Explor Anim Med Res,
Vol.10, Issue - 2, 2020, p. 134-140

ISSN 2277- 470X (Print), ISSN 2319-247X (Online)
Website: www.animalmedicalresearch.org

Research Article

HISTOPATHOLOGICAL AND MOLECULAR STUDIES ON CASEOUS LYMPHADENITIS IN SHEEP AND GOATS IN DUHOK CITY, IRAQ

Rezheen F. Abdulrahman[1*], Mahdi A. Abdullah[1], Kareem Herish Kareem[2], Zirak Dewali Najeeb[2], Hivi Miqdad Hameed[2]

Received 14 July 2020, revised 31 August 2020

ABSTRACT: Caseous lymphadenitis is an infectious bacterial disease of sheep and goats caused by *Corynebacterium pseudotuberculosis*. This study is aimed to estimate the frequency of caseous lymphadenitis in sheep and goats and to compare with the results of bacteriological analysis, histopathology and molecular diagnosis of suspected cases. In this study, 22 (2.1%) suspected cases of caseous lymphadenitis were diagnosed clinically from 1090 sheep and goats slaughtered at Duhok abattoir. The study showed grossly affected mediastinal lymph node was congested and highly oedematous compared to other lymph nodes. Histopathological examination showed presence of abscess in the centre of affected mediastinal lymph node. Based on standard bacteriological methods, *Corynebacterium pseudotuberculosis* was the only causative agent isolated from all suspected cases. The confirmation was performed by amplification of the target genes of *Corynebacterium pseudotuberculosis* including *16S rRNA, rpoB* and *pld*. The results showed successful amplification of *16S rRNA, rpoB* and *pld* among all suspected cases. Overall, the results of this study showed that the *Corynebacterium pseudotuberculosis* is still the main causative agent of CLA in sheep and goats. The results also revealed molecular and histopathological studies together could be used to identify *Corynebacterium pseudotuberculosis* directly from cases of CLA from sheep and goats. Further studies including study of virulence genes and antimicrobial susceptibility together with phylogenetic analyses are recommended to be done in the future for better understanding of the epidemiology of this pathogen in Duhok city.

Key words: CLA, *C. pseudotuberculosis*, Histopathology, Molecular diagnosis, Sheep and goat, Duhok.

INTRODUCTION

Caseous lymphadenitis (CLA) is an infectious disease that affects small ruminants including sheep and goats and the disease is caused by *Corynebacterium pseudotuberculosis* (*C. pseudotuberculosis*) (Guerrero *et al.* 2018, Parin *et al.* 2018). *C. pseudotuberculosis* is a Gram-positive, pleomorphic, facultative anaerobic, non-motile, non-spore forming, non-capsulated, and intracellular microorganism (Guimarães *et al.* 2011). The microorganism is classified into two biovars based on their ability to reduce nitrate and two biovars have been confirmed by biotyping and molecular techniques (Connor *et al.* 2000, Connor *et al.* 2007). The biovar Ovis is unable to reduce nitrate and it mainly affects sheep and goats causing caseous lymphadenitis. The biovar Equi on the other hand, is able to reduce nitrate and is mainly isolated from horses and cattle with ulcerative lymphangitis (Oliveira *et al.* 2016). CLA is characterized by formation of caseous abscesses in lymph nodes and visceral organs as well and it may also cause disorders of reproductive system including orchitis, abortion, and stillbirth (Umer *et al.* 2017). Infection with *C. pseudotuberculosis* is associated with necrosis, inflammation, and enlargement of lymph nodes. In addition, hair loss and chronic abscessation of affected lymph nodes may occur. Sometimes rupture of the affected node followed by pus discharge also is observed (Baird and Fontaine 2007). CLA is one of the economically important diseases in sheep and goats worldwide and the economic losses are due to poor

