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Molecular Detection of Glanders in horses in Baghdad, Iraq

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ABSTRACT: Glanders is, contagious, fatal, zoonotic, bacterial disease which caused by *Burkholderia mallei*. The aim of this study was to establish a fast and reliable method for identification of *B. mallei*, through molecular assay and determining the genetic relationship between the local and global isolates total of 100 nasal swabs were collected from horses of the Equestrian Club in Baghdad province (Iraq) during November (2021) to September (2023) and subjected for molecular examination using the conventional polymerase chain reaction (PCR). The results revealed that 29% of study animals were positive; including 21% in males and 8% in females. The result of phylogenetic analysis was showed a significant association between the study isolates and the NCBI-BLAST *B. Mallei* Turkish isolate (CP010349.1) and NCBI-BLAST *B. mallei* India isolate (CP009643.1).

Keywords: Keyword *Burkholderia mallei*, Polymerase chain reaction, Equestrian Club, Phylogenetic analysis

1. INTRODUCTION

Glanders it is one of the oldest animal diseases known to man, The disease was first defined by the Greek physician Hippocrates in the fourth century BC, and Aristotle gave it the Latin name malleus (1).
Burkholderia malei is a microorganism that is a cause of glanders disease in the equine family. In addition, it infects several mammals, including camels, dogs, lions, monkeys, and even humans are also susceptible to infection (2,3).
The bacteria do not survive for long period outside of body host and susceptible to heat, sunlight and common disinfectants but it prefers a warm and humid environment (4).
Glands are present and endemic in many countries such as North Africa, South America, Middle East and Asia and is still a matter of concern in these areas due to re-emerging disease but eradicated and restricted geographically from USA, Canada and Western Europe (5,6).
The prevalence of glanders in Iraq was estimated using the Malein test as a screening and diagnostic test for animals. In addition, it was confirmed that they were glandular using clinical, biochemical and serological studies (7,8).
The disease is transmitted by several ways, including by inhalation of bacteria in form of aerosols, ingestion of contaminated food and water and contact with respiratory secretions and skin exudates of infected animal, glanders typically manifests as acute or chronic, horses tend to develop the chronic form but donkeys get the acute phase of the disease (9,10).
Many tests used in the diagnosis for the purpose of disease in equine, however PCR is the most sensitive and accurate technique (11,12,13).
The use of treatment is not recommended due to the nature of the disease and to control it, requires death of affected equids to prevent spread of infection (14,15,16).

2. AIMES OF STUDY

Determining the prevalence of glanders in equine in Baghdad city via determination their positivity to *Burkholderia mallei* and molecular characterization of local strain of *Burkholderia mallei* and determine their heterogeneity to global isolate.

3.MATERIALS AND METHODS

3.1. Samples

The study was conducted at the Equestrian Club in Baghdad, which is the largest gathering of horses in Iraq, containing more than 800 animals, from the period extending from November 2021 to September 2023, 100 animals that were clinically diagnosed as suffering from respiratory diseases, nasal swabs were taken and immediately sent in a refrigerated and sterile condition to Internal Medicine Laboratory, University of Baghdad, College of Veterinary Medicine for DNA extraction according to the procedure mentioned in the extraction kit of Intron biotechnology company (G- Spin DNA Extraction Kit) then, PCR was done in the veterinary hospital laboratory in Babylon.

4.MOLECULAR EXAMINATION

Conventional PCR primers that used for detection *B. Mallei* was designed in this study based on NCBI-GenBank and primer 3 plus design. These primers were provided by Macrogen Company, (Korea), (table1).

Table (1) show primers use in this study

Gene	**Primers Sequence 5'-3'**		**PCR product size**
Fllagea	R:	CATCCCCATCGACATCAGCA	443bp
	L:	CGAGCTTCACGCGGATCAT	

Following the Maxime PCR PreMix Kit, Master mix tubes were prepared at a final volume of 25µl. Then, all the PCR tubes were vortexed and subjected to the thermocycler conditions as following (Table 2).

