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Genetic Variability and Antibacterial Sensitivity of *Dichelobacter nodosus* and *Fusobacterium necrophorum* Infection in Sheep Sulaimani Province, Kurdistan Region, Iraq

Rizgar R Sulaiman[1], Hardi F Marif[1]*, Basim A Ali[1], Kwestan N Ali[1], Mohammed O Baba Sheikh[2]

[1]Department of Clinic and Internal Medicine, College of Veterinary Medicine, University of Sulaimani, Sulaymaniyah, Kurdistan Region, Iraq, [2]Directorate of Veterinary Sulaimani, Salim Street, Sulaimaniyah, Kurdistan Region, Iraq

***Correspondence**:
hardi.marif@univsul.edu.iq
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A B S T R A C T

Footrot is a significant health problem in ruminants, especially sheep, caused by the bacteria *Dichelobacter nodosus* and *Fusobacterium necrophorum*. Infected animals may become lame and lose body condition. The aim of this study was to investigate whether sheep had mixed infections with *D. nodosus* and *F. necrophorum*, the global genetic relationships of both target genes (*lktA* and *16S rRNA* genes) with different reference genes, and the degree of genetic heterogeneity between the standard serogroups of *D. nodosus* were studied. In addition, to detect the antibacterial sensitivity against these two pathogens. One hundred and seventy-five sheep interdigital spaces were investigated (2016-2022). The *D. nodosus* field isolate (Dn Sul/016-KY399851) showed 96% compatibility with other global isolates (NR_104942/USA, JN008724/India, and DQ016290/Sweden) when its 16S rRNA sequence was compared to some reference genes. The local isolate (Fn Sul/016- KY399852) and other IktA gene strains from other nations (FJ230831/New Zealand, JX678872/Australia, JX648295/India) were compared, and the results showed 99% and 96% identity. Additionally, within the multiple sequence alignment, a single nucleotide variation at position 389 (CA) was seen in the partial *lktA* sequence when compared to the field isolate (Fn Sul/16-KY399852). This modification corresponds to a switch at residue 130 from alanine (Ala) to glutamic acid (Glu). This study showed the initial molecular identification of *D. nodosus* and *F. necrophorum* from sheep with footrot in the Iraqi province of Sulaimani.

Keywords: *Dichelobacter nodosus*, *Fusobacterium necrophorum*, PCR, sheep, footrot

INTRODUCTION

Ruminants can contract footrot, a contagious condition that affects the interdigital space and the capsule ungulate (1). The animals show lameness, loss of body condition, and decreased production of wool and meat (2). Because the disease spreads through contact with mud and feces as well as by eating roughages contaminated with foul discharges in grasslands, the outbreak mostly happens during wet seasons (1, 3). *Dichelobacter nodosus* and *Fusobacterium necrophorum* are the primary and secondary agents, respectively, that cause footrot in ruminants (3). The infection is unique to sheep and goats, though reports of its occurrence in cattle, horses, pigs, deer, and mouflons have also been made (4).

A Gram-negative, non-spore-forming rod, obligate anaerobic and fastidious bacterium called *D. nodosus* is the cause of sheep footrot (1, 5). Surface fimbriae and stable

extracellular proteases found in *D. nodosus* may enable the bacteria to colonize sheep foot interdigital epithelial tissue (5). Due to the high immunogenicity of fimbriae, *D. nodosus* is divided into nine major serogroups, A to I, based on agglutination reactions involving fimbrial antigens (6). The Gram-negative, anaerobic, pleomorphic, and nonspore-forming bacterium is more oxygen sensitive than *F. necrophorum* (7). It has been demonstrated that *F. necrophorum* produces a variety of endotoxins and exotoxins, which significantly increase its virulence (8).

Controlling footrot primarily involves reducing the frequency of severe infections in affected flocks, either prophylactically through vaccination or by using topical and systemic therapy in conjunction with vaccination. None of these interventions provides a long-term, simple-care method of disease management. Parenteral antibiotics were an effective treatment for lameness in sheep (9). The leukotoxin (*IktA*) gene, which is well characterized and distinctive, encodes the major virulence factor and best-described component of *F. necrophorum*. It can trigger immune response against *F. necrophorum* infection (10). The IktA gene has been used to identify and quantify strain variation in *F. necrophorum* (11). The goals of the current study were to identify whether *D. nodosus* and *F. necrophorum* can co-infect sheep, to examine the global genetic relationships between both target genes (*16S rRNA* and *IktA*) and various reference genes, and to assess the degree of genetic heterogeneity among the various standard serogroups of *D. nodosus*.

