Frontiers in Health Informatics
ISSN-Online: 2676-7104
www.healthinformaticsjournal.com
2024; Vol 13: Issue 3
Open Access

Pathological Changes of *Mycoplasma Agalactia* Infection in Albino mice

[a] Zainab Hashim. Rida, [a] Zainab Ismail Ibrahim

Department of Pathology and Poultry diseases, University of Baghdad, College of Veterinary Medicine

[a] Zainab.hashem1107b@covm.uobaghdad.edu.iq
ORCiD: https://orcid.org/0009-0006-9145-1609
[a] zainabaalrubaei@covm.uobaghdad.edu.iq
ORCiD : https://orcid.org/0009-0005-2586-0386

Cite this paper as: Zainab Hashim. Rida,Zainab Ismail Ibrahim (2024) Pathological Changes of Mycoplasma Agalactia Infection in Albino mice. *Frontiers in Health Informatics, 13* (3), 2988-3001.

Abstract

Mycoplasma agalactiae (M. agalactiae) is a significant pathogen known for causing contagious agalactia, primarily affecting sheep and goats, with widespread implications across the Mediterranean, including Iraq.Despite the prevalence of Mycoplasma agalactiae (M. agalactiae) in livestock in various regions of Iraq, as evidenced by limited yet focused survey studies, detailed understanding of the pathological changes induced by this pathogen, particularly in animal models, remains sparse. The aim of this study was to fill this knowledge gap by studying the pathological manifestations of M. agalactiae infection in a controlled laboratory. A total of 200 milk samples were collected from sheep and goats with or without mastitis and cultured in pleuropneumonia-like organism (PPLO) broth containing agar. Morphologically, the colonies of M. agalactiae had a "fried egg" colony morphology and were positive for mycoplasma bacteria in PPLO agar broth. PCR was performed using specific primers that amplify the 375 bp agalactiae gene. PCR results showed the mycoplasma 16S rRNA gene.20 female lactating mice were used in this study, divided in two group positive group(15mice) which infected with (1×10^4 CFU/ml)) in intraperitoneal injection route of mycoplasma agalactia and negative control. The clinical signs are reported from infected mice daily and after a month of infection the mice are euthanized and the joints and mammary glands are histopathological examined. Results: The results of this study showed that 15 (5.5%) of the 200 isolates were positiveinPPLO agar,and7 (20%) were positivein theM.agalactiae primer.Four of the five isolates (75%)camefrom sheep and two (25%) from goats. PCRtestsof 200 milk samples showed that 7 (3%) were positive forM. agalactiae's16S rRNAgene.Histopathological examination of the mammary gland and joints showed severe inflammatory cell infiltration and necrosis.

Keywords: *mycoplasma agalactia, PCR, mastitis, sheep, goats*

1-Introduction

Contagious agalactia is an important infectious disease in sheep and goats, characterized by mastitis, arthritis, pneumonia, keratoconjunctivitis and occasionally abortion.(Muhanad &Saleem., 2018; Migliore *et al*., 2021). The disease spreads rapidly through the contact of infected animals with healthy ones. Domestic sheep and goats of both sexes can be infected with these diseases at the same frequency (Madanat *et al*.,2001); however, morbidity is more frequently associated with pregnant and lactating female. (Damasceno *et al.,*2020). Contagious agalactia can occur in the form of acute, subacute, and chronic diseases (Santos e*t al*., 2015).

The presence of M. agalactiae in Iraq was first documented by Al-Aubaidi et al. in 1986, following the

isolation of the pathogen from fifteen herds of sheep and goats, marking a significant milestone in understanding the disease epidemiology in this region (Al-Aubaidi *et al*.,1986) Subsequent isolations and studies, such as those by Al-Graibawi *et al*. (1989) and Hasso (1990), further confirmed the pathogen's prevalence and highlighted the challenges posed by its diagnosis, which requires sophisticated laboratory techniques due to the organism's fastidious nature **(Khezri *et al.*,2015).**

In the Kurdistan region, M. agalactiae is endemic and poses continuous challenges, especially as no vaccination currently controls it, leaving antimicrobial therapy as the only option. This approach has unfortunately led to the development of antimicrobial resistant strains, further complicating treatment efforts (**Sulaiman.,2015)**.

Diagnosis of Mycoplasma diseases is complex, often requiring beyond clinical and serological evaluations to include advanced molecular techniques such as real-time PCR, which offers increased sensitivity, specificity, and quicker results compared to conventional PCR methods **(OIE., 2019**). Most researchers isolate and identify M. agalactiae using culture and molecular methods(Momani, 2006).M. agalactiae has been identified by molecular diagnostic testing over the past decade. In this regard, polymerase chain reaction (PCR)analysis was considered an effective method for detecting. agalactiae in milk samples **(Tola *et al.,* 1997).** M. agalactiae was isolated and identified by culture and PCR testing of goats and sheep in several Iraqi provinces. (Muhanad &Saleem., 2018).

2. Materials and Methods

2.1. Ethical Approval

Tests were directed as prescribed by the guidelines of the college of veterinary medicine. University of Baghdad reviewed and approved all procedures involved in the current study under the agreement number 369\P.G. dated on February 18,2024.

2.1. Collection of milk sample.

Approximately10 ml of milk samples from 200 sheep (170 samples) and30 samples from goats with or without mastitis were collected in aseptically collected sterile tubes. Samples were transported on ice to the laboratory of the Department of Culture Pathology.

