Summary of the Ph.D thesis No., **10201** Faculty of Veterinary Medicine, Urmia University.

The academic year: 2022-2023

Author: Zahra javadi

Title: Plasmid diversity of Coxiella burnetii in milk of cow, buffalo, sheep and goat in West Azerbaijan province

Summary:

In this investigation, we explored the genetic makeup of Coxiella burnetii isolates. These isolates were found to carry one of four large, well-preserved, autonomously replicating plasmids or a plasmid-like sequence integrated into the chromosome. Between 2020 and 2021, we gathered 400 milk samples from domestic ruminants, which included cows, sheep, goats, and buffaloes, all hailing from West Azerbaijan Province. To decode the genome of C. burnetii, we employed DNA extraction methods provided by Forgen in Taiwan. We utilized a Nested-PCR approach, employing specific primers for each gene, to amplify *IS1111* genes and plasmids (QPH1, QpRS, QpDV, and QpDG) from the collected samples. In our study, we analyzed a total of 400 milk samples obtained from cows, buffaloes, sheep, and goats, focusing on the presence of the *IS1111* gene as an indicator. Out of these samples, 62, or 15.5% (with a 95% confidence interval ranging from 12.3% to 19.4%), tested positive for the presence of C. burnetii. Among the 62 positive samples, 16, or 25.8% (with a 95% confidence interval ranging from 16.6% to 37.9%), were found to contain the QpH1 plasmid gene, while 5 samples, or 8% (with a 95% confidence interval ranging from 3.5% to 17.5%), were positive for the QpRS plasmid gene. Furthermore, there were 7 samples, or 11.3% (with a 95% confidence interval ranging from 5.6% to 21.5%), that tested positive for the presence of the QpDG gene, and 5 samples, or 11.3% (with a 95% confidence interval ranging from 3.5% to 17.5%), were positive for the QpDV gene. This research underscores the vital significance of plasmids in the enduring presence of C. burnetii within milk, thus enhancing our genetic investigation toolkit for this bacterium. The application of Nested PCR with plasmid-specific primers emerges as a valuable technique for directly characterizing C. burnetii plasmids in milk samples. Additionally, our phylogenetic analysis results displayed sequences with a 100% similarity rate. Furthermore, the construction of a phylogenetic tree using neighbor-joining analysis based on partial genes revealed that 20 isolates formed a closely clustered group, demonstrating a remarkable 99.9% similarity, essentially making them indistinguishable. We registered the gene sequences we acquired with appropriate accession numbers in the NCBI database. Furthermore, the results gleaned through the Basic Local Alignment Search Tool (BLAST) highlighted that these sequences exhibited a 100% match with numerous sequences in the gene bank obtained from diverse sources. These findings underscore the high sensitivity and precision of the nested PCR technique for detecting *C. burnetii* plasmids. Additionally, it proves to be a swift approach for the prompt diagnosis of C. burnetii in clinical samples.

Keywords: *Coxiella burnetii*, plasmid, QpH1 gene, QpRS gene, QpDV gene, QpDG gene, Nested PCR, West Azerbaijan