MATERIALS AND METHODS

Ethical Approval

The procedure performed in this study was approved by the College of Veterinary Medicine Scientific Research Committee, the University of Sulaimani, and the Kurdistan Regional Government of Kurdistan, Iraq under the approval number AVP-2023-1) dated on 7/5/2023.

Area of Study

The study was conducted in Sulaimani Province, Kurdistan Region, Iraq. The study area was located 34-35 °N and 45-46 °E (Figure 1). This region has four distinct seasons with different rainfall in autumn, winter, and early spring that ranged between 750 and 400 mm. This area is characterized by having different habitats, such as a river, meadow pastures, and agricultural lands with high biological diversity.

Sample Preparation

A total of 175 samples were taken from sheep over the years 2016–2022 in Sulaimani province, Kurdistan Region, Iraq. Twenty-five samples were investigated each year. The samples were taken with a swab from the interdigital spaces of sheep. Some sheep had symptomatic lesions on the inter-digital space, and others were healthy. They were collected by a private veterinary hospital and rapidly transferred to a veterinary diagnostic laboratory in Sulaimani (VDLS).

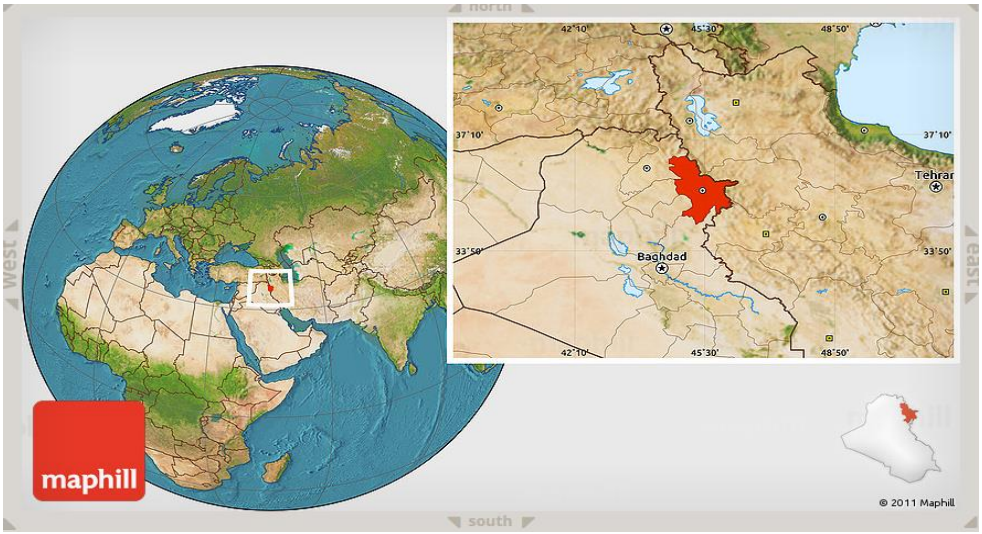

Figure 1. The geographical location of Sulaimani province on the map (red patch), from where samples were taken from sheep for PCR test (12)

DNA Extraction

DNA was obtained from dried swabs through the use of a DNA extraction kit (Genetbio Co., Korea). Briefly, dry swabs were inserted into sterile 1.5-mL tubes and mixed with 200 µL of PBS (prepared from 1 mL of 0.1 M PBS at pH 7), followed by thorough vertexing. A 100-µL sample was then employed for DNA extraction in accordance with the manufacturer's instructions.

Oligonucleotide Primers

In the current study, two primer pairs (Table 1) were used in the polymerase chain reaction (PCR): the first (FN1 and FN2) was specific for detection of the *F. necrophorum*

leukotoxin (*ItkA*) gene (13), and the second (ND1 and ND2) was specific for detection of the *D. nodosus 16S rRNA* (14, 15). The Republic of Korea's Macrogen Co. synthesized the primers.

