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Determination of Antibiotic Resistance Pattern and Molecular Characterization of *Corynebacterium pseudotuberculosis* from Lymph Node Samples of Sheep and Goats in Ethiopia

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ABSTRACT

Corynebacterium pseudotuberculosis consists of non-motile and pleomorphic rods. The frequent rout of entry of *C. pseudotuberculosis* into the body of animal is in punctures and scratches of the skin. It results in the development of caseous abscesses in external and internal lymph nodes. Therefore, the following were the objectives of this research: to identify and isolate bacteriologically, to characterize molecularly *C. pseudotuberculosis* from the external nodes of lymph of sheep and goats, and to determine multiple drug resistance of *C. pseudotuberculosis* in sheep and goats in Halal Food Industries, Modjo, Ethiopia. A total of 450 animals (100 sheep and 350 goats) were examined during antemortem and postmortem inspections. Bacteriological isolation and identification of the collected lymph nodes were performed and then confirmed by PCR. Then, the antibiotic resistance profile was revealed. Seven (17.7%) of goat isolates from Oromia, 5 (12.5%) from Southern Nations, nationalities and peoples regional states and 4 (10%) sheep isolates from Oromia were evaluated as positive for *C. pseudotuberculosis* in molecular diagnosis. *C. pseudotuberculosis* was moderately susceptible to gentamicin (68.75%) and highly susceptible to penicillin G (100%), doxycycline (100%), and ciprofloxacin (100%) had been observed. In conclusion, risk associated factors and seroepidemiology of caseous lymphadenitis in sheep and goats should be investigated in Ethiopia.

INTRODUCTION

In Ethiopia, about 42.9 million sheep and 52.5 million goats are estimated to be found. Among these, around 9.7 million sheep and 8.4 million goats are believed to be constituted by Oromia regional state where as 4.5 million sheep and 5.5 million goats by Southern, Nations, Nationalities and Peoples regional state (CSA, 2021). The national economy of Ethiopia and the livelihoods of many Ethiopians both greatly depend on the livestock subsector. It contributes roughly 35.6% of the country's agricultural GDP and 16.5% of the overall GDP. Additionally, it produces 15% of export revenue and 30% of agricultural employment. 80% of the rural population's livelihoods are currently supported and sustained by it. Among livestock, sheep and goats are economically important (Leta and Mesele, 2014).

Caseous lymphadenitis (CLA), a disease that affects sheep and goats, is widespread. CLA is caused by *Corynebacterium pseudotuberculosis.* CLA in sheep and goats shows clinical signs of abscess formation in the external lymph nodes at different sites and with variable sizes of either closed and opened which discharges whitish milky to creamy caseated pus. In some cases, alopecia over the lesion can be seen. Some infected sheep and goats show progressive emaciation and loss of appetite are observed (Al-Gaabary *et al*., 2009).

In vitro testing has shown that *C. pseudotuberculosis* is susceptible to numerous antibiotic compounds. Inhibition of *C. pseudotuberculosis* isolates growth and multiplication have been shown by the most commonly used antibiotic classes. Clinical CLA, however, typically

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resists antibiotic treatment. This is most likely caused by the caseous nature of the pus contained within lesions and the thick encapsulation around them. In addition, *C. pseudotuberculosis* is somewhat protected against several regularly used antibiotics due to the organism's intracellular presence during some of the disease cycle (Baird and Fontaine, 2007).

Treatment in sheep and goats affected by CLA consists of draining the abscesses and surgical removal of the affected external lymph nodes followed by cleansing and chemical cauterization, usually with 10% iodine. Due to the presence of internal abscesses this procedure might not be as effective as expected in the case of CLA disease even though it is a crucial treatment measure (Saeed and Alharbi, 2014)

Applying vaccination against CLA, which slows the spread of diseases, is the main method of CLA disease control in a number of nations. Despite this, there is currently no proprietary CLA vaccine that provides complete protection against *C. pseudotuberculosis* infection. As a result, the disease will continue to develop, but at a lower intensity. Through the culling/segregation of affected animals, the serological diagnosis offers a potent alternative to vaccination for disease prevention. This method is used to totally remove CLA from infected flocks, but because to its possible expense, it is not widely employed. It may only be useful in flocks where the disease does not occur frequently because the majority of farmers would not be prepared to give up the majority of their animals (Fontaine and Baird, 2008).

