason is not well known. Cut section of some of the affected lymph nodes revealed characteristic onion like appearance. The typical gross lesion is a discrete abscess distended by thick and often dry, greenish yellow or white, purulent exudates (Annex XI) indicating that the findings coincides with Fubini *et al*., (1983), Pugh, (2002) and Amini *et al*., (2008) . The researcher suspects that caseous lymphadenitis could occur along with Tuberculosis (TB), because there are similarities between the two diseases include gross postmortem findings, such as abscessation (Crawshaw *et al*., 2008, Baird and Fontaine 2007) and lesion distribution. The overall prevalence of caseous lymphadenitis is relatively low (10.68% or 82 out of 768). The probable reason could be that the source of most of the slaughter animals that came to the abattoir is from extensive husbandry system. Caseous lymphadenitis is prevalent worldwide but incidence is higher in areas where intensive husbandry is practiced (Brown and Olander, 1987; Lindsay and Lloyd, 1991; Literak *et al*., 1999). The epidemiology of caseous lymphadenitis seems to vary between different sheep production systems and fall into two broad forms of disease. In one form, the disease is prevalent in adults but much less so in lambs (for example in Australia, South Africa, Newzealand and some USA wool producing flocks). This form of disease appears to occur more frequently in extensive production system. The other form of disease is characterized by a moderate prevalence in lambs and adult sheep with some adults showing chronic wasting disease (for example in Saudi Arabia, Canada, France, United kingdom (UK) and more intensive production system in USA). This form of caseous lymphadenitis seems more common where sheep are raised intensively or where some housing is involved (Paton, 1997).

As indicated in Table 4 that when the abscess was cultured on the blood agar media pure and mixed colonies were isolated. This reveals that *C. pseudotuberculosis* was not the only bacteria that were isolated; there were some other bacterias which are not identified and included in this study as they were not the primary objective of this study. Unanian *et. al.*, (1985) reported that in 79 cases the isolated bacteria were in pure form and in 5 cases it was mixed by *Arcanobaterium pyogenes*, *Staphylococcus aureus*, and *Streptococcus alphahemolyticus.* Similarly Yosefbaigy *et al*., (2004) indicated that pure and mixed form of colonies were found from the culture of CLA; these isolated microorganisms include *C. pseudotuberculosis* in 79.3% of samples, *Arcanobaterium pyogenes* in 9.4%, *Staphylococcus aureus* in 7.5% and *Streptococcus alphahemolyticus* in 3.8% of samples. Other bacterial agents which also have been isolated from caseous lymphadenitis include *Staphylococcus epidermidis*, *Corynebacterium equi*, *Sterptococcus alphahemolyticus*, *Moraxella spp*., *Arcanobaterium pyogenes*, *Staphylococcus aureus*, *Pasturella spp*., *Pseudomonas aeroginosa*, *Escherichia coli. Micrococcus spp*., *Aeromonas* and different species of *Bacillus* (Renshaw *et al*., 1979; Al-Harbi, 2011) these microorganisms may cause the infection either primarily or secondarily.

Varied sensitivity of *Corynebacterium pseudotuberculosis* to different antimicrobials has been reported earlier (Muckle and Gyles, 1982; Skalka *et al.,* 1989; Connor *et al.,* 2007). Although *C. pseudotuberculosis* is sensitive to a wide range of antibiotics (Sargison, 2003), there have been a few published studies of the antimicrobial susceptibilities of *Corynebacterium* spp. (Garcia-Rodriguez *et al*., 1991, Martinez-Martinez *et al*., 1995, Soriano *et al*., 1995), there are presently no definite recommendations on the method or the criteria to use in order to determine the in vitro activities of the antibiotics commonly used to treat *Corynebacterium* infections (Weiss *et al*., 1996). Antimicrobial sensitivity pattern of this study revealed that the microorganisms were susceptible to the antibiotics norfloxacin, tetracycline, doxycyline HCl, kanamycine, and vancomycine. Resistance to ampicillin, clindamycin, and doxycyline HCl was observed while clindamycin, ampicillin, tetracycline and norfloxacin gave the intermediate sensitivity to some isolates of *C. pseudotuberculosis.*