Table 1. Sets of the primers used in the study

Gene	Primer	5' → 3'	Amplicon
ItkA	FN1	AAT CGG AGT AGT AGG TTC TG	402 bp
	FN2	CTT TGG TAA CTG CCA CTGC	
16S rRNA	DN1	AGA GAC AGC CGC ATC TTC TT	783 bp
	DN2	TCGGTACCGAGTATTTCTACCCAACACCT	

DNA Amplification

PCR premix (Genetbio, Korea) was used to amplify a partial sequence of the *lktA* and *16S rRNA* genes. Based on the following specifications, the reactions were conducted in a 0.2 ml PCR tube: 10 µL of premix PCR, 5 µL of DNA, 1 µL of forward primers (10 pmol), 1 µL of reverse primers (10 pmol), and 3 µL of ultra-pure water to make up the final volume of 20 µL. Thermocycler (Hercuvan, USA) was set up to perform the reaction: initial denaturation at 95 °C for 5 min, followed by 35 cycles of 95 °C for 30 sec, annealing at 58 °C for 30 sec, extension at 72 °C for 30 sec (the extension time for DN1 and DN2 for 50 sec), and final extension at 72 °C for 10 min.

A 1% agarose gel was used to load 10 µL of the amplified PCR products before being stained with a safe dye (Eurex) in Tris-acetate-EDTA (TAE) buffer (41). The PCR products were then electrophoretically run on the gel and examined under UV transilluminator (UVTEC, UK).

DNA Sequencing and Phylogenetic Tree Analysis

Sequences of the *IktA* gene fragments from *F. necrophorum* variants and *16S rRNA* of *D. nodosus* were analyzed using Clustal-W (CLUSTAL 2.1 multiple sequence alignment) (16). The phylogenetic tree based on *IktA* and *16S rRNA* sequences was constructed using the MEGA 6.0 program, employing the Maximum-likelihood with the Kimura 2-parameter nucleotide substitution model (17).

A 20 µL aliquot of the positive PCR product was forwarded in both forward and reverse directions for Sanger sequencing at Macrogen Co., Republic of Korea. The result of sanger sequencing both gene the *IktA* gene fragments from *F. necrophorum* variants and *16S rRNA* of *D. nodosus*.

Nucleotide Sequence Accession Numbers

The *IktA* gene fragments from *F. necrophorum* and the *16S rRNA* gene sequences from *D. nodosus* were registered and deposited in the GenBank under the accession numbers KY399852 and KY399851, respectively.

Antibacterial Sensitivity

Samples were transported to the laboratory in a cool container. Samples positive for PCR were checked for minimum inhibitory concentrations (MICs). BD-Wilkins-Chalgren anaerobic agar was used to conduct antimicrobial susceptibility tests on anaerobic bacteria, as recommended by the Clinical and Laboratory Standards Institute (CLSI) (18). The antibiotics used in the current study were benzylpenicillin, amoxicillin, cloxacillin, cefadroxil, cefuroxime, ceftiofur, kanamycin, gentamicin, streptomycin, spiramycin, tylosin, enrofloxacin, lincomycin, tetracycline, doxycycline, and trimethoprim. The plates were incubated at 36.5–37.5°C for 72 h using anaerobic sachets (CampyGen Sachet, Thermo Scientific Oxoid™, UK). Each run of antibiotic dilutions included inoculating two additional plates of BD-Wilkins-Chalgren agar for aerobic and anaerobic control (antimicrobial concentrations ranging from 0.05 to 256 mg/mL). Quality control (QC) was crucial for ensuring the quality of the medium and the stability of antimicrobial formulations. During each agar dilution test run, two strains were included as control strains: *Bacteroides fragilis* ATCC 25285 and *Bacteroides thetaiotaomicron* ATCC 29741. The MIC values from these dilutions were compared to the QC values prescribed by the CLSI protocols for these strains (18).

RESULTS

Identifications

Four samples out of 25 collected in 2016 were positive for *D. nodosus* by PCR. Four samples were detected positive in 2016-2018 and nine samples in 2019. Five samples were positive in 2020 and 2021 but six samples were positive in 2022 for *D. nodosus*. Regarding *F. necrophorum* nine samples were positive in 2016 and 2018 while thirteen and eleven positive samples were detected in 2019 and 2020, respectively. The remaining years 2017, 2021 and 2022 only twelve samples were positive. Totally, 37 samples of *D. nodosus* and 72 samples of *F. necrophorum* were positive (Tables 2). The PCR amplification using the *IktA* and *16S rRNA* genes specific primers generated PCR amplicons of the expected sizes (402 bp and 783 bp, respectively) (Figure 2).

