COMPARATIVE HISTOPATHOLOGIC CHANGES IN RABBIT (Oryctolagus cuniculus) (MAMMALIA: LAGOMORPHA: LEPORIDAE) SKIN IN RELATION TO DEGREE OF INFESTATION WITH Sarcoptes scabiei (ARACHNIDA: ACARI: SARCOPTIDAE)

RESEARCH NOTE

COMPARATIVE HISTOPATHOLOGIC CHANGES IN RABBIT (*Oryctolagus cuniculus*) (MAMMALIA: LAGOMORPHA: LEPORIDAE) SKIN IN RELATION TO DEGREE OF INFESTATION WITH *Sarcoptes scabiei* (ARACHNIDA: ACARI: SARCOPTIDAE)

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ABSTRACT

The aim of this research was to observe the histopathological changes in rabbit ear skin tissue caused by varying degrees of *Sarcoptes scabiei* infestation. This study used twelve 7-12 months old female local rabbits obtained from rabbit farms with poor sanitation. Clinical symptoms of ear infection include presence of papules, vesicles, erythema, crusta and alopecia in the ear, nose, eyes and feet. Twelve local rabbits infected by *S. scabiei* were divided into three groups with four rabbits each: P1 with mild scabies, P2 with moderate scabies and P3 with severe scabies. Histopathologic changes, which manifested in lesions, varied from parasitic infestation, parakeratosis, acanthosis, congestion, inflammation and cell degeneration. These were given scores from 0 to 4 (0, not seen; 4, highly visible). The mean score was highest in those with severe scabies. Histopathological changes in rabbit ear skin tissue using Mann-Whitney U test was significant (P<0.05): mild scabies (4.625±0.75), moderate scabies (8.8125±1.95) and severe scabies (17.5625±1.59). Severe scabies had the highest degree of damage, defined by parakeratosis, acanthosis, substantial cell degeneration and congestion and serious inflammation. This study suggests significant differences in histopathologic changes in skin tissue of rabbits with mild, moderate and severe scabies.

Key words: histopathologic changes, rabbit, *Sarcoptes scabiei*, scabies

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INTRODUCTION

To this day, scabies has been considered an emerging or re-emerging parasitic disease that threatens human and animal health worldwide because of its prevalence, and ways to control its spread are still limited, resulting to major economic losses (Tarigan, 2003; Walton and Currie, 2007; Alasaad *et al.*, 2013). Several areas of goat and rabbit farming in Indonesia have been reported for scabies infection, most likely caused by poor hygiene and sanitation. In addition, crammed cages and humidity contribute to the transmission of scabies to healthy animals (Wardhana *et al.*, 2006; Lastuti *et al.*, 2017). Scabies can be diagnosed by looking for clinical signs and performing laboratory tests, such as microscopic examination of skin scraping or through burrow ink test (Budiantono, 2004; Walton and Currie, 2007). Research in scabies diagnosis has been developed, and findings suggest that *S. scabiei* var. *caprae* in goats

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contains specific antigen protein around 205, 8, 57, 3 and 43 kDa, which can then be used to develop a candidate diagnostic kit (Lastuti *et al*., 2018). *Sarcoptes scabiei* is a parasite that requires a host to breed, and, once settled, creates tunnels in the stratum corneum, sucking the lymphatic fluid by tearing the epidermal layer and feeding on young epidermal cells. This causes intense itching and can lead to injury when the skin is scratched persistently, releasing an exudate which causes skin scabs (Soulsby, 1986; Espinosa *et al*., 2017).

If scabies infection reaches the connective tissue, along with skin fibrosis, epidermal hyperplasia and mononuclear cell become dominant in the perivascular, reaching chronic stage, which can be detrimental to the animal (Budiantono, 2004; Arlian *et al*., 2004; Espinosa *et al*., 2017). Determining the extent of skin damage caused by mild to severe scabies through histopathologic changes can be important, since chronic inflammation can lead to economic losses in rabbit livestock. Also, determining this can serve as reference for scabies control.

