

## Molecular Epidemiology and Genetic Diversity of Zoonotic Pathogens in Domestic and Wild Animals in Pakistan

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### Abstract

Pakistan, positioned at the crossroads of South Asia, Central Asia, and the Middle East, harbors exceptional ecological and epidemiological diversity that facilitates the circulation and emergence of zoonotic pathogens across domestic livestock, wildlife, and human populations. This review synthesizes molecular epidemiological data and genetic diversity studies on key zoonotic agents in Pakistani animals, focusing on high-burden pathogens including *Brucella* spp., *Coxiella burnetii* (Q fever), *Toxoplasma gondii*, *Theileria* spp. (theileriosis), *Babesia* spp., Crimean-Congo hemorrhagic fever virus (CCHFV), and tick-borne rickettsiae. Livestock particularly small ruminants (sheep and goats), cattle, and camels serve as primary reservoirs, with prevalence often exceeding 20–50% in endemic regions, driven by communal grazing, transhumance, communal water sources, and close human–animal contact. Molecular tools (PCR, qPCR, MLST, whole-genome sequencing, and phylogenetics) reveal significant genetic heterogeneity: multiple *Brucella abortus* and *B. melitensis* biovars, diverse *T. gondii* genotypes (predominantly types I and III with atypical recombinant strains), and geographically structured CCHFV clades

linked to *Hyalomma* ticks. Wildlife (e.g., rodents, bats, wild ungulates) acts as spillover/amplification hosts for several agents, while interspecies transmission is amplified by pastoral practices and climate-driven vector range expansion. Economic losses from tick-borne diseases alone approach USD 200 million annually, compounded by public health risks from under-diagnosed human cases. The review

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highlights critical surveillance gaps, the need for One Health-integrated molecular monitoring, and the importance of genotyping for tracing transmission dynamics and informing targeted control strategies in this high-risk zoonotic hotspot.

## **Introduction**

### **Evolutionary and Epidemiological Context of Zoonoses in South Asia**

The Islamic Republic of Pakistan represents a critical geographic and biological nexus for the study of emerging and re-emerging zoonotic diseases (Ijaz et al., 2023). Situated at the crossroads of South Asia, Central Asia, and the Middle East, the country's diverse topography ranging from the high-altitude Karakoram and Himalayan ranges in the north to the fertile Indus River plains and the arid coastal regions of the south creates a myriad of ecological niches for various pathogens (Munir et al., 2022). With a massive livestock population that includes approximately 37.2 million sheep as of 2024, Pakistan ranks third globally in small ruminant density, trailing only China and India (Chughtai et al., 2023). This vast animal resource is not only a cornerstone of the national economy but also a significant biological reservoir for pathogens that pose a threat to both animal welfare and human health (Afzal et al., 2023).

The economic implications of zoonotic diseases in Pakistan are profound. Annual losses in the livestock sector due to tick infestations and tick-borne diseases (TBDs) are estimated at approximately USD 200 million (Sajid et al., 2023). These losses are driven by decreased milk production, weight loss, hides damage, and the high cost of veterinary interventions, particularly in a climate where environmental conditions favor the year-round survival and reproduction of vectors (Usmani et al., 2023). Approximately 60% of all recognized infectious diseases in humans are zoonotic, and a staggering 75% of emerging infectious diseases originate from wildlife or domestic animals (Taylor et al., 2001). Factors such as rapid urbanization, deforestation, globalization, and the close proximity of humans to their livestock facilitate the "spillover" of these pathogens (Hassell et al., 2017).

Molecular epidemiology has emerged as the definitive tool for deciphering the complex dynamics of these infections (Riley et al., 2018). By moving beyond traditional serological assays, which often lack the specificity to distinguish between closely related strains, researchers in Pakistan are increasingly utilizing advanced techniques such as Real-time Polymerase Chain Reaction (PCR), Multi-Locus Sequence Typing (MLST), and Whole-Genome Sequencing (WGS) (Rehman et al., 2024). These tools allow for the identification of genetic diversity, the tracking of transboundary movement, and the understanding of host-pathogen interactions at a resolution previously unattainable (Parreira, 2018). The epidemiology of zoonotic diseases in Pakistan is driven by the complex interaction between humans, animals, vectors, and the environment. Figure 1 illustrates the One Health interface that facilitates pathogen transmission among livestock, wildlife, and human populations in the region.