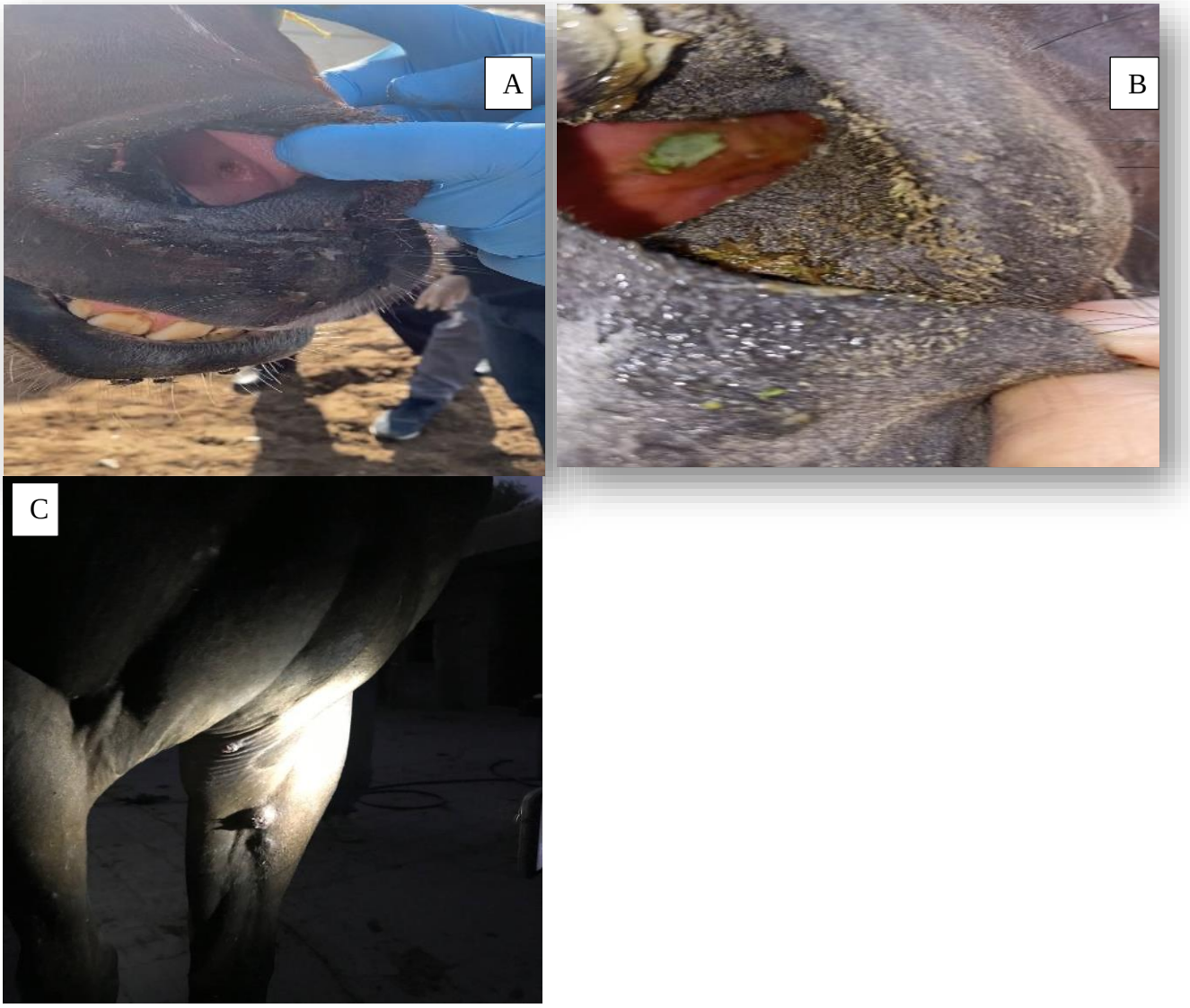
Table (2). PCR program for *Fllagea* gene of *B. mallei.*

No.	Step	Temperature C°	Time	No. of cycles
1	Initial Denaturation	95	5min.	1
2	Denaturation	95	30s	35
	Annealing	58	30s	
	Extension	72	1min.	
3	Final Extension	72	5min.	1
4	Hold	4 ºC	Forever	-

5.RESULTS AND DISCUSSION

5.1. Clinical examination

The current study examined 100 horses of different ages and sexes; these animals suffering from respiratory infections some of these horses showed severe nasal discharge and had a typical clinical sign (satellite shape), also some of them have skin lesion (farcy) as figures (1).



