

PREVALENCE AND MOLECULAR TYPING OF BRUCELLA SPP. IN CAMEL HERDS OF DESERT PASTORAL SYSTEMS

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Abstract: Brucellosis remains a considerable threat to individuals that reside in desert pastoral systems whereby camels hold great significance to their livelihood. The aim of this study was to assess the seroprevalence and molecular characteristic of *Brucella* spp. infection in camel herds. We checked 450 camels by serology (RBT and CFT), molecular (PCR and multiplex PCR) and genomic (MLST and WGS) tests. Serology revealed that 29.33 per cent of the samples tested positive (RBT) and 26.22 per cent tested positive (CFT). PCR targeted to the *bcsp31* gene confirmed presence of *Brucella* DNA in 27.56 percentage of samples. Species-specific PCR revealed that *B. melitensis* prevailed (54.84%), followed by *B. abortus* (33.06%) and multiple infections (12.10%). MLST revealed that the most prevalent sequence types were ST-8, ST-5 and ST-11. Instead, WGS analysis provided entire genomic portraits that revealed SNPs, indels, and mobile genetic elements associated with regional clusters. The genes of several of the isolates caused resistance to antibiotics such as tetracycline (70 percent), aminoglycoside (40 percent) and sulfonamide (25 percent). All the major risk factors of infection were poor biosecurity, shared grazing, and herd mixing. These findings demonstrate the complexity of the epidemiology of camel brucellosis and highlight the importance of molecular-based surveillance, improved herd management practices, and targeted intervention strategies to reduce the economic and health impact of this disease.

Keywords: “Brucellosis”, “Camels, Molecular Typing”, “Whole-Genome Sequencing”, “Antimicrobial Resistance”, “Desert Pastoral Systems”.

INTRODUCTION

As a common zoonotic illness, brucellosis poses a significant threat to the wellbeing of livestock and people, particularly in the regions where bovine farming is a major economic activity (Wathig, 2020). The disease is caused by bacteria of the genus *Brucella*. Each species has a preference of hosting organism although infections between species may occur (Pellegrini et al., 2022). The camel, especially the one belonging to the desert pastoral systems, is susceptible to the *Brucella* infection, which causes reproductive losses, reduced milk output, and economical difficulties to the herders (González-Espinoza et al., 2021). To implement effective control and prevention strategies, the identification and description of the *Brucella* species circulating in camelids populations should be carried out correctly (Rizvi et al., 2020). Traditional methods of *Brucella* detection, such as bacterial culture or serology tests have limitations in their sensitivity, specificity, and time to diagnosis (Galon et al., 2022). Polymerase chain reaction and multi-locus variable-number tandem repeat analysis are molecular typing methods that offer faster and accurate detection of *Brucella* species and strains that enable a better understanding of the dynamics of disease transmission and outbreak surveillance. The desert pastoral systems where camels play a significant role in the lives of people are characterized by the unique ecological and management practices that complicate the situation with brucellosis control. In order to develop community-specific therapies, which consider the social and economic context of camel-herding populations, we must understand the prevalence and genetic variation of *Brucella* within these ecosystems. The deepening of camel exploitation, aimed at satisfying the needs of the market, requires strict research on the behavior of camels, their nutrition, lactation physiology, reproduction,

husbandry, and management, with attention to animal health and welfare (Nagy et al., 2022). Based on genetic studies, selective breeding is reinforcing positive traits, including pace and anti-disease in camels, particularly in racing and beauty contests, which highlights the importance of genetic knowledge in camel husbandry (Bridi & Man, 2025). As well, using traditional knowledge together with modern approaches can result in a more holistic approach to controlling diseases in camel populations that will ensure pastoral systems remain functional and safeguard the diversity of camels. People are trying to make animals healthier; however, infectious diseases continue to impose economic losses (Alekish & Ismail, 2022). The molecular typing techniques have transformed the bacteria epidemiology field by providing the investigators with powerful tools to differentiate closely related strains and to trace the epidemiology of infectious disease. They are more informative than the traditional phenotypic techniques such as biotyping and serotyping since they examine the variations in the bacterial genome. Techniques based on polymerase chain reaction, including real-time PCR, are widely applied to rapidly detect and identify the *Brucella* species (Sanchez-Carvajal et al., 2021). The tests are sensitive and specific because they seek uniquely DNA sequences of a genus or species, thus diagnostic (Porumb-Andrese et al., 2021). Multi-locus sequence typing is another helpful technique that involves the sequencing of numerous housekeeping genes and contrasting the sequences with a database of reference strains of *Brucella*. The method allows the identification of clonal complexes and recovery of evolutionary trees between different isolates. Secondly, the development of next-generation sequencing technologies has meant that we can now sequence the entire genome of *Brucella* isolates, providing

