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Research Report

MOLECULAR IDENTIFICATION OF *SARCOPTES SCABIEI* VAR. *CUNICULI* FROM SURABAYA AND MALANG REGIONS OF EAST JAVA

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ABSTRACT

Scabies is a zoonotic skin disease caused by Sarcoptes scabiei mites. As an emerging/re-emerging parasitic disease, scabies represents a significant global threat to both human and animal health. Numerous cases of scabies in Indonesia have been reported, which support research on the prevalence of S. scabiei. However, most such studies have involved conventional morphological studies, with limited molecular diagnostic studies. The purpose of the present study was the genetic characterization of S. scabiei var. cuniculi in domestic rabbits to generate baseline genotypic data. S. scabiei var. cuniculi was isolated and identified from scabies-infected rabbits from the Surabaya and Malang regions of East Java. Molecular identification was performed using Polymerase Chain Reaction (PCR) using specific primers targeting the COX1 gene. We performed COX1 PCR using rabbit isolates of S. scabiei from Indonesia. To the best of our knowledge, no such study had been reported previously. This study was performed in the Laboratory of Veterinary Parasitology, Faculty of Veterinary Medicine and the Tropical Disease Diagnostic Center Laboratory, Universitas Airlangga. The results with agarose gel electrophoresis revealed a 289 bp PCR product amplified from the DNA of S. scabiei isolates from both Surabaya and Malang in accordance with the expected COX1 amplicon size, that indicated a single band 289 bp in length, demonstrating specific detection of S. scabiei var. cuniculi from Surabaya and Malang using COX1 primers. The results were consistent with the calculated amplicon size based on primer positions within the COX1 locus, with the forward primer spanning nucleotides 61–94, and the reverse primer spanning nucleotides 331–350 (350 – 61 = 289 bp). PCR genotyping of the isolates yielded an identical nucleotide length of 289 bp. Further studies are required to sequence the amplified fragments for homology assessment.

Keywords: *Sarcoptes scabiei, scabies, PCR, 289 bp, zoonosis*

ABSTRAK

Scabies disebabkan oleh tungau Sarcoptes scabiei adalah tungau penyebab penyakit kulit yang bersifat zoonosis. Scabies sebagai penyakit parasite yang muncul kembali dan mengancam kesehatan manusia maupun hewan di dunia. Kasus scabies yang tinggi pada beberapa hewan domestic seperti kelinci di Indonesia hanya didukung dengan hasil penelitian prevalensi S.scabiei, namun hanya bedasarkan tinjauan gejala klinis dan morphologi, sedangkan penelitian berbasis molecular seperti karakterisasi genetic dari tungau S.scabiei masih terbatas. Tujuan dari penelitian ini untuk mendeteksi karakterisasi genetik tungau Sarcoptes scabiei var.cuniculi dari kelinci domestik sebagai data dasar informasi genetik. Penelitian ini dilakukan isolasi dan identifikasi S.scabiei var.cuniculi dari kelinci yang menunjukkan gejala scabies dari daerah Surabaya dan Malang, selanjutnya dilakukan ektraksi DNA dari isolate S.scabiei kelinci Indonesia, untuk diproses dengan Polymerase Chain Reaction (PCR) menggunakan primer spesifik dengan target gen COX1 289 bp. Studi ini dilakukan di Laboratorium Parasitology Veteriner, Fakultas Kedokteran Hewan dan Laboratorium Pusat Diagnostik Penyakit Tropik, Universitas Airlangga. Hasil studi genetic S.scabiei isolate lokal Indonesia untuk menambah ilmu pengetahuan karena belum ada hasil studi yang dilaporkan sebelumnya. Hasil electrophoresis tungau S.scabiei var.cuniculi dari kelinci asal Surabaya dan Malang menunjukkan panjang nukleotida sebesar 289 bp sesuai dengan target. Hasilnya konsisten dengan ukuran amplikon yang

dihitung berdasarkan posisi primer di dalam lokus COX1, dengan primer ke depan mencakup nukleotida 61-94, dan primer terbalik mencakup nukleotida 331-350 (350 - 61 = 289 bp). Genotipe PCR dari isolat lokal menghasilkan panjang nukleotida identik 289 bp. Diperlukan penelitian lebih lanjut untuk mengurutkan fragmen yang diperkuat untuk penilaian homologi.

