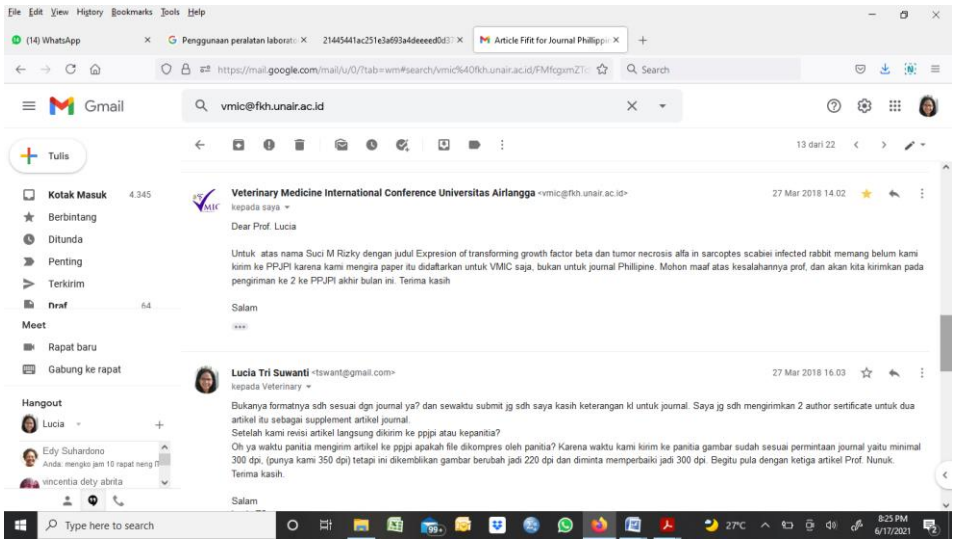
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kepada saya
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Dear Prof. Lucia
Untuk atas nama Suci M Rizky dengan judul Expresion of transforming growth factor beta dan tumor necrosis alfa in sarcoptes scabiei infected rabbit memang belum kami kirim ke PPJPI karena kami mengira paper itu didaftarkan untuk VMIC saja, bukan untuk journal Phillipine. Mohon maaf atas kesalahannya prof, dan akan kita kirimkan pada pengiriman ke 2 ke PPJPI akhir bulan ini. Terima kasih
Salam
Lucia Tri Suwanti <tswant@gmail.com>
kepada Veterinary
27 Mar 2018 16.03
Bukanya formatnya sdh sesuai dgn journal ya? dan sewaktu submit jg sdh saya kasih keterangan kl untuk journal. Saya jg sdh mengirimkan 2 author sertificate untuk dua artikel itu sebagai supplement artikel journal.
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Oh ya waktu panitia mengirim artikel ke ppjpi apakah file dikompres oleh panitia? Karena waktu kami kirim ke panitia gambar sudah sesuai permintaan journal yaitu minimal 300 dpi, (punya kami 350 dpi) tetapi ini dikemblikan gambar berubah jadi 220 dpi dan diminta memperbaiki jadi 300 dpi. Begitu pula dengan ketiga artikel Prof. Nunuk.
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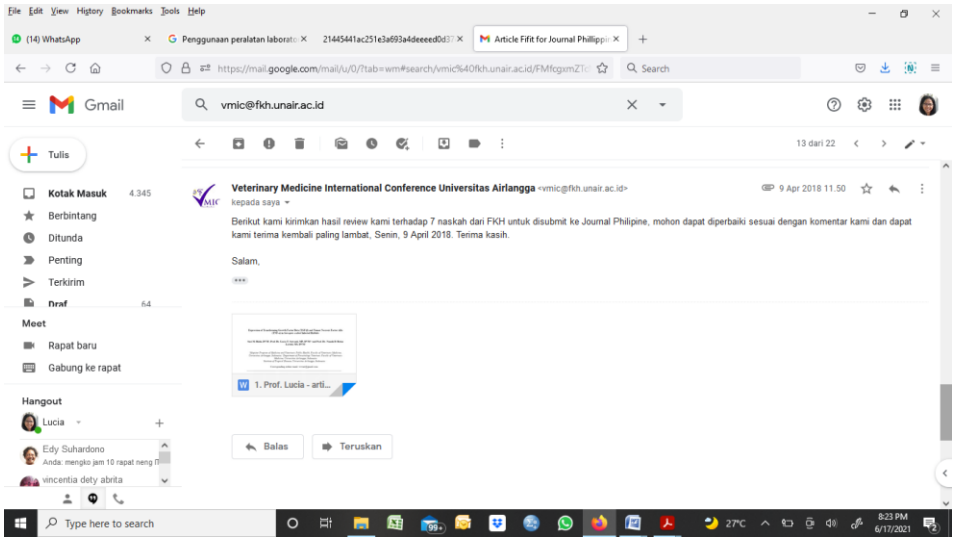
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Berikut kami kirimkan hasil review kami terhadap 7 naskah dari FKH untuk disubmit ke Journal Philipine, mohon dapat diperbaiki sesuai dengan komentar kami dan dapat kami terima kembali paling lambat, Senin, 9 April 2018. Terima kasih.
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Histochemical Expression of Transforming Growth Factor Beta (TGF-β) and Tumor Necrosis Factor Alfa (TNF-α) in *Sarcoptes scabiei* Infected Rabbits

Suci M. Rizki, DVM[1], Prof. Dr. Lucia T. Suwanti, MP, DVM[2, 3] and Prof. Dr. Nunuk D. Retno Lastuti, MS, DVM[2]

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Abstract

Sarcoptes scabiei mite infection caused type I and type IV hypersensitivity reactions. Type I and type IV hypersensitivity were played by cytokines Transforming Growth Factor Beta (TGF-β) and Tumor Necrosis Factor alfa (TNF-α). This study was conducted to analize the expression of cytokines TGF-β and TNF-α in *S. scabiei* infected rabbits. The sample was the ear skin of rabbits with clinical signs of scabies obtained from farms in some cities in East Java. The rabbits that used as samples were infected rabbits that haven't been given medical therapy. To make sure the rabbits are infected with scabies, a skin scraping is performed to detect the presence of *S. scabies* mites. Histological skin were stained with Haematoxilin Eosin (HE) and immunohistochemistry. The results showed that in all samples showed strong staining of TGF-β and moderate staining of TNF-α. TGF-β was observed on stratum granulosum to stratum basalis of epidermis layer, half of the dermis, sebaceous gland, and hair follicle. TNF-α expressions was visible from half of the epidermis layer, stratum spinosum to stratum basalis, and half of the dermis, but not on the sebaceous gland and hair follicle.