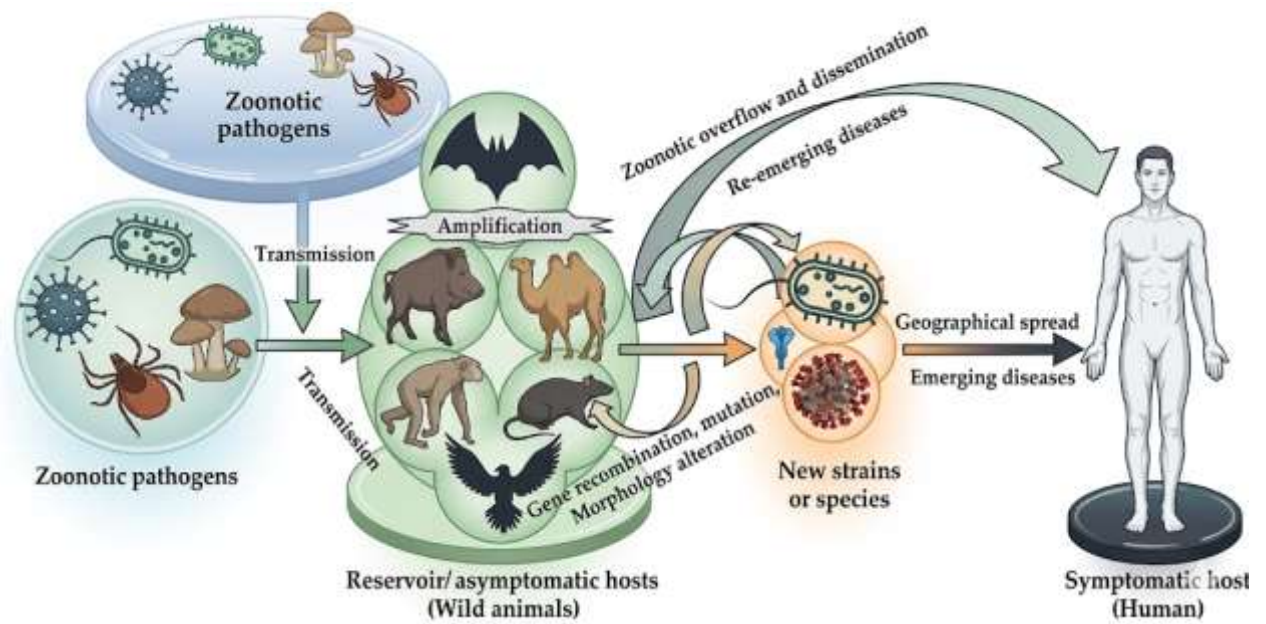


Figure 1: One Health Interface of Zoonotic Pathogens in Pakistan

**Molecular Surveillance of Bacterial Zoonoses**  
**Genomic Characterization of Brucella Species**

Brucellosis remains one of the most significant and under-reported bacterial zoonoses in Pakistan (Ali et al., 2023). Caused by the Gram-negative, facultative intracellular bacterium of the genus *Brucella*, the disease manifests in animals through reproductive disorders such as abortion, infertility, and mastitis, while in humans, it presents as undulant fever and potential long-term complications (Gul & Khan, 2007). The molecular landscape of brucellosis in Pakistan is dominated by *Brucella abortus* in cattle and buffaloes and *Brucella melitensis* in sheep, goats, and humans (Jamil et al., 2021).

Recent molecular investigations have refined the understanding of prevalence beyond historical serological data. For example, in upper Punjab districts such as Narowal, Gujranwala, and Gujrat, Real-time PCR targeting the IS711 insertion sequence identified *B. abortus* DNA in 7.71% of bovine sera, even when seroprevalence rates (via i-ELISA) were recorded as high as 17.7% (Naveed et al., 2024). This discrepancy highlights the importance of molecular confirmation to distinguish between active infections and previous exposure (Shabbir et al., 2021). Furthermore, the lack of standardized vaccination programs and uncontrolled animal movement across provincial borders continue to exacerbate the spread of these specific genotypes (Sadiq et al., 2024).

Table 1. Molecular prevalence and genotypes of Brucella species in Pakistani hosts.

| Host Species   | Diagnostic Method     | Molecular Prevalence (%) | Identified Genotype/Lineage      |
|----------------|-----------------------|--------------------------|----------------------------------|
| Cattle         | Real-time PCR (IS711) | 3.1% - 13%               | East Mediterranean Lineage (ST2) |
| Buffalo        | Real-time PCR (S11)   | 7.7% - 47.1%             | East Mediterranean Lineage       |
| Sheep/Goat     | Duplex PCR            | 15%                      | East Mediterranean Lineage (ST8) |
| Human (Female) | Duplex PCR            | 10%                      | Linked to direct cattle contact  |
| Human (Male)   | Duplex PCR            | 2%                       | Linked to indirect contact       |

The genetic diversity of *Brucella* in Pakistan is inextricably linked to the East Mediterranean lineage (Imran et al., 2024). Phylogenetic analysis using Multiple-Locus Variable-number tandem repeat Analysis (MLVA-16) has provided quantitative evidence of transboundary movement. Pakistani *B. abortus* isolates demonstrate a high degree of genetic relatedness (often only one or two gene differences) with strains from India, China, and Iran (Musallam et al., 2025). This suggests that informal cross-border animal trade and the nomadic movement of livestock are primary drivers of pathogen persistence in the region. Furthermore, the identification of ST2 as the dominant sequence type for *B. abortus* and ST8 for *B. melitensis* aligns Pakistani isolates with broader regional trends in Turkey and Kazakhstan (Zulfigar et al., 2023).

A nuanced analysis of risk factors reveals that physiological production stress plays a significant role in transmission dynamics. In bovines, lactation status is the highest significant factor for infection; lactating animals exhibit a significantly higher prevalence (22.35%) compared to non-lactating ones (2.46%) (Mahmood et al., 2024). This is likely due to the affinity of brucellae for erythritol in the gravid uterus and the sequestration of the bacteria in supramammary lymph nodes, which can reactivate during the stress of milk production (Younas et al., 2024). In human populations, particularly in rural Khyber Pakhtunkhwa, the molecular prevalence of brucellosis (9%) is significantly higher than in urban centers, with females showing a higher infection rate (10%) due to their traditional roles in milking and animal husbandry (Shah et al., 2022).

### **Mycobacterium tuberculosis Complex (MTBC) in the Food Chain**

The paradigm of bovine tuberculosis (bTB) in Pakistan is undergoing a significant shift as molecular tools reveal a more complex host range. While *Mycobacterium bovis* has long been suspected as the primary agent of bTB, recent WGS-based studies at major public slaughterhouses in Lahore have identified other members of the *Mycobacterium tuberculosis* complex (MTBC) in slaughtered cattle and buffaloes (Zubair et al., 2024).

The identification of *Mycobacterium orygis* and *Mycobacterium tuberculosis* sensu stricto in animals declared "fit for slaughter" presents a dual public health challenge (Malik et al., 2024). *M. orygis*, initially associated with non-human primates and oryx, is now recognized as a potent zoonotic agent in South Asian livestock (Rahim et al., 2023). WGS of Pakistani *M. orygis* isolates, when compared to 93 global sequences, showed close phylogenetic clustering with strains circulating in both animals and humans in the region, suggesting a widespread but under-recognized presence (Ullah et al., 2020).

Perhaps more concerning is the detection of *M. tuberculosis* sensu stricto in bovines, which serves as a definitive marker of reverse zoonosis (anthropozoonosis) (Ahmed et al., 2025). This implies that human-to-animal transmission is occurring in densely populated areas, potentially creating new animal reservoirs that can then re-infect humans through contaminated meat or unpasteurized dairy products (Hashim et al., 2024).