[1]Pathology and Microbiology Department, Collage of Veterinary Medicine, University of Duhok, Duhok, Kurdistan Region, Iraq, [2]Department of Medicine and Surgery, College of Veterinary Medicine, Duhok University, Iraq.
**Corresponding author. e-mail: rezheen_fatah@uod.ac*

growth of wool, decrease in meat, and milk production, reproductive disorders of affected animals and carcasses as well as skin condemnation in abattoirs (Arsenault *et al.* 2003, Guimarães *et al.* 2011, Umer *et al.* 2017). Diagnosis of CLA is based mainly on a post-mortem examination of the lesions, isolation and identification of *C. pseudotuberculosis* from the pus using phenotypic characterization and biochemical tests (Dorella *et al.* 2006). The characteristics of the species within the *Corynebacterium* genus are variable; accordingly advance technique such as polymerase chain reaction (PCR) is used to confirm the detection of the bacterium with high specificity, sensitivity and efficiency. For example, PCR based detection of *16S rRNA* (Çetinkaya *et al.* 2002), RNA polymerase β-subunit (*rpoB*) (Khamis *et al.* 2004) and phospholipase D (*pld*) (Pacheco *et al.* 2007) genes has been developed to identify *C. pseudotuberculosis* isolates. The aim of the study was to estimate the frequency of CLA in sheep and goat in Duhok city and to compare with the results of bacterial cultures, histopathology and molecular diagnosis of suspected cases.

MATERIALS AND METHODS

Sample collection

From September to January 2019-2020, 1090 sheep and goat carcasses in a Duhok abattoir were randomly selected for inspection at slaughter house. The samples were taken from the carcasses that had prominent enlargement of the following lymph nodes: submandibular, prescapular, prefemoral, or medistinal. Enlarged abnormal lymph nodes were collected with a sterile scalpel under aseptic conditions and placed in individual sterile containers. The samples were immediately transported in an ice box to the Duhok Research Centre at College of Veterinary Medicine and were processed for microbial isolation, histopathological processing and for molecular study to identify causitve agent(s).

Histopathology study

Suspected lymph nodes were inspected macroscopically for size, colour, consistency of exudate and presence of lamellate or onion ring morphology. Samples for histopathological study were fixed in 10% neutral buffered formalin (NBF). After 24-48 h the formalin solution was changed then tissue processing was performed manually and sectioning of the tissues was done using a sliding microtome to get 4-5 micrometers thick sections (Leica, Germany). Cut sections were stained with hematoxylin and eosin (H&E) (Luna1968).

Isolation and identification of bacteria using conventional method

C. pseudotuberculosis is the causative agent of CLA in sheep and goats, for the isolation of the bacterium, pus was collected aseptically to prevent contamination from either surrounding environment or surface of lymph node. Briefly, the surface of abscessed lymph nodes was cleaned with 70% ethanol and incised with a sterile disposable surgical blade. Cheesy substance or pus were collected using sterile disposable swabs and cultured onto 5% blood agar (brain heart infusion agar containing 5% sheep blood). The inoculated plates were incubated for 24–48 h at 37 °C. The plates were checked after 24 h for growth. Standard microbiology methods were used for primary identification and these methods included cultural characteristics, haemolytic activity after 24 and 48h incubation. Colonies with typical morphology of *C. pseudotuberculosis* were selected and streaked onto blood agar plate. Those isolates were further confirmed by using Gram staining and biochemical tests including catalase, urease and CAMP (Christie, Atkins, Munch-Peterson) tests with *Staphylococcus aureus*. Suspected *C. pseudotuberculosis* isolates were then maintained in 50% (v/v) glycerol in brain heart infusion broth (BHIB) (Dorella *et al.* 2006) and stored till further analysis at -20 °C.

Molecular detection of *C. pseudotuberculosis* by PCR

DNA extraction from suspected isolates

PCR was used to confirm the detection of suspected *C. pseudotuberculosis* isolates. To extract DNA, suspected isolates were culturedon 5% sheep blood agar from glycerol stocks. The plates were incubated for 24 h at 37 °C. Thermal lysis method as described by de Sá *et al.* (2013) was used to extract DNA. Briefly 5 to 10 colonies were resuspended in 500 µl of TAE buffer and mixed very well. Bacterial suspensions were kept in heating block for 15 min. The suspension was cooled at 4 °C for 5 min then centrifuged at 14,000 x *g* for 2 min. The supernatant was then used as the DNA template for PCR. NanoDrop 2000C spectrophotometer (Thermo Scientific, UK) was used to check DNA purity and concentration. For further studies, DNA was stored at -20 °C.

Primers and PCR conditions

Primers targeting the *16S rRNA*, *rpoB* and *pld* genes of *C. pseudotuberculosis* were considered from previously published work (Çetinkaya *et al.* 2002, Khamis *et al.* 2004, Pacheco *et al.* 2007). Oligonucleotide primers

Table 1. Details of primer sequence used for detection of *C. pseudotuberculosis*.