Key words: Cytokine, Immunohistochemistry, *Sarcoptes scabiei*, Scabies,TGF-β, TNF-α

1. Introduction

Scabies was a zoonosis disease that caused by *Sarcoptes scabiei* mites, highly contagious, infected people and wild animals, domestic animals and livestock (Bandi and Saikumar, 2013), and could affected on decreasing of the income in the livestock industry, e.g rabbit farming industry (Tarigan, 2003; Wardhana and Iskandar, 2006; Desiandura *et al*., 2017).

Scabies on rabbit caused by *Sarcoptes scabiei var*. *cuniculi*, with round or oval morphological shapes (Arlian and Morgan, 2017). Live cycles of *S. scabiei* mites consisted of several stages: egg, larva, *deutonymph, tritonymph,* and adult phases (Scoot *et al*., 2001; Arlian and Morgan, 2017). Mites mated on the host epidermis, and male mites will explore the skin continuously for several days to find female mites (Mellanby, 1985). Female mites will lay 2-4 eggs every day. Eggs were put 1 cm inside stratum corneum and sometimes mites will make a tunnel to reach stratum granulosum from the epidermis (Orion *et al*., 2006). Scabies, from its severe levelness, was divided into three stadium which was mild, moderate and severe stadium (Davis *et al*., 2013). At the severe stadium, there were crusts, pus, and excess hyperkeratosis on nose, muzzle, around the eyes, ears, and legs (Sofyan and Chrismanto, 2017).

The scabies transmission to human happened because direct contact with the infected animal and caused pruritic and irritation due to hypersensitivity reaction against mites and their product (Bandi and Saikumar, 2013), as the result of the involvement of type I and Type IV hypersensitivity reaction (Burns, 2004; Hick and Elston, 2009), TGF-β played an important role in type IV hypersensitivity reaction (Jyonouchi, 2015). Meanwhile, cytokine TNF-α played an important role in type I hypersensitivity reaction (Baratawidjaja and Rengganis, 2004; Burns, 2004; Mullins *et al*., 2009; Bhat *et al*., 2017). Bhat *et al*. (2017) stated the ones that have an important role during early inflammation response in scabies infection was active complement system, C3a, and C5a which activated mast cell to produced histamine and *tumor necrosis factor alpha* (TNF-α). Janeway *et al*. (2001), reviewed that in type I hypersensitivity reaction, Th2 cell have an important role in immediate hypersensitivity reaction initiation by stimulated IgE production and promoted inflammation. Th2 cell occurred because the presentation of antigen with T helper CD4+ cell, or maybe by dendritic cell caught antigen from the origin. The response

that occurred by antigen and another stimulus including cytokine (IL-4), T cell that differentiated into Th2 cell. Th2 cell will produce a large amount of cytokines (IL-4, IL-5, and IL-13). IL4 would react to B cell to stimulate the production from IgE and promoting more Th2 cell. Mast cell and basophil have a receptor called FcεR1, , that specifically into IgE and actively related to IgE.

First of all, antigen (allergen) will associated with IgE antibody, later IgE latched onto the mast cell. The bond of IgE with Fcε receptor will activated transduction signal to mast cell cytoplasm. This signal would cause degranulation of mast cell, so the active mediator in the mast cell granular released. Mast cell-produced cytokines (TNF, IL-1, IL-4, IL-5 and IL-6) and chemokine have an important role in type I hypersensitivity reaction through its ability to recruit and activate several kinds of inflammatory cell. (Janeway *et al*., 2001).

Mangan (2006) noted that type IV hypersensitivity reaction was divided into 2 stage. The first stage was proliferation and differentiation of T CD4 cells, and the second stage was T CD8+ cell reaction. Proliferation and differentiation of T CD4+ cells began with T CD4+ cell acknowledging peptide assembly that showed by dendritic cell and secreted IL-2 , as growth factor autocrine for the proliferation of T cell antigen-responsive. The difference between T cell antigen-stimulated with Th1 or Th17 seen at the cytokine production by APC during T cell activation. APC (dendritic cell and macrophage) sometimes will produced IL-12 that inducted T cell differentiation into Th1. In the development, IFN will be produced by the Th1 cell. If APC produced cytokines like IL-1, IL-6, and IL-23, and collaborated with TGF-β to stimulates T cell differentiation into the Th17 (Mangan, 2006).

This research used immunohistochemistry method in order to see the expression of TGF-β and TNF-α on rabbit skin that infected by *S.scabiei* at a severe stadium of infection. The goal

of this research is to see, from cytokine expression, how far cytokine takes a role in the inflammation process. This research used rabbits that obtained from rabbit farm in several cities in East Java regions and showed clinical symptoms of scabies, advanced stage.

2. Materials and Methods

Sample

The sample that used in this research was rabbits ears skin tissue that showed clinical symptoms of severe stage scabies. The rabbits obtained from several rabbit farms in East Java Region and The samples were taken during January until February 2018. Rabbits that used as samples were infected rabbits which haven't got any medical therapy. We used part of rabbit's earlobe as the sample of skin tissue. This research conducted in the Veterinary Pathology Laboratory and Veterinary Parasitology Laboratory, Faculty of Veterinary Medicine, Universitas Airlangga.

Ethical approval

This research has been approved by the ethical committee of the faculty of veterinary medicine, Universitas Airlangga, Number: 630-KE in correspondence of animal testing and usage rules.