6. CONCLUSION AND RECOMMENDATIONS

Livestock and livestock products constitute one of the major export resources and play a vital role in the economy of Ethiopia. Protecting Ethiopian livestock agricultural economy from endemic as well as exotic and emerging animal diseases, sufficient commitment to the production and handling of hygienic animal products to ensure competitiveness of our products in the global market place, and promote sustainable and profitable production systems at home. This requires investment and commitment in identifying major diseases problems prevalent in the country and those that can be introduced from the abroad and institute proper disease surveillance. Disease is the major concern to the goat and sheep industry as it causes extensive financial loss/waste as result of direct economic losses. In this study caseous lymphadenitis was more common on adult animals than young and it was mainly found on prescapular and prefemoral lymph nodes of small ruminants. Laboratory work reveals that *C. pseudotuberculosis* can be isolated along with other bacteria that might cause abscess disease. Moreover, these unidentified organisms may cause the infection either primarily or secondarily. *Corynebacterium pseudotuberculosis* shows different pattern of sensitivity for different antibiotics.

Therefore, based on the above conclusion the following recommendations are forwarded:

- ❖ Since the study was conducted on male small ruminants at abattoir and information that was collected didn't include about the field condition of caseous lymphadenitis, there is a need to conduct surveillance about the disease at sheep and goat production areas.
- ❖ Bacteriological study of the caseous lymphadenitis indicates that the presence of other bacteria other than *Corynebacterium pseudotuberculosis*, so these bacteria should be studied by other interested parties.
- ❖ Since it is difficult to diagnose the internal form of caseous lymphadenitis, other diagnostic methods should be implemented for the diagnosis and control of the disease such as ELISA.
- ❖ This study didn't include the economic impact of caseous lymphadenitis in Ethiopian condition, so it should be studied by other interested researchers.

- Indiscriminate use of antibiotics should be avoided not only for the treatment of CLA but also other diseases since it may result the emergence of antibiotic resistant strains of bacteria.

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8. ANNEXES

Annex I. Biochemical properties of Corynebacterium species and Gardnerella

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Motility	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Catalase	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
Metachromatic granules	D	-	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	d
Haemolysis	D	+	d	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
Growth improved by blood/serum	+	+	+	+	+	+	+	+	+	+	?	+	+	+	+	+	+	+
Carbohydrate breakdown-[F/O/-]F	F	F	F	F	F	F	F	F	F	F	F?	F	?	F	?	F	F	F
Carbohydrates, acid from:																		
Glucose	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+
Lactose	-	-	d	-	-	-	-	-	-	-	-	d	-	-	d	-	-	d
Maltose	+	+	+	+	+	d	+	+	+	+	-	+	d	-	+	-	+	+
Mannitol	-	-	-	-	-	-	-	-	?	-	-	?	?	-	?	-	-	-
Salicin	-	-	-	-	-	-	-	-	?	+	-	-	-	-	-	-	-	-
Starch	D	+	+	-	+	-	+	+	-	-	-	+	-	-	-	-	-	+
Sucrose	-	-	d	+	+	-	-	-	+	+	-	-	-	-	-	-	d	d
Trehalose	-	+	-	-	+	d	+	+	-	-	-	d	-	-	d	d	d	-
Xylose	-	-	-	-	-	-	-	+	-	-	-	-	?	-	?	-	-	d
VP	-	-	-	-	-	-	-	-	?	?	-	-	-	-	-	?	+	-
Aesculin hudrolysis	-	-	-	-	-	-	-	-	?	+	?	-	?	-	-	-	-	-
Nitrate reduced	+	-	d	+	+	-	+	-	-	+	+	-	-	+	-	-	?	-
Gelatin liquefaction	-	+	d	-	-	-	-	-	-	-	-	-	d	-	-	-	-	-
Urease	-	+	+	-	d	+	+	+	-	d	+	-	-	+	-	-	d	-
Arginine hydrolysis	-	-	+	-	-	?	?	?	?	?	?	?	?	-	-	?	-	-
Pyrazinamidase	-	-	-	+	+	+	+	+	+	+	?	?	?	+	+	?	?	?
Casein digestion	-	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	d
phosphatase	-	-	-	-	-	-	-	-	+	-	?	+	?	-	+	+	?	?