2.2. Mycoplasma isolation and identification

Milk samples were immediately cultured according to standard procedures in PPLO broth and PPLO agar media. The inoculated media were boiled at 37Oc in a humidifier incubator with5%CO2.The broth is periodically examined for signs of growth such as pH changes indicated by colour changes and media turbidity changes. After2–3 days of 100X magnification, the plate was examined for the typical “fried egg” appearance of mycoplasma colonies**.(Kizil and Ozdemir, 2006).**

2.3. DNA extraction

The DNA extraction of positive culture is done by the use of a DNA kit ((gSYNC™ DNA) extraction kit according to manufacturer instructions. The yield and purity of DNA preparations were determined by optical imaging at 260 nm and 280 nm. Five microliters of DNA model extracted were used in PCR amplification.

2.4. PCR systems

A fragment of 375 bp based on the sequence reported by Tola et al.In 1996,the FS1 sensual primer(5-AAAGGTGCTTGAGAAATGGC-3)and the anti-sense primer(5-GTTGCAGAGAAGTCCAATCA-3) were amplified. PCR products were electrophoresed with 1.2 percent agarose gels, stained with Invitrogen's Sibr®Safe DNA gels, and visualized with UV light filters.

2.5. Dose Preparation

2.5.1. The Preparation of Test Bacterial Culture

The test bacteria were cultured by taking one ose of a bacteria from Nutrient Agar (Oxoid, UK) and inoculating it in a liquid medium from Nutrient Broth (Oxoid) and ensuring homogeneity. The culture was incubated for 24 hours at 37°C. The experimental bacteria's susceptibility was measured in the incubated liquid medium of Nutrient Broth with a 625 nm wavelength spectrophotometer, and the optical density (OD)was 0.1, equivalent to 104CFU/ml. **(Murray *et al*., 1999)** .

2.5.2. Preparation of Mycoplasma agalactiae inoculums

To estimate the number of bacterial cells in each spiked sample included in this study, serial dilution from the original Mycoplasma culture was used. One ml of each log phase culture is distributed into the first 10 glass tubes containing 9 ml of PBS (phosphate buffer solution). After 3 minutes of vortex, 1 ml of the first dilution is transferred to the second tube. The process is repeated 10 times, resulting in a series of decimal dilutions.

2.6. Animals and Experimental Infection

Twenty (20) lactating female mice (3-6 months, weight 28-30g), were used for this experiment. Each animal receives 0.1 ml intraperitoneal injection of inoculum (1×10^4 CCU \ ml). The dosage of viable Mycoplasms injected into each experimental group was determined by titration before and immediately after the inoculation of the animal.

2.7. Clinical manifestation:

Post-challenge, animals were observed daily for any clinical signs indicative of *Mycoplasma agalactia* (MG) associated signs, such as, fever, pneumonia, mastitis, conjunctivitis, and arthritis.

2.8. Histopathological Examination

After 30 days of MG challenge, animals were scarified by an overdose of anaesthesia (Chlorophorm, HiMedia, India). Mammary glands and joint samples were fixed for 72 hours in 10 %formalin. The histopathological preparation includes the treatment of graduated ethyl alcohols (70%, 80%, 90%, 100% twice), and the subsequent elimination of xylene twice. Then block by liquid paraffin56oCandmicrotome at 5m. Then staining is made with haematoxylin and eosin. (**Abbas, 2022**)

3. RESULTS

3.1. Isolation and identification of *Mycoplasma Agalactia*

3.1.1. Bacterial isolation

The positive results of Mycoplasma from milk were obtained over two days with PPLO brothsupplementing20 ml of horse serum, yeast extract (25% w/v), 25 ml of thallous acetate, and penicillin (2000 units). In PPLO agar, isolated Mycoplasmas had the morphology of a colony"fried egg" (Figure 1). All of the Mycoplasma isolated were identified using conventional PCR technology.

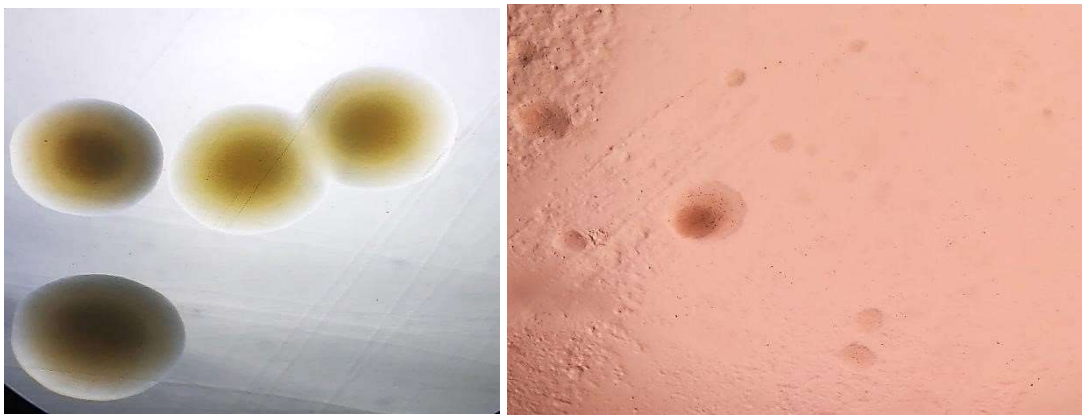

Figure 1. Mycoplasma Fried egg colonies on PPLO agar after 7 days of incubation with un aerobic condition (light microscope).