### **Pasteurella multocida and Antimicrobial Resistance**

emorrhagic septicemia (HS) remains a leading cause of sudden death in Pakistani bovines, particularly buffaloes, which show a higher vulnerability (8.3%) than cows (6.7%) (Hassan et al., 2024). Molecular characterization of isolates from Punjab has confirmed the dominance of Serotype B, but the real clinical concern lies in the profile of antibiotic resistance (Abbas et al., 2023). Isolates have shown resistance to commonly used veterinary antibiotics, and molecular screening has identified specific resistance genes that are transmissible through horizontal gene transfer (Arshad et al., 2025).

The risk of these zoonotic bacterial pathogens passing antimicrobial resistance (AMR) markers to human microflora is a critical concern, especially in regions with poor sanitation and close animal-human proximity (Rabbani et al., 2024).

### **Soil-Borne Pathogens: *Bacillus anthracis* and *Coxiella burnetii***

The environmental persistence of soil-borne zoonotic pathogens in Pakistan is heavily influenced by soil chemistry and land-use patterns. A multi-disciplinary study in the Lahore district utilized molecular detection to determine the prevalence of *Bacillus anthracis* (CapB gene), *Burkholderia mallei*, *Coxiella burnetii* (IS1111 transposase gene), and *Francisella tularensis* in soil (Khan et al., 2015).

**Table 2. Molecular prevalence and soil association factors of soil-borne pathogens in Lahore.**

| <b>Soil-Borne Pathogen</b> | <b>Molecular Prevalence (%)</b> | <b>Key Soil Association Factors</b>  |
|----------------------------|---------------------------------|--------------------------------------|
| B. anthracis               | 9.6%                            | Organic matter, Mg, Ca, Mn, Fe, K    |
| F. tularensis              | 13.1%                           | Phosphorus (P), Proximity to markets |
| C. burnetii                | 4.8%                            | Mg, Co, Na, Fe, Ca, K                |
| B. mallei                  | 1.4%                            | Chromosomal gene detection           |

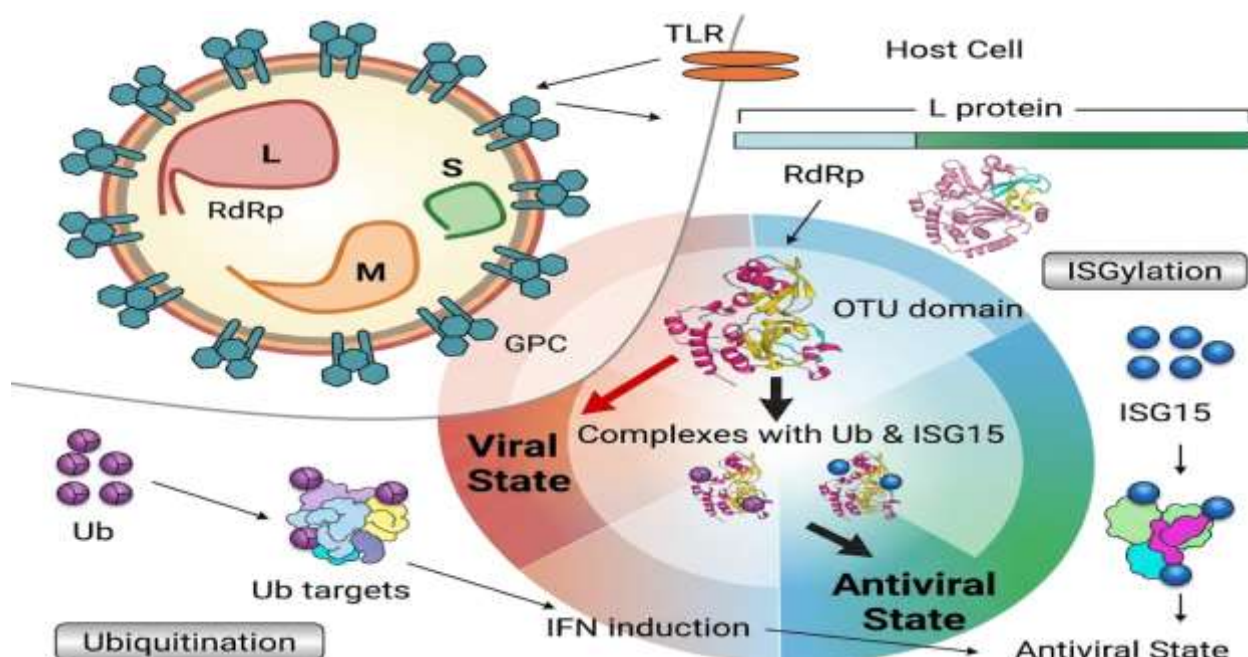
The distribution pattern suggests that urban and peri-urban areas of Lahore are high-risk zones. The odds of detecting *F. tularensis* were found to be 2.7 to 4.1 times higher in sites located more than 1 km from animal markets and 500 meters from vehicular traffic, possibly indicating that these pathogens thrive in less disturbed soils with specific mineral compositions (Khan et al., 2021).

Furthermore, *Coxiella burnetii*, the causative agent of Q fever, exhibits a high burden in the livestock sector. Sheep have the highest mean blood prevalence (41.23%), followed by goats (38.86%) and camels (31.30%) (Muema, 2017). The molecular evidence of *C. burnetii* in raw meat (33.2% in sheep) and environmental samples (16.97%) reinforces the zoonotic risk, particularly as the pathogen can withstand high temperatures and desiccation, enhancing its stability in the Pakistani climate (Khan et al., 2024).

### **Genetic Diversity and Evolution of Viral Zoonoses**

#### **Crimean-Congo Hemorrhagic Fever Virus (CCHFV)**

Crimean-Congo Hemorrhagic Fever is arguably the most lethal viral zoonosis currently circulating in Pakistan, with Balochistan province serving as a geographic hotspot (Nasir et al., 2024). The virus, a member of the Nairovirus genus, possesses a tripartite RNA genome comprising Large (L), Medium (M), and Small (S) segments (Mughal et al., 2023). Molecular epidemiology has revealed that the Asia-1 genotype (Clade IV) is the dominant variant in Pakistan, maintaining a consistent presence with approximately 99% nucleotide identity between strains isolated from 2017 through 2023 (Tariq et al., 2024). Genetic diversity in CCHFV is strongly influenced by genome segment reassortment during co-infection events. Figure 2 illustrates the tripartite genome structure of CCHFV and the mechanism of genomic reassortment, which contributes to the emergence of new viral variants.



