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Development of a qPCR for the detection of *Chorioptes bovis* in equine skin scrapings

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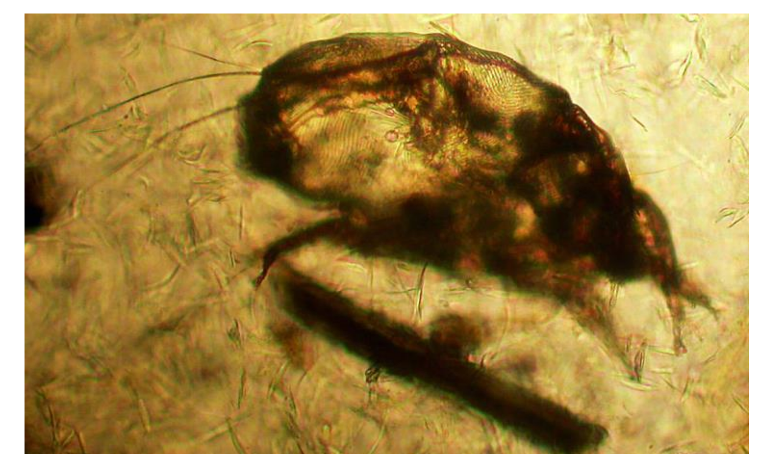
INTRODUCTION. Equine leg mange caused by *Chorioptes bovis*, often associated to pruritic pastern dermatitis, is a recognized welfare problem in horses. Diagnosis by direct microscopy can be challenging and frustrating in field practice. Our aim was to develop a reliable, molecular tool for the detection of *C. bovis* DNA in skin scrapings.

MATERIALS AND METHODS. A new set of primers and a Taqman probe were designed within the mitochondrial Cytochrome c Oxidase (COI) gene sequence of *C. bovis*. Before designing the Real-time PCR system, a qualitative PCR was first performed using generic COI primers (Gu et al., 2014 Parasites & Vectors 7:340; Fig. 1). DNA was extracted from *C. bovis*-positive (n=4) and *C. bovis*-negative (n=8) horses sampled with different techniques; mites were detected at microscopy (Figs. 2 and 3). PCR analysis was therefore run using 400 nM of each primer and 133 nM of the hydrolysis probe labelled with FAM-MGBNFQ (2 minutes of initial denaturation at 95 °C followed by 45 cycles of 95 °C for 5 seconds and 60 °C for 30 seconds). Samples were considered positive if the reaction curve (Fig. 4) exhibited the characteristic exponential shape and reached above the selected threshold based on the baselines in the samples and controls. To test the specificity of the assay, nine species of environmental mites were tested, all with negative results.

RESULTS AND CONCLUSIONS. The results from microscopy of the samples used for optimization of sampling were in general congruent with the PCR results except in one case when after acaricidal treatment a horse was still positive at PCR but negative at microscopy, suggesting residual DNA from *C. bovis* was still present in the sample. While our PCR method showed good specificity and sensitivity, it still needs to be tested on larger sample batches and the timing for post treatment follow-up analyses has to be set up.



Figs. 2 and 3: *Chorioptes mites* observed in skin scrapings (40x magnification).