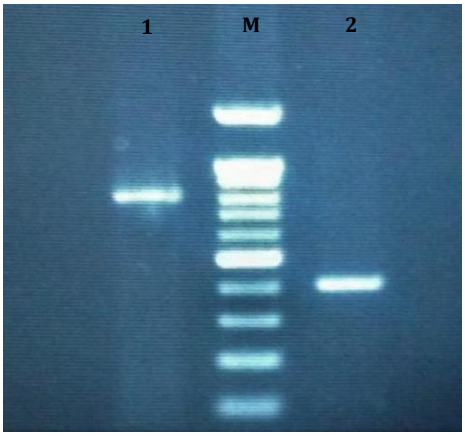

Figure 2. Agarose gel shows partial DNA amplification: Lane 1: partial 16S rRNA gene (783 bp), Lane 2: ItkA gene (402 bp) and Lane M = DNA marker

Table 2. PCR positive samples and infection rates for *D. nodosus* and *F. necrophorum* in sheep older than seven years

Year	Samples	*D. nodosus* Number (%)	*F. necrophorum* Number (%)
2016	25	4 (16)	9 (36)
2017	25	4 (16)	12 (48)
2018	25	4 (16)	9 (36)
2019	25	9 (36)	13 (52)
2020	25	5 (20)	11 (44)
2021	25	5 (20)	12 (48)
2022	25	6 (24)	12 (48)
Total	175	37 (21)	72 (41)

Sequencing and Phylogenetic Analysis

Phylogenetic algorithms were used to construct a phylogenetic tree. The Maximum-likelihood tree is shown in Figure 3. All the 48 isolates from *D. nodosus* are divided into eight serogroups (A, B, E. F, H, I. M, and 16S rRNA). The phylogenetic analysis of the partial *16S rRNA* gene sequence from isolates of *D. nodosus* (Figure 3A) in this study showed that this field isolate (Dn Sul/016-KY399851) was clustered with other strains (NR_104942/USA, JN008724/India, and DQ016290/Sweden). Similarly, the Figure 3B depicts the phylogenetic tree for the *IktA* gene. The Sulaimani isolate (Fn sul/016-KY399852) was clustered with FJ230831/New Zealand, JX678872/Australia, and JX648295/India.

Comparing the partial *16S rRNA* gene of the field isolate (Dn Sul/016-KY399851) to reference genes, it was found that the sequences most closely resemble those from the USA (NR_104942), India (JN008724), and Sweden (DQ016290), exhibiting a 96% identity. Similarly, when examining the sequences from Fn sul/016-KY399852 and comparing them to the IktA gene of strains from different countries, the highest similarities were observed with FJ230831/New Zealand, JX678872/Australia, and JX648295/India. Furthermore, within the multiple sequence alignment, when comparing the studied sample (Fu sul/016-KY399852), a single nucleotide variation at position 389 (C→A) was observed in the partial *IktA* sequence. This alteration corresponds to a change in amino acids from alanine (Ala) to glutamic acid (Glu) at residue 130 (Figure 4).