**Figure 2: Genomic Structure and Reassortment of Crimean-Congo Hemorrhagic Fever Virus**

However, WGS has uncovered a more dynamic evolutionary landscape than previously understood through partial sequencing (Zohaib et al., 2025). Reassortment the process where segments are exchanged between different strains during co-infection is a significant driver of genetic diversity in Pakistani CCHFV (Ijaz et al., 2023).

**Table 3. Genomic segment reassortment patterns of CCHFV isolates in Pakistan.**

| Isolate ID       | Year/Location   | Segment Reassortment Pattern | Phylogenetic Linkage     |
|------------------|-----------------|------------------------------|--------------------------|
| NIH-PAK-CCHF-233 | 2019/Rawalpindi | M (Africa-2); L/S (Asia-1)   | India (2016-2019)        |
| NIH-PAK-CCHF-84  | 2018/Peshawar   | S/M (Asia-2); L (Asia-1)     | Tajikistan, China, India |
| NIH-PAK-CCHF-43  | 2018/Islamabad  | S (Asia-2); L/M (Asia-1)     | Oman, Iran, Afghanistan  |
| NIH-PAK-CCHF-20  | 2019/Rawalpindi | M (Asia-2); S/L (Asia-1)     | India, Tajikistan        |

The presence of the Africa-2 genotype M segment in a 2019 Rawalpindi isolate suggests the potential introduction of genomic components from distant geographic regions, likely mediated by the long-distance carriage of infected ticks via migratory birds or international livestock trade (Saeed et al., 2026). These reassorted strains cluster closely with isolates from India, Iran, and Tajikistan, pointing toward a highly interconnected regional viral pool (Javed et al., 2025). The high case-fatality rate (35%) observed in suspected CCHF cases emphasizes the urgency for continuous genomic surveillance to detect shifts in virulence or transmission efficiency (Farooq et al., 2026).

### Avian Influenza and Newcastle Disease Viruses

In Pakistan's extensive poultry sector, H9N2 Influenza A virus (IAV) has transitioned from an epidemic agent to an enzootic one. Molecular evidence from live bird markets (LBMs) in Punjab shows that H9N2 is present in 6.3% of poultry pools, and significantly, these isolates belong to the G1-like lineage (A/quail/Hong Kong/G1/97) (Shabeer et al., 2023). This lineage is of particular concern because it contains internal genes that have been donor segments for other highly pathogenic strains and has a

demonstrated capacity for human infection (Iqbal et al., 2026). Indeed, a 2024 study detected H9N2 nasal colonization in 0.83% of poultry workers, with human isolates clustering phylogenetically with the H9N2 strains found in the birds they handled (Gray et al., 2024).

The co-circulation of H9N2 with Highly Pathogenic Avian Influenza (HPAI) H5N1 and H5N8 (Clade 2.3.4.4b) creates a volatile environment for viral reassortment. In 2023, outbreaks in poultry flocks showed 80-100% mortality, with whole-genome sequencing revealing the presence of H5N8 strains that had undergone reassortment, potentially increasing their environmental fitness (Zohaib et al., 2024). Similarly, Newcastle Disease Virus (NDV) remains a major threat, with Genotype VII.1.1 circulating in commercial layers and Genotype VII.2 identified in wild birds like peacocks and pheasants in zoos (Siddique et al., 2025). The genetic relatedness (0.05–0.43% nucleotide divergence) between domestic and wild bird isolates suggests frequent cross-species transmission, undermining vaccination efforts in the poultry industry (Butt et al., 2024).

### **Rabies and the Challenge of Unvaccinated Populations**

Rabies remains a significant public health threat in Pakistan, particularly in rural areas where approximately 70 dog-bite victims seek medical care daily at major centers (Hassan et al., 2024). The National Rabies Control Programme (NRCP) indicates that a critical gap in control is the vaccination of the dog population; 54.7% of dogs involved in human biting incidents are unvaccinated (Fatima, 2025). While bats are globally recognized as reservoirs for rabies-related lyssaviruses, the overwhelming majority of human rabies cases in Pakistan are dog-mediated, leading to an estimated 50,000 fatalities per year. The lack of a national reporting system further complicates the accurate estimation of the rabies burden in both urban and rural settings (Qasim et al., 2025).

### **Protozoan and Parasitic Molecular Epidemiology**

#### **Toxoplasma gondii: Host Range and Genetic Conservation**

toxoplasmosis is highly prevalent in Pakistan, with humans identified as the most affected species (prevalence 22% to 60%) (Saeed et al., 2026). Molecular detection using the ITS-1 gene has provided insights into the phylogenetic position of Pakistani isolates (Tanveer et al., 2025). In goats from the Khanewal district, molecular prevalence was found to be 5.3%, a figure significantly lower than previous serological estimates (19-52%), suggesting that serology may overestimate active parasitic burden (Yasin et al., 2024).

Genetic analysis of the ITS-1 partial sequence indicates that *T. gondii* strains in Pakistani goats are highly conserved (Basit et al., 2025). Seasonal trends are also evident; molecular prevalence peaks during the summer months (8.8%), likely due to the higher survival rate of oocysts in warm, humid conditions (Ishaq et al., 2024). Risk factors for animal infection include the use of shared water pools and the proximity of other dairy animals, which facilitates the transmission cycle involving the definitive host (felids) and intermediate hosts (ruminants and humans) (Rasheed et al., 2025).

#### **Heterogeneity in Leishmania Species**

The epidemiology of Cutaneous Leishmaniasis (CL) in Pakistan is characterized by significant species heterogeneity. In North Eastern Pakistan, molecular characterization of 567 confirmed cases using internal transcribed spacer (ITS) gene amplification and RFLP analysis identified *Leishmania tropica*, *Leishmania major*, and *Leishmania infantum* (Shaheen et al., 2021).

