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Molecular Detection of *Staphylococcus spp* in Ovine Actinomycosis

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Abstract

Ovine actinomycosis, also referred to as lumpy jaw, is a multifactorial illness that is characterized by a number of host-pathogen-environment interactions in which host immune and physiological processes (host) interact with a variety of causative agents, such as staphylococci spp. Oral infections are common in sheep. Periodontal disease, one of the most common diseases, can start early with periodontitis and progress to become a chronic condition that causes food debris to build up around the tooth below the level of the gums. *Staphylococcus spp.* are widely dispersed in the environment and is primarily discovered as a commensally micro-flora on the skin of animals and birds as well as the mucous membranes, particularly the nasal cavity. The PCR assay was applied for the detection of *Staphylococcus aureus* and *Staphylococcus haemolyticus* from three ovine actinomycosis cases based on the amplification of the 16S rRNA gene. In addition, the diagnosis depended on sequencing of the PCR products, and gene analysis was done to match the global isolates. The current study found that *Staphylococcus aureus* was isolated from all samples used 86 samples and that gene sequencing of this strain confirmed 99.88% global compatibility, whereas *Staphylococcus haemolyticus* revealed 100% global compatibility. Therefore, the aims of the study were to pinpoint *Staphylococcus spp*. as the secondary causative agent of lumpy jaw disease in Awasii sheep of Iraq.

Keywords: Lumpy jaw, ovine, *staphylococcus sp*.

Introduction

The *staphylococci* spp. was important human and animal pathogens that are known to produce a wide range of toxic compounds. *S. aureus* strains can be found in the environment as well as on the skin and mucous membranes of people and animals. They have the ability to spread a variety of animal illnesses, such as mastitis, suppurative conditions, and urinary tract infections. They can also result in food poisoning, septicemia, pneumonia, and wound infections in people (1,2,3). Staphylococci that are gram-positive are frequently grouped in clusters that resemble grapes. Based on their ability to form blood plasma clots and the coagulase enzyme, Staphylococci are divided into coagulase-positive and coagulase-negative species. (4,5). The majority of it is found as a commensally micro-flora on the skin of animals and birds, as well as the mucous membranes, notably the nasal cavity, in 20–40% of the general population. It is widely disseminated throughout the environment. *Staphylococcus aureus* is a substantial contributor to numerous community ailments in addition to infecting the skin and soft tissues, surgical sites, bones, and joints. (6,7). Ovine actinomycosis, also known as lumpy jaw, is a multifactorial disorder that is characterized by a variety of host-pathogen-environment interactions. In these interactions, host immune and physiological mechanisms (host) interact with a variety of causative agents, such as *staphylococci spp*. bacteria, environmental factors, and things like food that has accumulated in the teeth of affected animals. (8,9,10)

Materials and Methods

Study design

The Awasii sheep flocks showed that the infected animals varied in age from (3-6) years. The total number was 78. As soon as feasible after collection, samples were examined bacteriologically growth of *Staphylococcus spp.* on the nutrient Agar and incubated at (37°C) for (48) hours. Following incubation, 16S rRNA genes were used in PCR on the isolates to identify this pathogen before sequencing Table (1)

Need to mention routine bacteriological Generations the growth of *Staphylococcus spp.* Figure (3) on the nutrient agar or blood Agar Base or mannitol salt agar appears cocci, golden staph

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Figure 1: culture media and colonies of bacteria *S. aureus*

Table 1: The sequence and product size of primers used in this study. (Yugueros *et al*., 2001)

Name	Sequence	Amplicon size
GF-1	ATGGTTTTGGTAGAATTGGTCGTTTA	933 bp
GR-2	GACATTTCGTTATCATACCAAGCTG	

Results

It revealed that all isolates were positive as demonstrated in figure (1, 2). The percentages of two species were 7.17 % and 2.83% in *S. aureus* and *S. haemolyticus* respectively, figure (2). Two isolates of *S. haemolyticus* and one isolate of *S. aureus* were sent for gene analysis and the results matched the global results by 99.88% and 100%, figure (3) and table (3).

Detection of *staphylococcus spp*.

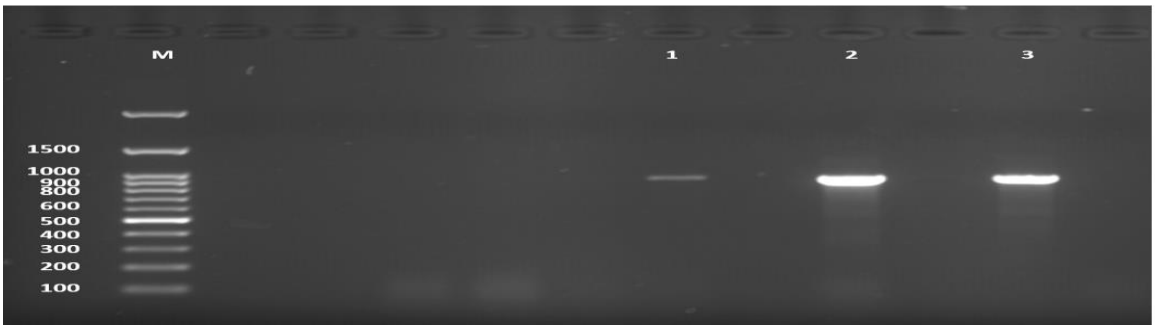

Figure 2: Gel electrophoresis image (1.5 %) shows positive samples (lanes 1,2,3) of *Staphylococcus spp*. (PCR amplicon size = 933 bp). M is molecular marker (ADDBIO, Korea).

