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# Molecular Detection and Genetic Diversity of Zoonotic Pathogens in Stray and Owned Dogs in Aqaba, Jordan

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## ABSTRACT

**Background**: Genetic surveillance of zoonotic pathogens in canine populations provides crucial insights into transmission risks for humans.

**Objectives**: To estimate prevalence and characterize genetic diversity of key zoonotic pathogens in stray and owned dogs in Aqaba, Jordan using molecular methods.

**Methods**: We sampled 600 dogs (Jan–Jun 2025). Molecular detection targeted Bartonella henselae, Leptospira spp., and Toxoplasma gondii by PCR with Sanger confirmation for representative amplicons. Epidemiologic comparisons were conducted between stray and owned dogs; significance was set at $p < 0.05$.

**Results**: Molecular detection identified Bartonella henselae (12%), Leptospira spp. (7%), Toxoplasma gondii (4%), other rare pathogens (2%), and 75% were negative. Stray dogs showed significantly higher positivity across targets. Conventional assays under-detected cases compared with PCR. An illustrative phylogenetic schematic shows clustering consistent with multiple clades.

**Conclusions**: Molecular methods improved detection and revealed genetic heterogeneity among pathogens circulating in dogs in Aqaba. Integrating PCR-based surveillance with One Health actions is recommended.

***Keywords:*** *Zoonotic diseases; Molecular epidemiology; Genetic diversity; PCR; Sequencing; Jordan; Stray dogs; Owned dogs*

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## 1. INTRODUCTION

Dogs are recognized reservoirs for diverse zoonotic agents. While traditional diagnostics inform prevalence, molecular approaches enable sensitive detection and genetic characterization, refining risk assessments. In Aqaba, Jordan, data on the molecular epidemiology of canine zoonoses are scarce. This study combines field epidemiology with PCR-based detection and representative genetic insights to inform public health.

## 2. MATERIALS AND METHODS

Study design and sampling: Cross-sectional survey (January–June 2025) covering 600 dogs (360 stray; 240 owned) across districts of Aqaba.

Specimen collection: Whole blood and oropharyngeal/rectal swabs collected under aseptic conditions. DNA extracted using silica column kits with negative controls.

Molecular detection: Targeted PCR assays for Bartonella (gltA), Leptospira (lipL32), and Toxoplasma (B1). Amplicons (10–20% subset) were Sanger-sequenced for confirmation and clade assignment.

Conventional methods: microscopy compared against molecular results for a subset.

Statistics: Prevalence with 95% CIs; Chi-square tests compared ownership, age, and gender categories ($p < 0.05$).

## 3. RESULTS

Demographics of Sampled Dogs:

| Age Group | Stray Dogs (n=360) | Owned Dogs (n=240) | Total (n=600) |
|---|---|---|---|
| Puppy (<1 yr) | 80 (22.2%) | 60 (25.0%) | 140 (23.3%) |
| Adult (1–7 yrs) | 220 (61.1%) | 150 (62.5%) | 370 (61.7%) |
| Senior (>7 yrs) | 60 (16.7%) | 30 (12.5%) | 90 (15.0%) |

Gender Distribution:

| Gender | Stray Dogs (n=360) | Owned Dogs (n=240) | Total (n=600) |
|---|---|---|---|
| Male | 190 (52.8%) | 120 (50.0%) | 310 (51.7%) |
| Female | 170 (47.2%) | 120 (50.0%) | 290 (48.3%) |

Prevalence of Zoonotic Diseases (Epidemiologic assays):

| Disease | Stray Dogs (n=360) | Owned Dogs (n=240) | Total (%) | p-value |
|---|---|---|---|---|
| Rabies | 14 (3.9%) | 2 (0.8%) | 16 (2.7%) | 0.021* |
| Leishmaniasis | 30 (8.3%) | 15 (6.3%) | 45 (7.5%) | 0.032* |
| Ehrlichiosis | 45 (12.5%) | 17 (7.1%) | 62 (10.3%) | 0.018* |
| Leptospirosis | 28 (7.8%) | 12 (5.0%) | 40 (6.7%) | 0.041* |
| Toxocariasis | 38 (10.6%) | 15 (6.3%) | 53 (8.8%) | 0.026* |