Gene	Primer name	Sequence (5'—3´)	Product size (bp)	Ref.
16S rRNA	16S-F 6S-R	ACCGCACTTTAGTGTGTGTG TCTCTACGCCGATCTTGTAT	815	(Çetinkaya *et al.* 2002)
rpoB	C2700-F C3130-R	CGTATGAACATCGGCCAGGT TCCATTTCGCCGAAGCGCTG	446	(Khamis *et al.* 2004)
pld	pld-F pld-R2	ATAAGCGTAAGCAGGGAGCA ATCAGCGGTGATTGTCTTCCAGG	203	(Pacheco *et al.* 2007)

were synthesised by Macrogen (South Korea) and the details are presented in Table1. The *16S rRNA*, *rpoB* and *pld* genes were amplified using ready-to-use master mixes for PCR (Ruby Taq Master®, Jena Bioscience, Thuringia, Germany). A total reaction volume of 25 µl was used. Each reaction contains the following reagents: 12.5 µl of master mix, 1 µl of each forward and reverse primer (10 pmol), 2.5 µl of template DNA and 8 µl dH_2O. PCR amplification was carried out in a Gene Amp PCR System 9700 Thermo Cycler.

The following PCR conditions (35 cycles) were used for amplification of *16S rRNA*, *rpoB* and *pld:* initial denaturation at 94 °C for 3 min, denaturation at 94 °C for 1 min, annealing at 56°C for 1 min, extension at 72 °C for 2 min and a final extension step at 72 °C for 7 min. PCR products were kept cold at 4°C until they were collected from the cycler. Amplified products were confirmed and separated by 1% (w/v) agarose gel electrophoresis made with TAE (1X) buffer stained with Prime Safe Dye (GeNet Bio, Korea). The fragment sizes were viewed under UV light.

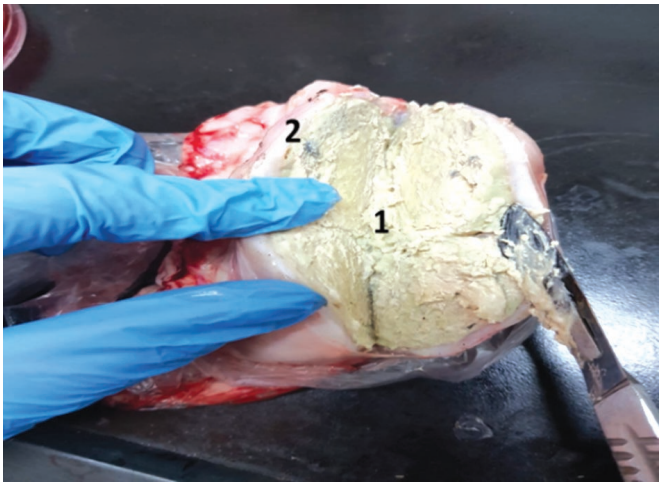

Fig.1. Macroscopic appearance of affected lymph node from sheep and goats showing lesion of abscess in the center (1) and thick fibrous connective tissue as lamellate layer (2).

RESULTS AND DISCUSSION

Macroscopical observation

C. pseudotuberculosis is the causative agent of CLA in sheep and goats and causes economic loses in many countries. The aim of this study was to determine the infection rate of CLA in sheep and goats slaughtered at Duhok slaughterhouse and to correlate with the results of bacterial isolation with histophathological and molecular detection. From 1090 examined sheep and goats slaughtered at Duhok abattoir, 22 (2.1%) suspected CLA cases of mediastinal lymph nodes were collected from sheep and goats. Previously it has been reported that the prevalence rate of CLA among sheep and goats was variable and it was varied from country to county. Similar infection rates including 2.2%, 2.31% and 2.55% were recorded in Turkey, India and Iraq, receptively (Al-Badrawi and Habasha 2016, Cetinkaya *et al.* 2002, Kumar *et al.* 2013). However, the prevalence was reported to be 4.81% in Egypt (Mubarak *et al.*1999) and it has also been shown to vary between 0.2% to 90.07% in different other studies (Al-Gaabary and Osman 2009, Al-Gaabary *et al.* 2010).