3.1.2. Molecular Detection

The amplified PCR products were used in the current study to characterize M. agalactiae isolates in molecular form. All bacterial colonies that grow in PPLO are positive for 16S proteins, 7(20 %) of the 200 grown samples were positive for 16S protein, response with species-specific primers for Mycoplasma agalactia (Figure 2 &3)

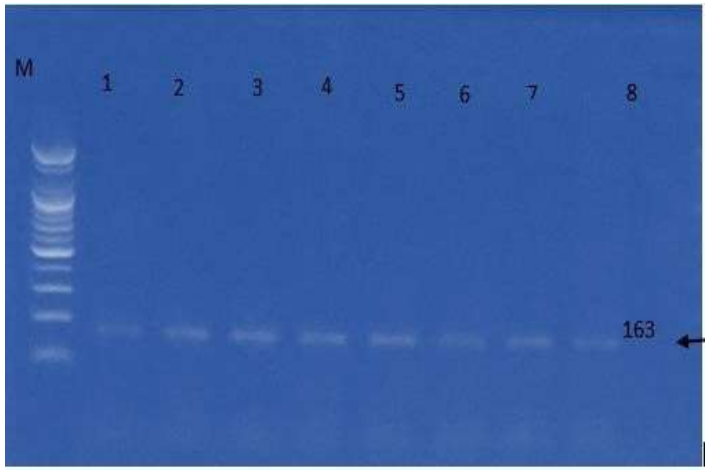

Figure 2. Mycoplasma polymerase chain reaction (PCR): PCR electrophoresis analysis of %1 gel agarose, M: marker (100bp DNA ladder) (163 bp band, Mycoplasma genus, NCTC 10123)

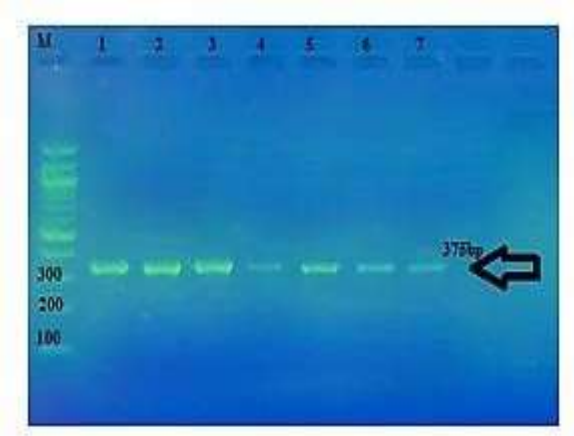

Figure 3. Ladder markers represent the amplification ofthe16s rRNA genes of unknown bacteria, which were divided into 1% agarose gel electrophoresis colored Eth. Br. M: 100bp. (Lane 1looks like a357bp PCR product)3.

3.2. Clinical Signs:

Throughout the experiment, many signs are observed there are fever, changes in the size, shape and consistency of the mammary glands. Body weight Loss of appetite and loss weight and after month there was swelling of articular joint, redness also ocular changes from glaucoma and blindness of infected mice. **(Migliore *et al*., 2021).**

Figure 4. Corneal opacity and conjunctivitis of eyes after infection of mycoplasma agalactia (arrow)

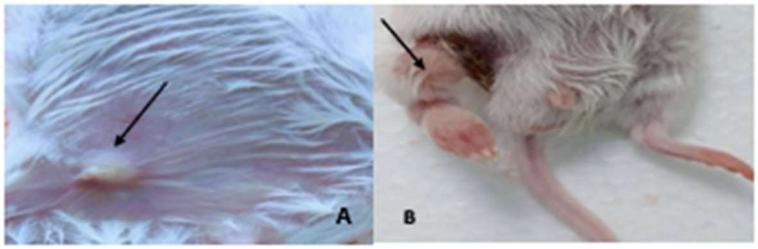

Figure5.Photographs from mice infected with Mycoplasma agalactia after one month. A: Mammary gland shows; swelling, enlarged gland with protruding the nipple and slight redness (arrow)

B:Articular joint: shows swelling, enlarged and redness of Knee-articular joint and swelling with redness of Right hind limb (arrow)

3.3. Histopathological examination:

3.3.1 Mammary gland

Mastitis was the prominent microscopic lesions in tissues of mammary glands from mice infected intraperitoneally with *Mycoplasma agalactia* (1×10^4 CFU/ml); characterized by marked of severe inflammatory reaction from heavy infiltration of inflammatory cells (neutrophils, lymphocytes, and macrophages), massive necrosis of acinar lining epithelium (Figure-6), steatites in (Figure-7); necrosis of the adipose tissue enlarged, irregular adipocytes and heavy infiltration of PMNS and MNCs extend to muscular tissue caused necrosis Of skeletal muscles; atrophied or swollen with deletion of sarcoplasm's and infiltration of inflammatory cells.The histopathological section in mammary gland of infected mouse also revealed hyperplasia of the lining epithelium of main duct and there was proliferation of fibrous connective tissue (Figure-8,9), there was also hyperplasia of acinar and ductal lining epithelium as papillae formation, vasculitis occurred represented by necrosis of endothelial cells and perivascular infiltration of inflammatory cells (Figure-10).