Sheep and goats in Ethiopia are affected by different diseases which hinder the economic benefit the country gains. Abscess forming diseases are more common post mortem findings in abattoirs which result in organ and carcass condemnation. There was insufficient study done on molecular characterization, assessment of molecular prevalence, and the significance of *C. pseudotuberculosis* as a cause of CLA in sheep and goats in Ethiopia, aside from the studies carried out to establish baseline data. Therefore, the purpose of this study is to characterize *C. pseudotuberculosis* from sheep and goat external lymph nodes bacteriologically and molecularly, as well as to identify antibiotic resistance profile of *C. pseudotuberculosis* in sheep and goats in Halal Food Industries, Modjo, Ethiopia.

MATERIALS AND METHODS

Study animals

The investigation was conducted on sheep and goats that were slaughtered at Halal Food Industries plc's export abattoir. In Ethiopia's Oromia Regional State's Eastern Shewa Administrative Zone, Modjo town, Lume District, is home to the abattoir. At an elevation of 1777 meters above sea level, Modjo Town is situated at 8°35' N and 39°10' E, 70 kilometres to the southeast of Addis Abeba. The animals originated in low-lying pastoral regions of the nation, specifically in the Southern Nations, Nationalities, and Peoples and Oromia regional states. Female animals are not slaughtered because of their production importance. Therefore, adult male sheep and goats were the only species that were slaughtered at the abattoir. The sheep and goats were raised in extensive farming systems, and when they were ready for slaughter, the sheep and goats were purchased and transported by merchants and sent to the abattoir.

This study used a cross-sectional study design on sheep and goats that were brought to the slaughterhouse for slaughter and appeared to be in good health. Systematic random sampling was applied during sampling in the abattoir line. Detail post mortem inspection of systematic random sampled lymph nodes and carcass were carried out by palpation, visualization, and incision immediately after the animals were slaughtered.

Sample size and sampling

Total of 450 animals (100 sheep and 350 goats) were examined during antemortem and postmortem inspections. Of 100 sheep from Oromia Regional state and 350 goats (200 goats from Oromia and 150 goats from Southern Nations, Nationalities and Peoples regional states (SNNPR)) were examined. During post mortem inspection at the slaughter line, the presence of gross abnormalities of lymph nodes were examined. Lymph nodes with swelling, enlargement and inflammation were cut, detached, and intact lymph nodes were transferred to sterile bottle and transported under the cold chain to National Veterinary Institute of Ethiopia.

Bacteriological isolation and identification

In the laboratory, swabs were taken aseptically while the collected lymph nodes were excised. The swabs were inoculated on 7% sheep blood agar (Biomerieux, 43041) for 72h at 37°C. After incubation and suspected colonies were stained by Gram staining (Biomerieux, 55542) and tested on reverse CAMP (with *Staphylococcus aureus*), catalase (Biomerieux, 55561), urease (Liofilchem, 30081) and sugar fermentation tests (TSI agar, Oxoid, CM0277) *C. pseudotuberculosis* is also identified different biochemical tests (Oreiby, 2015).

Molecular detection

A few pure colonies were put into eppendorf tubes

Table I. The oligonucleotides used in this study.

Target gene	Sequence (5′→3′)	Length of PCR products	Source/ reference
Putative oligopeptide/ dipeptide ABC transporter	F CCTTACCGAGACAACGTCAT R GCCTGGTGCTTATCATTGAT	285bp	(D'Afonseca *et al*., 2010)
NADP oxido_reductase coenzyme F420dependent	F CTGCGACATAGCTAGGCACT R CCGCCAGACTTTTCTCTACA	382bp	
Proline iminopeptidase	F AACTGCGGCTTTCTTTATTC R GACAAGTGGGAACGGTATCT	551bp	

from the isolated on blood agar plates. According to the manufacturer's instructions, the bacterial genomic DNA was extracted using the QIAGEN DNeasy blood and tissue kit (Qiagen, 69504). The DNA was kept at -20°C until usage and its purity were verified using 0.8% agarose gel electrophoresis.