There was variation in the size of infected lymph nodes. Amongst all affecetd lymph nodes, mediastinal lymph node revealed marked congestion and oedematous changes. The present observations were in consonance to other studies as reported previously in goats and sheep affected with *C. pseudotuberculosis* (Sonawane *et al.* 2016, Singh *et al.* 2017). On careful examination extensive abscessation of mediastinal lymph nodes was prominent. The cut surface(s) of the mediastinal lymph node exhibited greenish yellow, thick semi solid ?uid mixed with pus arranged and trapped in concentrically lamellate layers of fibrous connective tissue (Fig. 1). Onion-like appearance was also found in some of the affected lymph nodes. The presence of onion-like layering of fibrous tissue was due to progressive stages of necrosis and capsule formation within abscess which given its pathogonomic lesion. (West and Bruere 2002). This

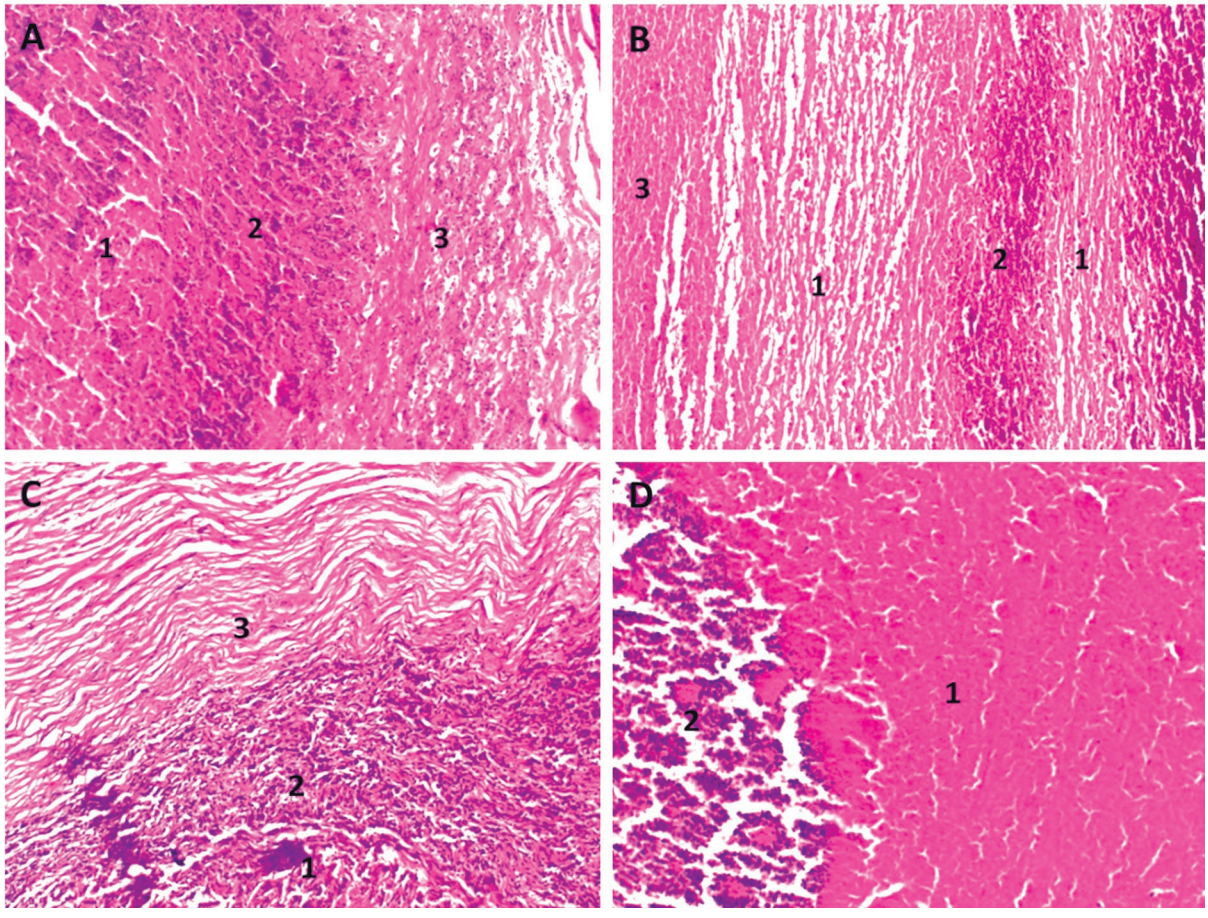

Fig. 2. (A) Microscopic section of affected lymph node showing lesion of abscess (1), pyogenic membrane (2) with concentric fibrous layers separated as fibrous connective tissue (3); (B) Microscopic section of affected lymph node showing concentrically layer as onion appearance (1) and zone of pyogenic membrane (2) with infiltration of inflammatory cells and necrotic area (3); (C) Microscopic section of lymph node abscess revealing central caseo-necrotic core (1) surrounded by pyogenic membrane with infiltration of polymorphonuclear and mononuclear inflammatory cells (2) and fibrous capsule (3); (D) Microscopic section of lymph node showing abscess as a caseous necrosis (1) surrounded by pyogenic membrane with infiltration of polymorphonuclear and mononuclear inflammatory cells (2) (H & E 20X).