1. **Corynebacterium diphtheria**
2. **‘Corynebacterium ulcerans’**
3. **‘Corynebacterim pseudotuberculosis;** *‘C.ovis’ ‘C.paseudotuberculosis-ovis’. Preisz-Nocard bacillus*
4. **Corynebacterium xerosis**
5. **Corynebacterium kutscheri**; *’C. murium ‘C. pseudotuberculosis-murium’*
6. **Corynebacterium ranale**;’ C. renale’ type I
7. **Corynebacterium pilosum**; *‘C. renale’ type II*
8. **Coyenebacterium Cystitidis**; *‘C. renale’ type III*
9. **Corynebacterium minutissimum**
10. **Corynebacterium matruchotii**; *‘Bacterionema matruchotii’*
11. **Corynebacerium ammoniagenes**; ‘Bacterionema ammoniagenes’
12. **Corynebacterium striatum**; *‘C. flavidum’*
13. **Corynebacterium jeikeium**; Group JK
14. **Corynebacterium pseudodiphtheriticum**; *C. hofmannii* (and variant spellings of both epithets)
15. **Corynebacterium bovis**
16. **Corynebacteium mycetiodes**
17. **Corynebacterium amycolatum**
18. **Gardnerella vaginalis** ‘*C. vaginale’*

D - Different results in the varieties (gravis,mitis and intermedius) of C.diphtheriae
d - Occasional strains positive

Source: (Cowan and Steel, 1974)

Annex II. Worksheet for recording postmortem findings, colony morphology and biochemical reaction for isolation and identification of *Corynebacterium pseudotuberculosis*

Sample No.	Sample type	Spp of animal	Colony characterstics on blood agar	Catalase	Urease	Glucose	Maltose	Xylose	Trehalose	Reverse CAMP test	Remark

Annex III. Estimation of the age of sheep from incisor teeth (Gatenby, 1991)

Permanent teeth	Age of the sheep
None	Less than 1 year 3 months
1 pair	1year 3 months -1year1 month
2 pairs	1year 1 month -2 years 4 months
3 pairs	2 years 4 months -3 years
4 pairs	More than 4 years

Annex IV. Estimation of the age of goats (Steele, 1996)

Age group	Teeth condition
Under 1 year	Eight sharp teeth
1-2 years	Central pair of baby teeth replaced by permanent once
2-3 years	4 permanent teeth
3-4 years	6 permanent teeth
4-5 years	8 permanent teeth
0ver 5 years	Worn teeth and some missing

Annex V. Medias used and preparation for the isolation and identification of *Corynebacterium pseudotuberculosis***.**

1. Blood Agar Base (Oxoid Ltd, BASINGSTOCK, HAMPSHIRE, ENGLAND)

Suspend 40 gm in 1 liter of distilled water; bring to the boil to dissolve completely, sterilized by autoclaving at 121ºC for 15 min. Cool to 45-50 ºC. For blood Agar add 7% defibrinated blood.

Typical formula (g/l)

'Lab-Lemco' power 10.0; Peptone 10.0; Sodium chloride 5.0; Agar 15.0.

PH 7.3 ± 0.2 at 25 ºC

2. Phenol Red Broth Base (HIMEDIA laboratories Pvt. Ltd. India)

Suspend 16.0 gms in 1000ml distilled water. Heat if necessary to dissolve completely. Mix well and dispense in tubes containing Durham's tubes and sterilize by autoclaving at 15lbs pressure (121ºC) for 15 minutes. Aseptically add filter sterilized or autoclave sterilized desired amount of carbohydrate solution (1% of Trehalose or Xylose or Maltose or Glucose solution) to sterile basal medium.

Standard formula

Ingredients	Gms/liter
Proteose peptone	10.00
Beef extract	1.00
Sodium chloride	5.00
Phenol red	0.018

Final PH (at 25ºC) 7.4 ± 0.2

Use – A basal medium to which carbohydrates may be added for determination of fermentation reaction of pure cultures of microorganisms.