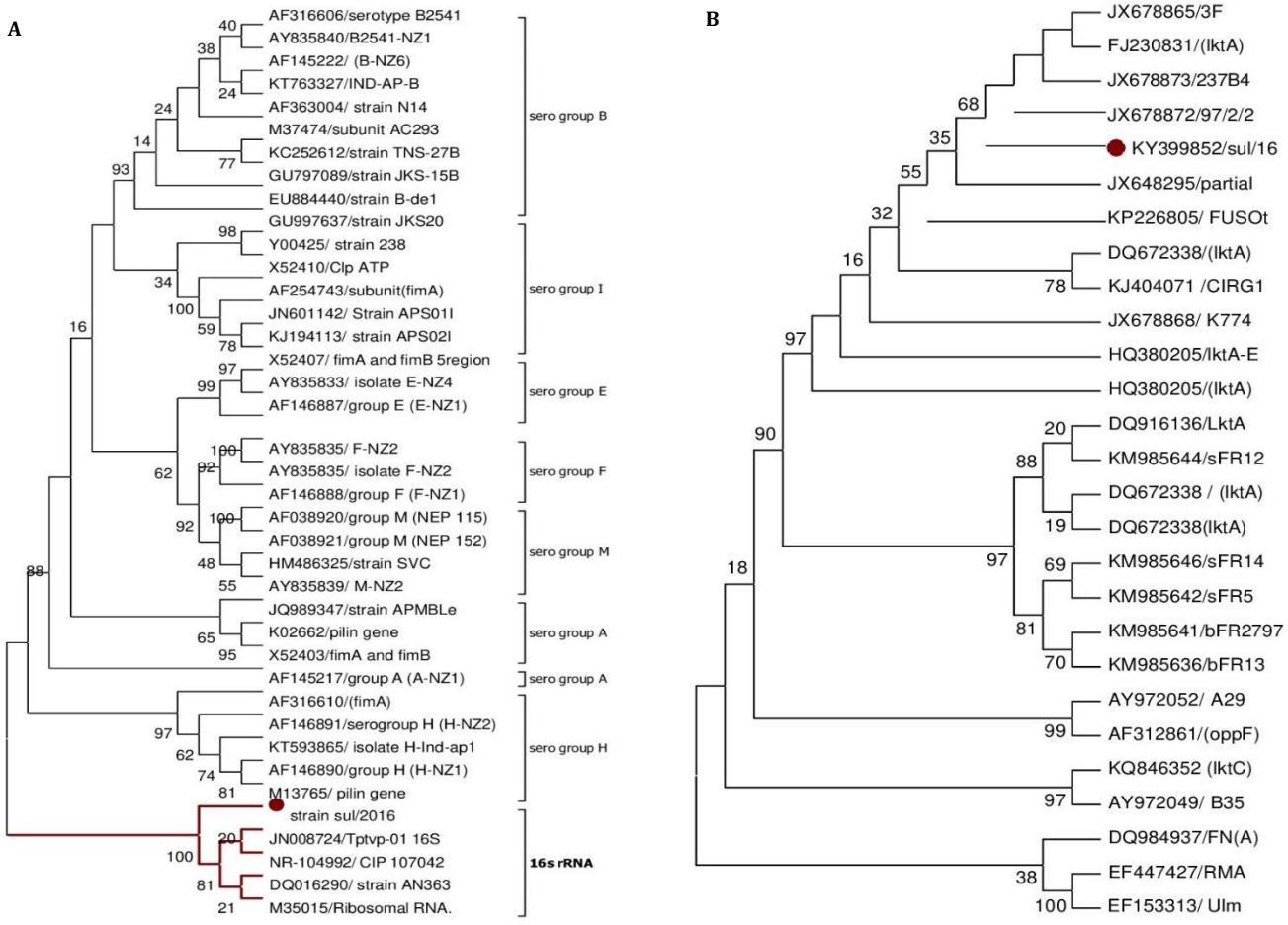

Figure 3. A maximum-likelihood tree created using Mega-6 software, incorporating partial sequences of the **(A)** *16S rRNA* gene from *D. nodosus* and **(B)** *IktA* gene from *F. necrophorum* along with reference sequences from various countries

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  CLUSTAL    2.0.10    multiple    sequence


  KY399852/fn-sul          -GVVGSVGVGGNVGVGASSDTNIIKRNTKTRVGKNTTMSDGGFGEEAEITADSKQGISSF
JX678873/Australia      59
FJ230831/NZ              --------------VGASSDTNIIKRNTKTRVGKNTTMSDGGFGEEAEITADSKQGISSF
  KP226805/In            IGVVGSVGVGGNVGVGASSDTNIIKRNTKTRVGKNTTMSDGGFGEEAEITADSKQGISSF
dia                     60
KJ404071/India           IGVVGSVGVGGNVGVGASSDTNIIKRNTKTRVGKNTTMSDGGFGEEAEITADSKQGIPSF
DQ672338/china          60


  KY399852/fn-sul          GVGVAAAGVGAGVAGTVSVNQFAGKTEVDVEEAKILVKKAEITAKRYSSVAVGNAAVGVA
JX678873/Australia      119
FJ230831/NZ              GVGVAAAGVGAGVAGTVSVNQFAGKTEVDVEEAKILVKKAEITAKRYSSVAVGNAAVGVA
  KP226805/In            GVGVAAAGVGAGVAGTVSVNQFAGKTEVDVEEAKILVKKAEITAKRYSSVAVGNAAVGVA
dia                     120
KJ404071/India           GVGVAAAGVGAGVAGTVSVNQFAGKTEVDVEEAKILVKKAEITAKRYSSVAVGNAAVGVA
DQ672338/china          120
JX648295/India           GVGVAAAGVGAGVAGTVSVNQFAGKTEVDVEEAKILVKKAEITAKRYSSVAIGNAAVGVA
                        120
                         GVGVAAAGVGAGVAGTVSANQFAGKTEVDVEEAKILVKKAEITAKRYSSVAIGNAAVGVA
  KY399852/fn-sul       119
JX678873/Australia       GVGVAAAGVGAGVAGTVCGNQFAGKTEGDVEEAKIGVEKDEITAKRYSSVAVGNAAEGVA
FJ230831/NZ             120
  KP226805/In            ******************. ******** ******* *:* ***********:**** ***
dia                      AKGAGIGAAVEVT- 132
                         AKGAGIGAAV - 116
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Figure 4. Alignment of amino acid sequences from the *lktA* gene of *F. necrophorum* sequences in this study with reference sequences from various countries

