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

MCM Couto, AP Sudre, MF Lima, TCB Bomfim

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Citation: Couto M., Sudre A., Lima M., Bomfim T. (2013): Comparison of techniques for DNA extraction and agarose gel staining of DNA fragments using samples of *Cryptosporidium*. Veterinarni Medicina, 58: 535-542.

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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 GelRedTM. 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 GelRedTM 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 $^{\text{TM}}$

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Contact

Mgr. Zuzana Karlíková Executive Editor phone: + 420 227 010 352 e-mail: vetmeo@cazv.cz

Address

Veterinární Medicína Czech Academy of Agricu Sciences

Slezská 7, 120 00 Praha 2, Republic

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