3. Nutrient Broth (HIMEDIA laboratories Pvt. Ltd. India)

Suspend 13.0 gm in 1000 ml of distilled water. Heat if necessary to dissolve completely. Sterilize by autoclaving at 121ºC for 15 min.

Standard formula

Ingredients	Gms/liter
Peptic digest of animal tissue	5.00
Sodium chloride	5.00
Beef extract	1.50
Yeast extract	1.50

Final PH (at 25ºC) 7.4 ± 0.2

4. Nutrient Agar (Oxoid Ltd, BASINGSTOCK, HAMPSHIRE, ENGLAND)

Suspend 28 gm in 1 liter of distilled water; bring to the boil to dissolve completely, sterilze by autoclaving at 121ºC for 15 min.

Typical formula (g/l)

'Lab-Lemco' power 10.0; yeast extract 2.0; Peptone 5.0; Sodium chloride 5.0; Agar 15.0.

PH 7.4 ± 0.2 at 25 ºC

5. Urea Agar Base (Oxoid Ltd, BASINGSTOCK, HAMPSHIRE, ENGLAND)

Suspend 2.4 gm in 95 ml of distilled water; bring to the boil to dissolve completely, sterilize by autoclaving at 115ºC for 20 min. Cool to 50ºC and aseptically add one ampule of sterile Urea solution mix well, distribute 10 ml amounts into sterile containers and allow to set in the slope position.

Typical formula (g/l)

Peptone 5.0; Glucose 1.0; Sodium chloride 5.0; Di-sodium phosphate 1.2; Potassium dihydrogen phosphate 0.8; Phenol Red 0.012; Agar 15.0.

PH 7.4 ± 0.2 at 25 ºC

Annex VI. Catalase test

Catalase is the enzyme that breaks hydrogen peroxide (H_2O_2) into H_2O and O_2. Hydrogen peroxide is often used as a topical disinfectant in wounds, and the bubbling that is seen is due to the evolution of O_2 gas. H_2O_2 is a potent oxidizing agent that can wreak havoc in a cell; because of this, any cell that uses O_2 or can live in the presence of O_2 must have a way to get rid of the peroxide. One of those ways is to make catalase.

Procedure

a. Place a small amount of growth from your culture onto a clean microscope slide. If using colonies from a blood agar plate, be very careful not to scrape up any of the blood agar—blood cells are catalase positive and any contaminating agar could give a false positive.

b. Add a few drops of H_2O_2 onto the smear. If needed, mix with a toothpick.

c. A positive result is the rapid evolution of O_2 as evidenced by bubbling.

d. A negative result is no bubbles or only a few scattered bubbles.

e. Dispose of your slide in the biohazard glass disposal container. Dispose of any toothpicks in the Pipette Keeper.

Annex VII. Reverse CAMP test

CAMP test can be used as quick presumptive test for *C. pseudotuberculosis,R. equi and C.renale* with β-hemolysin of *staphylococcus aureus.* The result is as follows

	***Staphylococcal* β-hemolysin**
C. pseudotuberculosis	Inhibition
R. equi	Enhancement
C.renale	Enhancement (Quinn *et al*., 1994)

Here, a *Staphylococcus aureus* is streaked in the center of sheep blood agar, and *Corynebacterium pseudotuberculosis* is streaked perpendicular to it. Following incubation at 37^{o}C for 24-48 hours in aerobic conditions, inhibition line is seen between growth of *Staphylococcus aureus* and *Corynebacterium pseudotuberculosis*. This is because of phospholipase D toxin produced by *Corynebacterium pseudotuberculosis* that inhibits the diffusion of *Staphylococcus aureus* hemolysin.

Annex VIII. Gram's staining technique

Procedure

1. Make a thin smear of the material for study and allow to air dry.
2. Fix the material to slide by passing the slide three or four times through the flame of Bunsen burner so that the material does not wash off during staining procedure.
3. Place smear on the staining rack and overlay the surface with crystal violet solution.
4. After 1-3 minutes of exposure to the crystal violet stain pour off and wash the remaining stain with iodine solution, leaving the slide covered for 1-2 minutes.
5. Drop off the iodine solution and wash in 95% alcohol unless no crystal violet dye is washed off anymore.
6. Counter stain with carbol fuchsin/safranin for 2 minutes.
7. Wash with water, place the smear in the upright position in the staining rack and allow the excess water to drain off and the smear to dry.