Proline iminopeptidase (551 bp), putative oligopeptide/ dipeptide ABC transporter (285bp), and oxidoreductase coenzyme F4200-dependent gene (382bp) were the three most conserved genes of *C. pseudotuberculosis* that the researchers synthesized and used for PCR amplification (D'Afonseca *et al*., 2010) (Table I). Table I lists the forward and reverse oligonucleotide primer sequences for amplification of these genes, as well as their product sizes. In three independent thermocyclers, PCR was conducted in a final volume of 25 µl of reaction mixture consisting of 1X PCR buffer, 2 mM MgCl2, 200 µM dNTPs mix, 0.2 mM of each forward and reverse primer, 1.25 units of Taq DNA polymerase, and 5l of DNA template. A total of 35 cycles of denaturation at 94 °C for 30 sec, annealing at 54°C for 45 sec, extension at 72°C for 45 sec, and final extension at 72°C for 5 min were utilized in each PCR condition used to amplify each of the three gene fragments.

The amplicons were separated on a 2% (wt/vol) agarose gel, stained with ethidium bromide, and visualized using UV light and photographed. A positive control was provided by the department of Microbiology, National Veterinary Institute of Federal Democratic Republic of Ethiopia.

Antimicrobial susceptibility testing

To determine antibiotic resistance *C. pseudotuberculosis,* four different antibiotic classes or drug categories (Beta-lactams, Tetracyclines, Aminoglycosides and Fluoroquinolones) were selected. Accordingly; Penicillin G (10IU), Doxycline (30µg), Gentamicin (10µg) and Ciprofloxacin (5µg) were selected from each antibiotic classes (Algammal, 2016). Kirby Bauer Disk Diffusion technique according to Clinical Laboratory Standards Institute (CLSI) was used to carry out antibiotic resistance test (CLSI, 2018). Growth inhibition zones were measured in millimeters and classified as resistant, moderate, or susceptible based on their sizes. Since specific guidelines for testing of *C. pseudotuberculosis* has not been published, Clinical and Laboratory Standards Institute (CLSI) breakpoints adapted from (Quinn *et al*., 2004).

Statistical analyses

The data in the tables were compared using statistical tests. The analyses were performed using IBM SPSS Statistics. Statistics were considered significant for P values under 0.05.

RESULTS

From 450 samples (100 sheep and 350 goats) collected from animals, suspicious lesions of CLA were observed in a total of 40 animals (8.9 %). Of these, 9 (2 %) were in sheep and 31 (6.9 %) were in goats. Of the goats 18 (4 %) were in Oromia and 13 (2.9 %) were in Southern Nations, Nationalities and Peoples regional states (Table II).

Table II. Relative prevalence of caseous lymphadenitis (CLA) in slaughtered sheep and goats in post mortem inspection findings.

Origin of animals	Number of examined		Proportion of affected caseous lymphadenitis No (%)	
	Goat	Sheep	Goat	Sheep
Oromia region	200	100	18(4)	9(2)
SNNPR	150	-	13(2.9)	-
Total	450		40 (8.9)	

Among the animals which were postmortem examined, suspected lesions of CLA were observed in only two different types of lymph nodes in this study. Forty suspected CLA lesions were observed in 35 prescapular (7.8%) and 5 prefemoral lymph nodes (1.1%) (Table III).

Table III. Relative prevalence of caseous lymphadenitis in sheep and goats in external lymph nodes.

Origin of animals	Proportion of affected lymph nodes No. (%)			
	Prescapular		Prefemoral	
	Goat	Sheep	Goat	Sheep
Oromia region	15(3.4)	9(2)	3(0.7)	-
SNNPR	11(2.4)	-	2(0.4)	-
Total	35(7.8)		5(1.1)	

Table IV. Relative prevalence of caseous lymphadenitis in slaughtered sheep and goats in bacteriological isolation and identification and PCR results.