findings corroborated similarly to other studies (Ali *et al.* 2016, Mahmood *et al.* 2015) who observed the presence of similar lesion(s) following experimental inoculation of *C. pseudotuberculosis* in goats.

Microscopical observation

Histopathological lesions of affected lymph node(s) was characterized by the presence of central zone of abscess surrounded by pyogenic membrane with severe infiltration of polymorphonuclear and mononuclear inflammatory cells and concentric zones of connective tissue (Fig. 2A, B, C, D). In CLA the development of chronic lesions with multiple abscesses formation and lamellate layer are attributed to the ability of bacteria to escape the immune system and its intention to spread to many other organs. Virulence factor like phospholipase D and mycolic acid (Baird and Fontaine 2007) determines its pathogenic ability, thus facilitates this organism to evade strong immune response from hosts. These findings were consistent to the previous reports (Sonawane *et al.* 2016, Singh *et al.* 2017) who stated the classical presentation of the histopathological feature of the pyogranulomatous in?ammation in lungs.

Isolation and identification using standard microbiology methods

In addition to the post-mortem examination of infected lymph nodes, conventional laboratory methods have also been used for the diagnosis of suspected cases of CLA in sheep and goats (Dorella *et al.* 2006, Guimarães *et al.* 2011). The causative agent was identified as *C. pseudotuberculosis* based on colony characteristic after