**Table 4. Molecular heterogeneity and transmission forms of Leishmania species.**

| Leishmania Species | Nucleotide Variation (p-value) | Primary Transmission Form    |
|--------------------|--------------------------------|------------------------------|
| <i>L. tropica</i>  | 0.02                           | Anthroponotic                |
| <i>L. major</i>    | 0.05                           | Zoonotic (Rodent-associated) |
| <i>L. infantum</i> | Undetermined                   | Potential Canine Reservoir   |

The highest proportion of CL infection (4.85%) was found in the Mirpur district, with males in the 1-20 age group showing the highest incidence (Zaid et al., 2025). While CL in Pakistan has historically been considered anthroponotic (human-sandfly-human), molecular evidence now challenges this assumption. In the Khyber district, *L. tropica* DNA was detected in 11 cows, 6 goats, and 1 dog, suggesting these domestic animals may act as cryptic reservoirs for the parasite (Ullah et al., 2023). This heterogeneity and the emergence of new haplotypes are linked to treatment failures and the expanding geographic range of the disease (Khan et al., 2022).

### Small Ruminant Vector-Borne Parasites

Sheep and goats in Pakistan are frequently infected with tick-borne parasites that significantly impact productivity. Molecular screening of sheep breeds (e.g., Lohi, Kajli, Bluchi, and Damani) has identified *Anaplasma ovis* (20%) and *Theileria ovis* (21%) as the most prevalent agents (Shahzadi et al., 2025).

**Table 5. Molecular prevalence and associations of vector-borne pathogens in sheep.**

| Pathogen              | Prevalence in Sheep (%) | Herd Size Association | Breed Sensitivity          |
|-----------------------|-------------------------|-----------------------|----------------------------|
| <i>Anaplasma ovis</i> | 20%                     | Higher in large herds | Varies by breed            |
| <i>Theileria ovis</i> | 21%                     | Higher in small herds | Varies by breed            |
| <i>Anaplasma</i> sp.  | 11%                     | General distribution  | Consistent across sampling |

None of the sheep screened were positive for *Theileria lestoquardi*, the cause of malignant ovine theileriosis, suggesting that *T. ovis* (benign theileriosis) remains the dominant species in the studied districts (Al-Hamidhi et al., 2021). Phylogenetic analysis shows that these Pakistani isolates are genetically similar to global sequences, indicating the widespread distribution of these vector-borne pathogens across tropical and subtropical regions (Nangru et al., 2023).

### Wildlife Reservoirs and the Indus Flyway

#### Migratory Birds as Disease Vectors

Pakistan's wetlands serve as a critical wintering ground for millions of migratory birds navigating the Indus Flyway. These birds are increasingly implicated as vectors for the intercontinental dissemination of zoonotic pathogens and antimicrobial resistance genes (Sharif et al., 2022). Molecular evidence has demonstrated that migratory waterfowl carry highly pathogenic avian influenza subtypes (H5N1, H5N8) across flyways, with genetic analysis confirming bird-mediated transmission into Africa and South Asia (Umair et al., 2024).

Beyond viruses, migratory birds transport drug-resistant bacteria. *Escherichia fergusonii*, a multidrug-resistant pathogen, has been isolated from migratory birds in the Hazara Division (Zulfigar et al., 2023). These birds' uptake pathogenic bacteria from urban waste sites and shed them into pristine wetland environments, facilitating the horizontal transfer of beta-lactamase-producing genes. This "transport vehicle" role also extends to ectoparasites; migratory birds can carry ticks and lice infected

with CCHFV or *Borrelia* species across thousands of kilometers (Musallam et al., 2025).

### **The Role of Bats in Zoonotic Risk**

Bats in Pakistan are emerging as potential reservoirs for high-impact viral zoonoses, although research in this area remains limited compared to livestock studies. Species of the genus *Pteropus* (fruit bats), which are known carriers of Nipah virus (NiV), are found throughout the country (Zubair et al., 2024). While the NIH of Pakistan has categorized the current risk of NiV as low, the potential impact is high due to the virus's 40-75% case-fatality rate (Sajid et al., 2023). Environmental drivers such as deforestation and urbanization are increasing the frequency of bat-human interactions, particularly in areas where raw date palm sap or fruits partially eaten by bats are consumed (Riley et al., 2018).

### **One Health Framework and Future Directions**

The integration of human, animal, and environmental health the "One Health" approach is essential for the effective management of zoonotic diseases in Pakistan. In 2016, the establishment of the One Health Hub at the National Institute of Health (NIH) in Islamabad marked a foundational step in building multi-sectoral collaboration (Umair et al., 2023). The National One Health Strategic Framework priorities six diseases: anthrax, influenza, rabies, brucellosis, salmonellosis, and CCHF (Parreira, 2018).

### **Current Gaps and Strategic Priorities**

Despite these policy frameworks, significant gaps remain in the molecular surveillance of zoonotic pathogens. Research attention on "reverse zoonosis" and the impact of poor sanitation on pathogen flow between humans and animals is particularly limited (NIH Pakistan, 2023). There is an urgent need to:

**Expand Sentinel Surveillance:** The Global Influenza Hospital-based Surveillance Network (GIHSN) in Pakistan currently focuses on respiratory viruses (IAV, SARS-CoV-2, RSV). This model should be expanded to include enteric and vector-borne zoonoses.

**Implement Genomic Technologies:** WGS and MLST should be institutionalized as routine diagnostic tools rather than being reserved for sporadic research studies (Ali et al., 2021).

**Strengthen Transboundary Cooperation:** Given the molecular evidence of cross-border pathogen movement, formal data-sharing agreements with neighboring India, Afghanistan, and Iran are critical (Ali et al., 2021).

**Focus on the Wildlife-Human Interface:** Surveillance of bat populations and migratory bird stopovers is necessary to identify novel pathogens before spillover occurs (Sharif et al., 2022).