The aim of this research is to explore the extent of damage to rabbit skin tissue infected with mild to severe scabies based on histopathologic changes. Assessing these changes by scoring method will be done for the first time in Indonesian samples. This study assumes significant differences in histopathologic changes in rabbit skin tissue with varying degrees of scabies infection. Reaching a certain threshold, *e.g*., displaying severe clinical symptoms can be an important factor when deciding to eliminate animals in a population. The results of this study can then be used as a reference for scabies prevention in rabbits.

MATERIALS AND METHODS

Animals

This research was approved by the Ethics Commission of the Faculty of Veterinary Medicine, Universitas Airlangga, No: 630-KE in accordance with the rules of experimental animal use. This research was done in the Veterinary Pathology Laboratory and Veterinary Parasitology Laboratory of the Faculty of Veterinary Medicine, Universitas Airlangga. Animals used were 12 female, 7 to 12 months old local rabbits infected with scabies, divided into three groups with four rabbits each (P1 with mild scabies, P2 with moderate scabies and P3 with severe scabies). Sampling criteria were based on a number of clinical signs: mild scabies has papules, vesicles, erythema and few crusta on the ear; moderate scabies shows mild hyperkeratosis or crusts on the ear, mild alopecia and thin scabs; meanwhile, severe scabies is characterized by the presence of crusts, pus and excessive lichenification, which causes the skin to look wrinkly and cracked, along with alopecia in almost all of the infected skin area (Espinosa *et al*., 2017).

Identification of *S. scabiei* var. *cuniculi*

To verify scabies infection, the ears were scraped then added with 10% KOH (Merck, Germany), and samples were examined under a microscope (Nikon E-100, Japan) at 100× magnification. *Sarcoptes scabiei* was identified using identification keys by Soulsby (1986). After being tested positive for scabies, the rabbits were euthanized by injecting 100 mg/kg ketamine (Mylan, Singapore) intramuscularly. Infected ears were incised with a diameter of 1 cm × 1 cm, and skin tissues were then immersed in 10% PBS solution (Merck, Germany) for histopathology preparations.

Preparation of histopathology specimens

Samples were fixed using 10% PBS solution, soaked for 24 h and washed with distilled water. Samples were dehydrated and cleared with alcohols 70%, 80%, 96%, absolute I-III, and xylol I and II (Merck, Germany) for 30 min. Tissues were submerged into paraffin I and II fluids, put into the oven (Memmert, Germany) at 80°C for 30 min, dipped back into paraffin fluids, then into the oven at same conditions. Paraffin blocks were made. Tissues that have expanded adequately after being dipped into warm water at 60°C were sliced with a thickness of 4-6 µm. These samples were then placed on a glass object (Sail Brand, China) previously smeared with

albumin, then dried over a hot plate at 60°C, and stained with hematoxyline eosine (HE) (Merck, Germany). Histological examination was done using a microscope (Nikon E-100, Japan) at magnifications 40×, 100×, 400×, followed by assignment of scores from 0 to 4 (0, not seen; 4, highly visible) on each lesion in terms of parasitic infestation, parakeratosis, acanthosis, cell degeneration, congestion and inflammation (Klopfleisch, 2013). Scoring results were analyzed using statistical tests Kruskal Wallis and Mann-Whitney U Test.

RESULTS AND DISCUSSION

Scraping of infected rabbit skin showing scabies symptoms identified the parasitic mite *S. scabiei* var. *cuniculi*. Histopathological changes in the skin tissue infected with scabies of varying severity, from mild, moderate and severe, were evident in the epidermis, defined by parakeratosis and acanthosis, and the dermis, characterized by parakeratosis, inflammatory cell infiltration, degeneration and congestion (Fig).

