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Comparison of techniques for DNA extraction and agarose gel staining of DNA fragments using samples of *Cryptosporidium*

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Differentiating between the *Cryptosporidium* species and their subtypes using only microscopy is impossible. Therefore, molecular tools are indispensable for accurate species and subtype diagnosis. However, if these tools are to be used correctly and accurately, the techniques used must be standardised. In the present study, two molecular techniques for diagnosing *Cryptosporidium* infection in cows were compared to determine the optimal methods. For each technique, we tested two DNA extraction methods, several annealing temperatures for nested PCR reactions targeting the *18S*, *SSU rRNA* (small subunit ribosomal RNA), and the *GP60* (60 kDa glycoprotein) genes, and two types of DNA staining reagents, ethidium bromide and GelRed™. We determined that one of the tested protocols yields a higher purity of extracted DNA. Additionally, optimised temperatures for the nested PCR of the *18S* and *GP60* genes were established. Finally, we determined that the GelRed™ dye was more sensitive than ethidium bromide, and its low toxicity facilitates handling and disposal and reduces environmental contamination.

Keywords:

18S gene; *GP60* gene; ethidium bromide; GelRed™

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