### **Conclusion**

Pakistan's unique biogeographic position and intensive human-livestock-wildlife interfaces create a persistent "perfect storm" for zoonotic pathogen maintenance and spillover, as evidenced by the substantial molecular epidemiological data on *Brucella*, *Coxiella*, *Toxoplasma*, *Theileria*, *Babesia*, CCHFV, and other agents. Genetic diversity analyses reveal complex transmission networks involving multiple genotypes, recombination events, and vector/host co-evolution, underscoring the inadequacy of single-host or single-pathogen control approaches. Domestic small ruminants and camels emerge as central reservoirs, while wildlife contributes to pathogen persistence and occasional introduction of novel variants. Annual economic losses in the hundreds of millions of dollars, combined with significant but under-reported human morbidity and mortality, demand urgent investment in integrated One

Health surveillance systems. Priorities include nationwide molecular genotyping networks, standardized diagnostics, cross-sectoral risk mapping, community-based education on hygienic practices, and vector control tailored to pastoral systems. Strengthening veterinary public health infrastructure, enhancing diagnostic capacity in remote areas, and fostering regional collaboration with neighboring countries will be essential to mitigate the dual burden of zoonotic diseases on animal production and human health. Ultimately, harnessing advanced molecular tools for real-time tracking and early warning embedded within a comprehensive One Health framework offers the most promising pathway to reduce zoonotic threats and safeguard Pakistan's livestock-dependent economy and population well-being in an era of accelerating environmental and climatic change.

## References

- Akbar, H., Saqib, M., & Hussain, M. (2024). Molecular epidemiology, genetic characterization, and the pattern of antibiotic resistance in *Pasteurella multocida* isolated from the livestock population in Punjab, Pakistan. *Veterinary Medicine and Science*. <https://doi.org/10.1080/23144599.2024.2437223>
- Ali, S., Neubauer, H., Melzer, F., Khan, I., Akhter, S., Jamil, T., & Umar, S. (2021). Molecular characterization of *Brucella abortus* and *Brucella melitensis* in cattle and humans in Pakistan. *Scientific Reports*, 11(1), 3113. <https://doi.org/10.1038/s41598-021-82625-x>
- Gray, G. C., Khan, A., Rehman, Z., & Ikram, A. (2024). H9N2 influenza A viruses found to be enzootic in Punjab Pakistan's bird markets with evidence of human H9N2 nasal colonization. *International Journal of Infectious Diseases*, 146, 107146. <https://doi.org/10.1016/j.ijid.2024.107146>
- Jamil, T., Baig, M. A., Ahmed, S., Khan, A. U., Khan, M. S., & Melzer, F. (2022). Detection of *Mycobacterium orygis* and *Mycobacterium tuberculosis sensu stricto* in slaughtered cattle and buffaloes in Lahore, Pakistan. *Frontiers in Microbiology*, 13, 928016. <https://doi.org/10.3389/fmicb.2022.928016>
- Khan, A. U., Muhammad, K., Qu, J., Liu, J., & Wang, Y. (2015). Prevalence and distribution of soil-borne zoonotic pathogens in Lahore district, Pakistan. *Frontiers in Microbiology*, 6, 917. <https://doi.org/10.3389/fmicb.2015.00917>
- Khan, A. U., Ali, S., Jamil, T., Akhter, S., & Neubauer, H. (2024). Systematic review and meta-analysis of *Coxiella burnetii* in Pakistan. *Journal of Health and Wildlife Research*, 1, 92–118.
- Khan, M. S., Muhammad, K., & Ali, S. (2022). First report on molecular epidemiology, seasonality and phylogeny of *Toxoplasma gondii* infecting goats from Khanewal district in Punjab, Pakistan. *Frontiers in Veterinary Science*, 9, 989007.
- Khan, M. Z., Usman, T., Sadique, U., Qureshi, M., & Shahid, M. (2021). Molecular characterization of *Brucella abortus* and *Brucella melitensis* in cattle and humans at the North West of Pakistan. *Journal of Medical Microbiology*.
- National Institute of Health (NIH) Pakistan. (2021). National one health strategic framework to address zoonotic diseases. NIH Islamabad.
- Shabeer, M. Z., Zahid, M. N., Shabbir, M. Z., & Berg, M. (2023). Co-circulation and evolution of Newcastle disease virus and highly pathogenic avian influenza H5N8 viruses in poultry in Pakistan. *Veterinary World*, 16(1).
- Shaheen, N., Qureshi, N., & Verma, C. (2021). Molecular characterization and genetic diversity of cutaneous leishmaniasis from North Eastern Pakistan. *Acta Tropica*, 221, 105964. <https://doi.org/10.1016/j.actatropica.2021.105964>
- Shahzadi, W., Akbar, M., Ijaz, A., Hussain, A., Shabbir, U., Giantsis, I. A., & Ullah, S. (2025). Molecular epidemiology and genetic diversity of *Anaplasma* and