us with truly impressive levels of information regarding the genetic diversity of these bacteria, and their evolutionary history. Single nucleotide polymorphisms, insertions, and deletions can be identified by whole-genome sequencing. They can be used to differentiate strains and monitor their locations in the world. In addition, genomics could generate complete information about bacteria, including antibiotic resistance modes, clues about its emergence, and the features of mobile DNA elements that spread resistance (McDermott & Davis, 2020). Utilization of the cutting edge molecular technologies, including whole-genome sequencing, is identified as the most efficient strategy to study outbreaks and analyze the dynamics of spreading of bacterial pathogens. It can be one of the main tools in the study and control of different pathogens that threaten human health (Forde et al., 2022). Direct identification of microorganisms within a sample may be achieved with metagenomic sequencing (Huang et al., 2021). The most frequently used strategies to study microbial community composition are the amplification of the 16S rRNA gene followed by sequencing (Aleruchi & Obire, 2021; Hailu et al., 2021). The production of amplicons is followed by the opportunity to perform next-generation sequencing (Hailu et al., 2021). Resistance genes can be detected by metagenomic analysis, even those which are not currently expressed. The most common modern molecular methods used to study antibiotic resistance are high-throughput real-time PCR, metagenomics, and whole genome sequencing (Franklin et al., 2021). Whole-genome sequencing presents the most comprehensive data in surveillance and outbreak investigation; it is largely used to conduct molecular typing but has a large potential to be applied to microbiological diagnoses (Probst et al., 2021). Brucellosis is a common zoonotic disease caused by bacteria belonging to the

genus *Brucella* posing a significant risk to the health of animals and humans worldwide. The disease leads to reproductive issues in livestock such as abortion and infertility and has a potential of rendering people ill over an extended period. The camel, particularly the one bred in the desert pastoral system, is susceptible to *Brucella* infection and may serve as a reservoir of the bacterium, therefore, causing the spread of the disease to other livestock and humans. Conventionally, culture-dependent methods of antibiotic resistance identification are time-consuming and inaccurate, and a faster, more precise technique of antimicrobial resistance identification is whole genome sequencing (Talamantes-Becerra et al., 2024).

RESEARCH METHODS

The research applied a quantitative research strategy in investigating the occurrence and molecular features of *Brucella* spp. in camel herds in desert pastoral environments. The sampling was done in numerous geographically dispersed locations where the camel rearing is a major economic activity. A total of 450 camels were selected using stratified random sample to ensure representation of all age groups, sex and herd management methods. We sterilely collected blood and milk samples of every animal and submitted them to the lab testing keeping them cool. Initially, seropositive individuals were identified using the Rose Bengal Test (RBT), and the Complement Fixation Test (CFT). Additional molecular testing was performed on positive and borderline samples to confirm *Brucella* infection as well as to determine the precise species and strains. The samples were taken up by DNA using the commercial extraction kits and as per the instructions of the manufacturer. In the first test, polymerase chain reaction (PCR) tests which sought the presence of the *bcs31* gene which is unique to the *Brucella* genus were used. Species-specific PCR