3.3.2. Joints:

The histopathological section in the articular region of the infected mouse (one month); shows marked thickening due to synovitis; characterized by periarticular infiltration of inflammatory cells polymorphoneutrophiles, lymphocytes, and cellular debris (Figure11,12,13), erosion of periosteal, deformity of bone trabeculae and the surrounding was thick due to thick inflamed fibrous connective tissue.: the microscopic section in articular region of infected mouse also showed the deformity of trabecular bones due to necrosis and erosion of lining mesenchymal cells (Figure-14), and the marrow cavities filled with inflammatory cells mainly from mononuclear cells and there was atrophy of trabeculae and proliferation of fibroblasts in marrow cavity with inflammatory cells (Figure-15). Figure-11; represents the necrosis of the cartilage cap and hypertrophy of chondrocytes.

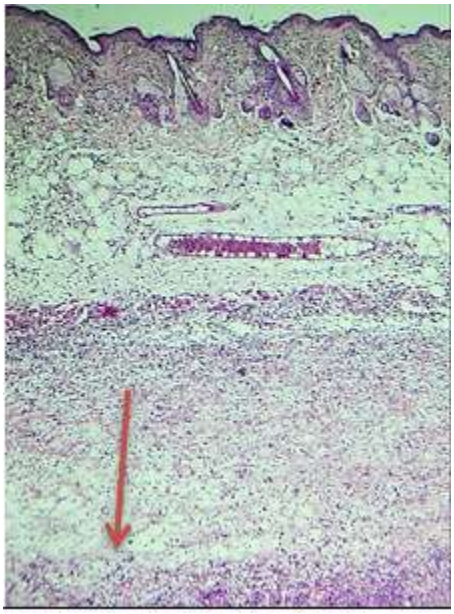

Figure-6: mammary gland section of infected mouse (one month); shows Severe damage (necrosis) and heavy infiltration of inflammatory cells and congestion of blood vessels also in subcutaneous tissue (arrow). (H&E stain, 40X).

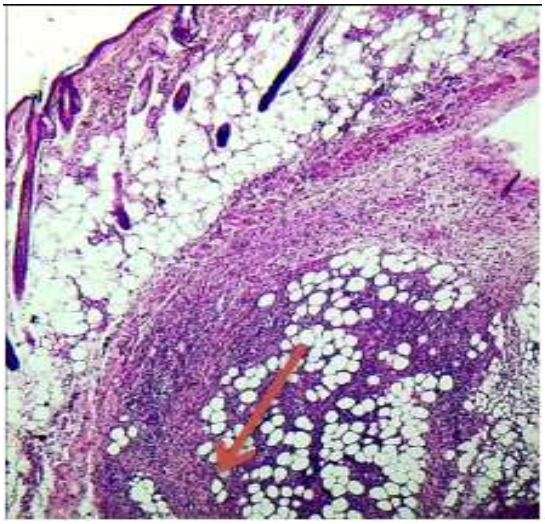

Figure-7: tissue section of mammary gland of an infected mouse (one month); shows steatites (arrow) with severe infiltration of inflammatory cells. (H&E stain, 100X).

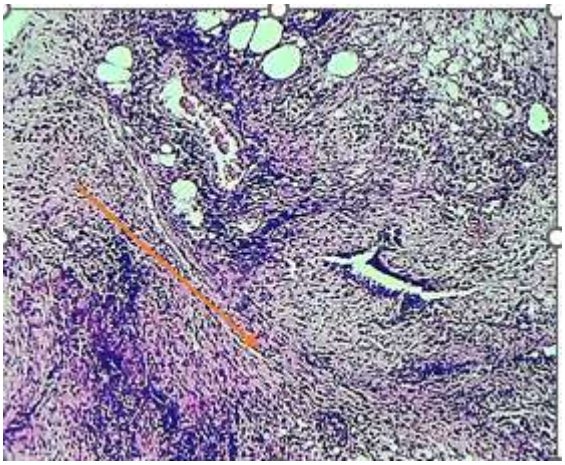

Figure-8: Tissue section of mammary gland of infected mouse (one month); shows hyperplasia of lining epithelium of main duct and healing with proliferation of fibrous connective tissue (arrow). (H&E stain, 200X).

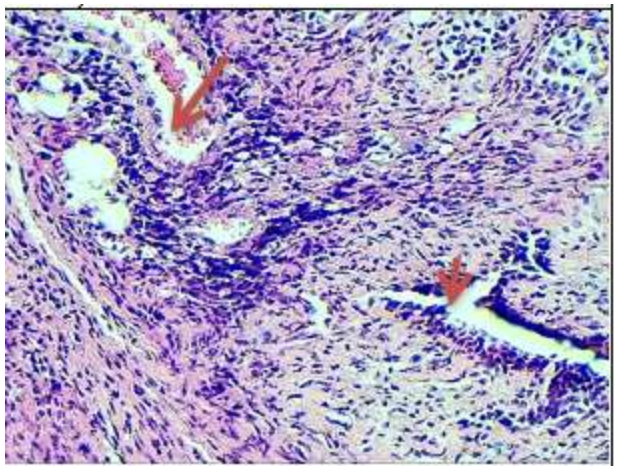

Figure-9: Histological section of mammary gland of infected mouse (one month); shows high magnified from figure-8 shows hyperplasia of ductal lining epithelium as papillae formation (arrow), vasculitis; necrosis of endothelial cells (arrow) and perivascular inflammatory cells. (H&E stain, 400X).

