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Mite *Sarcoptes scabiei* Varieties *Hominis* in South Sumatra: Specific Identification and Comparative Study

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Abstract

BACKGROUND: *Sarcoptes scabiei* mites have more than 15 genetically diverse varieties from various hosts. Identification of *S. scabiei* mite varieties *hominis* as an intervention in its prevention is still rarely done.

AIM: This study aimed to observe the genetic relationship of the mite *S. scabiei* varieties *hominis* compare to the parasite *S. scabiei* varieties *hominis* in other regions.

METHODS: This research used polymerase chain reaction (PCR) and sequencing methods with 16S gene-specific primers. From 32 *S. scabiei* samples, 22 samples were identified as varieties *hominis* that was marked by the appearance of the band at 132 bp.

RESULTS: *S. scabiei* mites *hominis* varieties from South Sumatra (Yessi Scabies A2 and Yessi Scabies B3) have similarities with deoxyribonucleic acid (DNA) strands with *S. scabiei hominis* varieties from China (KJ781377 and KJ781376). In contrast, Yessi Scabies A1 has similarities with DNA strands with mite *S. scabiei* varieties *hominis* from Australia (AY493402). Still, all the DNA strands, this research is different from *S. scabiei* mites DNA strands *hominis* from Panama and Pakistan.

CONCLUSION: The PCR method is advantageous and specific in identifying mites *S. scabiei hominis* varieties, the cause of scabies in humans.

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Introduction

Scabies is a contagious skin disease caused by mites *Sarcoptes scabiei* originating from human and animal hosts [1]. According to the World Health Organization, scabies is one of the most significant diseases in the world from six epidermal skin diseases with 300 million cases annually [1]. Scabies includes endemic skin diseases in tropical and subtropical regions, such as in Africa, Egypt, Central America, South America, Northern Australia, central Australia, the Caribbean Islands, India, and Southeast Asia [2]. Scabies caused by mites *S. scabiei* can enter the human skin layer (stratum corneum) in <30 min. The several reactions of insects in the skin are allergic reactions, inflammation, and the response of body immune in maintaining healthy skin [3].

Skin sensitivity to mite *S. scabiei* body and excretion results, mite *S. scabiei* secretion in the skin layer (stratum corneum) causes itching, resulting

in scratching activity on the skin. The results of scratching cause lesions on the surface [2]. The injury is the opening of the stratum corneum layer, which causes the bacteria *Streptococcus pyogenes* and *Staphylococcus aureus* enter, resulting in secondary infections [4]. Secondary infections cause impetigo, cellulitis, and abscesses and can cause life-threatening diseases, including sepsis, and glomerulonephritis [5]. Scabies examination through clinical symptoms was sometimes less accurate caused by similarities with other skin diseases. Scabies in humans is still ignored by the community, especially in students living in Islamic boarding schools because of a lack of knowledge about the causes, transmission, prevention, and complications of scabies.

There are various methods in diagnosing scabies, namely, skin scrapings, dermatoscopy, burrow ink, and tetracycline fluorescence. Grover and Jakhar (2017) use skin scrapings and dermatoscopy methods to identify scabies [6]. Examination of skin scrapings is done by putting the results of skin scrapings under

a microscope. On the other hand, an investigation by dermatoscopy is more practical and accurate because of mites. *S. scabiei* can be seen in the epidermis layer of the skin but cannot identify the mite varieties. Amanda *et al.* (2018) identified scabies by examining the burrow ink test by skin scrapping [7].

The polymerase chain reaction (PCR) method can detect genotype differences between *S. scabiei* mites from different hosts, including pigs, rabbits, foxes, hedgehogs, and humans [3]. Host populations that are adapted and located separately from *S. scabiei* are characterized using 16S primers [8]. Mites *S. scabiei* has more than 15 genetically diverse varieties from various hosts [9].

Research on the insects that cause scabies in human is still rarely done in Indonesia. Identification of parasites *S. scabiei* varieties *hominis* using PCR method with 16S primers has never been done in the South Sumatra region, especially in Islamic boarding schools. This study aims to identify the causes of scabies that occur in humans and genetically compare the mite *S. scabiei hominis* varieties in South Sumatra with other regions.

Methods

Study area

The research was conducted in boarding school in Palembang city (address: Depaten lama road 27 Iilir; 104°45'18"east longitude, 2°59'37" south latitude) and Indralaya district (address: Sakatiga village; 104°45'18"east longitude, 3°15'22" south latitude), South Sumatera, Indonesia. The analysis was carried out from August to December 2018.

Collecting samples

This research is an experimental analytic with a cross-sectional design. The sample came from the skin scrapings of respondents with scabies who lived in two Islamic boarding schools (RU boarding school and MS boarding school) located in South Sumatra. The sample examination was carried out in the laboratory of the Faculty of Medicine, Universitas Sriwijaya, Palembang, Indonesia. Respondents who suffer from scabies do skin scraping by lifting the papules or the roof of the tunnel that is skinned using a sterile scalpel number 15. A complete papule/tunnel is dropped with mineral oil or 10% KOH. Scrapings are placed on the slide and then given mineral oil or immersion oil and then given a cover glass and examined under a microscope with a magnification of 10–40× [10]. Examination with scrapings in diagnosing scabies is known to be 100% specificity and 90–95% sensitivity when scraping is

done at the right location. This study has received ethical approval at the Faculty of Public Health, Universitas Sriwijaya No. 289/UN9.1.10/KKE/2018.