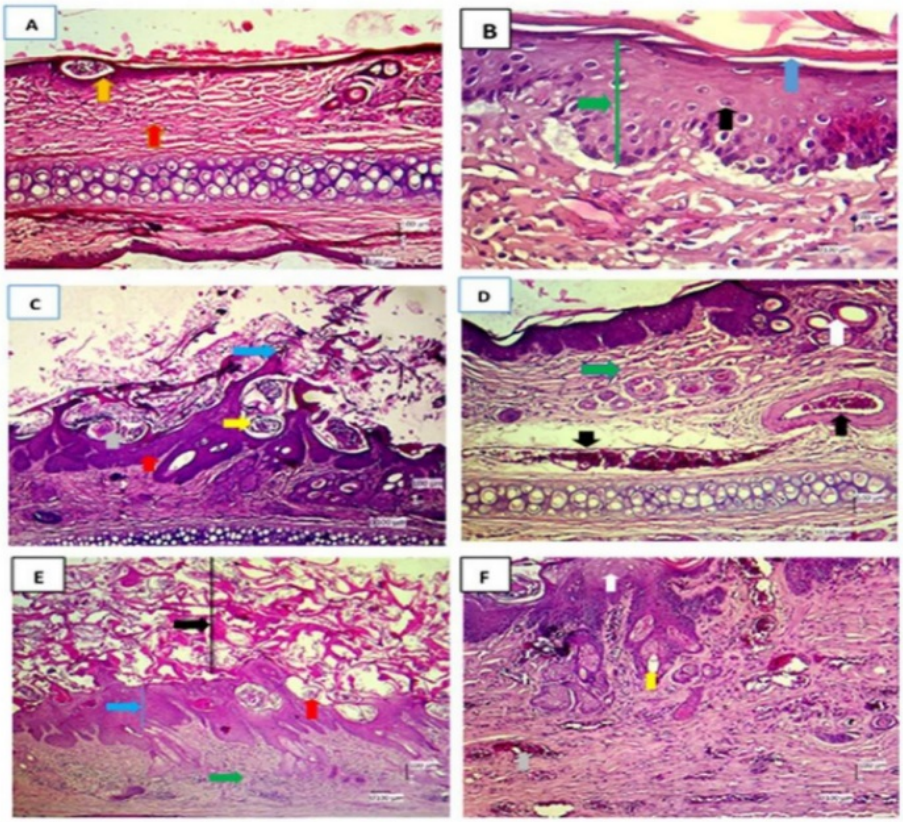

Fig. A and B: Histopathologic changes seen in rabbit with mild scabies: (yellow arrow) infestation of *S. scabiei,* (red arrow) inflammatory cell infiltration, (blue arrow) thin hyperkeratosis, (green arrow) acanthosis, (black arrow) cell degeneration. C and D: Histopathologic changes in rabbit with moderate scabies: (blue arrow) parakeratosis, (yellow arrow) mature mites, (gray arrow) mite larvae, (red arrow) acanthosis, (green arrow) infiltration of inflammatory cells, (white arrow) alopecia, (black arrow) congestion. E and F: Histopathologic changes in rabbit with severe scabies: (black arrows) severe parakeratosis, (blue arrows) acanthosis, (red arrows) mature infestation of mites, (green arrows) inflammatory cell infiltration, (white arrow) cell degeneration, (yellow arrow) alopecia, (gray arrow) congestion. (Bar = 100 µm) Nikon® E-100 microscope.

Histopathological changes in the rabbits were evaluated based on the descriptions of parakeratosis, acanthosis, congestion, inflammation and degeneration of cells. These were given scores of 0-4 (Klopfleisch, 2013). Results of the mean scores are presented in Table 1. Kruskal Wallis test, followed by Mann-Whitney U test, indicates a significant difference ($P<0.05$) between groups (Table 2).

Histopathologic changes induced by parasitism caused an immune response (protection against foreign agents) and disturbances in keratinization, in the form of parakeratosis and acanthosis. Keratinization, in particular, has resulted to thickened and layered skin and hair loss, leading to secondary infection (Espinosa *et al.*, 2017). Based on its life cycle, *S. scabiei* begins its settlement by penetrating and sucking on the lymph, feeding on young epidermal cells, which then causes irritation and intense itching. Scratching leads to formation of a solid exudate and a crust on the skin's surface. Clinical symptoms of severe itching seem to be associated with type I, III and IV hypersensitivity reactions in humans (Arlian *et al.*, 2004), and it appears that *S. scabiei* creates a substance that activates type 1 T-cells to produce IL-10, which plays anti-inflammatory and immune suppression roles (Arlian *et al.*, 2004; Lastuti *et al.*, 2018).