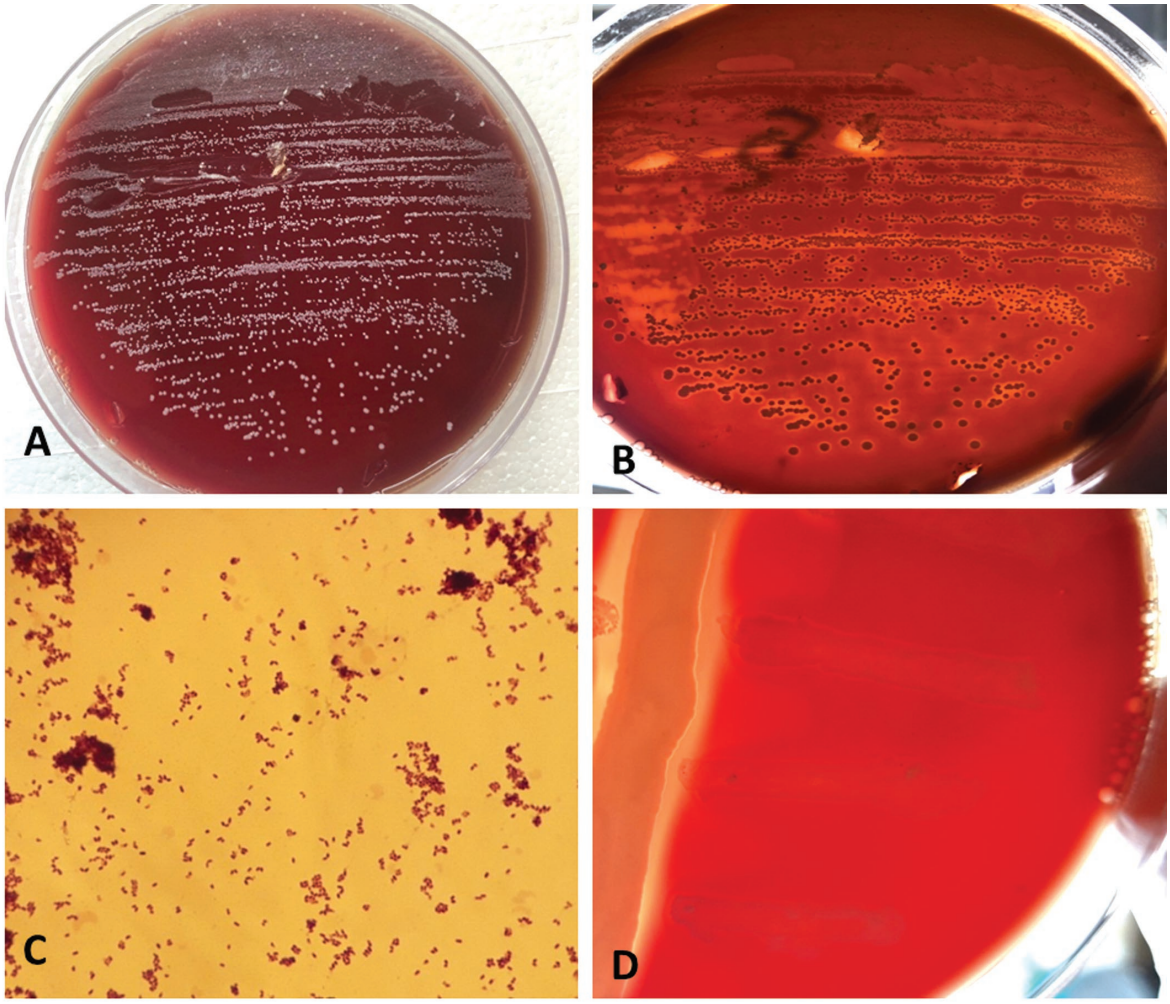

Fig. 3. Colony characteristics, Gram stained smear and CAMP test of *C. pseudotuberculosis* isolates from sheep and goats.
A, non-hemolytic, dry and small white colonies after 24 h.B, narrow zone of â-hemolysis appeared 48 h incubation on %5 sheep blood agar. C, Gram-positive and club-shaped rods detected in stained smear with Gram stain. D, CAMP test of *C. pseudotuberculosis* (right) with *S. aureus* (center) shows inhibition of the effect of the beta-haemolysin produced by *S. aureus*.

24 to 48 h incubation, and also on the basis of morphological characteristics by Gram staining. On sheep blood agar small, dry and non-haemolytic white colonies were observed (Fig. 3A) and the colonies turned cream-colored colonies, with a narrow β-hemolysis zone, after further 48h of incubation (Fig. 3B). Gram-positive pleomorphic rods arranged as chinese letters were observed in Gram stained smear (Fig. 3C). The presumptive identification was confirmed by biochemical features. The isolates were catalase positive and they were able to hydrolyse urea. CAMP test showed inhibition of β-hemolysis by *Staphylococus aureus*as shown in Fig. 3D. The results of this study revealed that all caseous lymph nodes examined were found to be positive for *C. pseudotuberculosis*. It has been showed previously that the lymph nodes are the primary sites for replication of *C. pseudotuberculosis* and it has been identified from lymph nodes of sheep and goats (Çetinkaya *et al*. 2002, Ilhan 2013, Guerrero *et al*. 2018). Furthermore, *C. pseudotuberculosis* was isolated and identified in lymph nodes of mice in another study by Firdaus Jesse *et al*. (2013).

Molecular detection of *C. pseudotuberculosis*

The conventional bacteriological methods including colony morphology, biochemical test are usually not accurate due to the species variation within the genus *Corynebacterium* (Dorella *et al*. 2006). Therefore, fast and specific diagnostic tools are developed for early diagnosis of *C. pseudotuberculosis* especially from cases

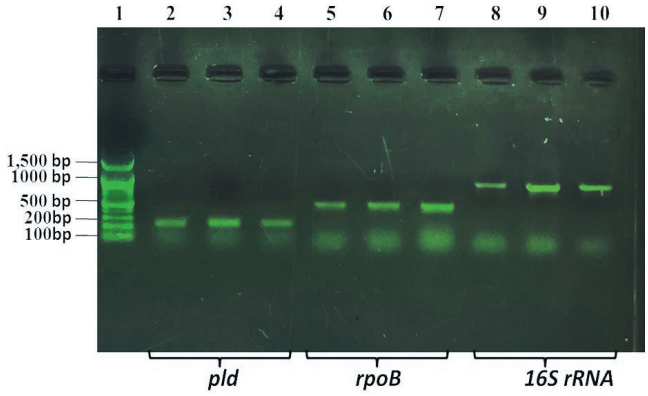

Fig. 4. Molecular detection of *C. pseudotuberculosis* isolates from sheep and goats.
1% agarose gel electrophoresis shows amplification of *pld, rpoB and 16S rRNA* genes specific for *C. pseudotuberculosis* from cases of CLA in sheep and goats. Lane 1: 100 bp DNA marker, Lane 2-4: amplification of 203 bp fragment of *pld*, Lane 5-7: amplification of 446 bp fragment of *rpoB*, lane 8-10 amplification of 815 bp fragment of *16S rRNA*.