Deoxyribonucleic acid (DNA) extraction

Human skin samples were placed in a tube. Ethanol added, then put into a 1.5 ml microcentrifuge tube and added with a 180 µl ATL buffer, 20 µl proteinase K, mixed with vortex and incubated at 56°C for 24 h until lysis, occasional vortex during incubation, vortex 15. Samples were then added 200 µl AL buffer, vortex, and incubated at 56°C 10'. The next sample is added with 100% ethanol and mixed with a vortex. The mixture is then taken with a dropper pipette and put in a Dnase Mini spin column 2 ml collection tube, 6000 × g (8000 rpm) centrifuge for 1', and then the collection tube is discarded. A 2 ml spin column tube was added with 500 µl Buffer AW1 and centrifuged 1' 6000 × g (8000 rpm), and then the collection tube was removed. A 2 ml spin column tube was added with 500 µl AW2 buffer and centrifuged for 4' at 13,000 rpm, and then the collection tube was removed. The 2 ml spin column tube is centrifuged 1' at 13,000 rpm. Then, the collection tube is removed. 1.5–2 ml of the spin-column machine is transferred into the microcentrifuge tube. DNA elution is carried out by adding 200 µl of AE buffer in the middle of the spin column membrane tube. Incubation is performed at 1' room temperature (15–25°C), while centrifugation is at 1' 6000 × g (8000 rpm). The process is repeated in eight steps to increase the amount of DNA.

PCR and sequencing

The PCR application lasts for 40 cycles. DNA amplification results were detected by electrophoresis for 45 min with 85 volts using 2% agarose (1 g agarose in 50 ml TAE and added 2.5 µl ETBR). The amplicons from samples and markers are electrophoretic, and the electrophoretic DNA can be seen under ultraviolet light on the Biorad doc gel. First pair of PCR reaction, that is, Forward: 5' – GGG TCT TTT TGT CTT GGA ATA AA – 3'. Reverse: 5' – CTA AGG TAG CGA AAT CAT TAG C – 3' using 16S primers [11]. Sequencing products were analyzed using ABI 3730 xl Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). The sequences that have been collected are stored in the NCBI GenBank database.

Phylogenetic tree construction

Phylogenetic tree analysis was built using Mega 6.0 software according to Tamura *et al.* and Harnelly *et al.* [12], [13]. The phylogenetic tree was used to search for the comparison of mite *S. scabiei* varieties *hominis* from various regions taken from NCBI GenBank data.

Results

From the results of PCR analysis using primers used 16S F/R on 2% agarose gel, there is a specific band of 132 bp which identified the presence of mite *S. scabiei* varieties *hominis* in human samples.

From 12 DNA specimens derived from scrapings of human skin suffering from scabies, the samples RU1, RU3B, RU4A, RU7A, RU7B, RU8, and RU10A showed that *S. scabiei* mite DNA was positive which was indicated by the appearance of bands on the target band at 132bp (Figure 1a). The samples of RU2A, RU2B, RU3A, RU5, and RU6 show that the DNA of the mite. *S. scabiei* is negative, which is indicated by the absence of bands on the target band at 132 bp. From 13 DNA specimens derived from scrapings of human skin suffering from scabies, in samples with codes RU.10B, RU.11, MS.2, MS.3, MS.4, MS.5A, MS.5B, MS.6, MS.7, MS.8A, and MS.8B showed that the mite *S. scabiei* varieties *hominis* was positive which was indicated by the appearance of bands on the target band at 132 bp (Figure 1b). The RU12 and MS.1 code samples show that the mite *S. scabiei* varieties *hominis* of DNA is negative, which is indicated by the absence of bands on the target band at 132 bp. From 7 DNA specimens derived from scrapings of human skin suffering from scabies, in code samples of MS.10, MS.11, MS.12, and MS.13A showed that mite *S. scabiei* varieties *hominis* DNA was positive which was marked by the appearance of bands on the target band, that is, at 132 bp (Figure 1c). The MS9A, MS9B, and MS13B code samples show that the mite *S. scabiei* DNA of *hominis* varieties is negative, which is characterized by the absence of bands on the target band at 132 bp.