Table 3. In vitro activities of 16 antimicrobial agents against clinical isolates of *D. nodosus* and *F. necrophorum*

	D. nodosus (37)[a]				*F. necrophorum* (72)[a]			
Antibiotic	**Concentration (Range)**	**MIC_{50}[b]**	**MIC_{90}[b]**	**% S[c]**	**Concentration (Range)**	**MIC_{50}**	**MIC_{90}**	**% S**
Benzylpenicillin	0.065-1.20	0.28	1.1	28.7	0.068–2.20	0.068	0.35	27.7
Amoxicillin	0.138-8.90	0.60	8.0	41.0	0.050–67.0	0.150	48.0	56.9
Cloxacillin	0.122-5.10	0.70	5.0	65.0	0.131-4.80	0.900	3.10	62.5
Cefadroxil	0.320-9.10	1.00	9.0	91.0	0.135–8.80	1.030	4.70	67.7
Cefuroxime	0.068-5.40	0.33	5.0	83.4	0.078–7.80	0.350	1.20	78.8
Ceftiofur	0.125-5.00	0.26	3.0	98.0	0.500–2.00	0.800	1.99	98.1
Kanamycin	0.300-44.0	1.00	40	100	4.700–262	71.00	262	100
Gentamicin	0.600-38.0	1.20	38	79.0	5.000–271	68.00	271	47.8
Streptomycin	2.800-18.0	4.00	18	88.0	9.000–261	66.00	261	33.5
Spiramycin	0.068-2.00	0.28	2.0	69.0	0.078-278	3.700	78.0	58.9
Tylosin	0.135-5.00	1.00	4.0	96.0	0.075-8.50	3.100	8.50	61.8
Enrofloxacin	0.078-0.80	0.35	0.8	86.2	0.580-8.60	2.000	8.60	77.3
Lincomycin	0.600-18.0	4.00	18	76.5	0.133-8.50	0.800	6.00	76.7
Tetracycline	0.380-265	4.00	38	33.4	0.127-340	0.800	21.0	46.3
Doxycycline	0.134-138	0.40	80	67.0	0.068-8.30	0.350	8.30	63.8
Trimethoprim	2.000-41.0	4.00	41	82.6	9.000-261	128.0	261	85.9

[a]Number of isolates tested. [b]MIC of all agents except penicillin G was given micrograms per milliliter. Penicillin G MICs are in units per milliliter. [c]Percentage of strains susceptible at the MIC breakpoint

Antibacterial Sensitivity

The MICs of the 16 different antimicrobial agents for preventing the *in vitro* growth of *D. nodosus* and *F. necrophorum* strains of bacteria are shown in Table 3. All 37 isolates of *D. nodosus* and 72 isolates of *F. necrophorum* were 100% susceptible to kanamycin, and 98% susceptible to ceftiofur. At the same time, a lower sensitivity rate was detected against benzylpenicillin, 27.7% and 28.7%, respectively.

DISCUSSION

Conformation of hooves plays an important role in animal welfare mitigation and economic impacts associated with footrot (19). This paper is the first molecular study to investigate the detection and characterization of both *D. nodosus* and *F. necrophorum* in sheep in Sulaimani province, Kurdistan Region, Iraq. Best et al. (19) highlighted that poor conformation of hooves is likely to act as a reservoir for *D. nodosus* infection and the transmission of infection within