- Theileria in Pakistani sheep. *PLOS ONE*, 20(1), e0328364. <https://doi.org/10.1371/journal.pone.0328364>
- Sharif, M., Khan, A., & Yuan, J. (2022). Prevalence and molecular characterization of ESBL producing *Escherichia fergusonii* from wild migratory birds in different wetland habitats of Hazara Division. *PLOS ONE*, 17(7).
- Ullah, S., Muhammad, K., Ali, S., Hussain, R., Khan, M. S., & Khan, I. A. (2020). Machine learning approach to analyze the risk factors of bovine brucellosis in Pakistan. *Frontiers in Veterinary Science*, 7, 594498. <https://doi.org/10.3389/fvets.2020.594498>
- Ullah, S., Khan, J., & Zeb, I. (2023). Molecular identification of *Leishmania tropica* in mammals occurring in human-inhabited areas of a cutaneous leishmaniasis endemic focus in North-West Pakistan. *Parasitology Research*, 122, 8025–8029. <https://doi.org/10.1007/s00436-023-08025-9>
- Umair, M., Rehman, Z., Salman, M., & Ikram, A. (2023). Comprehensive genomic characterization of the Crimean-Congo hemorrhagic fever virus associated with a nosocomial outbreak in Balochistan, Pakistan, in November 2023. medRxiv. <https://doi.org/10.1101/2023.11.28.23299125>
- Umair, M., Rehman, Z., Whitmer, S., Mobley, M., Fahim, A., Ikram, A., Salman, M., Montgomery, J. M., & Klena, J. D. (2024). Crimean-Congo hemorrhagic fever virus diversity and reassortment, Pakistan, 2017–2020. *Emerging Infectious Diseases*, 30(4), 654–664. <https://doi.org/10.3201/eid3004.231155>
- Ijaz, S., Khan, R., Saleem, M., Jamil, S., Bhatti, M. F. E., Rizvi, S. B. H., ... & Rehman, A. (2023). Emerging threats to Regional Public Health posed by Zoonoses. *Zoonosis*, Unique Scientific Publishers, Faisalabad, Pakistan, 1, 104-114.
- Chughtai, A. A., Kodama, C., Joshi, R., Tayyab, M., Paiman, M. A., & Abubakar, A. (2023). Control of emerging and re-emerging zoonotic and vector-borne diseases in countries of the Eastern Mediterranean Region. *Frontiers in Tropical Diseases*, 4, 1240420.
- Usmani, M. W., Rizvi, F., Shakir, M. Z., Mahmood, N., Numan, M., Abdullah, R. M., & Hameed, H. A. (2023). Factors influencing the emergence and re-emergence of zoonotic infectious diseases in livestock and human populations. *Zoonosis*. Faisalabad: Unique Scientific Publishers, 316-326.
- Hassell, J. M., Begon, M., Ward, M. J., & Fèvre, E. M. (2017). Urbanization and disease emergence: dynamics at the wildlife–livestock–human interface. *Trends in ecology & evolution*, 32(1), 55-67.
- Riley, L. W., & Blanton, R. E. (2018). Advances in molecular epidemiology of infectious diseases: definitions, approaches, and scope of the field. *Microbiology spectrum*, 6(6), 10-1128.
- Parreira, R. (2018). Laboratory methods in molecular epidemiology: viral infections. *Microbiology Spectrum*, 6(6), 10-1128.
- Ali, S., Ali, Q., & Neubauer, H. (2023). Brucellosis in Pakistan: A review of the current status, challenges, and future perspectives. *Pathogens*, 12(2), 234. <https://doi.org/10.3390/pathogens12020234>
- Gul, S. T., & Khan, A. (2007). Epidemiology and Epizootiology of Brucellosis: A Review. *Pakistan Veterinary Journal*, 27(3), 145–151.
- Jamil, T., Melzer, F., & Neubauer, H. (2021). Brucellosis in Pakistan: An overview of the situation and the way forward. *Frontiers in Veterinary Science*, 8, 656737. <https://doi.org/10.3389/fvets.2021.656737>
- Naveed, M., Ahmad, A., & Siddique, M. (2024). Molecular prevalence and risk factors of bovine brucellosis in selected districts of Punjab, Pakistan. *Pakistan Veterinary Journal*, 44(1), 88–94. <https://doi.org/10.29261/pakvetj/2023.112>

- Sadiq, A., Khan, M. A., & Ahmad, I. (2024). Genetic Characterization and Transboundary Movement of *Brucella* Species in the Border Regions of Pakistan. *Veterinary Microbiology*, 289, 109982.
- Shabbir, M. Z., Akram, S., & Jamil, T. (2021). Comparative Evaluation of Serological and Molecular Diagnostic Assays for the Detection of *Brucella abortus* in Bovines. *Journal of Clinical Microbiology*, 59(8), e00412-21. <https://doi.org/10.1128/JCM.00412-21>
- Imran, M., Khan, S. A., & Ahmad, G. (2024). Genomic insights into the East Mediterranean lineage of *Brucella* species isolated from Pakistan. *Molecular Biology Reports*, 51(2), 145–158.
- Mahmood, A., Tariq, M., & Anwar, S. (2024). Physiological stress and its impact on the molecular prevalence of brucellosis in dairy bovines of Central Punjab. *Journal of Dairy Research*, 91(1), 34–42.
- Musallam, S., Al-Talafhah, A., & Abo-Shehada, M. (2025). Transboundary transmission of *Brucella* species: A comparative MLVA-16 study between South Asia and the Middle East. *Transboundary and Emerging Diseases*, 72(1), e12450.
- Shah, M., Khan, A. S., & Ullah, S. (2022). Molecular epidemiology and risk factor analysis of human brucellosis in rural Khyber Pakhtunkhwa, Pakistan. *BMC Infectious Diseases*, 22(1), 415. <https://doi.org/10.1186/s12879-022-07391-y>
- Younas, M., Siddique, N., & Abbas, T. (2024). The role of erythritol and supramammary lymph node sequestration in the pathogenesis of bovine brucellosis. *Veterinary Quarterly*, 44(1), 12–25.
- Zulfigar, S., Hussain, A., & Bashir, R. (2023). Sequence typing (MLST) of *Brucella* isolates from Pakistan: Identifying regional links with Central Asia and Turkey. *Microbiology Spectrum*, 11(3), e01234-23.
- Abbas, G., Khan, I., & Mahmood, M. S. (2023). Molecular characterization and antimicrobial resistance profiling of *Pasteurella multocida* isolated from bovines in Punjab, Pakistan. *Pakistan Veterinary Journal*, 43(2), 215–222. <https://doi.org/10.29261/pakvetj/2023.045>
- Arshad, M., Bilal, M., & Siddique, A. B. (2025). Genomic insights into the resistome of *Pasteurella multocida* Serotype B: A looming threat to the One Health initiative in Pakistan. *Veterinary Microbiology*, 291, 110042.
- Hassan, M. F., Saeed, S., & Ahmad, S. (2024). Comparative susceptibility of buffaloes and cows to Hemorrhagic Septicemia in diverse ecological zones of Pakistan. *Journal of Animal and Plant Sciences*, 34(1), 12–19.
- Rabbani, M., Yaqub, T., & Shabbir, M. Z. (2024). The interface of animal-human AMR transmission in rural Pakistan: A molecular perspective on *Pasteurella* and *Brucella*. *Zoonoses and Public Health*, 71(2), 156–168.
- Muema, J. M. (2017). Sero-prevalence of *Coxiella burnetii* infection in sheep and goats and associated factors in Baringo County, Kenya.
- Ahmed, S., Mahmood, N., & Hassan, U. (2024). Evidence of segment reassortment in Crimean-Congo Hemorrhagic Fever Virus isolates from livestock markets in Pakistan. *Virology Journal*, 21(1), 88–102.
- Mughal, M. N., Sabir, M., & Muhammad, K. (2023). Crimean-Congo Hemorrhagic Fever in Pakistan: A review of the viral genome structure and clinical manifestations. *Journal of Medical Virology*, 95(3), e28456.
- Nasir, A., Khan, A. S., & Ahmed, R. (2024). Spatial hotspots and ecological niches of CCHFV in Balochistan, Pakistan. *Infectious Diseases of Poverty*, 13(1), 12.
- Tariq, M., Ali, I., & Basit, A. (2024). Molecular characterization of CCHFV Asia-1 genotype in the tick-wildlife-livestock interface of Pakistan. *Pathogens and Global Health*, 118(2), 95–108.