Interpretation

- Gram positive bacteria appear blue
- Gram negative bacteria stained pink

Annex IX. McFarland 0.5 turbidity standard preparation

Solution A (0.048 M $BaCl_2$):

1.75 gram $BaCl_2.2H_2O$

Make up to 100 ml with distilled water.

Solution B (0.36N H_2SO_4):

1 ml H_2SO_4

Make up to 100 ml with distilled water.

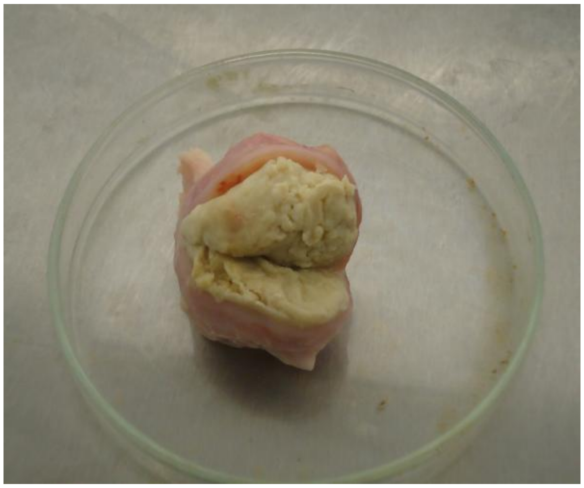
McFarland 0.5 turbidity standard was prepared by mixing 0.5 ml of solution A (0.048 M $BaCl_2$) and 99.5 ml of solution B (0.36N H_2SO_4). Shake vigorously and dispense into 4-6 ml sealed tubes or screw capped vials. Store it in the dark at room temperature. The turbidity standard should always be agitated before use (Quinn *et al*., 1994)

Annex X. Zone size interpretation chart adapted from (Quinn *et al*., 1994) and the manufacturers' manual

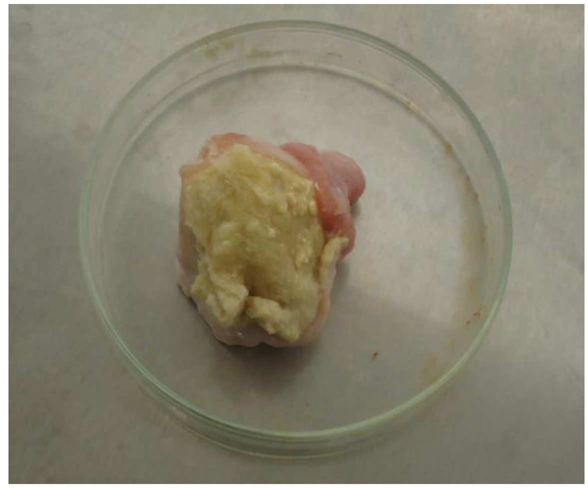
Antimicrobial agents and their codes	Disc content in µg	Diameter of zone of inhibition to nearest mm		
		Resistant ≤	Intermediate	Susceptible ≥
Clindamycin (DA)	2 µg	14	15-20	21
Vancomycin (VA)	30 µg	9	10-11	12
Kanamycin (K)	30 µg	14	15-17	18
Ampicillin (AMP)	10 µg	28	----	29
Norfloxacin (NOR)	10 µg	12	13-16	17
Tetracycline (TE)	30 µg	14	15-18	19
Doxycylin Hcl (DO)	30 µg	12	13-15	16

Annex XI. Pictures of abattoir and laboratory work

Caseous lymphadenitis of Prefemoral lymph node (indicated by black arrow)

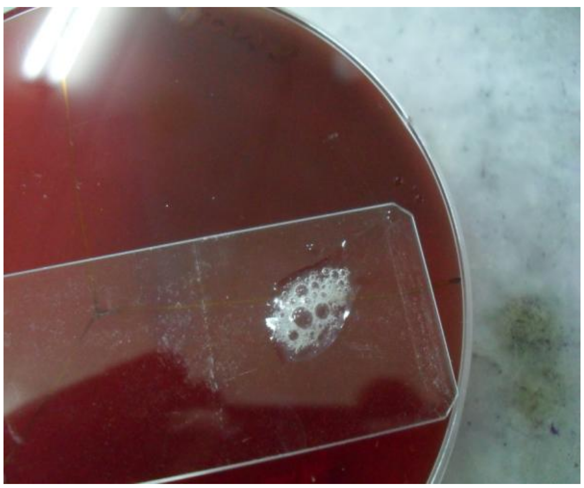


A.