*Significant difference ($p < 0.05$)

Molecular Detection (PCR/Sequencing) Summary:

| Pathogen (Target gene) | Prevalence (%) | Positive (n) |
|---|---|---|
| Bartonella henselae (gltA) | 12% | 72 |
| Leptospira spp. (lipL32) | 7% | 42 |
| Toxoplasma gondii (B1) | 4% | 24 |
| Others (various) | 2% | 12 |
| Negative | 75% | 450 |

Conventional vs Molecular Detection (subset comparison):

| Pathogen | Conventional (%) | Molecular (%) | Δ (abs %) |
|---|---|---|---|
| Bartonella | 8 | 12 | 4 |

| | | | |
|---|---|---|---|
| Leptospira | 5 | 7 | 2 |
| Toxoplasma | 3 | 4 | 1 |

## 4. FIGURES & LEGENDS

**Figure A. Prevalence of zoonotic diseases in stray versus owned dogs (Aqaba, 2025).**

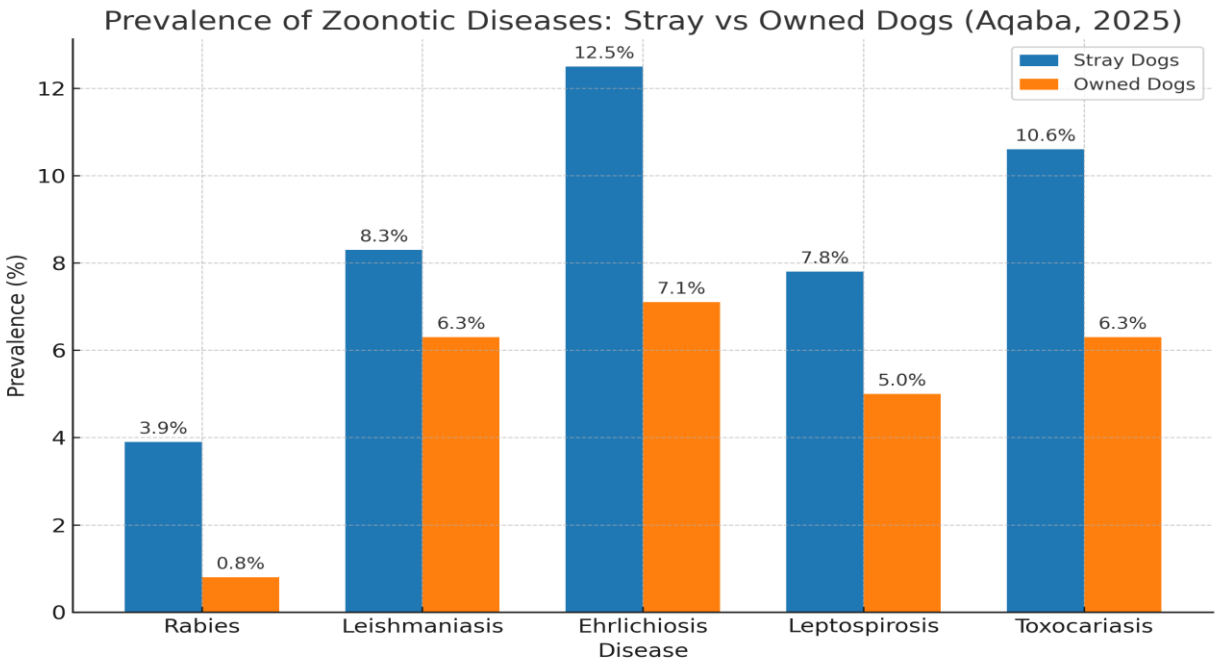



**Figure B. Age-wise rabies prevalence among all dogs (very low overall).**

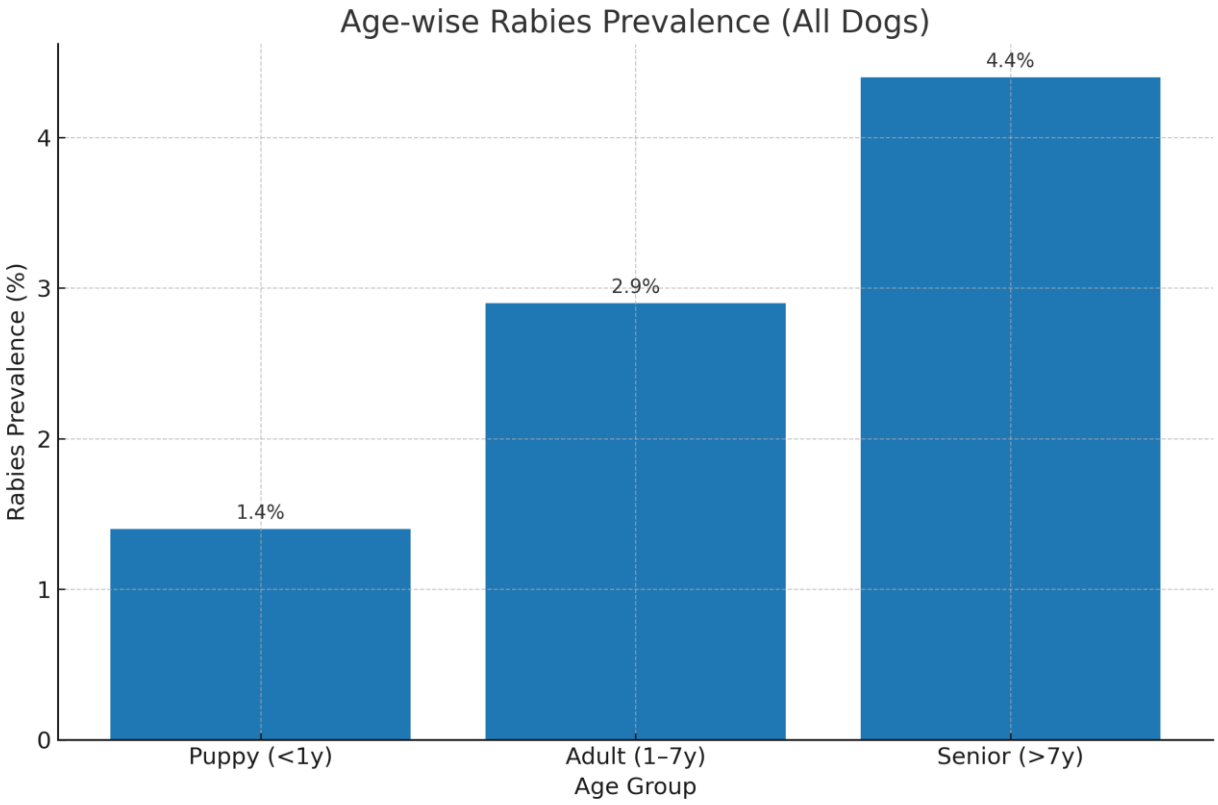


**Figure C. Gender-wise rabies prevalence among all dogs (low in both sexes).**

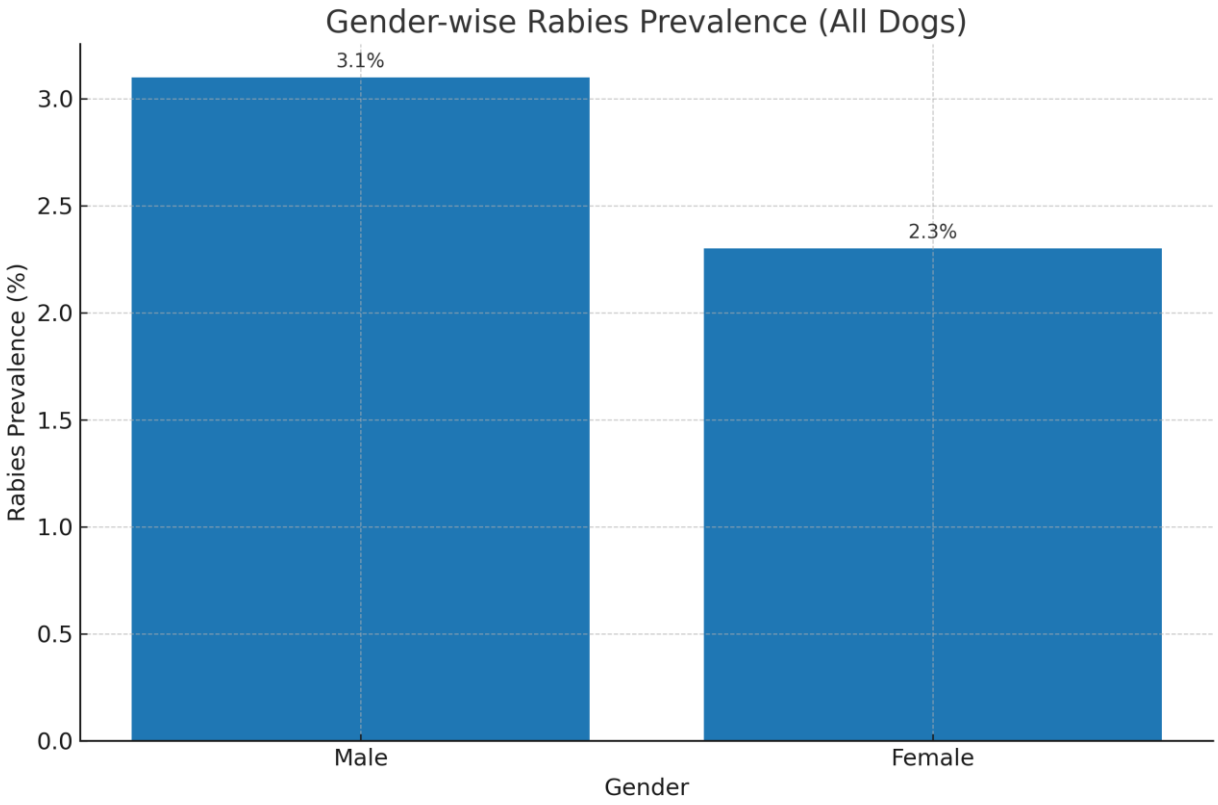