Skin scraping and parasitological examination

Skin scraping was conducted by scraping the scabies infected skin. First, the scraped skin, purified using 10% KOH solution and put on slide object glass, and examined under a microscope with 100x magnification. Identification of *Sarcoptes Scabiei* according to Soulsby (1982).

Histopathological examination

Tissue was fixated in 10% formalin and to make the histopathology slides by embedded rabbit skin tissue size 1 cm x 1 cm in paraffin block. Afterward the piece of skin tissue was cut by

microtome with 4 μm thickness and put into the 55^{o} C water bath. The floated piece of tissue in water bath was picked up by slide object glass and dried at 40^{o} C hot plate for over night. Then deparaffinization and rehydration tissue slides were done. The slide was put on the slide holder. Next, the slide was soaked gradually in xylol solution 2 times, each for 5 minutes, incrementally alcohol (96%, 90%, 80%, 70%), 4 minutes each of it, and then washed (immersed) with water for 5 minutes, then coloring using Hematoxylin Eosin (HE), and lastly dehydrated, clearing and mounting.

Immunohistochemical examination

Immunohistochemical was done by LSAB Kit from Dako®. The skin tissue in paraffin block was cut with 4 μm thickness, and later on, it got deparaffinated, rehydrated and incubated with primary antibody (polyclonal TGF-β or TNF-α). After that, slide was carried out using a commercially available kit and following the manufacture's protocols. Finally tissue was given *counterstain* using Haematoxylin.

Image analysis

The observation of histopathology slide and immunohistochemical slide with Nikon® E-100 Microscope and photos were taken by OptiLab® MTN001 with 100x and 400x magnification. Klopfleisch (2013) semiquantitative modified scoring system were used at histopathology slide. At immunohistochemical slide, positive tissue marked by the change of color, which was brown. The intensity of coloring from cytokine TGF-β and TNF-α were grouped into 4 group: strong (≥50% colored cell) score 3, moderate (20-50% colored cell) score 2, weak (<20% colored cell) score 1 and negative (no colored cell) score 0 (Nassef *et al*., 2016).

3. Result and Discussion

Scabies was the common skin disease that found in the environment with poor sanitation, especially in the developed country. The common symptoms of scabies were pruritus, scabs, and scratching might result in secondary infection (Feldmeier *et al*., 2009). At the severe cases, there were crusts, pus, and excess hyperkeratosis on nose, muzzle, around the eyes, ears, and legs (Sofyan and Chrismanto, 2017).

The histopathological result could be seen on Figure 1. On all samples, it was found there were *S. scabiei* mites in the stratum corneum layer until stratum granulosum from epidermis (Fig.1 b,c), hypergranulosis (Fig.1 b), spongiosis (Fig.1 b,d), acanthosis (Fig.1 b,c), epidermal tunnel (Fig.1 c), hyper-keratosis (Fig.1 b), abscess, elongation of rete ridges, dermal infiltrate and vascular proliferation. From this finding, we could concluded that rabbits in this studi were infected scabies in severe stage. Similiar findings also been reported by Mittal *et al.* (2004); Orion *et al*. (2006); Falk and Eide, (2008); Bhattacharjae and Glusac (2010); Nassef *et al*. (2016); Espinosa *et al*. (2017); Salvadori *et al*. (2017), they said that obsevered scabies patients, it was found there were *S. scabiei* mites on the epidermis, hyperkeratosis, hypergranulosis,

spongiosis, acanthosis, epidermal tunnel, elongation of rete ridges, dermal infiltrate, vascular proliferation, and parakeratosis.

The immunohistochemical images of scabies infected rabbit severe stadium revealed strong staining on TGF-β and moderate staining on TNF-α. The illustration of cytokine TGF-β expression was shown in Figure 2. Strong staining towards cytokine TGF-β found in epidermis layer (Fig.2 b,c,d), in stratum granulosum to stratum basalis (Fig.2 c,d), part of the dermis (Fig.2 b,d), part of the sebaceous gland (Fig.2 c,d) and hair follicles (Fig.2 b).

Strong expression of TGF-β in this research was equal with the research conducted by Walton *et al*. (2008) in human patient and the cases in the same level of severeness, meanwhile the research by Singh *et al*. (2014) used severely scabies infected dogs. Bhat *et al*. (2017) reviewed that TGF-β produced by Treg whom role was to push the inflammation response caused by scabies infection.

Research by Nassef *et al*. (2016) and Morgan and Arlian (2011). Nassef *et al*. (2016), noted that the expression of TGF-β on scabies patient was mild. In the research by Nassef *et al*. (2016), the host was human that naturally infected scabies, but it was unknown the rate of severity and length of infection. Arlian and Morgan (2011) reasearched with in-vitro method on human skin and the resulth showed light staining against cytokine TGF-β, and the *S.scabiei* mites entered in the human skin tissue cultured 48 hours after infection. The difference between this research and Arlian and Morgan's research were the host method and length of infection.

Figure 3 showed the expression of cytokine TNF-α was a moderate amount in the scabies infected rabbits severe stadium. Cytokine TNF-α was found in epidermis (Fig.3 b,c,d), on stratum spinosum to stratum basalis (Fig.3 b,c) and part of the dermis (Fig.3 b,c,d) but wasn't found in the hair follicle and sebaceous gland. The moderate amount of expression in TNF-α

might be caused by the decreasing amount of TNF-α, This result to strenght the opinion by Bandi and Saikumar, (2013), that said the expression of TNF-α high at the beginning of infection but not in the severe cases, even research conducted by Walton *et al.* (2008) didn`t found cytokine TNF-α in the severely infected patient. In in-vitro method research reflected that peripheral blood mononuclear cells (PBMCs) from healthy human that stimulated with *S.scabiei var.canis* extract increased the secretion in TNF-α, different with research that held by Arlian *et al*. (2004). Similarly to the research by Mullins *et al*. (2009), who stated that the extract of *S.scabiei var*. *canis* inducted cytokine TNF-α inflammation, although the research conducted by Walton *et al.* (2008) and Mullins *et al.* (2009) were done in different research method with this research, it still could give images of cytokine TNF-α expression.