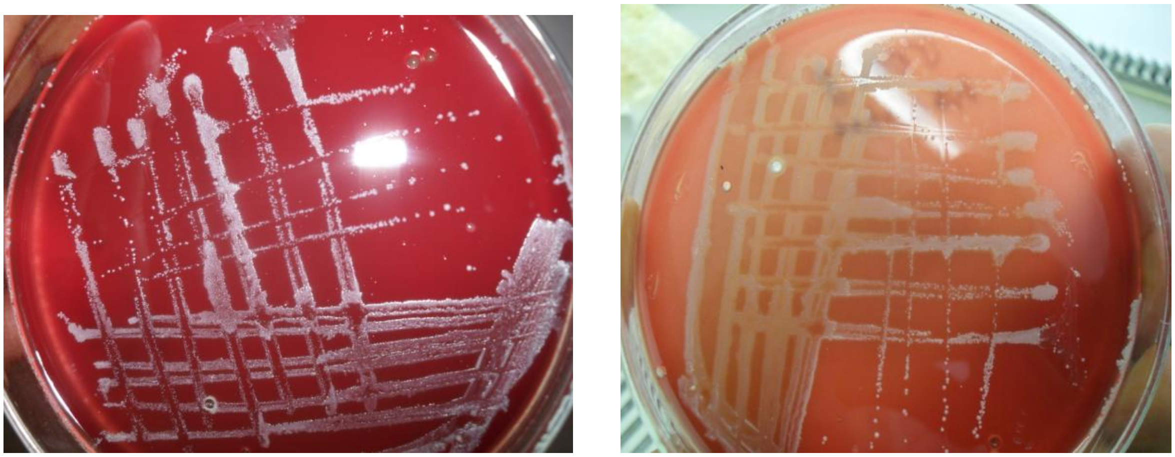
B.

Greenish yellow pus from prescapular (A) and prefemoral (B) lymph nodes of caseous lymphadenitis