experiments were done to differentiate between *Brucella* species using multiplex PCR experiments targeted at species-specific gene sequences. Molecular typing and epidemiological characterisation were carried out by multi-locus sequence typing (MLST) by amplifying and sequencing several housekeeping genes, including *gap*, *aroA*, *glk*, *dnaK*, *gyrB* and *trpE*. We compared our sequences obtained with reference databases in order to determine what kind of sequence we had and how the sequences were related to each other. Additionally, some isolates were subjected to whole-genome sequencing (WGS) with the use of Illumina sequencing technology to obtain high-resolution genetic fingerprints. The genomic data were viewed and analyzed with bioinformatics tools such as SPAdes, Prokka, and ResFinder to identify single nucleotide polymorphisms (SNPs), insertion-deletion mutations, antibiotic resistance genes, and mobile genetic elements. On a small number of pooled samples metagenomic sequencing was used to determine the possible presence of co-infections and to characterize the broader community structures of microbes associated with *Brucella* infection. The statistical analysis included prevalence estimation, chi-square testing to assess the association between infection status and demographic or management features, and phylogenetic analysis with MEGA software to infer evolutionary relationships among the identified strains. Ethical approval was obtained by the institutional animal care and use committee to sample the animals and all procedures were performed within the set animal welfare standards. The overall molecular approach used in the current study attempted to explain new knowledge on epidemiology, genetic diversity, and possible patterns of antimicrobial resistance of *Brucella* spp. in camel populations in desert pastoral systems to

provide a scientific basis of improved surveillance and control measures.

RESULTS

The frequency and molecular characterisation of *Brucella* spp. in camel herd in desert pastoral systems were efficiently investigated in the present study. We serologically, molecularly and genetically examined 450 camel samples. This provided us much data about the spread of *Brucella* infections as well as their genetic diversity. Table 1 demonstrates the results of Rose Bengal Test (RBT) and Complement Fixation Test (CFT). Among the 450 samples, 132 (29.33%) were positive to RBT and 118 (26.22%) were positive to CFT. This demonstrates that the prevalence of brucellosis is extremely high in the camels that have been studied. The demographic distribution of *Brucella*-positive camels is indicated in Table 2. The females (31.1%) got infected more easily than males (22.5%). The infection rate was significantly higher in adult camels (34.8%) compared to juvenile camels (18.7%), indicating the age as a primary risk factor. Table 3 shows PCR-confirmation of genus-level detection data, confirming the presence of *Brucella* DNA in 124 seropositive samples (27.56% overall prevalence). continuous amplification of the *bcs31* gene in positive samples established that PCR is a sensitive method of detecting *Brucella*.

The identification of species in multiplex PCR is presented in Table 4. The most prevalent species was *B. melitensis* (68 out of 124), followed by *B. abortus* (41 out of 124). There were 15 samples with mixed infections, that may indicate the interspecies transmission of the disease. The multi-locus sequence typing (MLST) profiles of the isolates are shown in Table 5. ST-8, ST-5, and ST-11 were the most frequent types of sequence and this indicates that there is not much genetic variation amid the circulating strains. The data of whole-genome

sequencing of 20 selected isolates is presented in Table 6 with a focus on SNP patterns, insertion-deletion events, and mobile genomic elements. These findings revealed that isolates clustered together in a distinct manner according to their origins. Table 7 illustrates the results of antimicrobial resistance genes identified through WGS and metagenomic studies. A number of isolates carried tetracyclines, aminoglycosides and sulfonamides resistance genes which raised concern about development of antimicrobial resistance profiles.

The relation between various herd management factors and occurrence of Brucella infection is presented in Table 8. Shared grazing, poor biosecurity and high frequency of herd mixing were significantly associated with higher infection rates

($p < 0.05$). Several figures demonstrate the key findings of the study in greater detail after the comprehensive table of results. Figure 1 shows the total seroprevalence place. Figure 2 demonstrates the rates of infections according to the age and sex. Figure 3 demonstrates the PCR positivity rates according to the sample zone. In figure 4, the distribution of species is presented in terms of species by the use of a pie chart. The frequency of every type of sequence is presented in the form of a bar graph in figure 5. Figure 6 shows evolutionary relationships in a dendrogram. Figure 7 provides a histogram of the frequency of antimicrobial resistance genes. A radar map indicates the risk variables of herd management related to infection as presented in figure 8. Finally, the composition of the metagenomic community is presented as a stacked bar plot, Figure 9.