The result of this research revealed the high level of cytokine TGF-β and the moderate level of cytokine TNF-α caused by on the scabies patient involving the engagement of type I and type IV hypersensitivity reaction. On type I reaction, the encounter of mites antigen and Immunoglobulin E on mast cell in epidermis causing mast cell degranulated (Burns, 2004). Several cytokines released by mast and basophil cell e.g cytokine TNF-α (Baratawidjaja and Rengganis, 2014). On type IV reaction Th1 or Th17 was the activated T cell, where the involved cytokine in Th1 cell was IFN-γ while the involved cytokine in Th17 was TGF-β (Jyonouchi, 2015). From the high level of TGF-β in this research, we concluded that on type IV hypersensitivity reaction, the activated T cell was Th17 cell not Th1 cell. This was stated by Mounsey *et al*. (2015) on their research where they found the increasing amount of cytokine IL-17, which was a cytokine that produced by Th17 cell, on severe scabies infected pigs. Based on research by Bhat *et al.* (2017), the secretions of IL-6, TGF-β and IL-23 triggered Th17 or Tc17 differentiation, and production of IL-17 (Bhat *et al*., 2017; McGeachy and Cua, 2008). TGF-β

and IL-2 inducted Tregs. Tregs produced TGF-β and IL-10 and might contribute to delayed inflammatory response in scabies and curbed inflammatory (Bhat *et al*., 2017). Ohno *et al*. (1996) said that eosinophils expressed TGF-β and could be depressed the local inflammation response, to regulate the activity and the growth of T cell (Tregs) cell regulatory. Eosinophil that produced TGF-β could inhibited the differentiation from naive T lymphocytes to Th1 or Th2 (Jacobsen *et al*., 2007). They found that on scabies patient, the number of eosinophils was high (Sluzevich and Lucky, 2007), on the other hand, there was research where the high number of eosinophils increased the number IL-17 that produced by Th17 (Dias and Banerjee, 2013). We can conclude that on severe scabies infected rabbits with high eosinophils will produce high TGF-β, which resulted in high number of IL-17 that produced by Th17.

Conclusion, infection scabies severe stadium triggered strong expression of TGF-β and moderate of TNF-α in rabbits. Expression of TGF-β was found on stratum granulosum to stratum basalis of epidermis layer, half of the dermis, sebaceous gland, and hair follicle. Expressions of TNF-α was found from on half of the epidermis layer, on stratum spinosum to stratum basalis, and half of the dermis.

Acknowledgements

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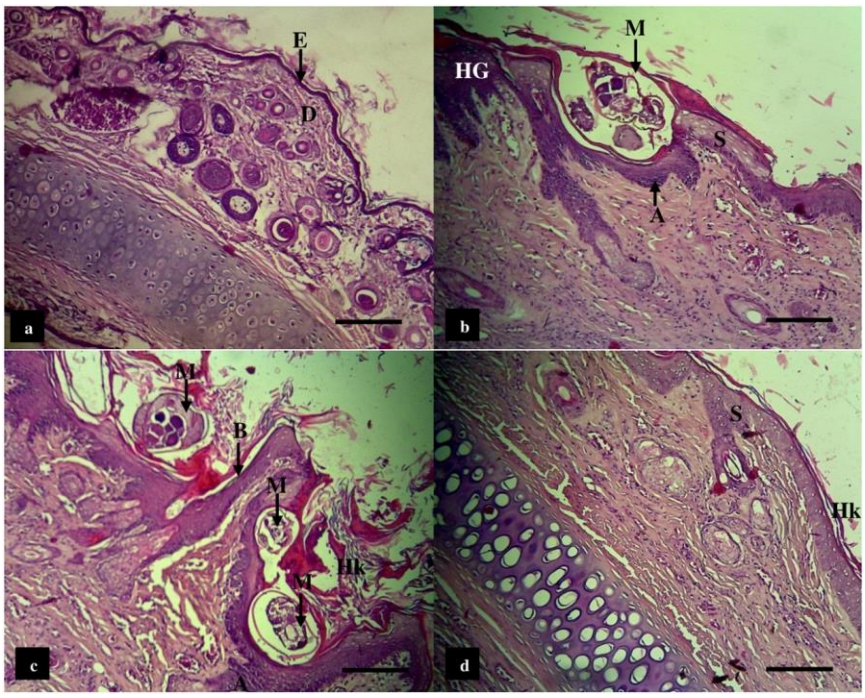


Figure 1. Histology of rabbit`s skin with Hematoxylin and Eosin staining. (a) control, (b-d) *S. scabiei* infected skin, (A) acanthosis, (B) epidermal tunnel, (D) dermis, (E) epidermis, (HG) hypergranulosis, (HK) hyperkeratosis, (M) *S.scabiei* mites, (S) spongiosis, (bar=100µm)

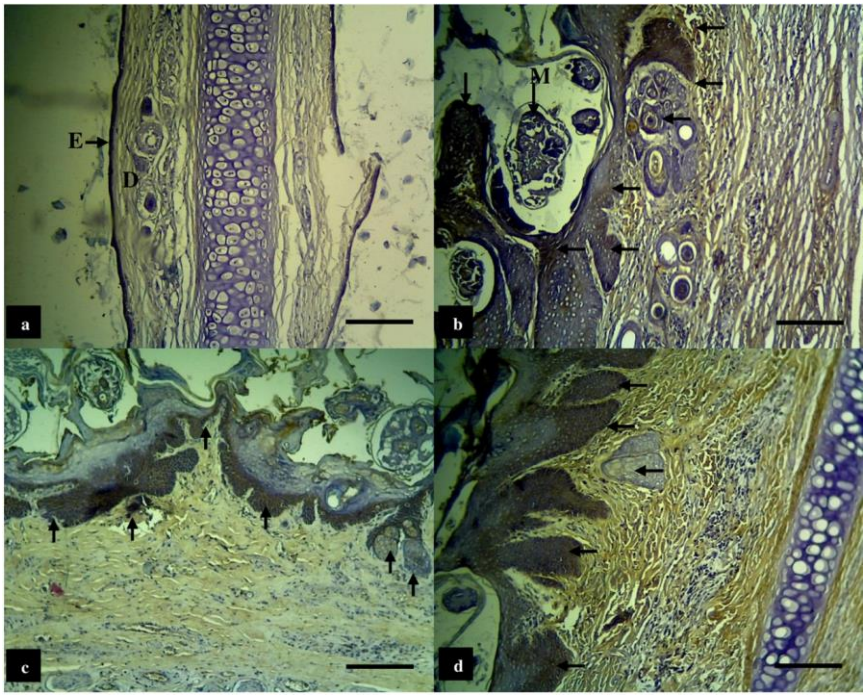


Figure 2. Histology of rabbit`s skin with immunohistochemical staining (anti TGF-β antibody); (a) control, (b-d) *S.scabiei* infected skin. The arrow was cell that expressed TGF-β, (bar=100µm).