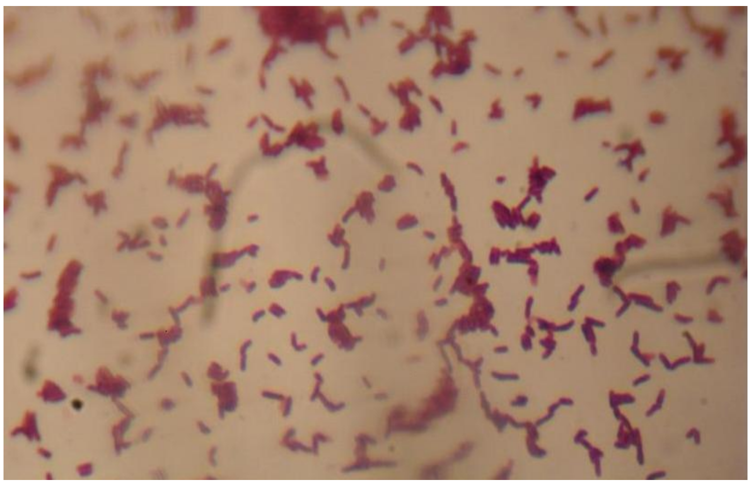
Catalase test

Mixed colonies

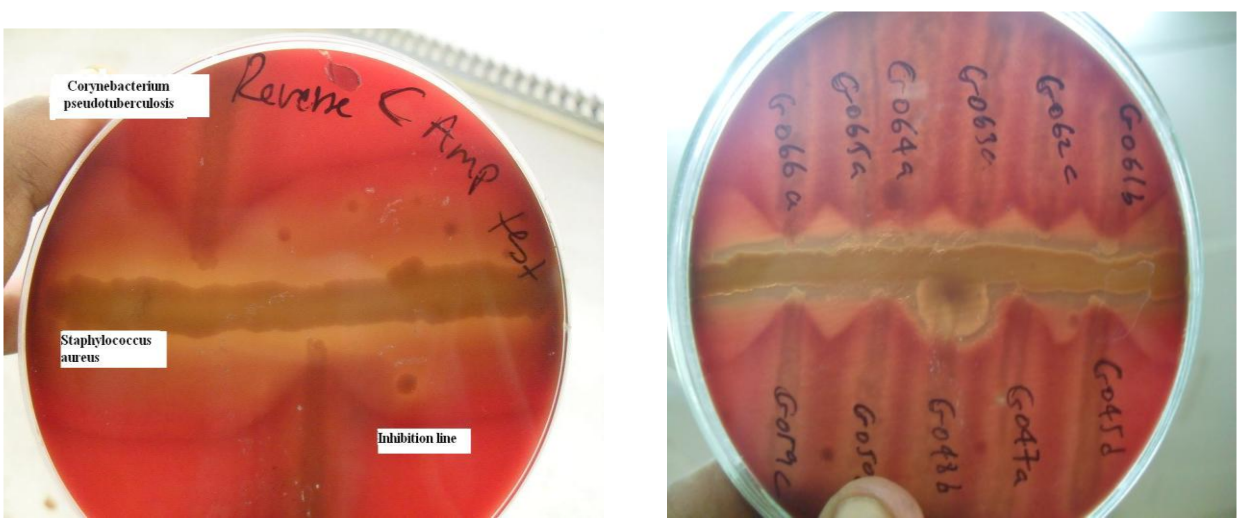


A **B**

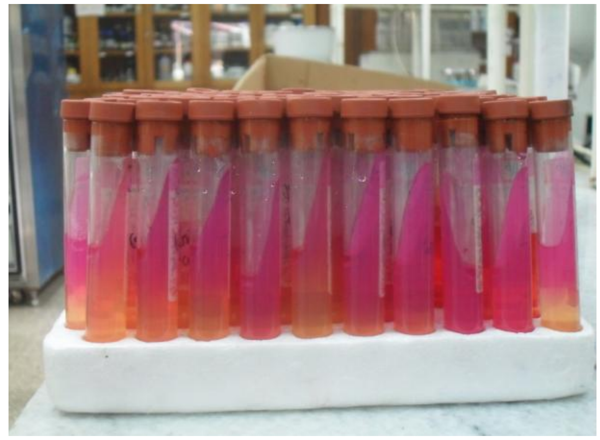
***C.pseudotuberculosis* on blood agar (after 24 hours (A) and 72 hours of incubation (B))**



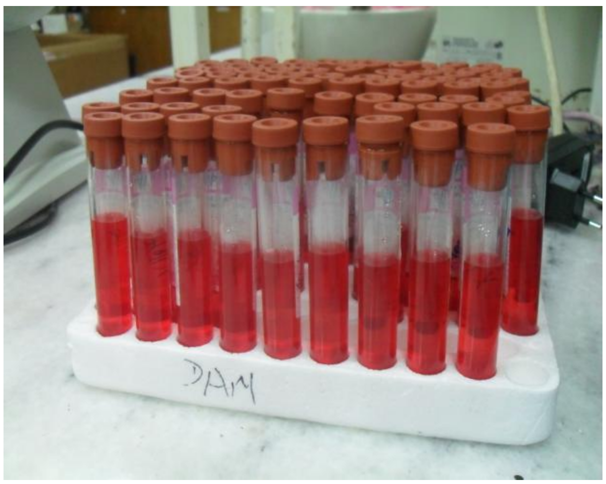
Gram stain of *C.pseudotuberculosis* (Chinese letter arrangement)



