Universal conventional and real-time PCR diagnosis tools for *Sarcoptes scabiei*

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Abstract  

**Background:** The mite *Sarcoptes scabiei* has a known host-range of over 100 mammal species including humans. One of the prime objectives of the *Sarcoptes*-World Molecular Network (WMN) is to design and develop universal *Sarcoptes* PCR-based diagnosis methods.  

**Methods:** We describe here for the first time two universal mitochondrial-based diagnosis methods: (i) conventional end-point PCR and (ii) TaqMan real-time PCR. The design of both of these universal diagnosis methods was based on *Sarcoptes* samples collected from 23 host species in 14 countries.  

**Results:** These methods, based on skin scrapings, were successfully used to etiologically confirm the diagnosis of different clinical degrees of sarcoptic mange in 48 animals belonging to six species. These universal PCR-based diagnosis methods are highly specific, technically sensitive and simple, and are based on the amplification of 135 bp from the Mitochondrial 16S rDNA. The method based on TaqMan real-time qPCR was more sensitive than the conventional end-point PCR.  

**Conclusions:** Two universal PCR-based diagnosis methods for *S. scabiei* were successfully designed and applied; one based on conventional end-point PCR and the other on TaqMan real-time PCR. We recommend further testing and the application of these new universal methods worldwide.  

**Keywords:** Sarcoptic mange, Scabies, Host species, Mitochondrial 16S rDNA, Conventional end-point PCR, TaqMan real-time PCR, *Sarcoptes* WMN

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Scabies control in humans, eradication programs of sarcoptic mange in farm animals [9, 10] and studies of the epidemiology and pathology of this condition in a range of animal species (including vulnerable wildlife) [11] would clearly benefit from improved methods that are more sensitive to infection by Sarcoptes mites. The aim of this paper was to report the design and application of two new universal diagnosis methods of Sarcoptes scabiei based on traditional end-point PCR and real-time TaqMan PCR following the recommendations made by the Sarcoptes-World Molecular Network [1].

Methods

Sample collection
A total of 39 Sarcoptes mites were individually collected using different isolation methods [12] from the skin of 23 host species from 14 countries (Table 1). To test the new tools, we also collected 48 skin scrapings from the following animals (samples were collected from dead animals for post-mortem diagnostic purposes): (a) two healthy unexposed roe deer (Capreolus capreolus); (b) four healthy unexposed badgers (Meles meles); (c) five red foxes (Vulpes vulpes) with different degrees of mange; (d) 14 northern chamois (Rupicapra rupicapra) with different degrees of mange; (e) a mange-affected red deer (Cervus elaphus); and (f) 22 Iberian ibex (Capra pyrenaica), of which three were healthy and unexposed, and 19 mangy (Table 2). All skin samples from mangy animals were microscopically confirmed to be S. scabiei positive.

DNA extraction
The HotSHOT Plus ThermalSHOCK technique [13] and NucleoSpin Tissue kit procedure (Macherey-Nagel, Düren, Germany) [14] were employed to extract genomic DNA from all individual mites. The success rate of DNA extraction from parasites was about 70 % depending on the method used (live or dead mites) and the type of preservation (frozen or in ethanol) [14, 15]. DNA was extracted from the skin scrapings using the two above-mentioned methods with minor modifications (e.g. we used twice as much reagent as we used to extract genomic DNA).

Amplification and sequencing of a fragment from the Mitochondrial 16S rDNA
A fragment from the Mitochondrial 16S rDNA (407 bp) was amplified by PCR using primers 16S-F and 16S-R as reported previously [10] in a 2720 thermal cycler (Applied Biosystems, Foster City, California). The amplicons were examined on 1.5 % agarose gel stained with ethidium bromide for DNA visualization under UV light. The purified PCR products were directly cycle-sequenced from both directions on ABI PRISM 310 Genetic Analyser (Applied Biosystems, Foster City, California) using the BigDye Terminator Cycle Sequencing Kit 1.1 (Applied Biosystems, Foster City, California). Individual mite consensus sequences were manually trimmed of primer sequences, aligned, compared and edited using BioEdit v7.0.9.0 [16].