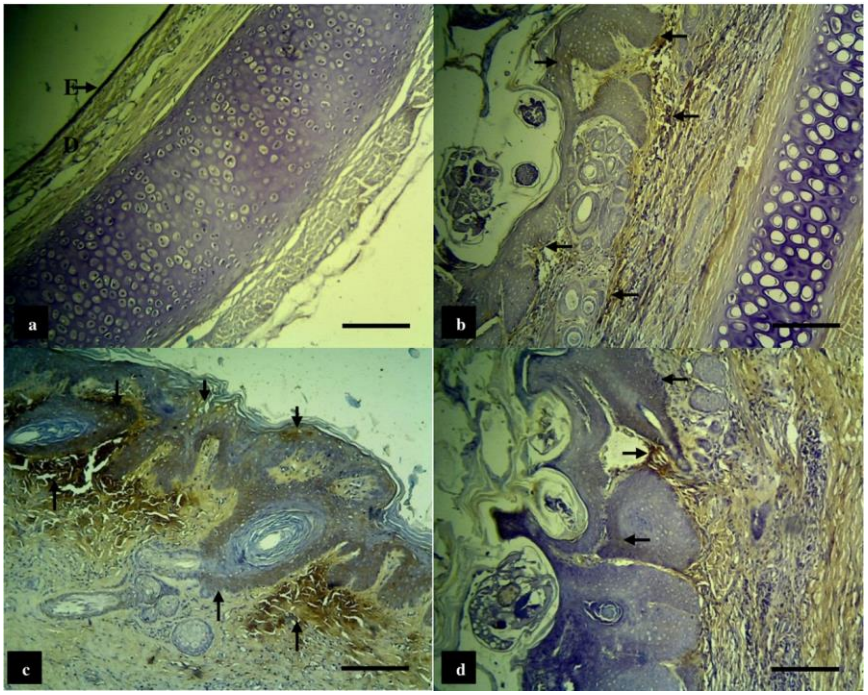
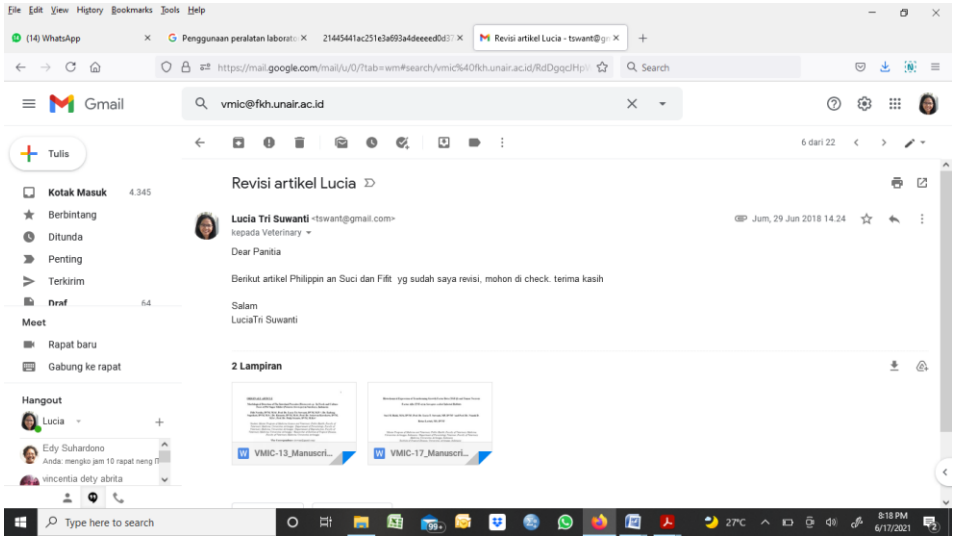


Figure 3. Rabbit skin with immunohistochemical staining (anti TNF-α antibody); (a) control, (b-d) *S.scabiei* infected skin, the arrow was the cell that expressed TNF-α, (bar=100μm).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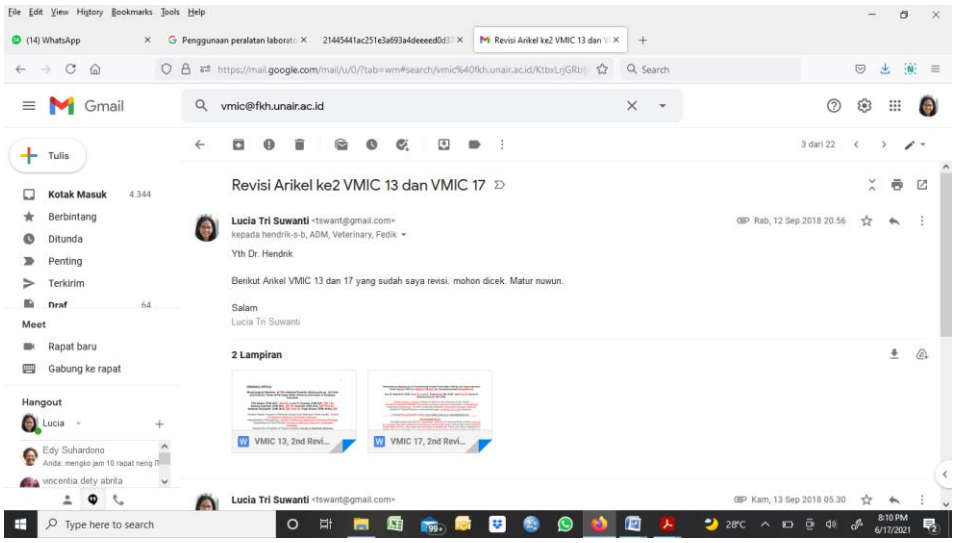
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Histochemical Expression of Transforming Growth Factor Beta (TGF-β) and Tumor Necrosis Factor Alpha (TNF-α) in Rabbits Infected with *Sarcoptesscabiei*