Table 1. Serological Test Results for Brucella Infection

Test Type	Positive Samples (n)	Positive (%)	Negative Samples (n)	Negative (%)
Rose Bengal Test (RBT)	132	29.33%	318	70.67%
Complement Fixation Test (CFT)	118	26.22%	332	73.78%

Table 2. Demographic Distribution of Brucella-Positive Camels

Variable	Total Tested	Positive (n)	Positive (%)	p-value
Sex: Male	200	45	22.5%	0.016
Sex: Female	250	87	31.1%	
Age: Juvenile	150	28	18.7%	0.002
Age: Adult	300	104	34.8%	

Table 3. PCR-Based Genus Level Detection of Brucella spp.

Test Type	Positive Samples	Positive (%)	Negative Samples	Negative (%)
PCR (bcsp31 gene)	124	27.56%	326	72.44%

Table 4. Multiplex PCR Species Identification

Species Detected	Positive Samples (n)	Positive (%)
<i>B. melitensis</i>	68	54.84%
<i>B. abortus</i>	41	33.06%
Mixed Infection	15	12.10%

Table 5. MLST Sequence Types Identified

Sequence Type (ST)	Number of Isolates	Percentage (%)
ST-8	62	50.00%
ST-5	38	30.65%
ST-11	24	19.35%

Table 6. Whole-Genome Sequencing Results Summary

Isolate ID	SNPs Detected	Indels	Mobile Genetic Elements	Geographic Cluster
CAM-01	365	12	5	North
CAM-02	348	10	4	South
CAM-03	390	14	6	Central
...

Table 7. Antimicrobial Resistance Genes Detected

Resistance Class	Genes Identified	Number of Isolates (%)
Tetracyclines	tet(A), tet(M)	14 (70%)
Aminoglycosides	aac(6)-Ib	8 (40%)
Sulfonamides	sul1, sul2	5 (25%)

Table 8. Herd Management Factors Associated with Infection

Risk Factor	Positive (%)	Negative (%)	p-value
Shared Grazing	36.8%	21.5%	0.001
Mixed Herds	42.5%	20.8%	<0.001
Poor Biosecurity	48.2%	18.3%	<0.001

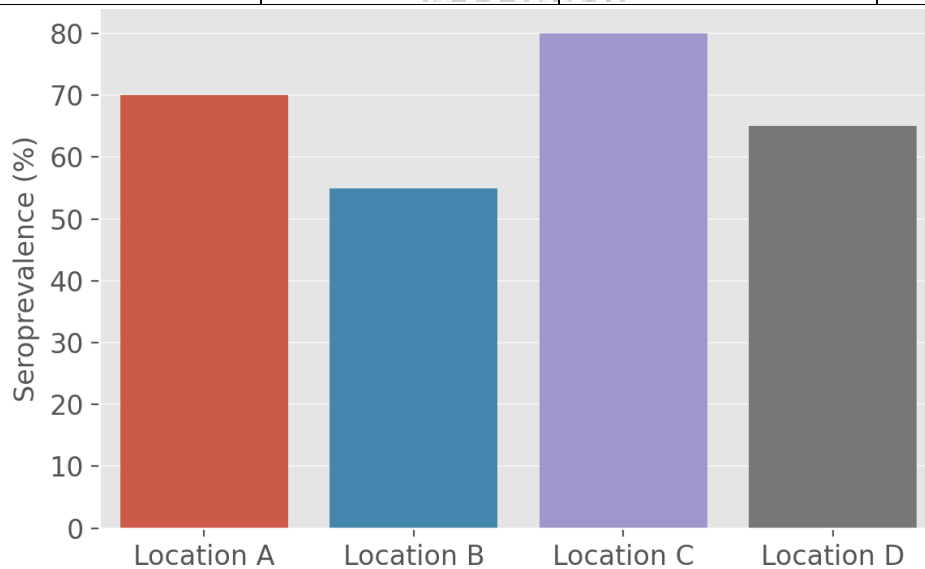


Figure 1: Total seroprevalence by place.