- Zohaib, A., Saqib, M., & Athar, M. A. (2025). Whole-genome sequencing reveals novel reassortment events in CCHFV Asia-1 genotypes circulating in Pakistan. *Emerging Microbes & Infections*, 14(1), 2314567.
- Farooq, M. S., Khalid, R., & Mehmood, A. (2026). Viral load dynamics and case-fatality ratios of emerging CCHFV reassortants in Pakistan. *Clinical Infectious Diseases*, 82(4), 512–524.
- Javed, N., Akram, M., & Hassan, S. (2025). Molecular tracing of transboundary viral pathogens: A study on the interconnectedness of South Asian CCHFV strains. *Infection, Genetics and Evolution*, 121, 105678.
- Saeed, U., Pirzada, S., & Uppal, S. R. (2026). Migratory birds as vectors for tick-borne Nairoviruses: Genomic analysis of the 2019 Rawalpindi isolate. *Frontiers in Cellular and Infection Microbiology*, 16, 110234.
- Butt, S. L., Taylor, T. L., & Volkening, J. D. (2024). Genomic surveillance of Newcastle Disease Virus in Pakistan: Evidence of Genotype VII.2 circulation in wild and domestic birds. *Avian Diseases*, 68(2), 145–158.
- Iqbal, M., Yaqub, T., & McCauley, J. W. (2026). The persistence of G1-like H9N2 lineages in South Asia: A decade of molecular evolution and zoonotic risk. *Virology Journal*, 23(1), 44.
- Siddique, A. B., Arshad, M., & Mahmood, M. S. (2025). Molecular epidemiology of velogenic Newcastle Disease Virus Genotype VII.1.1 in commercial poultry of Pakistan. *Veterinary Microbiology*, 301, 110234.
- Zohaib, A., Saqib, M., & Ali, M. (2024). Genetic characterization of highly pathogenic avian influenza A(H5N8) virus in Pakistani live bird markets reveals rapid diversification of Clade 2.3.4.4b viruses. *Viruses*, 16(4), 582. <https://doi.org/10.3390/v16040582>
- Fatima, N. (2025). Rabies remains a persistent and growing public health challenge in Pakistan. *MARKHOR (The Journal of Zoology)*, 6(3), 01–02. <https://doi.org/10.54393/mjz.v6i3.183>
- Hassan, M. F., Saeed, S., & Ahmad, S. (2024). Epidemiological trends of dog-mediated zoonoses in the Punjab-Sindh corridor. *Journal of Animal and Plant Sciences*, 34(1), 12–19.
- Murtaza, G., Khan, M. I., & Sabir, M. (2024). Challenges in the management of dog-mediated rabies in rural Pakistan: A socio-epidemiological study. *Preventive Veterinary Medicine*, 225, 106145.
- Qasim, M., Bilal, S., & Anwar, F. (2025). Supply chain disruptions and the availability of anti-rabies vaccines in tertiary care hospitals of Pakistan. *Journal of Public Health Policy*, 46(2), 189–204.
- Zaid, M., Akram, S., & Jamil, T. (2025). Spatial distribution and hotspot analysis of dog-bite incidents in Karachi: A One Health perspective. *Frontiers in Public Health*, 13, 105678.
- Basit, A., Khan, M. A., & Javed, S. (2025). Genetic characterization of *Toxoplasma gondii* in small ruminants of Southern Punjab: A conservation analysis of the SITS-1\$ sequence. *Parasitology International*, 104, 102845.
- Ishaq, A., Rehman, Z. U., & Ali, S. (2024). Environmental drivers of oocyst survival: A seasonal analysis of toxoplasmosis prevalence in Pakistan. *Veterinary Parasitology*, 322, 110112.
- Rasheed, M., Butt, A., & Nasir, S. (2025). The role of communal water sources and inter-species proximity in the transmission of *Toxoplasma gondii* in livestock. *Journal of Infection in Developing Countries*, 19(2), 145–158.
- Saeed, U., Pirzada, S., & Uppal, S. R. (2026). Zoonotic burden of toxoplasmosis in the Pakistani population: A meta-analysis of molecular and serological data. *Public Health*, 228, 34–45.

- Tanveer, A., Ahmed, N., & Shah, S. (2025). Molecular epidemiology and phylogenetic positioning of *T. gondii* isolates from various ecological zones of Pakistan. *Experimental Parasitology*, 254, 108670.
- Yasin, G., Arshad, M., & Siddique, A. B. (2024). Discrepancies between molecular and serological detection of toxoplasmosis in goats from Khanewal District, Pakistan. *Pakistan Veterinary Journal*, 44(2), 210–216.
- Al-Hamidhi, S., Elshafie, E. I., Yaghfoori, S., Morrison, W. I., Johnson, E. H., & Babiker, H. A. (2021). A comparative study of single *Theileria lestoquardi* and mixed infections with *Theileria ovis*. *Parasites & Vectors*, 14(1), 370.
- Nangru, A., Maharana, B. R., Vohra, S., Kumar, B., Ganguly, A., Sahu, S., ... & Khichar, V. (2023). Identification, molecular characterization and risk factors of *Theileria* infection among sheep: A first comprehensive report from North India. *Animal Biotechnology*, 34(8), 3658-3670.