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ABSTRACT

Sarcoptesscabiei infection causetype I and IV hypersensitivity reactions induced by cytokines TGF-ß and TNF-α. This study was conducted to analyze the TGF-ß and TNF-α expression in rabbits with severe scabies. Five mixed-bredrabbits (3 males and 2 females), with age 12-18 months, were obtained from farms in East Java. Rabbit ear skin samples that showed clinical symptoms of severe scabies (without medical therapy), such as crusts, pus, excessive hyperkeratosis on nose, muzzle, around the eyes, ears, and legs were collected.Skin scraping was done to detect the presence of *S. scabies* mites, andskin samples were stained with hematoxylin eosin (HE) and subjected to immunohistochemistry. Strong staining of TGF-ß and moderate staining of TNF-α were evident in all samples. TGF-ß was expressed on stratum granulosum to stratum basalis of epidermis layer, half of the dermis, sebaceous gland, and hair follicle, while TNF-α was expressed on half of the epidermis layer, stratum spinosum to stratum basalis, and half of the dermis. This study illustrates thatseverescabiesinfection triggered increased expression of TGF-ß and TNF-α in rabbit ear skin,whereTGF-ß expression was more pronounced than TNF-α.

Key words: cytokine,immunohistochemistry,*Sarcoptesscabiei*, scabies,TGF-ß, TNF-α

INTRODUCTION

Scabies is a zoonotic disease caused by the parasitic mite *Sarcoptesscabiei.* It isahighly contagious infection that affects humans, wild animals, domestic animals and livestock (Bandi and Saikumar, 2013). Damage incurred from this infectioncan result to economicloss for the livestock industry, including rabbit farming industry (Tarigan, 2003; Wardhana*et al.*, 2006; Desiandura*et al.*, 2017).

*Sarcoptesscabiei*var. *cuniculi*, a round/ oval mite, specifically causes scabies in rabbits (Arlian and Morgan, 2017). Its life cycle spans several stages: egg, larva, deutonymph, tritonymph, and adult phase (Scoot *et al.*, 2001; Arlian and Morgan, 2017). Mites mate on the host epidermis, and male mites explore the skin continuously for several days to find female mites (Orkin and Maibach, 1985). Female mites lay 2-4 eggs every day. Eggs are placed 1 cm inside the stratum corneum, and, sometimes, mites make a tunnel to reach the stratum granulosum of the epidermis (Orion *et al*., 2006). Scabies infection can be classified as mild, moderate, and severe (Davis *et al*., 2013). Severe stage is characterized by presence of crusts, pus, and excessive hyperkeratosis on the nose, muzzle, around the eyes, ears, and legs (Sofyan and Chrismanto, 2017).

Scabies transmission to humans happens because of direct contact with the infected animal,causing pruritus (itch) and irritation due to hypersensitivity reactionsto mites (Bandi and Saikumar, 2013). Specifically, types I and IV hypersensitivity reactionsare

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involved in this process (Rook *et al.*, 1972; Hick and Elston, 2009),which are mediated bythe cytokine tumor necrosis alpha (TNF-α) (Rook *et al.*, 1972; Baratawidjaja and Rengganis, 2014;Mullins *et al.*, 2009; Bhat *et al.*, 2017) andtransforming growth factor-beta (TGF-ß), respectively(Jyonouchi, 2015) (Tizard, 2004).

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Bhat *et al.* (2017) stated the important role of the activated mast cell in producing histamine and TNF-αas an early inflammatory response to scabies infection. Janeway *et al.* (2001)reviewed that in type I hypersensitivity reaction, Th2 cell is involved in immediate hypersensitivity reaction initiation by stimulatingIgE production and promoting inflammation. It thenproduces a large amount of cytokines (IL-4, IL-5, and IL-13). Interleukine (IL) 4 stimulates B cell to produce IgE. Mast cell and basophil have a receptor called FcεR1 that specifically recognizesIgE, OnceIgEis bound to FcεR1, thisactivates transduction signal to mast cell cytoplasm, causing degranulation of mast cell and release of the active mediator in cytokines (TNF, IL-1, IL-4, IL-5, and IL-6) and chemokines, which have an important role in type I hypersensitivity reaction.

Mangan (2006) noted that type IV hypersensitivity reaction hastwo stages: first stage is proliferation and differentiation of CD4T cells, and the second stage isCD8+ T cell reaction. Proliferation and differentiation of CD4+ T cells will recognize the antigen presented by the dendritic cell (APC). APC produces the cytokines IL-1, IL-6, IL-12 and IL-23. When these cytokinesinteract? with TGF-ß,this will stimulate the T cell to differentiate into Th17 cell, and the activated Th17 cell will produce IL-17 (Mangan, 2006).

Research on immunohistochemical cytokine expression in animal skin infected with scabies, especially in rabbits, has never been done.Therefore, this researchintended to determine the extent of TGF-ß and TNF-α expressionin rabbit skin infected with severe scabies.

MATERIALS AND METHODS

Samples

This research was carried out from January to February 18 usingfive,12-18 month old,mix-bred rabbits (3 males and 2 females).obtained from farms in East Java, Indonesia. Tissue histology was done on rabbit ear skin tissues that showed clinical symptoms of severe scabies but without medical therapy. Symptoms include presence of crusts, pus, and excessive hyperkeratosis on nose, muzzle, around the eyes, ears, and legs. This research was conducted at the Veterinary Pathology Laboratory and Veterinary Parasitology Laboratory, Faculty of Veterinary Medicine, UniversitasAirlangga.