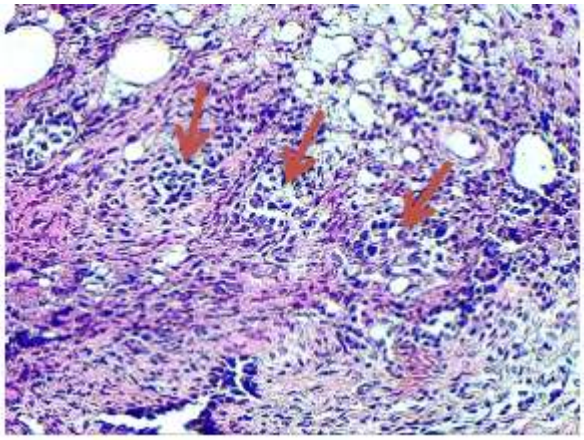

Figure-11: Histological section of mammary gland of infected mouse (one month); shows severe necrosis of acinar epithelium and occluded with inflammatory cells and cellular debris (arrow) localized with fibrous connective tissue. (H&E stain, 400X).

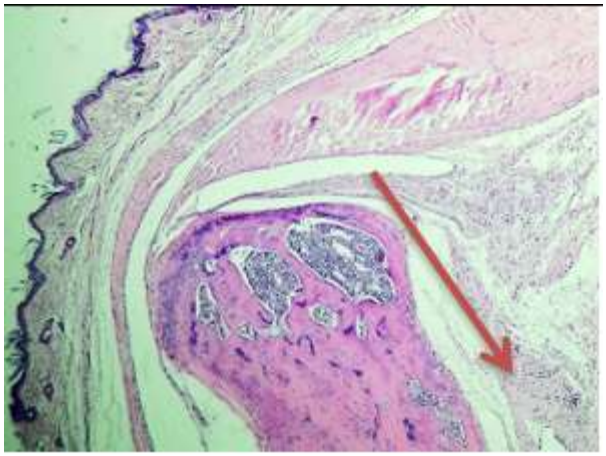

Figure-12: Histological section in the articular region of the infected mouse (one month); shows synovitis, periarticular infiltration of inflammatory cells (arrow) and cellular debris. (H&E stain, 40X).

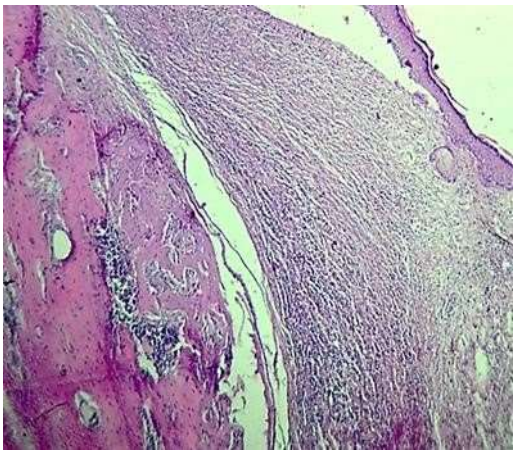

Figure-13: Histological section in the articular region of infected mouse (one month); shows erosion of periosteal, deformity of bone surrounded by thick inflamed fibrous connective tissue. (H&E stain, 100X).

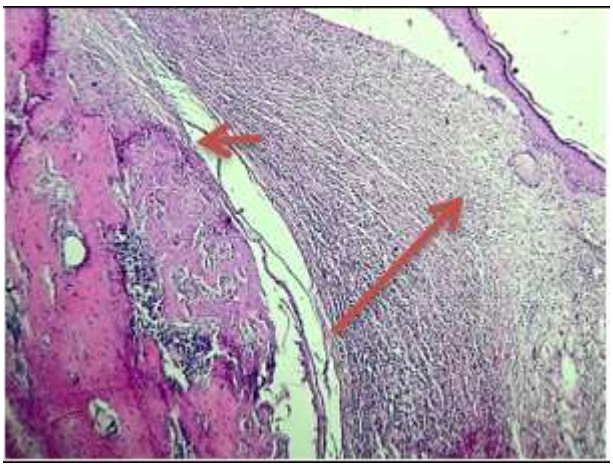

Figure-14: Histological section in the articular region of the infected mouse (one month); shows erosion of periosteal (arrow), deformity (necrotic) of bone surrounded by thick inflamed fibrous connective tissue

(arrow). (H&E stain, 100X).

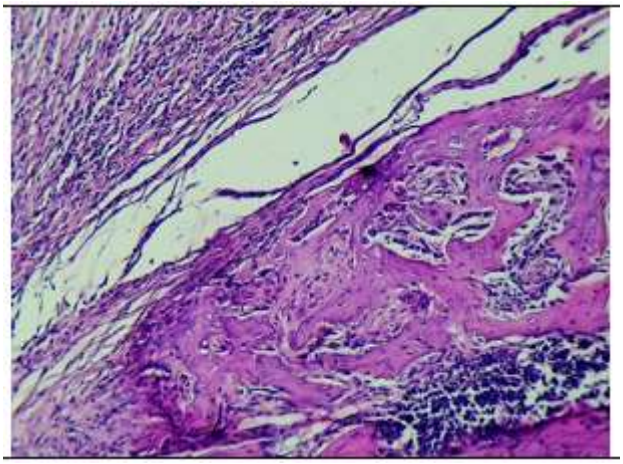

Figure-15: Histological section in the articular region of an infected mouse (one month); high magnified from figure 7 shows deformity of trabeculae and erosion of periosteal. (H&E stain, 200X).