Origin of animals	Bacteriological isolation and identification results No. (%)		Molecular characterization (PCR results) No, %	
	Goat	Sheep	Goat	Sheep
Oromia region	7(17.5)	4(10)	7(17.7)	4(10)
SNNPR	5(12.5)	-	5(12.5)	-
Total	16(40)		16(40)	

A total of 16 colonies (40%) suspected to be *C. pseudotuberculosis* were isolated from 40 CLA suspected lesion samples in bacteriological isolation and identification, providing a total prevalence of 3.6% among the total number of animals examined in post mortem. Around 11 isolates (27.5%) were isolated from prescapular and 5 isolates (12.5%) from prefemoral lymph nodes (Table IV). Of total 100 sheep, bacterial growth was observed in 4 (10%) samples and identified as *C. pseudotuberculosis* then isolates were confirmed to be positive in the PCR. Of total 350 goats, bacterial growth was observed in 12 samples (30%) (7 samples (17.5%) from Oromia and 5 samples (12.5%) from SNNPRS) and identified as *C. pseudotuberculosis* then isolates were positive in the PCR (Table IV). Amplifications of 285, 382 and 551 bp fragments were produced for each of the 16 isolates using the *C. pseudotuberculosis*-specific PCR to identify the isolates (Figs. 1, 2 and 3).

All the 16 *C. pseudotuberculosis* isolates all of which were positive to PCR were tested for susceptibility to 4 antibiotics that were selected from 4 drug classes or categories. As shown in Table V, the antibiotic sensitivity test revealed that most of the examined isolates of *C. pseudotuberculosis* were highly susceptible to penicillin G (100%), doxycycline (100%), ciprofloxacin (100%), and moderately susceptible to gentamicin (68.75%) had been observed (Table V).

Table V. The resistance pattern of *C. pseudotuberculosis* isolates to antimicrobial agents from their respective drug families.

Drug classes	Antimicrobials	Susceptibility pattern of *C. pseudotuberculosis*, No. (%)		
		Resistant	Intermediate	Susceptible
B-lactams	Penicillin G	-	-	16(100)
Tetracyclins	Doxycycline	-	-	16(100)
Fluoroquinolones	Ciprofloxacin	-	-	16(100)
Aminoglycosides	Gentamicin	5(31.25)	-	11(68.75)

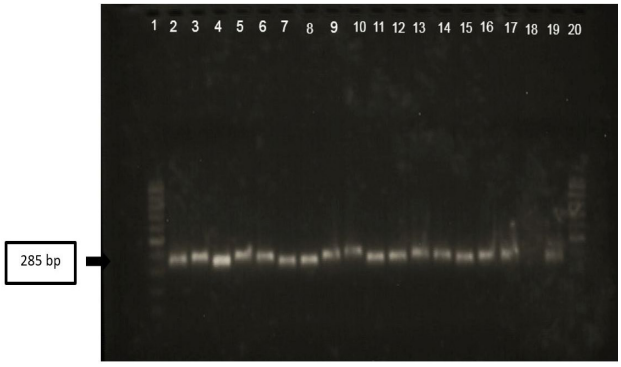

Fig. 1. Electrophoretic pattern of Putative oligopeptide/dipeptide ABC transporter gene in 2% agarose gel showing the amplified product at 285 bp, lane (1- 20): 100 bp DNA ladder, lanes (2-17): positive samples, lane (18): negative control, lane (19): positive control *C. pseudotuberculos* strain.

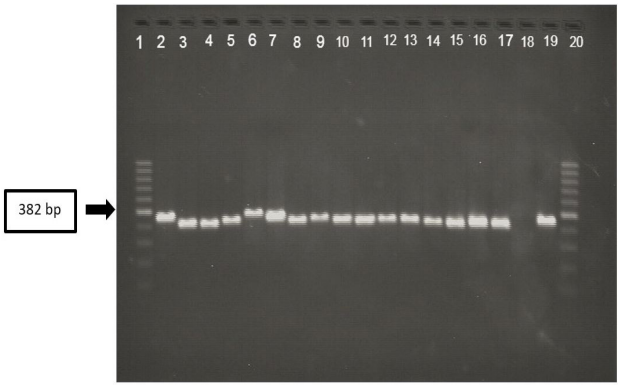

Fig. 2. Electrophoretic pattern of NADP oxido-reductase coenzyme F420 dependent gene in 2% agarose gel showing the amplified product at 382 bp, lane (1- 20):100 bp DNA ladder, lanes (2-17): positive samples, lane (18): negative control, lane (19): positive control *C. pseudotuberculosis* strain.