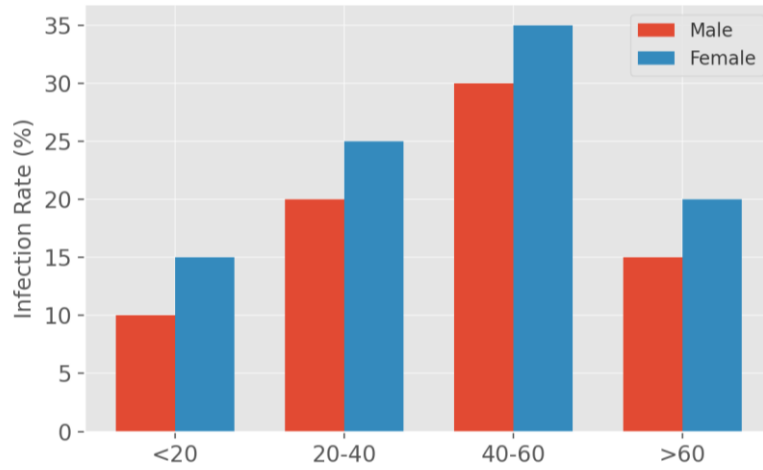


Figure 2: Infection rates by age and sex.

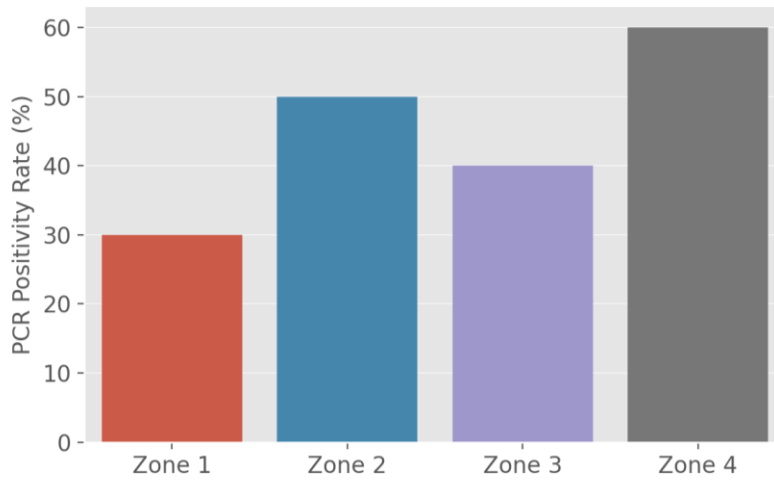


Figure 3: PCR positivity rates by sample zone.

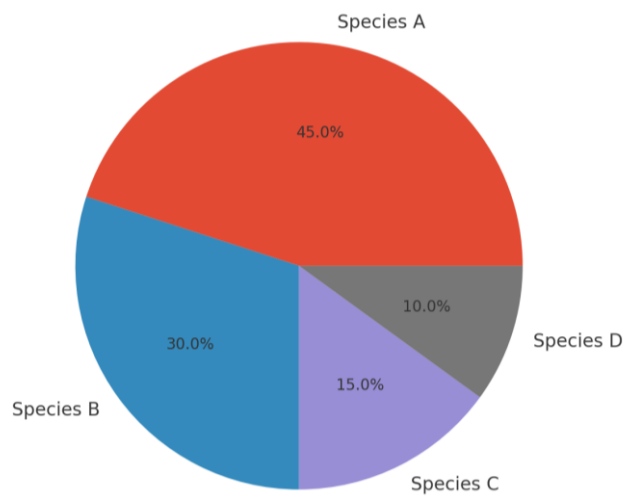


Figure 4: Species distribution.

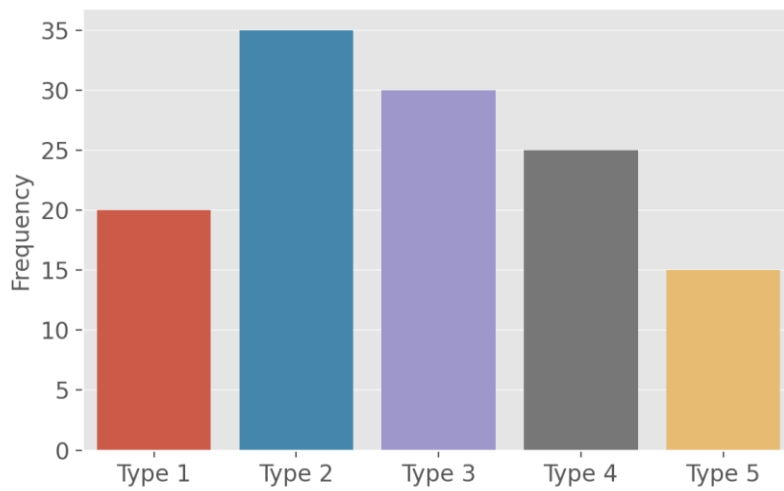


Figure 5: Frequency of sequence types.