flocks. They also suggested that overgrown feet might appear healthy clinically but act as a subclinical carrier for *D. nodosus*. From this suggestion, we can say that hoof trimming will be one of the most important risks of transmission in flocks. DNA sequences generated in this study confirmed that *D. nodosus* and *F. necrophorum* are present in some sheep flocks in Sulaimani province. Few studies have been conducted in surrounding countries, such as Turkey, and no documented papers have been conducted on footrot in the Kurdistan Region. Footrot cases have been documented in some countries globally, such as India in central Kashmir, New Zealand, the United Kingdom, and Turkey. *D. nodosus* isolation and molecular characterization was found in central Kashmir, with the highest percentage (15.84%) found in Srinagar and the lowest percentage (10.89%) in Budgam, while the infection rate was 13.28% in district Ganderbal (20). Another study in India used PCR to detect footrot by taking swabs from infected sheep feet. The overall prevalence was 16.19% and ranged from 13.69% to 19.71%, respectively (21). In New Zealand, a study conducted on footrot in sheep detected 14 hoof scrapings collected from six farming regions contained *D. nodosus*. Furthermore, DNA analysis of the New Zealand farms revealed 15 strains covering 8 serogroups (22). In Turkey, a PCR-confirmed study detected 247 cases of footrot among 8,970 sheep from 10 flocks (23).

In the phylogenetic tree, the sequences of *F. necrophorum* clustered with Australia, New Zealand, and India (Figure 2), while the sequences of *D. nodosus* clustered with the USA, India, and Sweden (Figure 1). These results can epidemiologically prove that footrot has the same source. Many animals have been imported from India; this may contaminate the soil and acts as a main source of infection. Footrot can be transmitted from contaminated ground to the damaged interdigital space (24). According to this study, there was little variation among the field sequences of the *F. necrophorum lktA* gene in New Zealand, India, and China, but much more variation was found in Australia (Figure 3). There are no mutations or differences in the genes used to construct the phylogenetic tree or sequence alignment across the six years of the study; therefore, alignment of the first year only was included. As can be noticed from the phylogenetic trees, the genes detected in this study have 96% - 99% identity with those in other countries.

The study additionally evaluated the effectiveness of some antimicrobial agents involved in footrot in sheep in order to select high-quality treatments for sheep suffering from this infectious disease. The results showed that all 72 and 37 isolates of *F. necrophorum* and *D. nodosus* were 100% susceptible to kanamycin, which belongs to the aminoglycoside group, while other antimicrobial agents (gentamicin and streptomycin) in the same group were not effective (47% and 33%, respectively) against *F. necrophorum*. This might be because of how aminoglycosides work, which depends on how they enter the bacteria partly through active transport and partly through passive diffusion. Under anaerobic conditions, such active transport typically does not occur (25).

The rise in the number of microorganisms with antimicrobial agent resistance poses a serious threat to the control of infectious diseases. Due to resistant microorganisms, this leads to infections that do not respond to standard medical care, prolonging illness and raising the risk of death. The sensitivity of benzylpenicillin was found to be between 27.7% and 28.7% in this study, which is consistent with (25). Aside from the fact that approximately 30% of *F. necrophorum* and 40% of *D. nodosus* isolated from the United States and Central Europe produced β-lactamase, which results in the destruction of the β-lactam ring in the structure of benzylpenicillin (26) or may be due to the overuse or misuse of benzylpenicillin, which is the method for increasing resistance to antimicrobials, a lot of studies have confirmed the terrible financial magnitudes of antimicrobial resistance, including exceedingly high healthcare costs due to an increase in hospital admissions and drug usage (27). The result of the current study disagrees with (28) because their study showed that *F. necrophorum* was sensitive to penicillin. Furthermore, amoxicillin, cloxacillin, cefadroxil, and cefuroxime are ineffective against the two bacteria, whereas ceftiofur sensitivity was 98% against both microorganisms because it is the newest 3rd generation cephalosporin that was recently launched in our region.

Good activity was obtained from the macrolide group, which included tylosin and spiramycin, with 61.8%, 58.9% sensitivity against *F. necrophorum* and 96%, 69% sensitivity against *D. nodosus*, which is consistent with (26, 29). It is crucial to draw attention to the positive effects of tylosin, whose ability to resist macrolides is typically caused by rRNA methylases that alter the 23S component of the ribosome and have been discovered in both Gram-positive and Gram-negative anaerobic rods. Both levofloxacin and enrofloxacin performed well against *F. necrophorum* and *D. nodosus* in this study (77.3% and 86.2%, respectively), which is consistent with the findings of (30). Enrofloxacin is the third generation of fluoroquinolones, a broad-spectrum bactericidal antimicrobial agent that acts by inhibiting two enzymes used for bacterial DNA synthesis, both of which are DNA topoisomerases used for DNA replication. All 72 and 37 isolates of *F. necrophorum* and *D. nodosus* were not susceptible to tetracycline and doxycycline, and this result agrees with (25, 31).