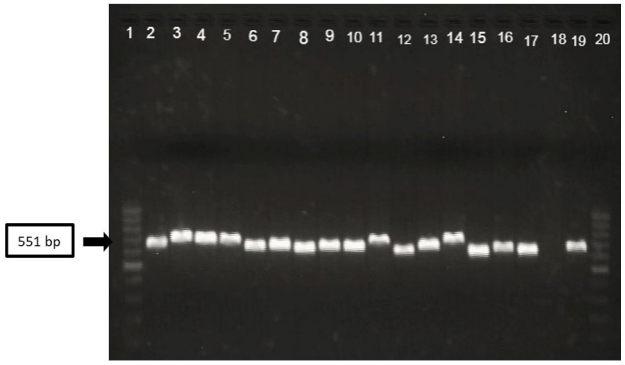

Fig. 3. Electrophoretic pattern of Proline iminopeptidase gene in 2% agarose gel showing the amplified product at 551 bp, lane (1-20): 100bp DNA ladder, lanes (2-17): positive samples, lane (18): negative control, lane (19): positive control *C. pseudotuberculosis* strain.

DISCUSSION

The present study had revealed 8.9% lesions suspicious of CLA among postmortem examined animals in which sheep (9%) and goats (8.9%) ((goats (9 %) Oromia and goats (8.7%) Southern Nations, Nationalities and Peoples regional states) and these suspicious lesions of CLA had been observed in prescapular (7.8%) and prefemoral (1.1%) lymph nodes. The previous study conducted in Ethiopia had found 10.67% abscesses or CLA in which, like the current study identified, prescapular (5.54%) and prefemoral lymph nodes (3.91%) in goats were the most frequent sites of abscesses (Abebe and Tessema, 2015). In a study conducted at the Tanta abattoir in Egypt to determine some epidemiological and histological aspects related to CLA on slaughtered animals (sheep and goats), CLA was found to be present in 26.92% of the animals, with prevalence rates of 33.23% and 10.74% for sheep and 10.74% for goats, respectively (Al-Gaabary *et al*., 2010). On the basis of a clinical examination, a different study carried out in Egypt to evaluate some epidemiological, clinical, and preventive strategies related to CLA reported a prevalence of 19.23% (Al-Gaabary *et al*., 2009). Study in Tabrith had revealed 42.09% cases were suspected to have CLA with signs of prominent enlargement in one of the lymph nodes (Zavoshti *et al*., 2012).

In bacteriological isolation, *C. pseudotuberculosis* growth was observed in giving an overall prevalence of 7% and all the isolates were confirmed to be positive in the PCR, providing a total prevalence of 7% in sheep. In the same fashion in goats, bacteriological isolation results had shown growth of *C. pseudotuberculosis* providing a total prevalence of 3.4% (3.5% in Oromia and 3.3% SNNPRS) and the whole isolates subjected to PCR were positive providing a total prevalence of 3.4% (3.5% in Oromia and 3.3% SNNPRS) with a total prevalence of 3.4% in goats. From the processed samples only 72% (59/82) *C. pseudotuberculosis* isolates were recovered in bacteriological isolation with a total prevalence of 7.68% in previously carried out study in Ethiopia which was relatively a little higher than this study revealed (Abebe and Tessema, 2015). According to Al-Gaabary *et al*. (2010), who discovered prevalence rates higher than those found in the present study 32.65% and 5.55% in sheep and goats, respectively on the basis of bacteriological investigation, the overall prevalence was 25.05%. According to a bacteriological study, there was a 17.32% overall prevalence, with 22.10% in sheep and 7.77% in goats (Al-Gaabary *et al*., 2009). *C. pseudotuberculosis* isolates were identified with the frequency of 11.3% in sheep in Kosovo (Robaj *et al*., 2017). The frequency of CLA based on bacteriological culture was 12.60% was revealed (Zavoshti *et al*., 2012). The isolation rate of 39.22% *C. pseudotuberculosis* in abscess of goats was reported for the first time in southwestern China (Li *et al*., 2018). *C. pseudotuberculosis* was isolated from 55.1% of abscessed lymph nodes of sheep in Turkey (Ilhan, 2013). In contrast to the current study, the Dakahlia governorate in Egypt reported an isolation rate of 1.7% *C. pseudotuberculosis* in sheep abscess (Ahmed *et al*., 2021). Studying the morphological, cultural, and biochemical traits of Sirohi breed goats on a managed farm in Rajasthan, India's semi-arid tropical region, revealed 2.4% *C. pseudotuberculosis* (Kumar *et al*., 2012). On the basis of physical, cultural, and biochemical traits, the same research was done on sheep. In Rajasthan, India's semi-arid tropical region, 1.1% of bacterial isolates from abscesses were *C. pseudotuberculosis*, which was lower than the prevalence found in the current investigation, which was conducted in extensive (pastorals). The management system may be the cause of this discrepancy.