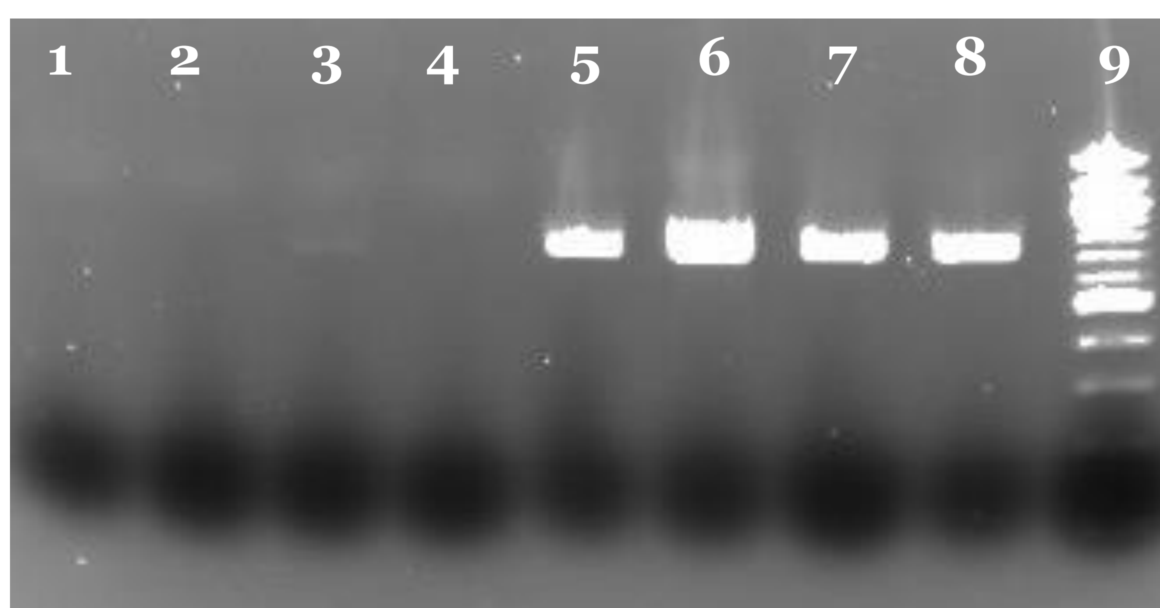


Fig. 1: qualitative PCR for mitochondrial Cytochrome c Oxidase (COI) gene target. **1-4**: DNA from one clinical sample, a weak positive signal (n. 3) can be seen and has been sequenced. **5-7**: DNA from another clinical sample: n. 5 DNA from a *Chorioptes* mite, n. 6-7 DNA from skin scraping. **8**: positive control from a previously sequenced sample. **9**: DNA ladder (100 bp).

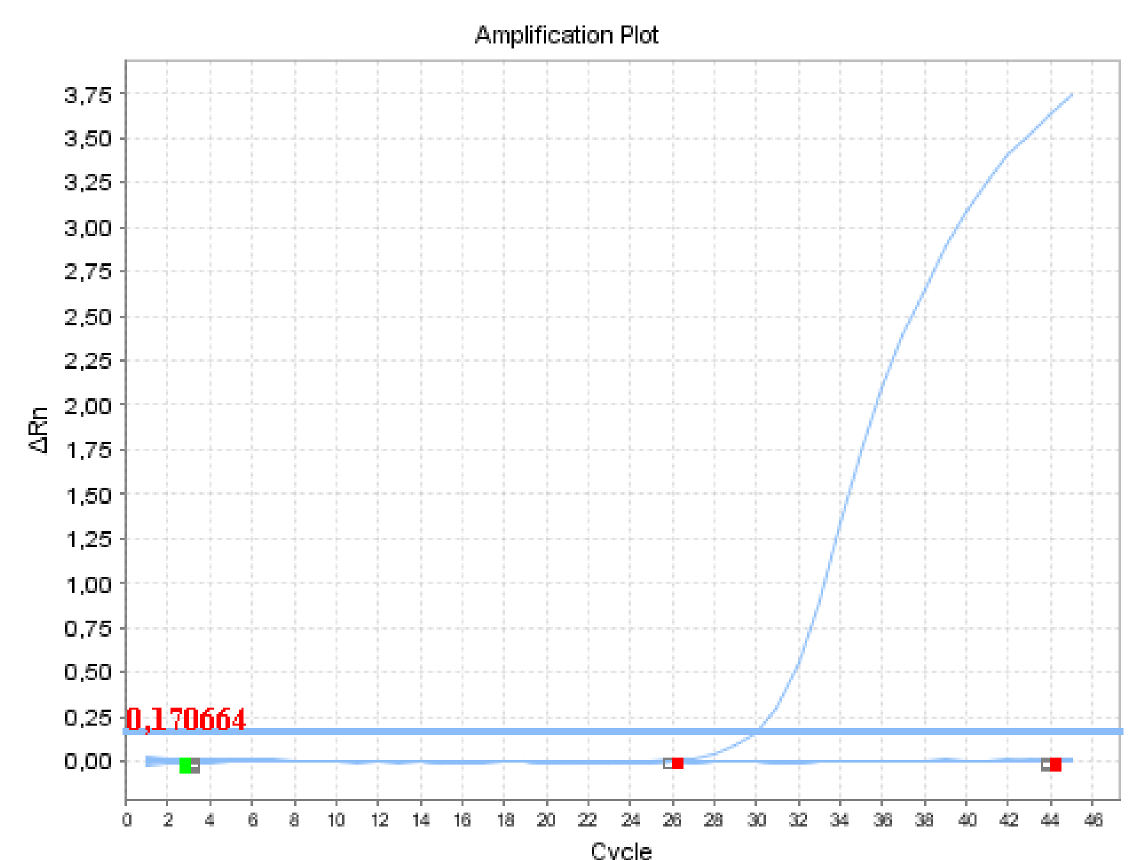


Fig. 4: amplification curve obtained from one of the *Chorioptes*-positive samples.

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