Table 1. Mean scores of histopathologic changes in *S. scabiei* var. *canaliculi* based on degree of infestation in rabbit.

Parameter	Mean score		
	Mild scabies	Moderate scabies	Severe scabies
No. of parasites	0.3	1.4	3.4
Parakeratosis	0.3	0.9	2.6
Acanthosis	0.5	1.6	3.5
Cell degeneration	1.7	2.2	3.2
Congestion	0.4	0.6	1.0
Inflammation	1.4	2.1	3.8

Table 2. Statistical test scores of histopathological changes in *S. scabiei* var. *canaliculi* based on degree of infestation in rabbit.

	Mild scabies	Moderate scabies	Severe scabies
Total score (mean±SD)	4.625 ± 0.7500^{c}	8.812 ± 1.9512^{b}	17.562 ± 1.5898^{a}

Different superscripts on the same column show significant difference ($P<0.05$).

Mitosis in the epidermal layer starts from the basal stratum which increases the production of keratin cells rapidly, resulting to acanthosis, the thickening of the stratum spinosum due to increased cell division. In severe scabies, parakeratosis and acanthosis occur very significantly compared with moderate and mild scabies due to increased infestation by *S. scabiei.* (Nanney *et al.*, 1986; Espinosa *et al.*, 2017). Parakeratosis, characterized by the presence of of pyknotic nuclei that aggregate in the stratum corneum, occurs since the epidermal cells fail to differentiate completely. Further change in the structure of the epidermis caused by *S. scabiei* infestation is injury to the cell membrane of epithelial cells, especially in the granular layer and stratum spinosum. This decreases the cell's permeability because of the antigenic proteins released by the mites

during penetration, as the parasites suck on blood and fluid lymph and feed on epidermal cells. Such conditions allow K^+ ions to be easily transported outside the cells and vice versa; Ca^+, Na^+ and water easily pass through the cells, causing the cytoplasm to swell (Hennings *et al.*, 1983).

Hydropic degeneration that attacks epithermal epithelial cells in severe scabies is more significant compared with moderate and mild scabies, since higher antigen production means reduced cell permeability and formation of hydropic degeneration in epidermal epithelial cells. Lesion on the epidermal layer will then activate the inflammatory mediator, which stimulates the vasoactive amine, increasing vascular permeability, vasodilation and activation of histamine and serotonin. Blood flow out of the veins is reduced, and blood accumulates in the vein, a condition called congestion (Charles *et al.*, 1967).

Accumulation of erythrocytes also influences the the movement of leukocytes, becoming attached to the edge of the endothelium in an attempt to exit the site of lesion. There were significant differences in congestive veins in severe, moderate and mild scabies. High inflammatory reaction in severe scabies occurs because of higher degree of infestation, leading to increased blood flow, vasodilation and congestion.

Prolonged and worsened *S. scabiei* infestation will activate type IV hypersensitivity reaction due to tissue damage from the accumulation of macrophages, monocytes and lymphocytes in exposed areas (Walton *et al.*, 2010; Singh *et al.*, 2014; Lastuti *et al.*, 2018). Skin tissue of rabbits with severe *S. scabiei* infestation shows evidence of chronicity: many mononuclear cells dominated the surface of the dermis, and the perivascular was inflamed; meanwhile, polymorphonuclear cells predominated in the dermis layer of rabbits with moderate and mild scabies. Morever, other histopathologic changes include alopecia (hair loss in animals), a condition which disrupts the body's immune system due to infection caused by parasites, viruses, bacteria or stress (Bandi and Saikumar, 2013; Kuty-Pacheck, 2015; Espinosa *et al.*, 2017).

This study reveals significant differences in histopathological changes in rabbit skin tissue with mild, moderate and severe scabies based on the presence of parakeratosis, acanthosis, substantial cell degeneration and congestion, and high level of inflammation. Further studies through immunochemistry is suggested to investigate cytokines in damaged skin caused by scabies.

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