Ethical approval

This research has been approved by the ethical committee of the Faculty of Veterinary Medicine, UniversitasAirlangga, Number: 630-KE, in accordanceto the rules of animal testing and usage.

Parasitological examination

Rabbit ear skin was scraped for *S. scabiei* mites. Scraped skin sample was purified using 10% KOH solution, placed on a glass object, and examined under a microscope at 100x magnification. Identification of *S.scabiei*was based onSoulsby (1982).

Histopathological examination

Skin tissue was fixated in 10% formalin, and the histology slides were made byembedding rabbit skin tissue (1 cm x 1 cm)on paraffin block. Afterwards, skin tissue was cut 4 µm thick with a microtome, then samples weresubmerged in water bath at 55˚C. Tissues were picked up with aglass object slide and dried in 40˚C hot plate overnight, deparaffinated, and rehydrated.A slide was placed on a slide holder, soaked gradually in xylol solution twice, each for 5 min,then soaked incrementally in alcohol (96%, 90%, 80% and 70%) for 4 min.

Then slide was washed (immersed) with water for 5 min and stained with hematoxylin eosin (Merck, Germany), and finally dehydrated, cleared and mounted.

Immunohistochemical examination

Immunohistochemistry was performed using LSAB kit from Dako® (Carpinteria, California, USA). The skin tissue in paraffin block was cut 4 µm thick, deparaffinated, rehydrated, and incubated withprimary polyclonal antibody TGF-ß or TNF-α (Santa Cruz Biotechnology Inc., Dallas, Texas, USA) and antibody titer at 1:100.Procedure was carried out, following the manufacturer's protocols?. Finally, tissue was counterstained with hematoxylin (Merck, Germany).

Histologic slides were observed under a light microscope (Nikon® E-100, Japan) at 100x and 400x magnification, and photos were taken with a camera (OptiLab® MTN001, Indonesia). Histology data of rabbit skin with immunohistochemical staining was presented according to Nassef*et al.* (2016). Positive expression of cytokines TGF-ß or TNF-α was denoted by brown colored cells in histologic tissue. Intensity of cytokine expression denoted bybrown colored cells wascategorized into strong, moderate, weak, and negative, or, by percentage, corresponded to ≥50%, 20-50%, <20%, and absenceof brown colored cells per field, respectively (Nassef*et al.*, 2016).

All histologic data were presented descriptively.

RESULTS AND DISCUSSION

Scabies is a common skin disease that can be easily transmitted in environments with poor sanitation, especially inless? developed countries. Common symptoms of scabies are pruritus, scabs, and scratching could result in secondary infection (Feldmeier*et al.*, 2009). Severe cases of scabies are marked by crusts, pus, and excessive hyperkeratosis on nose, muzzle, around the eyes, ears, and legs (Sofyan and Chrismanto, 2017).

As seen in Figure 1, in all samples manifested cytokine expression: *S. scabiei* mites were found in the stratum corneum layer up to thestratum granulosum of the epidermis (Fig.1 b,c), characterized by hypergranulosis (Fig.1 b), spongiosis (Fig.1 b,d), acanthosis (Fig.1 b,c), epidermal tunnel (Fig.1 c), hyperkeratosis (Fig.1 b), abscess, elongation of rete ridges, dermal infiltrate, and vascular proliferation. It can be said then that rabbits in this study were infected with severe scabies. Similar findings have also been reported by Mittal *et al.* (2004), Orion *et al.* (2006), Falk and Eide, (2008), Bhattacharjee and Glusac (2010),Nassef*et al.* (2016), andSalvadori*et al.* (2016).These studies found *S. scabiei*mites on the epidermis, and histologic changes seen were hyperkeratosis, hypergranulosis, spongiosis, acanthosis, epidermal tunnel, elongation of rate ridges, dermal infiltrate, vascular proliferation, and parakeratosis.

Immunohistochemical images of rabbits infected with severe scabiesrevealed strong staining on TGF-ß and moderate staining on TNF-α. Cytokine TGF-ß expression is illustratedin Figure 2. Strongly stained TGF-ß was evident in the epidermis layer (Fig.2b,c,d), stratum granulosum to stratum basalis (Fig.2c,d), part of the dermis (Fig.2b,d), part of the sebaceous gland (Fig.2c,d), and hair follicles (Fig.2b).

Strong expression of TGF-ß in this research is consistent withthe research conducted in humans (Walton *et al.*,2008) anddogs (Singh *et al.*,2014). According to Bhat *et al.* (2017), TGF-ß expression triggers inflammation response in scabies infection.

Figure 3 shows the moderate expression of cytokine TNF-α in rabbits with severe scabies. Cytokine TNF-α was found in the epidermis (Fig.3b,c,d), stratum spinosum to stratum basalis (Fig.3b,c), and part of the dermis (Fig.3b,c,d), but none was found in the hair follicle and sebaceous gland. This result differs with the research done by Bandi and Saikumar (2013), wherein they reported strong expression of TNF-α during the early stage

of infection. Notably, Walton *et al.* (2008) did not find cytokine TNF-α inseverely infected patients, implying thatTNF-αexpression depends on the progression of the disease and the infected host.