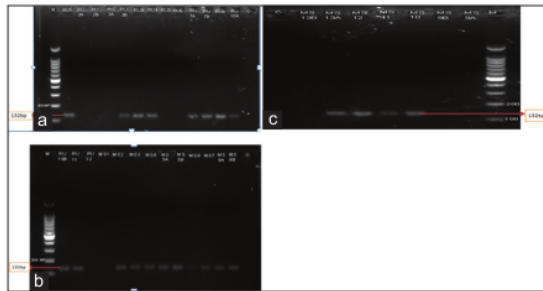


Figure 1: (a-c) Positive band of *Sarcoptes scabiei*

Phylogenetic tree

Sequencing analysis of 16S *S. scabiei* primer varieties *hominis* originating from different hosts from Panama 3 sequence types, from Australia 7 sequence types, from Pakistan 1 sequence type, and from China 2 sequence types form a cluster on a phylogenetic tree together with reference sequences from GenBank are the broad host and geographic distribution of *S. scabiei* mites *hominis* varieties (Figure 2). Mite *S. scabiei*

varieties *hominis* from South Sumatra (Yessi Scabies A2) and (Yessi Scabies B3) is genetically related to parasite *S. scabiei* varieties *hominis* China (KJ781377 and KJ781376). Furthermore, mites *S. scabiei* from South Sumatra (Yessi Scabies A1) is genetically related to insect *S. scabiei* varieties *hominis* from Australia (AY493402).

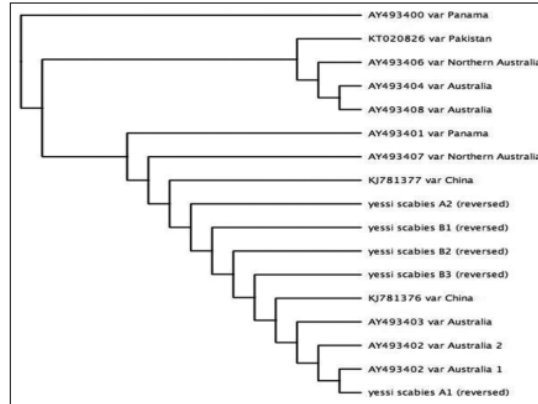


Figure 2: Identification of mite *Sarcoptes scabiei* *hominis* varieties with 16S gene primers based on nucleotide sequences

Discussion

Based on PCR test results with a sample of 32 mites *S. scabiei*, the parasite *S. scabiei* DNA was positive with a 132bp band of 22 insects *S. scabiei* varieties *hominis*. This study is in line with the results of Naz *et al.*, 2018, that samples from hospital patients in Pakistan are positive if there are mite *S. scabiei* varieties *hominis* DNA marked by the presence of band bands at a target of 600 bp using 16S primers and harmful if no band was found from the mite *S. scabiei* varieties *hominis* [11]. This study was also supported by research by Walton and Currie, Naz *et al.*, Walton *et al.* and Walton *et al.* [2], [11], [14], [15], the length of band fragments on DNA mite *S. scabiei* varieties *hominis* with the band between 138 bp and 178bp. Insects have a minimum body size. Female ticks are with a capacity of 0.3 mm and width of 0.25 mm, and male bugs are with a size of half of the size of female insects [16]. Mite size was <0.4 mm [11]. Mites *S. scabiei* *hominis* varieties *hominis* can be able to survive in the environment outside the human body, for 24–36 h with normal room conditions that have a temperature of 21°C. Mites *S. scabiei* varieties *hominis* are associated with the environment with residential density [17]. High risk of transmission of scabies to dense residential settings such as Thai orphanages (87%) [5], [18], refugee camps in Sierra Leone (67%) [5], and Islamic religious schools in Bangladesh (61%) [19]. Research conducted by Walton *et al.* [15] randomized phylogenetic grouping of several *S. scabiei* mites originating from dogs and human hosts.

The results obtained are that there are different forms of groups that prove that in endemic areas, scabies transmission occurs through from human to human.

Sequencing analysis with 16S primers from mites *S. scabiei* varieties *hominis* that come from different hosts from Panama (three sequence types), Australia (seven sequence types), Pakistan (one sequence type), and China (two sequence types) formed a cluster in phylogenetic trees with reference sequences from a gene bank. This reflects the broad host and distribution of the location of the *S. scabiei* population variety of *hominis*. Mites *S. scabiei* varieties *hominis* from Southern Sumatra (Yessi Scabies A2 and Yessi Scabies B3) genetically has similarities with *S. scabiei* mites varieties *hominis* originating from China (KJ781377 and KJ781376). Yessi Scabies A1 is genetically similar to *S. scabiei hominis* variety from Australia (AY493402). Mite *S. scabiei* varieties *hominis* originating from Pakistan (KT020826) do not have the genetic similarity of all mite *S. scabiei* varieties *hominis* in Southern Sumatra. Based on the history of the origin of scabies, the first incident of Scabies in Australia occurred in 1854 due to the presence of Chinese people who brought scabies to come to Victoria. Based on medical records the first Scabies incident occurred in Northern Australia, which was located more than 3000 km from Victoria in 1944 by Lt Col, the first shipment of Chinese people in the golden age of Victoria in 1954 [19].

Conclusion

The PCR method is advantageous and specific in identifying mites *S. scabiei hominis* varieties, the cause of scabies in humans. Epidemiological studies of parasites *S. scabiei* are needed in the prevention and control of scabies in humans in residential areas, especially in Islamic boarding schools.

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