of CLA by amplification of specific target genes including 16S rRNA, RNA polymerase β-subunit (*rpoB*) and phospholipase D (*pld*) genes (Çetinkaya *et al*. 2002, Khamis *et al.* 2004, Pacheco *et al.* 2007). In order to confirm the identification of the 22 suspected isolates by bacteriological methods, the isolates were further analysed using PCR. Detection was confirmed by amplification of the specific target genes of *C. pseudotuberculosis* including 16*S rRNA, rpoB* and *pld* respectively. All isolates were confirmed as *C. pseudotuberculosis* based on PCR amplification of 16*S rRNA* (815bp), *rpoB* (446 bp) and *pld* (203 bp) as shown in Fig. 4. Similar findings have also been confirmed in previous studies (Cetinkaya *et al.* 2002, Khamis *et al.* 2004, Hexian *et al.* 2018).

CONCLUSION

Molecular and histopathological studies together could be used to identify *C. pseudotuberculosis* directly from cases of CLA from sheep and goats. Further studies including study of virulence genes and antimicrobial susceptibility together phylogenetic analyses are recommended to be done in the future for better understand the epidemiology of this pathogen in Duhok city.

ACKNOWLEDGEMENT

The authors would like to thank Collage of Veterinary Medicine and Duhok Research Center (DRC) for supplying facilities to carry out this research work. Thanks to director of Duhok abattoir for providing facilities to collect samples from animals.

REFERENCES

Al-Badrawi TYG, Habasha FG (2016) Bacteriological and molecular studies of ovine caseous lymphadenitis in Iraq. J Agri Vet Sci 9(5): 46-51.

Al-Gaabary MH, Osman SA, Ahmed MS, Oreiby AF (2010) Abattoir survey on caseous lymphadenitis in sheep and goats in Tanta, Egypt. Small Rum Res 94: 117-124.

Al-Gaabary MH, Osman SA, Oreiby AF (2009) Caseous lymphadenitis in sheep and goats: clinical, epidemiological and preventive studies. Small Rum Res 87: 116-121.

Ali AD, Mahmoud AA, Khadr AM, Elshemey TM, Abdelrahman AH (2016) *Corynebacterium pseudotuberculosis*: disease prevalence, lesion distribution, and diagnostic comparison through microbiological culture and molecular diagnosis. Alex J Vet Sci 51: 189-198.

Arsenault J, Girard C, Dubreuil P, Daignault D, Galarneau JR *et al.* (2003) Prevalence of and carcass condemnation from maedi–visna, *paratuberculosis* and caseous lymphadenitis in culled sheep from Quebec, Canada. Preventive Vet Med 59: 67-81.

Baird GJ, Fontaine MC (2007) *Corynebacterium pseudotuberculosis* and its role in ovine caseous lymphadenitis. Comp Path 137: 179-210.

Çetinkaya B, Karahan M, Atil E, Kalin R, De Baere T *et al.* (2002) Identification of *Corynebacterium pseudotuberculosis* isolates from sheep and goats by PCR. Vet Microbiol 88 (1): 75-83.

Connor KM, Quirie MM, Baird G, Donachie W (2000) Characterization of United Kingdom isolates of *Corynebacterium pseudotuberculosis* using pulsed-field gel electrophoresis. J Clin Microbiol 38(7): 2633-2637.

Connor K, Fontaine M, Rudge K, Baird G, Donachie W (2007) Molecular genotyping of multinational ovine and caprine *Corynebacterium pseudotuberculosis* isolates using pulsed-field gel electrophoresis. Vet Rec 38(4): 613-623.

de Sá MCA, Gouveia GV, da Costa KC, Veschi JLA, de Mattos-Guaraldi AL *et al.* (2013) Distribution of PLD and FagA, B, C and D genes in *Corynebacterium pseudotuberculosis* isolates from sheep and goats with caseus lymphadenitis. Gen Mol Biol 36(2): 265-268.

Dorella FA, Pacheco LGC, Oliveira SC, Miyoshi A, Azevedo V (2006) *Corynebacterium pseudotuberculosis*: microbiology, biochemical properties, pathogenesis and molecular studies of virulence. Vet Rec 37: 201-218.