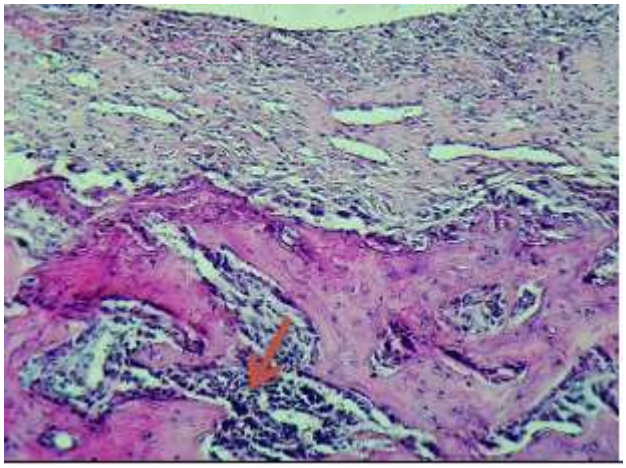

Figure 16: Histological section in the articular region of the infected mouse (one month); shows irregular trabeculae, erosion of periosteal, and the marrow cavities filled with inflammatory cells (arrow) mainly from mononuclear cells. (H&E stain, 200X).

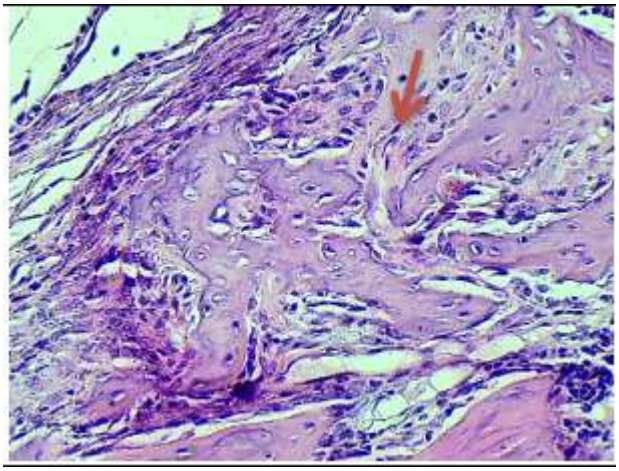

Figure-17: Histological section in the articular region of the infected mouse (one month); shows atrophy of trabeculae and proliferation of fibroblasts (arrow) in marrow cavity with inflammatory cells. (H&E stain, 200X).

4. DISCUSSION

Mycoplasma agalactiae is the cause of infectious agalactia (CA) in OIE-recognized diseases, causing mastitis in dairy goats and causing substantial financial losses due to arthritis and the decline or complete suspension of milk secretion, cachexia, and corneal opacity, which can cause total blindness. **(Santos *et al.*, 2015).** Mastitis reduces milk production, changes the composition of milk, shortens the productive life of affected cows, sheep, and goats, and is very costly for dairy farmers. **(Agharid *et al*,. 2022).**

The isolation of Mycoplasmas is considered one of the most difficult tasks for diagnostic laboratories, as the laboratory media cannot grow easily despite significant improvement in the formulation of the medium. The preferred samples for the cultivation of mycoplasma are milk, nose secretions, joints, and pleural fluids depending on clinical diseases. **(Momani *et al*,. 2006).** This study found that 20 (5.5%) ofthe200 milk samples had positive effects on the PLO agar plate. Similarly, in a study conducted in Turkey, 17 M. agalactiae isolates were obtained from 47 milk samples collected from sick goats**. (Kizil & Ozdemir, 2006).**

The PCR test is often used in many laboratories and is extremely sensitive. When clinical samples are used, it can provide a quick early warning system, allowing a complete investigation to take place when results are positive. **(Foddai *et al*,.2005).**

In Kurdestan-Iraq the isolates *Mycoplasma agalactia* by **(Sulaiman ., 2015),** reported in his studies (KC 594646) and (KC 594647) from the Iraqi governors of Arbil and Soleimani that GenBank's accession number for multiple random nucleic acids and amino acids had developed a new diagnostic test based on the PCR of the 16 Sr RNA genes and that it was a genetically specific primer for Mycoplasma, and that DNA products were separated by the PCR product on the primary sequence using DNA products by denaturing gradient gel. **(Abd Alameer.,2017).** PCRs can also be used more reliably in cultured mycoplasmas; 24 hours of mycoplasma enrichment in appropriate media greatly facilitates the detection of PCR seven in the presence of bacterial contamination. **(Nicolas., 2002).**

The use of mice as laboratory animals for infection of *Mycoplasma agalactia occurred* for the first time in 1967 by Smith, who used 25.2 x 10^6 dose for intraperitoneal injection and found that, this dose was harmless without the use of mucin. He also found that the virulence of bacteria was much less in mice compared to sheep and goats, That agreed with the present results the intraperitonial infection of mice with 10^4 CFU/ml, doesn't give immediate signs of disease but after one month.