Figures 1. (A and B) show nasal lesion of glanders. C show skin lesion of glanders.

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5.2. Total results of PCR

29 samples showed positive for glanders disease **29%** as Table (3)**,** prevalence rates of *glanders* and the variations between males and females. The results indicate that females had the lower rate of infection at 8%, whereas males had a considerably highest infection rate of 21%**.** While Prevalence based on the age showed non-significant variation**,** where the bacteria infect all ages**.**

Table (3) show result of PCR and comparative between male and female

100 horses	PCR results			
	-ve Male	+ve Male	-ve Female	+ve Female
	41	21	30	8
total	62		38	

Specific primers were utilized to distinguish of *Burkholderia mallei* in horses using the PCR technique, fllagea designed to amplify a 443 bp and showed positive results in figure 2

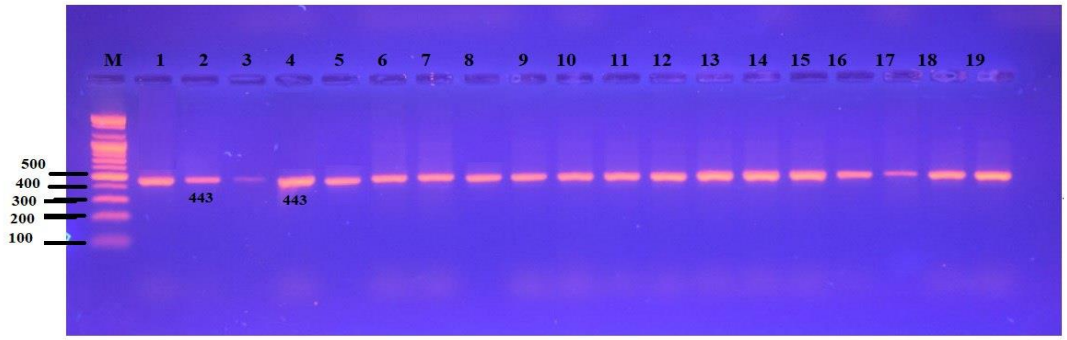

Figure 2. Agarose gel electrophoresis of Amplified DNA for *B. mallei* show positive results.

This study is considered the first work using the PCR technique in Iraq to diagnose glanders disease. The research has previously been diagnosed using serological methods such as ELISA, or diagnosis by using maline test or collecting research on the presence of this disease, A high incidence rate with this microorganism in horses (7,8,17).

A several studies were reported incidence of Glanders in Iraq, since 1977 Al-Kafawi, *et al.*, has study about glanders disease, then the researcher in the college of Veterinary Medicine in Baghdad studies disease by the histopathological and electron microscope, recently many studies conducted present glanders in Iraq by using ELISA kit (18, 19,8,17).

Furthermore, the disease is endemic in the neighboring countries of Iraq, such as Iran, Turkey and Kuwait, the distribution of horse glanders in the world according to OIE data from 2011 to 2021(20).

5.3. Results of sequence analysis

The DNA sequencing method was carried out to genetic relationship analysis in 16S ribosomal RNA gene in *B. mallei* Iraq isolates related NCBI-Gen bank country *B. mallei* strains.

The phylogenetic tree genetic relationship analysis was showed that The *B. mallei* Iraq isolates (IQ.No.1-IQ.4) were showed closed related to NCBI-BLAST *B. mallei* Turkey and India strain at total genetic changes (0.1%) as showed in figure (3), the phylogenetic tree was constructed using Unweighted Pair Group method with Arithmetic Mean (UPGMA tree) in (MEGA 6.0 version). The *B. mallei* Iraq isolates (IQ.No.1-IQ.4) were showed closed related to NCBI-BLAST *B. mallei* Turkey and India strain at total genetic changes (0.1%).

The homology sequence identity between *B. mallei* Iraq isolates and NCBI-BLAST *B. mallei* Turkey isolate (CP010349.1) were showed genetic homology sequence identity ranged from (99.70-99.80%) as showed in table (4).