Kata kunci: *Sarcoptes scabiei, scabies, PCR, 289 bp, zoonosis*

INTRODUCTION

Scabies or mange is an infectious zoonotic skin disease caused by the mite *Sarcoptes scabiei* and is considered an important disease in both humans and animals. It is estimated that more than 300 million people are infected each year.[1,2] The Sarcoptes mite is an obligate parasite of the skin; the mites burrow into the stratum corneum complete their life cycle starting from the egg to the adult stage.[2–4] *S. scabiei* infection may trigger multiple reactions including allergic reactions, inflammation, innate immune reactions, and activation of immune components in the skin.[5] Significant clinical symptoms of scabies include thickening of skin, crust formation, alopecia involving the eyes, ears, mouth, legs, and itching accompanied by the formation of red spots (rash). Occasionally, 3-cm long lines or grooves are formed on the skin, which lead to crust, papule, or vesicle formation.[6–8] Scabies mainly occurs in areas with high poverty rates and low nutritional status and is transmitted via direct contact with infected humans or animals.[9] Numerous cases of scabies in Indonesia have been reported, which support research on the prevalence of *S. scabiei*. However, most such studies have involved conventional morphological studies, with limited molecular diagnostic studies.[10,11] Esther et al. (2015) reported PCR based detection of genotypic differences between *S. scabiei* from different hosts including pigs, rabbits, foxes, jackals, and porcupines in comparison with the Genbank database using three different genes, including cytochrome C oxidase 1 (*COX1*) with a 467 bp amplicon and Glutathione-S-Transferase class 1 (*GST1*) with a 670 bp amplicon. The highest percentage identity of database-reported sequences, at 99%, was with *S. scabiei* from rabbits, obtained using *COX1* PCR. Another study reported an *S. scabiei COX1* amplicon of 250 bp in an isolate from Hong Kong.[11] The purpose of this study was the genetic characterization of *S. scabiei var. cuniculi* from domestic rabbits to generate baseline genotypic data. We performed *COX1* PCR using rabbit isolates of *S. scabiei* from Indonesia. To the best of our knowledge, no such study had been reported previously.

MATERIAL AND METHOD

S. scabiei mites (adults, nymphs, and larvae) were isolated from domestic rabbits from two different regions in East Java, including four rabbits from Surabaya and two from Malang. Domestic rabbits showed clinical signs of scabies such as thickening of skin, crust formation, and alopecia involving the eyes, ears, mouth, and legs. Mites were morphologically identified using identification keys.[6,7] This study was performed in the Laboratory of Veterinary Parasitology , Faculty of Veterinary Medicine and the Tropical Disease Diagnostic Center Laboratory, Universitas Airlangga.

PCR Based Detection of *S. scabiei* in Rabbits

DNA extraction was performed using mini kit QIAamp DNA (Qiagen, Hilden, Germany), following the manufacturer's protocol as follows: 20 µl of Qiagen protease was added to a microcentrifuge tube with a 200 µl suspension of *S. scabiei*; 180 µl of buffer tissue lysis (ATL buffer) was then added and the tube was centrifuged at 8,000 rpm for 3 min, vortexed for 15 s, and incubated at 56°C for 24 h. Further, 200 µl of buffer lysis (AL buffer) was added to the tube and vortexed for 15 s; 200 µl of 96% ethanol was added to the tube, vortexed for 15 s, and centrifuged at 8,000 rpm for 1 min to pellet DNA. Next, 500 µl of buffer washing 1 (AW1 buffer) was added to the pellet and centrifuged at 8000 rpm for 1 min; 500 µl of AW2 buffer was then added to the pellet and the tube was centrifuged at 13,000 rpm for 3 min, at 13,000 rpm for 1 min, and 50 µl of elution buffer (AE buffer) was added to the final DNA pellet; following an incubation at 15–25°C for 1 min, the contents were centrifuged at 8,000 rpm for 1 min to collect DNA.[12]

Next, PCR to amplify a 289 bp region of the *COX1* gene was performed using the forward primer 5'-TCTTAGGGGCTGGATTTAGTATG-3' and the reverse primer 5'-AGTTCCTCTACCAGTTCCAC-3'. PCR was performed in an automatic thermocycler (Biorad) with an initial denaturation step at 95°C for 5 min, and 35 cycles of 94°C for 1 min, 55°C for 1 min, and 72°C for 1 min, followed by a final extension step at 72°C for 5 min.[13] The PCR reaction product was then resolved using 2% agarose gel electrophoresis.

RESULT AND DISCUSSION

S. scabiei var. *cuniculi* mites were isolated from rabbits that showed clinical signs of scabies including thickening of the skin, crust formation, alopecia involving the eyes, ears, mouth, and legs. Morphological appearance of mites is spherical, transparent, and oval.[6,7] The dorsal surface of their body contains fine-grooved lines equipped with a plastron, conical scales, and spines. They have four pairs

of short legs; two pairs of legs on the anterior (notothorax) with a sucker and ambulacra, and two pairs of posterior legs (notogaster). The mouth consists of a chelicera, capitulum, and hypostome [6] (Figure 1).

Agarose gel electrophoresis revealed a 289 bp PCR product amplified from the DNA of *S. scabiei* isolates from both Surabaya and Malang in accordance with the expected *COX1* amplicon size. PCR reactions from both samples showed a band between the 200 bp and 300 bp molecular weight markers. The results were consistent with the calculated amplicon size based on primer positions within the *COX1* locus, with the forward primer spanning nucleotides 61–94, and the reverse primer spanning nucleotides 331–350 (350 – 61 = 289 bp). Electrophoresis results are shown in Figure 2 and Figure 3.