A PCR assay targeting the putative oligopeptide-dipeptide ABC transporter, nicotinamide adenine dinucleotide phosphate, oxidoreductase coenzyme F420, and proline iminopeptidase genes of *C. pseudotuberculosis* was developed and revealed 14 abscess samples as positive with an overall prevalence of 1.29% (Kumar *et al*., 2013). This study found lower prevalence than the current study, which may be related to the management strategy previously mentioned. Another investigation that confirmed all 14 bacterial isolates with an overall incidence of 2.4% used a PCR assay targeting the proline iminopeptidase gene unique to *C. pseudotuberculosis* (Kumar *et al*., 2012).

In this study, the antibiotic sensitivity test revealed that, most of the examined isolates of *C.*

pseudotuberculosis were highly susceptible to penicillin G (100%), doxycycline (100%), ciprofloxacillin (100%), and moderately susceptible to gentamycin (68.75%). The previous study conducted in Ethiopia by Abebe and Tessema (2015) confirmed that *C. pseudotuberculosis* isolates were susceptible to doxycycline. Contrary to the present study, *C. pseudotuberculosis* isolates showed resistance to penicillin and they were highly sensitive to ciprofloxacin (Algammal, 2016) similar to the present study. Similar to the present study by (Robaj *et al*., 2017) revealed that *C. pseudotuberculosis* isolates were highly susceptible to gentamicin.

CONCLUSIONS

By this research, for the first time in Ethiopia, *C. pseudotuberculosis* was identified with PCR assay targeting three conserved genes specifically, nicotinamide adenine dinucleotide phosphate oxidoreductase coenzyme F420-dependent, proline iminopeptidase and Putative oligopeptide/-dipeptide ABC transporter. No multiple drug resistance *C. pseudotuberculosis* isolates were observed. This research concludes that risk associated factors and seroepidemiology of caseous lymphadenitis in sheep and goats should be investigated.

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IRB approval and ethical statement

Not applicable

Statement of conflict of interest

The authors have declared no conflict of interest.

REFERENCES

Abebe, D. and Tessema, S.T., 2015. Determination of *Corynebacterium pseudotuberculosis* prevalence and antimicrobial susceptibility pattern of isolates from lymph nodes of sheep and goats at an organic export abattoir, Modjo, Ethiopia. *Lett. appl. Microbiol.*, **61**: 469–476. https://doi.org/10.1111/lam.12482

Ahmed, M.S., Magdy, A.S., Gedawy, E., Abdullah, A. and Elsayed, Y.E., 2021. Epidemiological , bacteriological and molecular studies on caseous lymphadenitis in sheep of. *Anim. Biotechnol.*, **0**: 1–6.

Al-Gaabary, M.H., Osman, S.A., Ahmed, M.S. and Oreiby, A.F., 2010. Abattoir survey on caseous lymphadenitis in sheep and goats in Tanta, Egypt. *Small Rumin. Res.*, **94**: 117–124. https://doi.org/10.1016/j.smallrumres.2010.07.011

Al-Gaabary, M.H., Osman, S.A. and Oreiby, A.F., 2009. Caseous lymphadenitis in sheep and goats: Clinical, epidemiological and preventive studies. *Small Rumin. Res.*, **87**: 116–121. https://doi.org/10.1016/j.smallrumres.2009.10.008

Algammal, A., 2016. Molecular characterization and antibiotic susceptibility of *Corynebacterium pseudotuberculosis* isolated from sheep and goats suffering from caseous lymphadenitis. *Zagazig Vet. J.*, **44**: 1–8. https://doi.org/10.21608/zvjz.2016.7826

Baird, G.J. and Fontaine, M.C., 2007. *Corynebacterium pseudotuberculosis* and its role in ovine caseous lymphadenitis. *J. comp. Pathol.*, **137**: 179–210. https://doi.org/10.1016/j.jcpa.2007.07.002

CLSI, 2018. *Performance standards for antimicrobial susceptibility testing; twenty eight informational supplement*. Clinical Laboratory, Wayne, PA, USA.

