# Characterization of circulating miRNA signature in water buffaloes during brucellosis and evaluation as potential biomarkers in early and non-invasive diagnosis in vaginal secretion

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Brucellosis is one of the most important zoonotic disease in ruminants. It is caused by Brucella species, which is a Gram negative, facultative, intracellular pathogens. Water buffaloes (*Bubalus bubalis*) are very susceptible to both species of Brucella, namely *Brucella abortus* and *Brucella melitensis*. Both can be transmitted to humans mainly by raw milk consumption, as well as by means of direct contact with infected animals [1]. Serological tests are used for the initial diagnosis of Brucellosis, but the results can be negative, especially during the early phases of the disease [2]. A throughout understanding of Brucella biology and the identification of novel biomarkers is essential for early diagnosis and establishing prophylaxis protocols. The role of microRNA (miRNA) has been recently highlighted in pathogen-host interactions [3]. At present, their role in brucellosis is virtually unknown. The present study aimed to a) delineate miRNA expression in blood serum of water buffaloes; b) evaluate the miRNA expression in vaginal secretion; c) determine whether miRNA can be used as biomarkers for water buffaloes affected by brucellosis; d) integrate miRNA to their target genes and to categorize target genes for biological processes.

Blood and vaginal secretion samples were collected from Brucella-positive and Brucella-negative animals collected from Brucella-free farms. The profiles of blood circulating miRNA were characterized by NGS and the sequences were mapped against bovine miRNA available in mirBASE. Differentially expressed miRNAs were validated by qPCR using TaqMan® probes on both blood serum and vaginal secretion samples. Predicted targets of the significant differentially expressed (DE)-miRNAs were computationally retrieved from the TargetScan database (http://www.targetscan.org/vert_71/), DAVID (https://david.ncifcrf.gov/) and KEGG (http://www.genome.jp/kegg/) bioinformatics resources. Differentially analysis revealed that 11 known miRNA exhibited significant alterations in expressions, among which 6 miRNA were upregulated, namely miR-let7f, miR-let7i, miR-126-5p, miR-92a, miR-92b, miR215, and 5 were downregulated, namely miR-30e-5p, miR-320a, miR-339b, miR-127, miR-133a. Five of these miRNAs were selected for further qRT-PCR validation both in blood serum and in vaginal secretion. The comparative analysis in blood serum demonstrated that the level of miR-133a ($p$ = 0.013) was significantly lower in Brucella-positive compared to negative animals. In vaginal secretion the level of miR-92a, miR-126-5p, miR-320a, miR-let7i and miR-let7f were significantly higher in Brucellapositive compared to negative animals. The AUCs were good for miR-let7f (0.880) and let7i (0.800) and fair for miR-320a (0.727) and miR-92a (0.760). Computational target prediction and

functional genes enrichment identified common biological pathways between different miRNAs, among which metabolic pathway, PI3K-Akt pathway, MAPK pathway and cytokine-cytokine receptor interaction are at the top.

In conclusion, our study investigated, for the first time, the dynamic expressions of circulating miRNAs during brucellosis in water buffaloes. ROC analysis suggested that miR-let7f and miR-let7i may be considered novel and promising biomarkers for identification of Brucella-positive animals starting from vaginal secretion. The detection of miRNA in vaginal secretion paves the way for an early and non-invasive diagnostic procedure.