There is no doubt that additional samples must be tested, and additional work is required to be documented as many different genotypes are feasible and to cover all of the nation's provinces. Furthermore, gathering such data and comparing it to information stored in international

databases will help with strain genotyping of the pathogen as well as ongoing active surveillance.

We document the first molecular characterization of *D. nodosus* and *F. necrophorum* in sheep in Sulaimani Province, Iraq. However, this will be very alarming as it can heavily affect economic losses for the sheep industry. Over the six years of the study, no differences in the gene alignments have been seen. Future investigations are needed to characterize the genotype and serogroups of the causative agents and identify a potential vaccine so that the diseases can be eradicated.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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التباين الجيني والحساسية المضادة للبكتيريا للإصابة ببكتيريا ديكيلوباكتر نودوسوس وفيوزوباكتيريوم نيكروفورم في الأغنام في محافظة السليمانية، إقليم كردستان، العراق

رزكار رحيم سليمان[1]،هه ردى فتاح مارف[1]، باسم عبدالواحد علي[1]،كويستان نجم علي[1]، محمد عمر بابه شيخ[2]

[1]فرع الطب السريري والطب الباطني، كلية الطب البيطري، جامعة السليمانية، السليمانية، إقليم كردستان، العراق، [2]مديرية بيطرة السليمانية، شارع سالم، السليمانية، إقليم كردستان، العراق

الخلاصة

تعفن القدم هو مشكلة صحية كبيرة في المجترات، وخاصة الأغنام، وتسببه جرثومتي ديكيلوباكتر نودوسوس و فيوزوباكتيريوم نيكروفورم. قد تصبح الحيوانات المصابة عرجاء وتفقد حالة الجسم نتيجة لذلك. كان الهدف من هذه الدراسة معرفة ما إذا كانت الأغنام مصابة بعدوى مختلطة لكل من ديكيلوباكتر نودوسوس و فيوزوباكتيريوم نيكروفورم، وتحديد العلاقات الوراثية بين جينات هاتين الجرثومتين مع جينات مرجعية موجودة في عزلات عالمية مختلفة، فضلا عن دراسة درجة عدم التجانس الوراثي بين المجموعات المصلية القياسية لـ ديكيلوباكتر نودوسوس. فضلا عن ذلك، جرى الكشف عن الحساسية المضادة للجرثومتين تحت الدراسة. تضمن البحث دراسة ١٧٥ منطقة بين ظلفي حافر الاغنام للفترة ٢٠١٦-٢٠٢٢. أظهرت العزلة الحقلية ديكيلوباكتر نودوسوس (Dn Sul/016-KY399851) توافقًا بنسبة ٩٦٪ مع العزلات العالمية الأخرى (NR_104942/USA، وJN008724/India، وDQ016290/Sweden) عندما جرى مقارنة تسلسل قواعد الجين 16S rRNA ببعض الجينات المرجعية. وجرى مقارنة العزلة المحلية (Fn Sul/016-KY399852) من ناحية تسلسل قواعد الجين *IktA* مع ذلك الموجود في سلالات جرثومية معزولة من دول أخرى (FJ230831/نيوزيلندا، JX678872/أستراليا، JX648295/الهند)، وأظهرت النتائج وجود تطابق بنسبة ٩٩٪ و٩٦٪. فضلا عن ذلك، عند مقارنة تتابعات قواعد الجين *lktA* في عزلات متعددة، لوحظ وجود تباين بنيوكليوتيد واحد في الموضع ٣٨٩ (CA) مقارنة بالعزلة الحقلية (Fn Sul/16-KY399852). ادى هذا التغيير الحاصل في تسلسل النيوكلوتيد الى استبدال الفضلة ١٣٠ الموجودة في الحمض الاميني الالنين (Ala) ليحوله إلى حمض الجلوتاميك (Glu). اظهرت هذه الدراسة التشخيص الجزيئي الأولي لكل من ديكيلوباكتر نودوسوس و فيوزوباكتيريوم نيكروفورم في الأغنام التي تعاني من تعفن القدم في محافظة السليمانية العراقية.

الكلمات المفاحية: ديكيلوباكتر نودوسوس، فيوزوباكتيريوم نيكروفورم، تفاعل البوليميراز المتسلسل،الاغنام، التعفن القدمي