CSA (Central Statistical Authority), 2021. *Ethiopian agricultural sample survey. Vol II. Report on livestock and livestock characteristics.* Statistical Bulletin, 589. CSA, Addis Ababa, Ethiopia.

D'Afonseca, V., Prosdocimi, F., Dorella, F.A., Pacheco, L.G.C., Moraes, P.M., Pena, I. and Azevedo, V., 2010. Survey of genome organization and gene content of *Corynebacterium pseudotuberculosis*. *Microbiol. Res.*, **165**: 312–320. https://doi.org/10.1016/j.micres.2009.05.009

Fontaine, M.C. and Baird, G.J., 2008. Caseous lymphadenitis. *Small Rumin. Res.*, **76**: 42–48. https://doi.org/10.1016/j.smallrumres.2007.12.025

Ilhan, Z., 2013. *Detection of* Corynebacterium pseudotuberculosis *from sheep lymph nodes by PCR*, pp. 60–66.

Kumar, J. and Tripathi, B.N., 2013. *Rapid detection of* Corynebacterium pseudotuberculosis *in clinical samples fromF sheep.* https://doi.org/10.1007/s11250-013-0381-8

Kumar, J., Singh, F., Tripathi, B.N., Kumar, R., Dixit, S.K. and Sonawane, G.G., 2012. Epidemiological, bacteriological and molecular studies on caseous lymphadenitis in Sirohi goats of Rajasthan, India. *Trop. Anim. Hlth. Prod.*, **44**: 1319–1322. https://

doi.org/10.1007/s11250-012-0102-8

Kumar, J., Tripathi, B.N., Kumar, R., Sonawane, G.G. and Dixit, S.K., 2013. Rapid detection of *Corynebacterium pseudotuberculosis* in clinical samples from sheep. *Trop. Anim. Hlth. Prod.*, **45**: 1429–1435. https://doi.org/10.1007/s11250-013-0381-8

Leta, S. and Mesele, F., 2014. Spatial analysis of cattle and shoat population in Ethiopia: Growth trend, distribution and market access. *Springer Plus*, **3**: 1–10. https://doi.org/10.1186/2193-1801-3-310

Li, H., Yang, H., Zhou, Z., Li, X., Yi, W., Xu, Y. and Hu, S., 2018. Isolation, antibiotic resistance, virulence traits and phylogenetic analysis of *Corynebacterium pseudotuberculosis* from goats in southwestern China. *Small Rumin. Res.*, **168**: 69–75. https://doi.org/10.1016/j.smallrumres.2018.09.015

Oreiby, A.F., 2015. Diagnosis of caseous lymphadenitis in sheep and goat. *Small Rumin. Res.*, **123**: 160–166. https://doi.org/10.1016/j.smallrumres.2014.11.013

Quinn, P.J., Carter, M.E., Markey, B. and Carter, G.R., 2004. *Clinical veterinary microbiology* (6th ed.). Elsevier, USA.

Robaj, A., Hamidi, A., Bytyqi, H. and Sylejmani, D., 2017. Frequency and antimicrobial susceptibility of bacterial isolates from caseous lymphadenitis in sheep in Kosovo. *Bulgar. J. agric. Sci.*, **23**: 1033–1036.

Saeed, E. and Alharbi, K., 2014. Morel's disease and caseous lymphadenitis: A literature review with special reference to Saudi Arabia. *IOSR J. agric. vet. Sci.*, **7**: 76–86. https://doi.org/10.9790/2380-07537686

Zavoshti, F.R., Khoojine, A.B.S., Helan, J.A., Hassanzadeh, B. and Heydari, A.A., 2012. Frequency of caseous lymphadenitis (CLA) in sheep slaughtered in an abattoir in Tabriz: Comparison of bacterial culture and pathological study. *Comp. Clin. Pathol.*, **21**: 667–671. https://doi.org/10.1007/s00580-010-1154-7