**TITLE:** DEVELOPMENT OF A REAL-TIME PCR ASSAY FOR DETECTION OF *Pasteurella multocida* IN POULTRY

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**ABSTRACT:**

*Pasteurella multocida*, causative agent of fowl cholera (FC), is one opportunistic pathogen associated with respiratory diseases in domesticated and wild birds. FC infection is generally diagnosed by bacteriological isolation and identification of the organism from infected tissues. However, bacteriological isolation process takes several days to conclude the diagnosis. Sometimes, suspected samples for *P. multocida* can remain undetected on culture plates in the presence of heavy loads of contaminants. An alternative to bacteriological diagnosis is the polymerase chain reaction (PCR), which can detect low number of DNA target in contaminated samples. In this study, a real-time PCR method was developed for fast and accurate detection of *P. multocida* DNA of naturally and experimentally infected chickens. In addition, isolates from clinical cases of FC in Brazil between 2014 and 2016 were tested. The primer and probe sequences for amplifying a 130-bp fragment of the *ompH* gene was based on the 18 nucleotide sequence from the *ompH* gene available in GenBank. *P. multocida* DNA was detected by real-time PCR in head swab, liver, spleen, lung and femur bone marrow.
The assay detected a minimum of approximately 10 CFU of *P. multocida* per reaction. Of 59 samples submitted to the laboratory for routine bacteriological culture, 6 were positive in both methods, real-time PCR assay and bacteriological isolation. Of the 16 isolates belonging to the laboratory strain bank, all were positive by PCR and culture. The sensitivity, specificity, and reproducibility of the FC real-time PCR assay revealed its suitability for detection of FC in samples from clinically infected chickens as well as rapidity in diagnosis when compared to bacteriological isolation.

**Keywords:** diagnosis, pasteurella multocida, poultry, real-time PCR

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