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Comparative Efficacy of Next-Generation Sequencing and Traditional Diagnostic Methods for Bovine Tuberculosis Water Buffaloes

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Abstract | The dairy and meat industries face a significant zoonotic and financial challenge because of bovine tuberculosis (bTB) which is caused by *Mycobacterium bovis*. Bovine tuberculosis transmission dynamics heavily depend on water buffalo (*Bubalus bubalis*). Traditional diagnostic techniques such as molecular genotyping (e.g., spoligotyping and MIRU-VNTR) and culture-based identification are time-consuming and often lack sufficient resolution for distinguishing closely related *M. bovis* strains. For direct identification and characterization of *M. bovis* from infected buffalo tissue samples, this study assesses the efficiency of next-generation sequencing (NGS), particularly short-read Illumina whole-genome sequencing (WGS) and long-read Oxford Nanopore Adaptive Sampling (NAS). Seven buffaloes under government supervision at slaughterhouses received sequencing performed with both traditional culture-based and independent sequencing approaches. All tissue samples tested positive for *M. bovis* DNA by the GeneXpert MTB/RIF Ultra assay while Illumina sequencing showed the maximum read count and deepest sequencing which yielded highly detailed genome-wide data. Direct-from-tissue sequencing through NAS brought two major benefits that combined quick pathogen strain detection with reduced need for bacterial cultures. The phylogenetic study uncovered one clade of isolates which indicates they come from a single transmission source. The genetic similarity displayed by isolates through SNP analysis confirmed previous research about low *M. bovis* population divergence under laboratory settings. Key genomic markers including hsp65, rpoB and 16S rRNA showed concordance between amplicon sequencing results and various experimental methods. Long-read sequencing demonstrates significant promise for real-time bovine tuberculosis monitoring because it provides more powerful disease management approaches. The integration between Next Amplification Sequence and standard Whole Genome Sequencing offers Iraqi veterinary facilities enhanced options for diagnosing *M. bovis* and conducting epidemiological monitoring in endemic zones.

Keywords | *Bovine tuberculosis*, *Mycobacterium bovis*, Iraqi water buffalo, Whole-genome sequencing, Oxford nanopore, Illumina sequencing, Molecular epidemiology

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INTRODUCTION

Mycobacterium bovis (*M. bovis*) infects cattle as well as other animals worldwide thus becoming a severe zoonotic pathogen and causing economic strain. The complex nature of the illness makes control efforts and eradication difficult because *M. bovis* has the ability to survive in numerous animal reservoirs (Arnot and