Phylogenetic studies on *S. scabiei* from rabbits using the *COX1* sequence are limited; Wong et al. (2005) reported that the *COX1* amplicon size using *S. scabiei* var. *cuniculi* isolated from Hong Kong was 250 bp, while that using *S. scabiei* var. *cuniculi isolated* from Indonesia, specifically Malang and Surabaya, was 289 bp. Zhao et al. (2015) reported that it is unclear whether the same species of Sarcoptes mites parasitize humans and animals, and data on genetic diversity of these mite populations in humans is scarce.[14] In a study conducted in China to identify *S. hominis* and *S. canis*, genomic DNA was extracted from ten individual mites (five isolated from patients with scabies and five from dogs with mange) for amplification of ITS2 rDNA, 16S mtDNA, and *COX1* fragment sequences. Future studies assessing the sequence of PCR products from *S. scabiei* are required to determine the level of homology among *S. scabiei* var. *cuniculi* originating from different countries, which can aid the development of subunit vaccines.

Scabies poses a significant threat to public health and causes economic loss because of high prevalence and the lack of efficient prevention measures. Dissatisfaction with use of insecticides is increasing because of the emergence of resistant parasites, environmental pollution, and consumer rejection of livestock products containing pesticide residues.[15] Vaccination is the best available alternative because it causes least damage to the environment and is safe for consumer use, and potentially more effective than scabicide and inexpensive; however, several obstacles hinder large scale vaccine production. Therefore, several studies have been aimed at developing molecular subunit vaccines using recombinant DNA cloned from *S. scabiei*.[16–19]

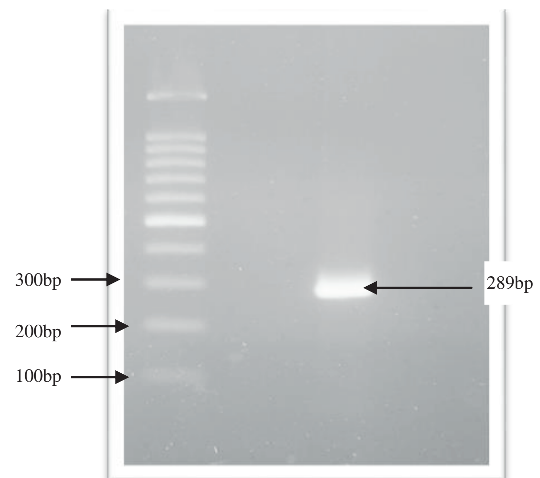


Figure 3. Agarose gel electrophoresis of the PCR product from *S. scabiei var. cuniculi* isolated from Malang

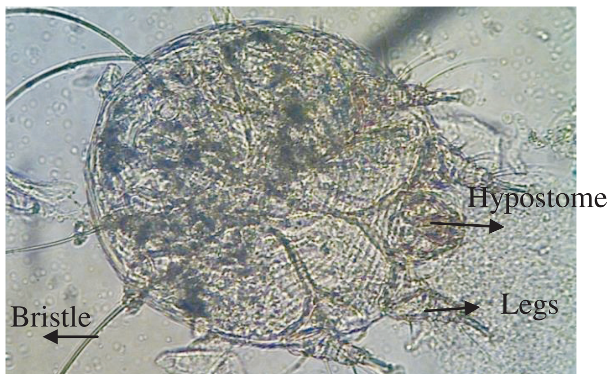
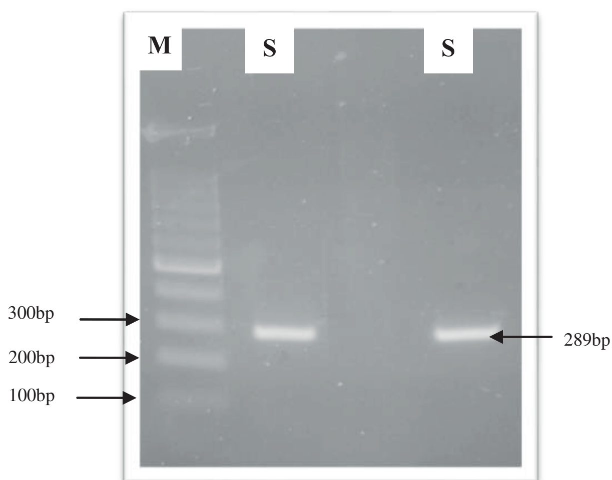


Figure 1. *Sarcoptes scabiei* ventral view (Olympus Optilab Camera Microscope, Magnification: 400×)



Information: M = Marker S = Sample

Figure 2. Agarose gel electrophoresis of the PCR product from *S. scabiei var. cuniculi* isolated from Surabaya

CONCLUSION

Sarcoptes scabiei isolated from rabbits that were infected with scabies was detected using *COX1* PCR with the forward primer 5'-TCTTAGGGGCTGGTATTAGTATG-3' and the reverse primer 5'-AGTTCCTCTACCAGTTCCAC-3', yielding the expected amplicon size of 289 bp.

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