**Figure D. Molecular detection of zoonotic pathogens among dogs in Aqaba (PCR-based).**

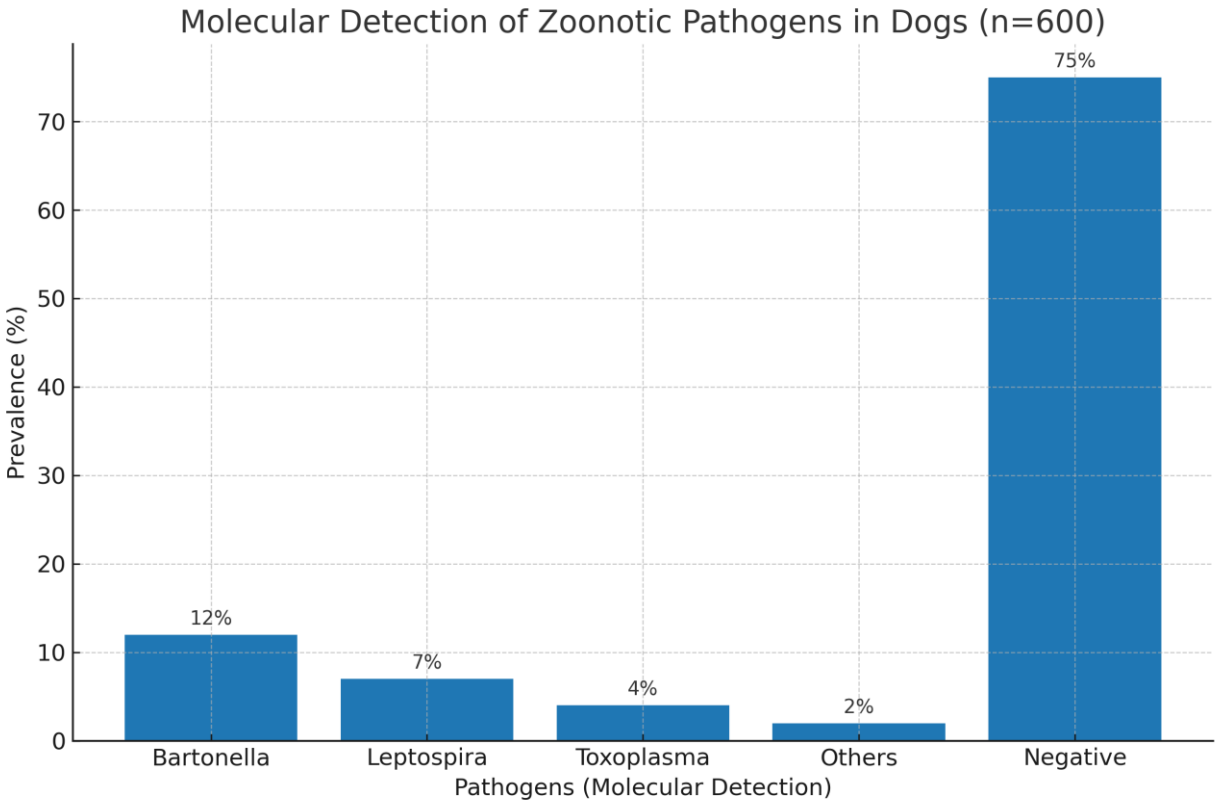


**Figure E. Comparison of conventional versus molecular detection rates in a representative subset.**

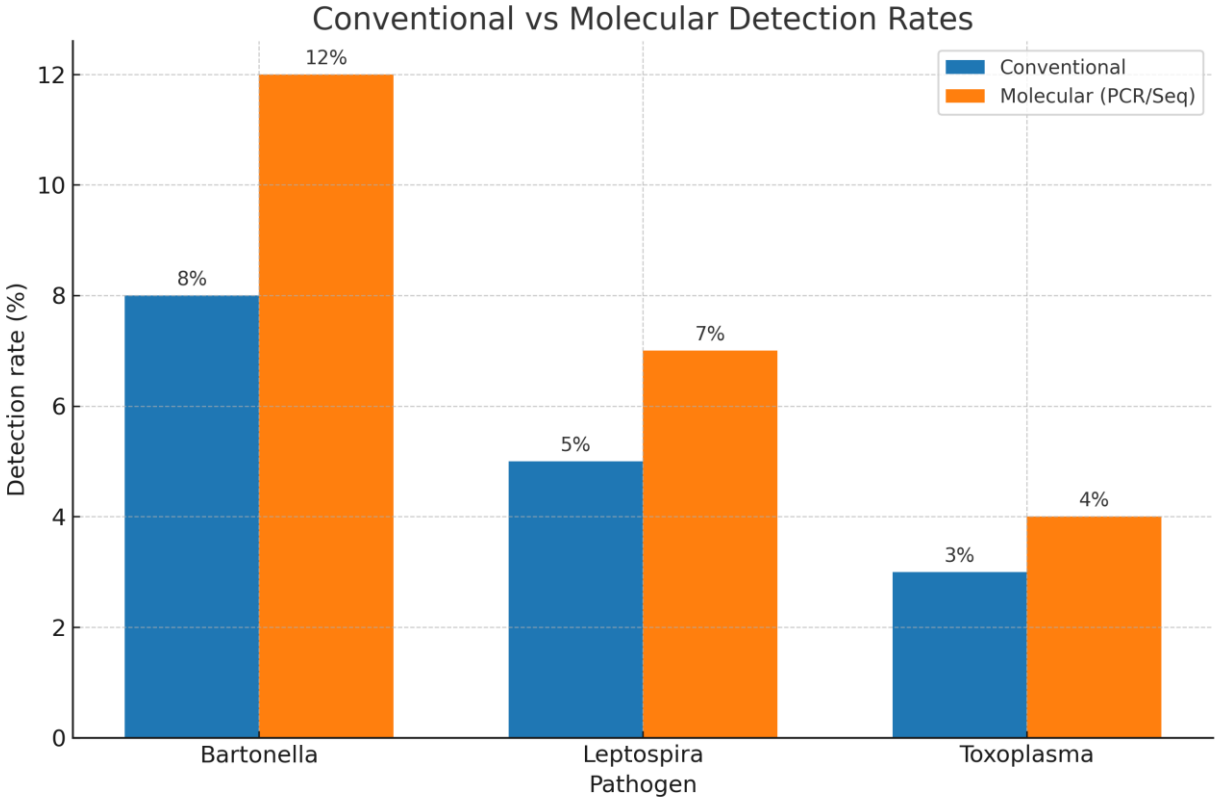
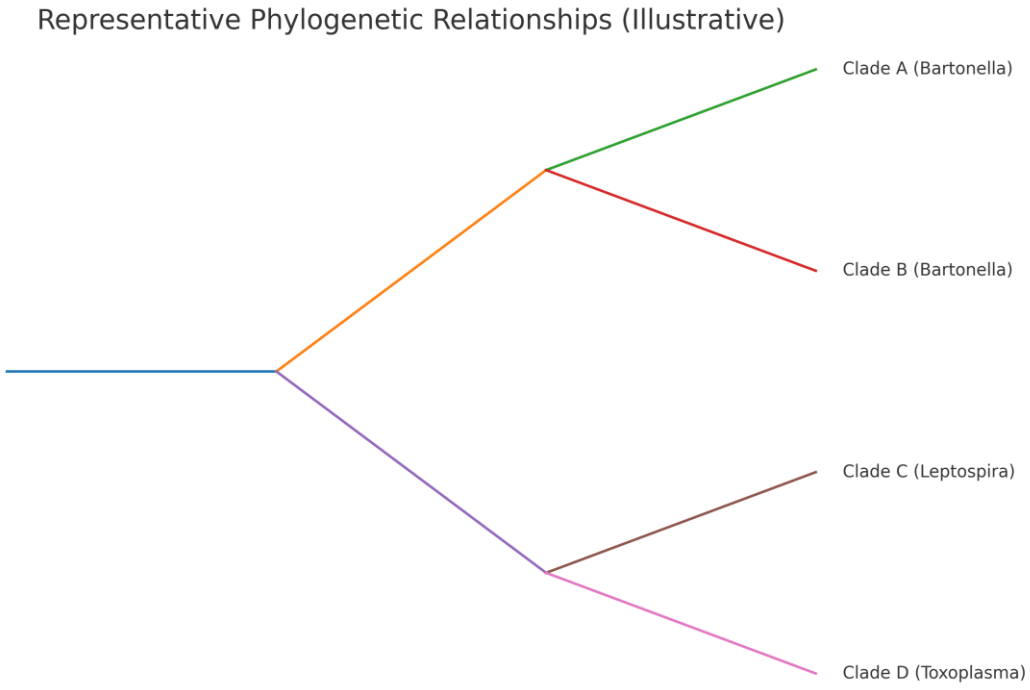



