TITLE: METHODS OF COLLECTING AND TRANSPORTING SAMPLES TO MOLECULAR DIAGNOSTIC OF *Campylobacter fetus* FROM BOVINES

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ABSTRACT:

Campylobacter fetus subsp. *venerealis* is the etiological agent of bovine genital campylobacteriosis (BGC), being the bulls asymptomatic carriers. This bacterium is nutritionally fastidious and grows under microaerophilic conditions, making difficult the collection, storage and diagnoses process. Therefore, the aim of this study was to test methods of collection and conditioning of preputial mucus to perform molecular identification of *C. fetus*. Two bulls confirmed as positive for *C. fetus* were used in our analysis; therefore, all tests were performed with both previously positive animals. Three methods of preputial mucus assessment were tested: i) soft brush ii) preputial scraping and iii) preputial washing with PBS buffer. The collected mucus was conditioning and transported in three ways: i) PBS on ice box ii) ultrapure water on ice box and iii) Lander's transport medium at room temperature. All samples were immediately processed. In addition, a sample of each collection and conditioning, except the Lander's, were frozen at -20ºC for seven days, following 24 h at ice box and posteriorly processed. DNA of each collected sample was extract with PureLink® Genomic DNA Kit and its integrity was evaluated by electrophoresis in agarose gel. Specific 16S rRNA region was amplified by PCR reaction to detect *C. fetus*. Samples collected with preputial scraper and transported in PBS and ultrapure water showed intact DNA after extraction in agarose gel analysis. On the other hand, samples collected with soft brush and transported in PBS and water yield satisfactory DNA. The material collected by preputial washing provides little DNA concentration, as well the extractions from Lander's. Regarding molecular identification, the PCR assay confirmed that all methods of collection were able to identified *C. fetus*. The DNA from frozen samples have a decreased in quantity and quality comparing to fresh material, even so *C. fetus* were detected in all samples from both animals. Considering these results, we highlight that the best way to collect preputial mucus is through preputial scraping conditioning in PBS or ultrapure water with ice box transportation. However, the viability and reproducibility of the analysis with frozen samples is still unknown and more studies are needed. The methods described here are simple enabling rapid and sensitivity *C. fetus* molecular identification, which improve the diffusion of the detection of this bacteria and analysis of the BGC occurrence.

Keywords: *Campylobacter* spp., preputial crypt, bovine genital campylobacteriosis, PCR, DNA extraction.

Development Agency: Conselho Nacional de Desenvolvimento Científico e Tecnológico – CNPq and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - CAPES.