Comparison of PCR-Reverse Line Blot and real-time PCR for the detection of dermatophytes in clinical samples.

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Introduction
In a previous study PCR-Reverse Line Blot (PCR-RLB) was compared with culture and the potassium hydroxide test (KOH) for the detection of dermatophytes. Culture, KOH and PCR-RLB analysis yielded 17/99, 39/99 and 57/99 positive results respectively. Drawbacks of the PCR-RLB are the laborious nature of the test, the difficult standardization and the interpretation of weak results. Therefore a multiplex real-time PCR was developed. The aim of this study was to compare PCR-RLB analysis with multiplex real-time PCR for the detection of dermatophytes.

Objectives
In a previous study PCR-Reverse Line Blot (PCR-RLB) was compared with culture and the potassium hydroxide test (KOH) for the detection of dermatophytes. PCR-RLB showed to be more sensitive than culture and KOH. Drawbacks of the PCR-RLB are the laborious nature of the test, the difficult standardization and the interpretation of weak results. Therefore a multiplex real-time PCR was developed. The aim of this study was to compare PCR-RLB analysis with multiplex real-time PCR for the detection of dermatophytes.

Methods
Both PCR-RLB and real-time PCR targeted the ITS1 region located between the genes coding for 18S and 5.8S rRNA. The RLB membrane harboured 13 different probes to identify and discriminate between 9 different dermatophyte species. Real-time PCR consisted of two multiplex assays. One assay targeted T. rubrum, T. violaceum and T. tonsurans. The second targeted Microsporum spp., T. interdigitale group and the whole group of dermatophytes. Phocine herpes virus-1 was used as internal control for the real-time assays. Samples were processed using QI Amp® DNA mini kit (Qiagen, Germany) with a separate pre-lysis step. Totally 100 clinical samples (52, 38, 10 respectively nail-, skin- and hair samples) were analysed retrospectively by real-time PCR and compared with PCR-RLB.

Results
Of the 100 samples 60 were positive with the PCR-RLB (27 T. rubrum, 14 T. interdigitale, 6 T. tonsurans, 3 T. violaceum, 1 M. canis and 9 Trichophyton spp.). All samples identified as T. rubrum, T. interdigitale, T. tonsurans and T. violaceum by the PCR-RLB were confirmed by the real-time PCR. The sample which tested positive for M. canis by PCR-RLB was identified as Microsporum spp. by real-time PCR. The 9 samples which scored positive for Trichophyton spp. in the PCR-RLB yielded weak results. Of these 9 samples real-time PCR identified 3 samples as T. interdigitale, 1 as T. rubrum, 1 as T. tonsurans, 1 as dermatophyte positive and 3 samples remained negative. The real-time PCR detected 8 additional samples which scored negative with the PCR-RLB. Of these 8 samples real-time PCR identified 4 samples as T. rubrum, 3 as T. interdigitale and 1 as dermatophyte positive.

Conclusion
These data show that real-time PCR is a sensitive method for detection of the most prevalent dermatophytes in nail-, skin- and hair samples. Furthermore real-time PCR is more standardized and less laborious than PCR-RLB, making it a useful tool in routine diagnostics.

Materials and Methods
Both PCR-RLB and real-time PCR targeted the ITS1 region located between the genes coding for 18S and 5.8S rRNA. The RLB membrane harboured 13 different probes to identify and discriminate between 9 different dermatophyte species. Real-time PCR consisted of two multiplex assays. One assay targeted T. rubrum, T. violaceum and T. tonsurans. The second targeted Microsporum spp., T. interdigitale group and the whole group of dermatophytes. Phocine herpes virus-1 was used as internal control for the real-time assays. Samples were processed using QiAmp® DNA mini kit (Qiagen, Germany) with a separate pre-lysis step. Totally 100 clinical samples (52, 38, 10 respectively nail-, skin- and hair samples) were analysed retrospectively by real-time PCR and compared with PCR-RLB.

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