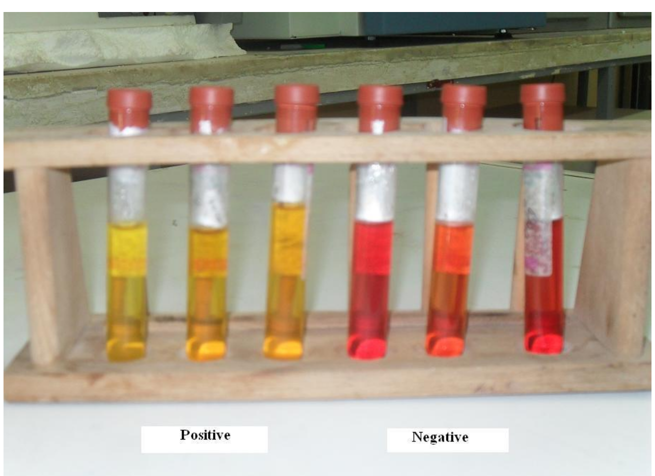
Reverse CAMP test (inhibition of *S.aureus* hemolysin with *C.pseudotuberculosis* toxin)



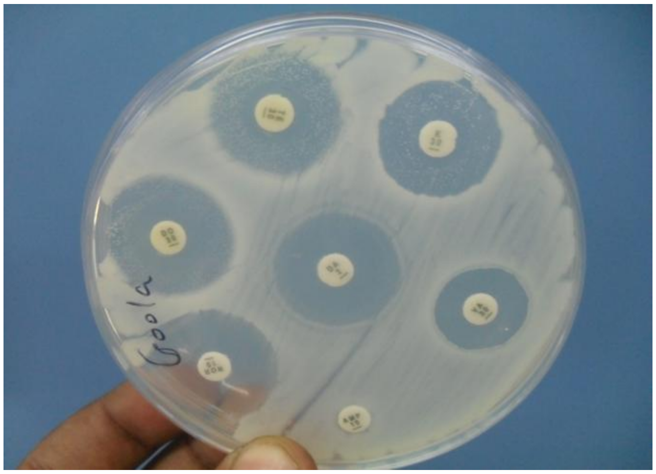
Uninnoculated urea test media **Urease positive**



Uninnoculated sugar fermentation broth (Phenol red trehalose or xylose or glucose or maltose broth)



Sugar fermentation test positive (left) negative (right)



Antimicrobial sensitivity test

9. CURRICULUM VITAE

1. **Personal Information:**

Name:	Daniel Abebe Mekonnen
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Marital Status:	Single

2. **Language :**

Language	**Read**	**Write**	**Speak**
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B. English	Excellent	Excellent	Excellent

3. **Current Address**: Addis Ababa

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4. **Educational background**

Elementary & Junior School:	Tesfa Kokeb primary school
High School:	Higher 4 high school
Undergraduate study:	Haramaya University
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5. **Work experience:**

   From April 2009 till now Lecturer at Alage ATVET College

6. **Trainings:** Pedagogy at Alage ATVET College by Hawassa TTI College.
7. **Hobbies:**

   Reading books and magazines, walking, helping others, experience sharing, discuss about different ideas with other, and strongly believe in discussion.

8. **Membership**
   - Ethiopian Veterinary Association (EVA).
9. **Reference:**

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   Mobile -0921477483 or 0912112647

10. SIGNED DECLARATION SHEET

I declare that this thesis is my original work and that all sources of material used for this thesis have been duly acknowledged. This thesis has been submitted to Addis Ababa University in partial fulfillment of the requirements of an M.Sc. degree and is deposited at the university's library to be made available to borrowers under the rules of the library. I solemnly declare that this thesis is not submitted to any other institution anywhere for the awards of any academic degree, diploma, or certificate.

Brief quotations from this thesis are allowable without special permission provided that accurate acknowledgment of source is made. Request for this manuscript in whole or in part may be granted by the Head of the Department of Microbiology, Immunology, Epidemiology and Public Health or the Dean of the School of Graduate studies when in his or her judgment the proposed use of the material is in the interest of scholarship. In all other instances, however, permission must be obtained from the author.

Name: Daniel Abebe Signature: _______________

Place: Addis Ababa University, Debre Zeit, Ethiopia

Date of submission: June 18, 2012

This thesis has been submitted for examination with my approval as University advisor.

Dr Tesfaye Sisay (DVM, MSc, PhD, Assistant Professor) Signature: __________________