Universal primer design
Based on the comparison of the obtained sequences, we used Primer 3 (v. 0.4.0) [17] to design a set of universal

| Geographical origin | Host species | No. of samples |
|---------------------|--------------|----------------|
| Korea               | Human (Homo sapiens sapiens) | 1 |
| Brazil              | Human (Homo sapiens sapiens) | 1 |
| France              | Human (Homo sapiens sapiens) | 1 |
| Italy               | Northern chamois (R. rupicapra) | 2 |
| Spain               | Southern chamois (R. pyrenaica) | 1 |
| Spain               | Spanish ibex (Capra pyrenaica) | 2 |
| Italy               | Alpine ibex (Capra ibex) | 1 |
| Italy               | Red fox (Vulpes vulpes) | 2 |
| Spain               | Red fox (Vulpes vulpes) | 1 |
| Italy               | Wild boar (Sus scrofa) | 3 |
| Spain               | Rabbit (Oryctolagus cuniculus) | 3 |
| Germany             | Raccoon (Procyon lotor) | 3 |
| Tanzania            | Wildebeest (Connochaetes taurinus) | 1 |
| Japan               | Raccoon dog (Nyctereutes procyonoides) | 1 |
| West Indies         | Dog (Canis lupus familiaris) | 1 |
| Argentina           | Capybara (Hydrochoerus hydrochaeris) | 1 |
| Italy               | Bovine (Bos taurus) | 1 |
| Italy               | Red deer (Cervus elaphus) | 1 |
| Spain               | Red deer (Cervus elaphus) | 1 |
| Italy               | Stone marten (Martes foina) | 1 |
| Switzerland         | Eurasian lynx (Lynx lynx) | 1 |
| Italy               | Mouflon (Ovis aries musimon) | 1 |
| Spain               | Grey wolf (Canis lupus) | 1 |
| Egypt               | Sheep (Ovis aries) | 1 |
| Kenya               | Thomson’s gazelle (Eudorcas thomsoni) | 1 |
| Kenya               | Lion (Panthera leo) | 1 |
| Kenya               | Cheetah (Acinonyx jubatus) | 1 |
| Kenya               | Dog (Canis lupus familiaris) | 1 |
| Kenya               | Reticulated giraffe (Giraffa camelopardalis reticulata) | 1 |
| Tunisia             | Dromedary camel (Camelus dromedarius) | 1 |
The end-point PCR universal diagnosis method protocol
The final protocol for the diagnosis of *S. scabiei* after adjusting the PCR mixture and the annealing temperature consisted of a total volume of 30 µL. PCR mixture composed of 3 µL of single *Sarcoptes* DNA, 200 µM of each dNTP, 0.1 µM of each primer, 3 µL of 10X PCR buffer (100 mM Tris–HCl, pH 8.3 and 500 mM KCl), 1.5 mM MgCl₂ and 0.3 µL (1.5 U/reaction) Hot-Start Taq DNA polymerase (Qiagen, Milano, Italy). Samples were submitted to the following thermal profile for amplification in a 2720 thermal cycler (Applied Biosystems, Foster City, California): 15 min at 95 °C (initial denaturing), followed by 35 cycles consisting of three steps of 30 s at 94 °C (denaturation), 45 s at 53 °C (annealing) and 1.5 min at 72 °C (extension), before a final elongation of 7 min at 72 °C. The amplicons were examined on 2 % agarose gel and stained with ethidium bromide for DNA visualization under UV light.

The TaqMan real-time PCR universal diagnosis method protocol
The TaqMan real-time PCR probe relies on the 5′–3′ exonuclease activity of Taq polymerase, which cleaves a dual-labelled probe in the hybridization phase to the complementary target sequence and fluorophore-based detection [18]. The resulting fluorescence signal allows quantitative measurements of the accumulation of the PCR-product in the exponential stages to be made [18].

The set of universal primers for the amplification of *S. scabiei*, SSUDF and SSUDR (generating 135 bp amplicons), was used with a newly designed species-specific TaqMan probe for the identification of *S. scabiei* (ProSc: 5′-GGTAACTTGTATGAAGGGACTAATAA-3′).

The probe was designed using Primer 3 (v. 0.4.0) [17]. The TaqMan probe was labelled with a BHQ1 quencher dye (Eurofins Genomics) at 3′-end, and with FAM reporter dye at 5′-end. Amplification reactions contained 0.4 µM of each primer (SSUDF and SSUDR), 0.25 µM of probe (ProSc), 1X Master Mix (TaqMan Universal Master Mix, Applied Biosystems by Life Technologies), 1.5 µL of DNA solution (replaced by water in No Template Controls) and nuclease-free water in a final volume of 15 µL. Cycling conditions for the PCR consisted of a 10-min start-up denaturation step at 95 °C, followed by 45 cycles of amplification for 15 s at 95 °C and 1 min at 60 °C.