The homology sequence identity between *B. mallei* Iraq isolates and NCBI-BLAST *B. mallei* India isolate (CP009643.1) were showed genetic homology sequence identity ranged from (99.30-99.34%) as showed in table (5).

Finally, local *B. mallei* Iraq isolates (IQ.No.1-IQ.4) were submitted into NCBI Gen bank and identified by accession numbers (OQ691633.1 into OQ691633.1).

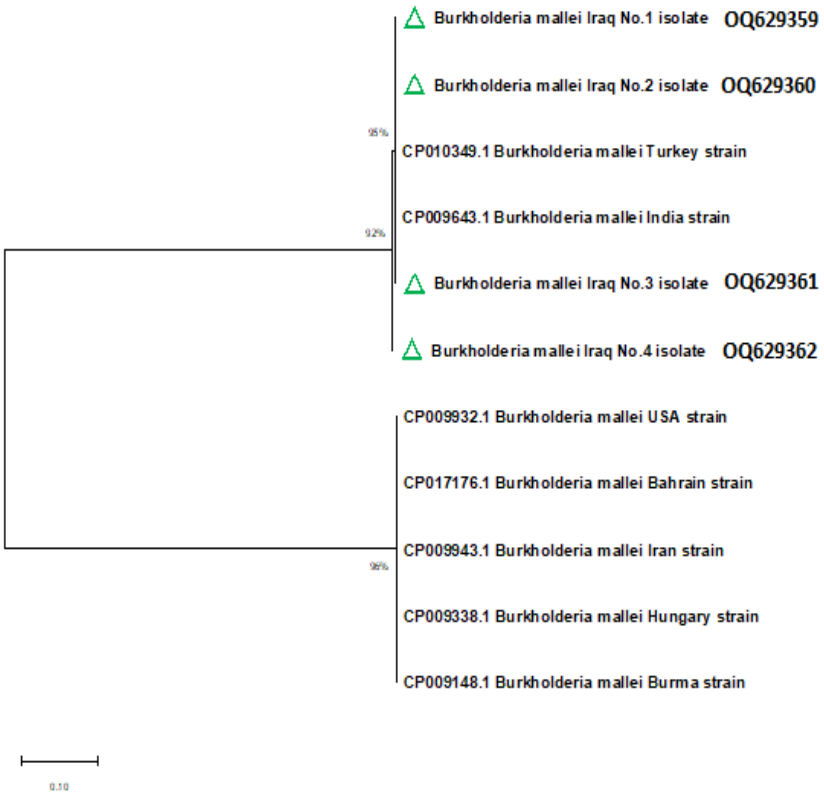

Figure 3: Phylogenetic tree analysis based 16S ribosomal RNA gene partial sequence in *B. mallei* Iraq isolates that used for genetic relationship analysis.

Table (4) the NCBI-BLAST Homology Sequence identity percentage between *B. mallei* Iraq isolates and *B. mallei* Turkey strain

Burkholderia mallei Iraq isolates	Accession number	Homology sequence identity (%)		
		Country	Accession number	Identity (%)
IQ.No.1	OQ691633.1	Turkey	CP010349.1	99.70%
IQ.No.2	OQ691634.1	Turkey	CP010349.1	99.70%
IQ.No.3	OQ691635.1	Turkey	CP010349.1	99.70%
IQ.No.4	OQ691636.1	Turkey	CP010349.1	99.80%

Table (5) the NCBI-BLAST Homology Sequence identity percentage between *B. mallei* Iraq isolates and *B. mallei* India strain:

Burkholderia mallei Iraq isolates	Accession number	Homology sequence identity (%)		
		Country	Accession number	Identity (%)
IQ.No.1	OQ691633.1	India	CP009643.1	99.30%
IQ.No.2	OQ691634.1	India	CP009643.1	99.30%
IQ.No.3	OQ691635.1	India	CP009643.1	99.30%
IQ.No.4	OQ691636.1	India	CP009643.1	99.34%

6.CONCLUSION

Finding *Burkholderia mallei* isolates in Iraqi horses using Polymerase Chean Reaction technology.

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