The initial clinical signs that suggestive diagnosis involve fever, mastitis, conjunctivitis, arthritis, and pneumonia; These observations were confirmed by post-mortem examination which showed signs of inflammation due to congestion, collection of hemorrhage fluids in the mammary gland, and inflammation due to pneumonia these findings agreed with **(Jay &Tardy., 2019).**

In the case of *M.agalactiae* arthritis, mice showed less severe symptoms compared to sheep and goats, including inflammatory reactions and joint capsule thickening, with liquid aspects of exudates and other organ abscesses associated with high prevalence of polyarthritis indicating an infection of *Mycoplasma agalactiae* this agree with (**Hajizadeh *et al*., 2018).** The microscopic lesion of the mammary gland and joints tissues were examined H&E staining sections which appeared severe infiltration of inflammatory cells with necrosis which indicates mastitis, one of the most main clinical signs of *Mycoplasma agalactia* and OIE approved this; **(OIE, 2019).**steatites and fat necrosis occur together as two extremes of an inflammatory condition which compromise fine, lipid-containing cytoplasmic vacuoles this approved by (**Begg *et al.*,2000).** Rodríguez and colleagues (2015) reported that the histopathological findings included acute diffuse interstitial pneumonia, arthritis, and multifocal necrotic purulent splenitis, the present study confirmed the

findings about synovitis, periarticular infiltration of inflammatory cells and cellular debris of particular region of infected mice refers to inflammation of joints (arthritis) which caused by Mycoplasma agalactia . The main mechanism(s) by which the infection caused by Mycoplasma agalactiae affects the host cells are not entirely understood, although the most widely accepted theory attributes excessive inflammation and subsequent tissue destruction to the host immune system. In this sense, morphological alterations such as membrane blebbing, cytoplasm shrinkage, and cell elongation were brought about by the in vitro infection of HeLa cells. These modifications point to an apoptosis-like process that reduces cell viability and increases cell lysis, along with chromatin condensation and enhanced caspase-3 activation **(Hegde et al., 2016).**

CONCLUSIONS AND RECOMMENDATIONS

Explain the complex epidemiology and stop livestock from getting sick by developing therapeutic approaches or Mycoplasma agalactia vaccinations, a deeper understanding of the molecular mechanisms underlying the variability and pathogenicity of *Mycoplasma agalactiae* is required.

Acknowledgments

The authors express their gratitude to the pathology department staff at the University of Baghdad, Iraq's College of Veterinary Medicine for their assistance and the facilities they offered for the processing of the samples.

Novelty statement

The study novelty is focused on the histopathological change of mycoplasma agalactia in infected mice after isolation from sheep and goat milk and identification by PCR system.

Authors contribution

Each author has contributed significantly, directly, and intellectually to the work and given their approval for publishing.

REFRENCES

Abbas L, Batah, Shakir M, Mirhish (2022). Histomorphological and Histochemical Study of Adrenal Gland in Adult Male of Guinea Pigs (Cavia porcellus) .The Iraqi Journal of Veterinary Medicine., 43(1): 59-66. https://doi.org/10.30539/iraqijvm.v43i1.475 (Original work published 2019)

Abd Alameer EM, Fadel AS, Al-Adhad BN, Khazaal KA (2017). Identification by culture, DGGE-PCR, of Mycoplasma from ruminants and poultry. Iraqi J. Agric. Res. (Special Issue)., 22 (3): 180-186.

Agharid AA, Sana'a SA, Karim AA, Bashiru G (2022). Immunopathological Responses to the Bovine Mastitis Associated with Staphylococcus Species Infection. The Iraqi Journal of Veterinary Medicine., 46(2): 7-11. https://doi.org/10.30539/ijvm.v46i2.1398

Al-Aubaidi JM, Saed OM, Al-Shammari JN, Hayran KA (1986). Contagious agalactia of sheep and goats, isolation and identification of Mycoplasma agalactiae clinical and pathological confirmation of the disease. the six international congress of the M10, PII-100. (August): 26-31.

Al-Graibawi MAA, AlShammari AJN, Sharma VK, Saed CM (1989). Isolation and identification of Mycoplasma agalactiae from Angora goats in Iraq. Indian Journal of Comparative Microbiology., 10: 156-158.

Al-Momani W, Halablab MA, Abo-Shehada MN, Miles K, McAuliffe L, Nicholas, RA (2006). Isolation and molecular identification of small ruminant mycoplasmas in **Jordan. Small Ruminant Research**.,*65*(1-2): 106-112. https://doi.org/10.1016/j.smallrumres.2005.05.022

Begg GS, Bruno DW, McVicar A H (2000). The histopathology and ultrastructure of steatitis affecting common dab Limanda limanda. Diseases of Aquatic Organisms.,*41*(2): 123-133. doi: 10.3354/dao041123.