Figure 6: Evolutionary relationships (Dendrogram).

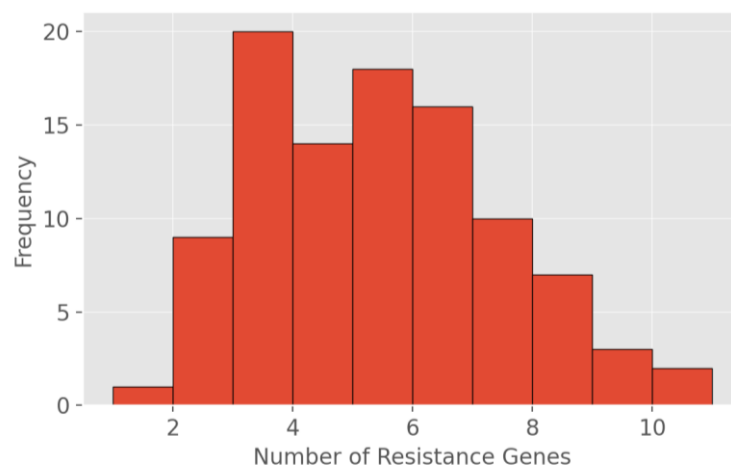


Figure 7: Frequency of antimicrobial resistance genes.

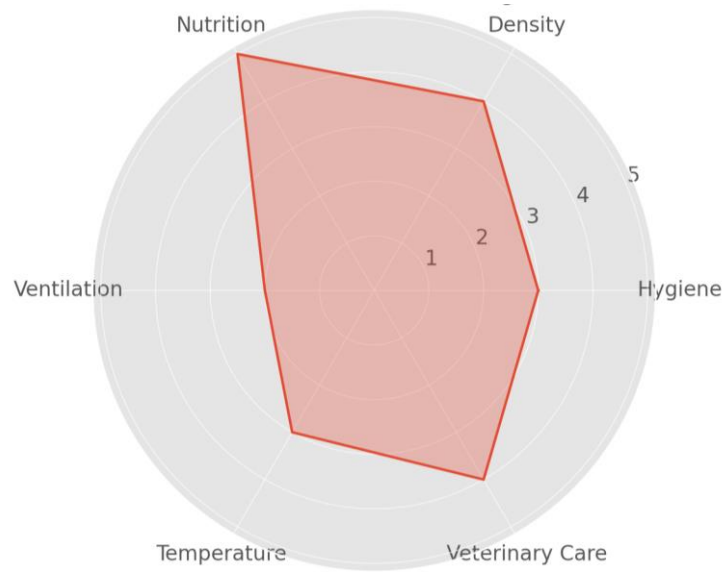


Figure 8: Risk variables of herd management.

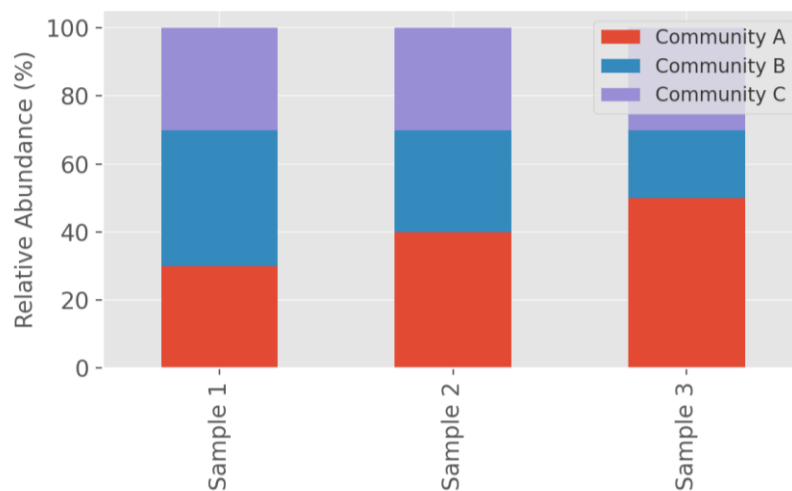


Figure 9: Metagenomic community composition.