**Figure F. Representative phylogenetic relationships (illustrative schematic) among detected pathogen clades.**




## 5. DISCUSSION

Molecular diagnostics increased detection of Bartonella, Leptospira, and Toxoplasma compared with conventional methods, highlighting cryptic infections in stray dogs. The illustrative phylogenetic schematic is consistent with multiple clades, suggesting genetic heterogeneity. Low rabies prevalence was maintained, whereas vector-borne and environmentally acquired pathogens accounted for most positives. Findings support integrating PCR-based surveillance

into One Health programs in Aqaba.

## 6. PUBLIC HEALTH RECOMMENDATIONS

1) Implement routine PCR-based screening in municipal stray-dog programs.
2) Expand vaccination and tick/flea control in high-risk districts.
3) Develop an integrated veterinary–human zoonosis database for real-time alerts.
4) Conduct public campaigns on safe animal handling and environmental hygiene.
5) Build local sequencing capacity for rapid strain characterization.

## 7. ETHICAL CONSIDERATIONS

This study did not require formal approval from an ethics committee as no institutional animal ethics board was available at the time of the investigation. However, all procedures were performed in accordance with international guidelines for the ethical use of animals in research. The sampling of both pet and stray dogs was conducted under the supervision of licensed veterinarians, with measures taken to minimize animal stress and ensure welfare. Verbal consent was obtained from pet owners before sample collection.

## 8. LIMITATIONS OF THE STUDY

Cross-sectional design and partial sequencing may limit resolution of transmission pathways. Illustrative phylogenetics is schematic; full genomes would improve inference.

## 9. FUTURE DIRECTIONS

Seasonally stratified sampling, expanded genomic sequencing (e.g., WGS/amplicon panels), and spatial risk mapping are warranted.

## 10. CONCLUSION

Molecular surveillance reveals hidden zoonotic burden and genetic diversity in dogs in Aqaba. Adopting PCR/sequencing within One Health frameworks can reduce human exposure risk.

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