Damasceno EM, Pinheiro RR, Andrioli A, Alves FSF, Lima AMC, Peixoto RM, Brandão IS (2020). Seroprevalence and associated risk factors of Mycoplasma agalactiae and investigation of coinfection with the caprine lentivirus in Rio Grande do Norte, Brazil. Tropical Animal Health and Production, *52*(4): 2111-2117. doi:10.1007/s11250-020-02234-5

Foddai A, Idini G, Fusco M, Rosa N, De la Fe C, Zinellu S, Tola S (2005). Rapid differential diagnosis of Mycoplasma agalactiae and Mycoplasma bovis based on a multiplex-PCR and a PCR-RFLP. Molecular and Cellular Probes., *19*(3): 207-212. https://doi.org/10.1016/j.mcp.2004.11.007

Hajizadeh A, Ghaderi R, Ayling RD (2018). Species of Mycoplasma causing contagious agalactia in small ruminants in Northwest Iran. Veterinaria Italiana., *54*(3): 205-210. https://doi.org/10.12834/VetIt.831.4072.2

Hasso HAH (1994). Possibility of new sites as sources of infection for Mycoplasma Agalactiae. Iraqi J Vet Me.,18(2):44-49. Available from: https://doi.org/10.30539/ijvm.v18i2.1594

Hegde PS, Karanikas V, Evers S (2016). The where, the when, and the how of immune monitoring for cancer immunotherapies in the era of checkpoint inhibition. Clinical Cancer Research. *22*(8): 1865-1874. https://doi.org/10.1158/1078-0432.CCR-15-1507

Jameel MM, Hasso SM (2018). Molecular detection of Mycoplasma agalactiae and Mycoplasma capricolum in mastitic and non mastitic milk of goats by using Real Time Polymerase Chain Reaction. Iraqi J Vet Med., 42(1):1-6. https://doi.org/10.30539/iraqijvm.v42i1.22

Jaÿ M, Tardy F (2019). Contagious agalactia in sheep and goats: current perspectives. Veterinary Medicine: Research and Reports: 229-247.

Khezri M, Pourbakhsh SA, Ashtari A, Rokhzad B (2015). Investigation of Mycoplasma agalactiae in sheep in Kurdistan province by PCR. Archives of Razi Institute., *70*(2): 73-80. DOI: 10.7508/ari.2015.02.001

Kizil O, Ozdemir H (2006). WITH MYCOPLASMA AGALACTIAE. Bull Vet Inst Pulawy., *50*: 325-328.

Madanat A, Zendulkova D, Pospíšil Z (2001). Contagious agalactia of sheep and goats. A review. Acta Veterinaria Brno., *70*(4): 403-412.

Migliore S, Puleio R, Nicholas R A, Loria GR (2021). Mycoplasma agalactiae: The sole cause of classical contagious agalactia?. Animals, 11(6): 1782. **https://doi.org/10.3390/ani11061782**

Mohanad M, Jameel & Saleem MH (2018). Molecular detection of Mycoplasma agalactiae and Mycoplasma capricolum in mastitic and non mastitic milk of goats by using Real Time Polymerase Chain Reaction. The Iraqi Journal of Veterinary Medicine., 42(1): 1-6. https://doi.org/10.30539/iraqijvm.v42i1.22

Mohanad MJ, Saleem MH (2018). Molecular detection of Mycoplasma agalactiae and Mycoplasma capricolum in mastitic and non mastitic milk of goats by using Real Time Polymerase Chain Reaction. The Iraqi Journal of Veterinary Medicine., *42*(1): 1-6. https://doi.org/10.30539/iraqijvm.v42i1.22

Murray PR, Baron EJ, Jorgensen JH, Pfaller MA, Yolken RH (1995). Manual of clinical microbiology. 6th edn Washington. *DC:* American Society for Microbiology.

Nicholas R, Ayling R, McAuliffe L (Eds.) (2008). Mycoplasma diseases of ruminants. CAB International. https://doi.org/10.1079/9780851990125.0000

Rodríguez F, Castro P (2015). Differential cytokine expression in natural and experimental mastitis induced by Mycoplasma agalactiae in dairy goats. Reproduction in Domestic Animals., 50(1): 159-163.**https://doi.org/10.1111/rda.12457**

Santos OM, Campos AC, Santos JP, Santos, POM, Caldas ELC, Santos ADF, Azevedo EO (2015).

Agalaxia contagiosa em ovinos e caprinos do Estado de Sergipe: dados preliminares. Scientia Plena, *11*(4).

Smith GR (1967). Experimental infection of mice with Mycoplasma agalactiae. Journal of Comparative Pathology., 77(2): 199-202. https://doi.org/10.1016/0021-9975(67)90011-4

Sulaiman R (2015). Isolation, Molecular Identification and in vitro Antibiotic Susceptibility Testing of Mycoplasma agalactiae From Goats in Two Provinces of Kurdistan Region-Iraq. Journal of Zankoy Sulaimani-Part A., *17*(3): 127-136.

Thiaucourt F, Bölske G (1996). Contagious caprine pleuropneumonia and other pulmonary mycoplasmoses. Rev. sci. tech. Off int. Epiz., *15*(4): 1397-1414.

Tola S, Angioi A, Rocchigiani AM, Idini G, Manunta D, Galleri G, Leori G (1997). Detection of Mycoplasma agalactiae in sheep milk samples by polymerase chain reaction. Veterinary microbiology., *54*(1): 17-22. https://doi.org/10.1016/S0378-1135(96)01269-2

World Organization for Animal Health (OIE)(2019). Chapter 3.7.3 Contagious Agalactia. In Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (Terrestrial Manual), 8th ed.; OIE: Paris, France; Volume 1, pp: 1430–1440. Available online:https://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/3.07.03_CONT_AGALACT.pdf (accessed on 1 May 2021).