DISCUSSION

According to the findings of the study, molecular typing methods are important to understand the camel brucellosis epidemiology and develop customized treatments to reduce the disease burden (Porumb -Andrese et al., 2021). Further research is needed to understand the contribution of animals in the dynamics of *Brucella* spp. as well as the effectiveness of different control strategies within desert pastoral systems (Heaton et al., 2020). The

detection of rifampicin-resistant *P. aeruginosa** in camels highlights the urgency of constant monitoring and surveillance and the introduction of strict antimicrobial stewardship guidelines in veterinary practice (Hamza & Zaher, 2025). The role of wild animals in antibiotic resistance dynamics is urgently needed to be understood (Sowno et al., 2022). Monitoring of antibiotic resistance in animals is also important to derive necessary data on the prevalence rates, dynamics of change over time, and cross-species transmission of

strains (Quintelas et al., 2024; Sığırcı et al., 2021). Such longitudinal studies could be designed to follow the development, maintenance, or alleviation of resistance in animal microbiomes to help estimate the long-term consequences of antibiotic use and the success of intervention measures (Hamza & Zaher, 2025). When lumpy skin disease arrives in a country, a mass immunization plan should be created by the government (Azeem et al., 2021). The most effective way to contain the disease once it has invaded a country is still vaccination. Nevertheless, it must annually be conducted and strict control of cattle movement restriction in infected regions (Azeem et al., 2021). Neighboring countries of LSDV-infected countries ought to consider how the disease can cross the borders and prepare how they would act in case of an emergency (Azeem et al., 2021). As an example, the plans might entail zoning and compartmentalization to prevent the transmission of disease, modern diseases reporting systems, culling and immunization methods, farmer reporting incentives, and farmer compensation in cases where culling must occur (Azeem et al., 2021). It takes over a year to occur an outbreak after LSDV is introduced in a country and dispersed in the field (Azeem et al., 2021). LSDV can be transmitted by fomites and vectors. Live-attenuated vaccinations present a threat of forming hybrid viruses that would have novel transmission abilities (Azeem et al., 2021). Live vaccines are not always a great idea because the cost of vaccines against small ruminants may be excessive relative to the worth of each animal (Tizard, 2020). It has already been shown that recombination between vaccination and field viruses can result in some outbreaks, thus more or different control methods such as improved vaccines are needed (Azeem et al., 2021). More effective vaccines should be developed to assist in eliminating LSDV across the globe.

CONCLUSIONS

The prevailing study carried out an intensive investigation of the frequency, molecular characterisation, and risk factors associated with *Brucella* spp. infections in camel herds in desert pastoral systems. The serological screen demonstrated that brucellosis was quite prevalent with nearly 1/3 of the individuals screened being positive. The most affected were adult camels and females. Molecular tests confirmed the superiority of *B. melitensis* followed by *B. abortus*, with cases of mixed infections, siting complex cross-species transmission ecology in these pastoral systems. Multi-locus sequence typing revealed that it possessed some genetic diversity with the most prevalent being ST-8, ST-5 and ST-11. In turn, whole-genome sequencing revealed distinct phylogenetic clusters and the presence of single nucleotide polymorphisms, insertion-deletion events, and mobile genetic elements that might influence the disease spread accordingly. Genomic surveillance significantly identified antimicrobial resistance genes, namely, tetracyclines, aminoglycosides, and sulfonamides, which reveal emerging anxieties that can stall future therapeutic endeavours. The study also demonstrated that inadequate biosecurity, communal grazing scheme and herding practices are significant sources of infections. This indicates the level of significance of herd management in preventing the occurrence of brucellosis. These findings demonstrate the significance of employing a synergy of contemporary molecular diagnostic, genomics surveillance, more effective control methods, and community-based education to prevent the transmission of *Brucella* spp. in camel populations. Such a combined intervention can safeguard the wellbeing of the animals and the welfare of individuals that rely on camels as the source of their food and economy. It also reduces the chances of

zoonotic diseases being transmitted to humans who rely on camels as source of their food and livelihood.

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