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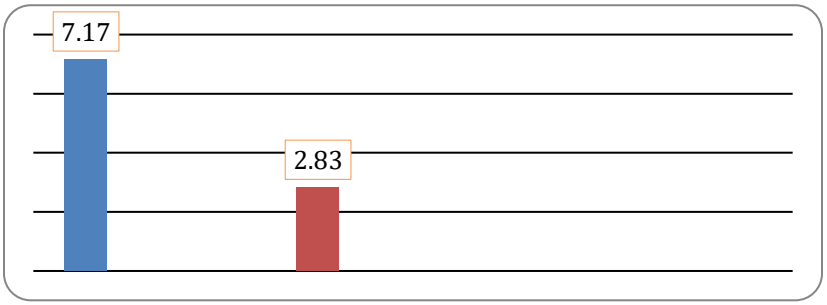

Figure 3: The percentages of two species were 7.17 % in *Staphylococcus aureus* and 2.83% in *Staphylococcus haemolyticus*.

Calculations of the evolutionary divergence between the *Staphylococcus spp.* Base substitution rates per site between sequences are displayed. Using the Maximum Composite Likelihood model, analyses were performed. There were 5 nucleotide sequences in this analysis. The final dataset contained 867 locations altogether. In MEGA11, evolutionary analyses were carried out.

Table 2: the NCBI-BLAST Homology Sequence identity (%) in local *staphylococcus sp*. These sequences were deposited in gene bank under the following accession numbers, and these were being compared with other global sequences.

Sequence No.	Accession No.	NCBI-BLAST Homology Sequence identity (%)			
		Identical to	Genbank Accession No.	Country	Identity
1	OQ750818	***Staphylococcus haemolyticus***	CP045137	India	100 **%**
2	OQ750819	***Staphylococcus haemolyticus***	CP045137	India	100**%**
3	OQ750820	***Staphylococcus aureus***	CP038229	China	99.88**%**

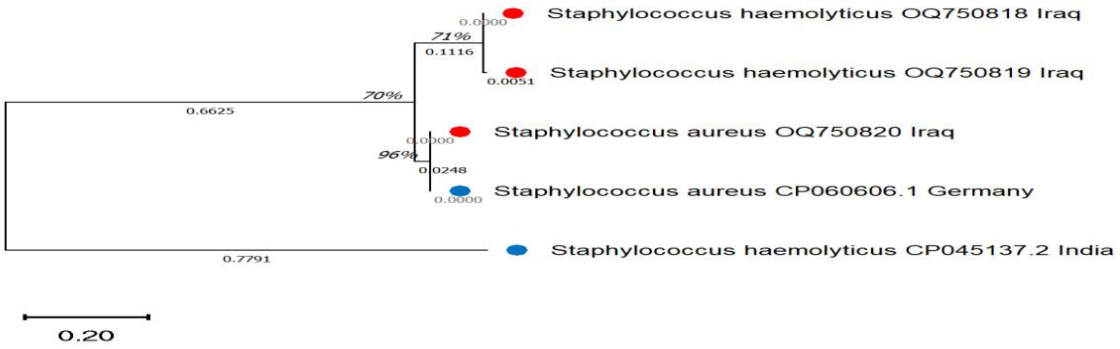

Figure 4: Evolutionary relationships of taxa *Staphylococcus spp.*

The Neighbor-Joining approach was used to infer the evolutionary history. The ideal tree is displayed. The tree is depicted with branch lengths and is scaled. The Phylogenetic tree of these isolates was built using the MEGA11 and NCBI programmed showed 100% identification with China and 99.88% identity with worldwide isolates (figure 3), including Iraq and India. Finally, Germany and compatibility were 96% (Figure 3). It is possible to obtain the strains at OQ750818, OQ750819, and OQ750820 (16S rRNA gene), which were published on NCBI.

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Discussion

Clinical disorders caused by *S. aureus* and *S. haemolyticus* can range from minor skin infections to life-threatening illnesses such septic shock, endocarditis, and osteomyelitis. The most typical source of community-acquired bacteremia is *S. aureus*. Endocarditis, osteomyelitis, and abscesses are some of the symptoms of metastatic infection that can result from persistent bacteremia (11). The animals, which naturally carry *Staphylococcus* species on their bodies, serve as the main source of *S. aureus* and, in the right conditions, can spread the bacteria to hosts who are vulnerable. (12). Additionally, animals with subclinical inflammatory illnesses are a significant source of disease and a reservoir in the dairy environment. (13). Direct or indirect transmissions are also possible. Opportunistic commensal Staphylococcus aureus typically lives on or inside the host without causing disease. (14). Based on the amplified 16S rRNA gene, the PCR assay was used in the current study to identify *S. aureus* and *S. haemolyticus.* In terms of this gene's 16S rRNA sequence, the isolates had 99.88% and 100% compatibility with international isolates from China and India, respectively.

Conclusion

Staphylococcus spp. is regarded as the secondary infection in ovine actinomycosis, and its identification by PCR and sequencing was reliable and produced positive results.

Acknowledgments

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Ethical Approval:

This study was approved by the ethical and research committee of college of veterinary medicine, University of Al-Qadisiyah.

Conflict of interest

There is no conflict of interest, according to the authors.

Author's contribution

Each author made an equal contribution to every aspect of this manuscript.

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