Firdaus JF, Adamu L, Osman AY, Bin Muhdi AF, Haron AW *et al*. (2013) Polymerase chain reaction detection of *C. pseudotuberculosis* in the brain of mice following oral inoculation. Inter J Ani Vet Advan 5(1): 29-33.

Guerrero JAV, de Oca Jiménez RM, Dibarrat JA, León FH, Morales-Erasto V, Salazar HGM (2018) Isolation and molecular characterization of *Corynebacterium pseudotuberculosis* from sheep and goats in Mexico. Microbial Path 117: 304-309.

Guimarães AS, Carmo FB, do Pauletti RB, Seyffert N, Ribeiro D *et al.* (2011) Caseous lymphadenitis: epidemiology, diagnosis, and control. IIOAB Journal 2(2): 33-43.

Hexian L, Yang H, Zhou Z, Li X, Yi W *et al.* (2018) Isolation, antibiotic resistance, virulence traits and phylogenetic analysis of *Corynebacterium pseudotuberculosis* from goats in southwestern China. Small Rum Res168: 69-75.

Ilhan Z (2013) Detection of *Corynebacterium pseudotuberculosis* from sheep lymph nodes by PCR. Rev de Med Vet 164(2): 60-66.

Khamis A, Raoult D, Scola BL (2004) *rpoB* gene sequencing for identification of *Corynebacterium* species. J Clin Microbiol 42(9): 3925-3931.

Kumar J, Tripathi BN, Kumar R, Sonawane GG, Dixit SK (2013) Rapid detection of *Corynebacterium pseudotuberculosis* in clinical samples from sheep. Trop Anim Health Prod 45(6): 1429-1435.

Luna L (1968) Manual of histological staining methods of the Armed Forces Institute of Pathology. 3rd edn. McGraw-Hill Book Company, New York.

Mahmood ZKH, Jesse FF, Saharee AA, Jasni S, Yusoff R *et al.* (2015) Clinicopathological changes in goats challenged with *Corynebacterium pseudotuberculosis* and its exotoxin (PLD). Am J Anim Vet Sci 10: 112-132.

Mubarak M, Bastawrows AF, Abdel-Hafeez MM, Ali MM (1999) Caseous lymphadenitis of sheep and goats in Assiut farms and abattoirs. Ass Vet Med J 42: 89-112.

Oliveira A, Teixeira P, Azevedo M, Jamal SB, Tiwari S *et al.* (2016) *Corynebacterium pseudotuberculosis* may be under anagenesis and biovar *Equi* forms biovar *Ovis*: a phylogenic inference from sequence and structural analysis. BMC Microbiol 16: 100.

Pacheco LGC, Pena RR, Castro TLP, Dorella FA, Bahia RC *et al.* (2007) Multiplex PCR assay for identification of *Corynebacterium pseudotuberculosis* from pure cultures and for rapid detection of this pathogen in clinical samples. J Med Microbiol 56(4): 480-486.

Parin U, Kirkan S, Ural K, Savasan S, Erbas G *et al.* (2018) Molecular identification of *Corynebacterium pseudotuberculosis* in sheep caseous lymphadenitis (CL) is a bacterial disease that causes considerable economic loss in sheep and goat industries. Acta Vet Brno 87: 03-08.

Singh R, Kumar P, Sahoo M, Bind RB, Kumar MA *et al.* (2017) Spontaneously occurring lung lesions in sheep and goats. Indian J Vet Path 4: 18-24.

Sonawane GG, Kumar J, Sisodia SL (2016) Etio-pathological study of multiple hepatic abscesses in a goat. Indian J Vet Path 40(3): 257.

Umer M, Abba Y, Firdaus JFA, Monther MSW, Umer M *et al.* (2017) Caseous lymphadenitis in small ruminants. Int J Vet Sci Ani Hus 2: 23-31.

West DM, Bruere AN, Ridler AL (2002) Caseous lymphadenitis, In: The sheep: health, disease and production 2nd edn. Palmerston North: Foundation for continuing education. 274-279.

***Cite this article as:** Rezheen FA, Abdullah MA, Kareem KH, Najeeb ZD, Hameed HM (2020) Histopathological and molecular studies on caseous lymphadenitis in sheep and goats in Duhok city, Iraq. Explor Anim Med Res 10(2): 134-140.