Specificity and technical sensitivity of the conventional end-point PCR universal diagnosis method
The specificity of the generic primers for the universal diagnosis of *S. scabiei* infection was evaluated using reference samples of *S. scabiei* preserved in the authors’ mite collection, as well as heterologous samples of *Psoroptes cuniculi* and Notodectes cattae. *Psoroptes cuniculi* and *Notodectes cattae* were chosen since they are phylogenetically close to *Sarcoptes* mites and are not difficult to obtain. DNA samples extracted from skin biopsies of unexposed badgers (*Meles meles*) and roe deer (*Capreolus capreolus*) were used as negative controls. Specificity was verified by comparing with these negative control samples and by the DNA sequencing of the PCR products.

The sensitivity of our assay was assessed using a two-fold dilution series (between 5 ng/µL and 0.01 ng/µL) of *S. scabiei* gDNA. The detection limit was based on the final dilution at which the amplified 135 bp band was still visible in the agarose gel.

Specificity and technical sensitivity of the TaqMan real-time PCR universal diagnosis method
The specificity of the TaqMan real-time PCR diagnosis method was tested with the same samples as used for testing the specificity of the conventional end-point PCR diagnosis method and with the same criteria.

The sensitivity of our assay was assessed using a two-fold dilution series (between 5 ng/µL and 0.0005 ng/µL) of *S. scabiei* gDNA. The limit of detection was based on the final dilution at which the signal of the TaqMan probes was still exponentially amplified.

Results and discussion
We obtained positive diagnoses for all samples from manky animals with both diagnosis methods (conventional
end-point PCR and TaqMan real-time PCR). No false positives were generated by either test for the heterologous samples from *P. ovis*, *O. cynotis* and *N. catti*, for the healthy badger and roe deer DNA samples, or for skin scrapings from healthy unexposed animals (Fig. 1).

The technical sensitivity of the end-point PCR diagnosis was lower than that of the TaqMan PCR diagnosis. The minimum amount of *Sarcoptes* gDNA detected with conventional end-point PCR was about 80 pg/μL (Fig. 2), whereas only 10 pg/μL was needed for the TaqMan PCR technique (Fig. 3). The higher sensitivity of the TaqMan real-time PCR diagnosis method was expected and can be attributed to the fact that the detection limit in a conventional end-point PCR is based on the final dilution at which a PCR product is still visible in agarose gels, while the fluorophore signal in the TaqMan probes is still detectable at much lower concentrations. The PCR mixtures/conditions of the
TaqMan PCR and conventional end-point PCR may also have contributed to this difference.

Both diagnostic methods were successfully applied to all of the 48 skin scrapings obtained from the six host species. We obtained nine negative results (no amplicons) for skin scrapings from healthy animals and 39 positive results (presence of the amplicons) for the skin scrapings from mangy animals (Fig. 4).

Even given the known advantages of the TaqMan PCR over the end-point PCR [19], this latter diagnosis method is still a good alternative option, above all because the majority of scabies/mange infections occur in countries with poor economic resources where the equipment, materials and expertise needed for the TaqMan qPCR diagnosis method may be lacking.

Additional experimental steps are clearly needed to test how the new methods may contribute to fine-tuning current knowledge of the epidemiology of sarcoptic mange in domestic and wild animals, and of scabies in man, including further studies to test the true sensitivity of the methods, using suspected cases and a gold standard method (or a combination of methods, such as the burrow ink test and handheld dermatoscopy in the case of scabies) as reference [20, 21]. We foresee that, beyond the clinical diagnostic and therapeutic context, the potentialities disclosed by the two methods may find promising...
application in experiments aimed to investigate the mechanisms of resistance/immunity to infection by *S. scabiei*, including spontaneous recovery in naïve and previously exposed individuals/populations, and the subclinical carrier state of *S. scabiei* in livestock and wildlife, amongst other topics.

**Conclusions**

We successfully designed and applied two universal PCR-based diagnosis methods for *S. scabiei*, one based on conventional end-point PCR and the other on TaqMan real-time PCR. These new methods were standardized and found to have high specificity and technical sensibility in 23 host species from 14 counties. They successfully diagnosed (based on skin scrapings) different clinical degrees of sarcoptic mange affecting several animal species. We recommend further testing and the application of these new universal methods worldwide.

**Competing interests**

None of the authors have any competing interests regarding this manuscript.

**Authors’ contributions**

SA, LR, ARM, MP conceived and designed the experiments; ARM, MP, SA LR, ANA, SD, FB, VO, MG, RCS performed the fieldwork and the experiments. The manuscript was analysed, discussed and written by all co-authors. All authors read and approved the final version.

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