As stated earlier,cytokines TGF-ß and TNF-α play important roles in type I and IV hypersensitivity reactions, respectively (Jyonouchi, 2015 Tizard, 2004; Bhat *et al*., 2017). Thereby, their increased expression in rabbits with scabiescan potentially triggers these hypersensitivity reactions. With type I reaction, mites antigen and immunoglobulin E on mast cell in the epidermis causethe degranulated mast cell (Rook *et al.*, 1972) to release TNF-α, along with other cytokines (Baratawidjaja and Rengganis, 2014). Meanwhile, type IV hypersensitivity reaction is mediated by the activation of Th1 or Th17 cells, where Th1 cells link with IFN-γas a result of TGF-ß activity (Jyonouchi, 2015) (Tizard, 2004). Considering thefindings of Mounsey*et al*. (2015), the researchers hypothesized that high level of TGF-ß in this study activated the Th17 cell to release IL-17, causing type IV hypersensitivity reaction. Mounsey*et al.* (2015) found increasing amount of cytokine IL-17 in pigs with severe scabies. Based on Bhat *et al.* (2017), secretions of IL-6, TGF-ß, and IL-23 trigger Th17 or Tc17 differentiation and production of IL-17 (McGeachy and Cua, 2008). In detail, TGF-ß and IL-2 induceTregs cell. Tregs produce TGF-ß and IL-10,possibly contributing to delayed inflammatory response in scabies and thus curb inflammation? (Bhat *et al*., 2017). Ohno *et al*. (1996) said that eosinophils express TGF-ß that can depress local inflammation response andregulate the activity and growth of T cell (Tregs). Eosinophils that produce TGF-ß can inhibit the differentiation from naive T lymphocytes to Th1 or Th2 (Jacobsen *et al*., 2007). If number of eosinophilson scabies patient is high,as seen in the study of Sluzevich and Lucky (2007), this could translate to a concomitant increase in IL-17 produced by Th17 (Dias and Banerjee, 2013), causing type IV hypersensitivity reaction.

This study showed that severe scabies in rabbits enhanced the expression of cytokines TGF-ß and TNF-α, whereinTGF-ß expression was more pronounced. Since this expression is tied to hypersensitivity reaction types I and IV, it is necessary to consider treatment in rabbits by suppressingcytokine expression, especially TGF-ß, as a means to control scabies.

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ACKNOWLEDGMENT

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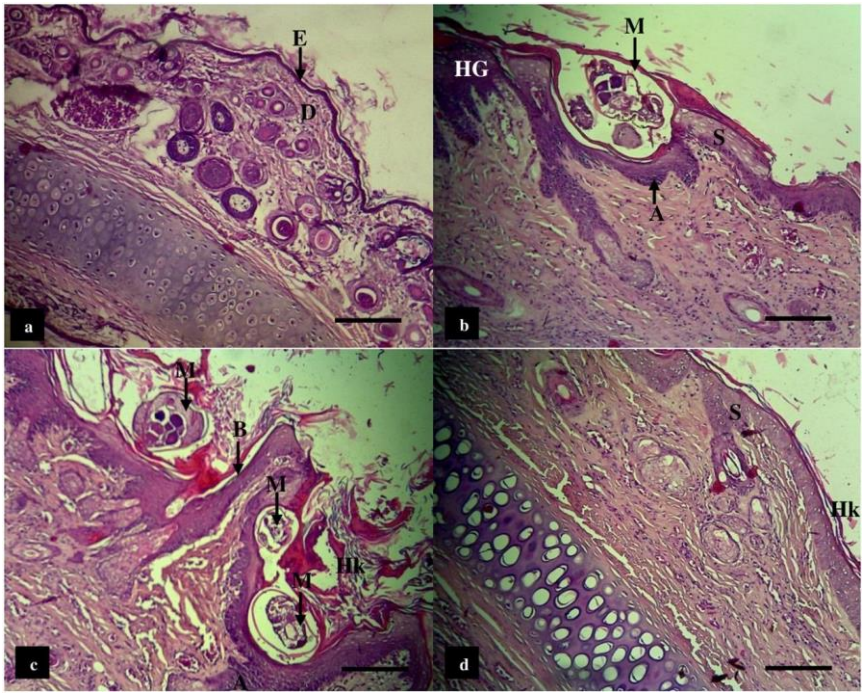

Fig. 1. Histology of rabbit skin stained with hematoxylin and eosin. (a) control, (b-d) *S. scabiei* infected skin; (A) acanthosis, (B) epidermal tunnel, (D) dermis, (E) epidermis, (HG) hypergranulosis, (HK) hyperkeratosis, (M) *S.scabiei* mites, (S) spongiosis (bar=100µm).

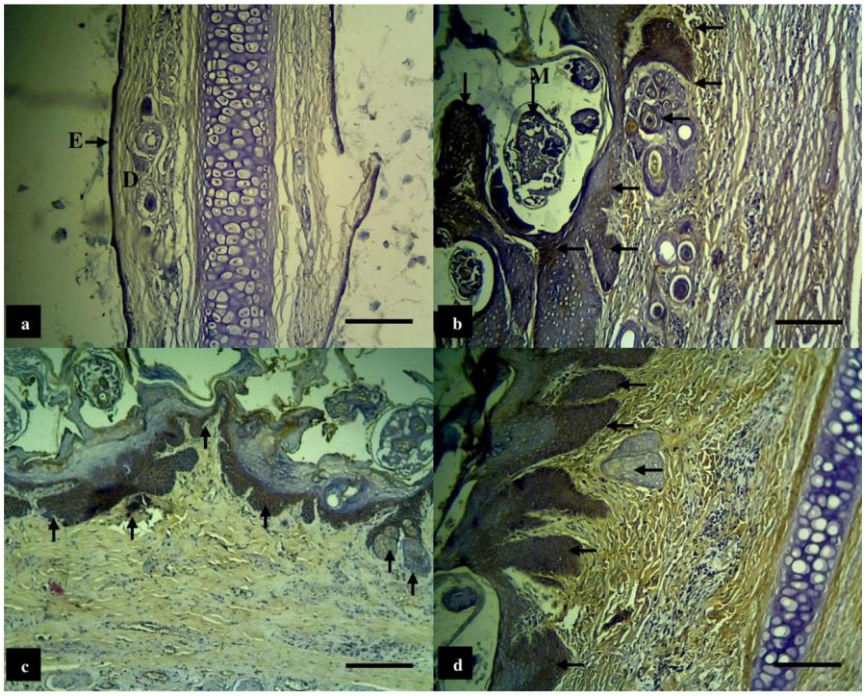

Fig 2. Histology of rabbit skin stained with anti TGF-ß antibody: (a) control, (b-d) *S.scabiei* infected skin. Arrow pertains to cells that expressed TGF-ß (bar=100µm).

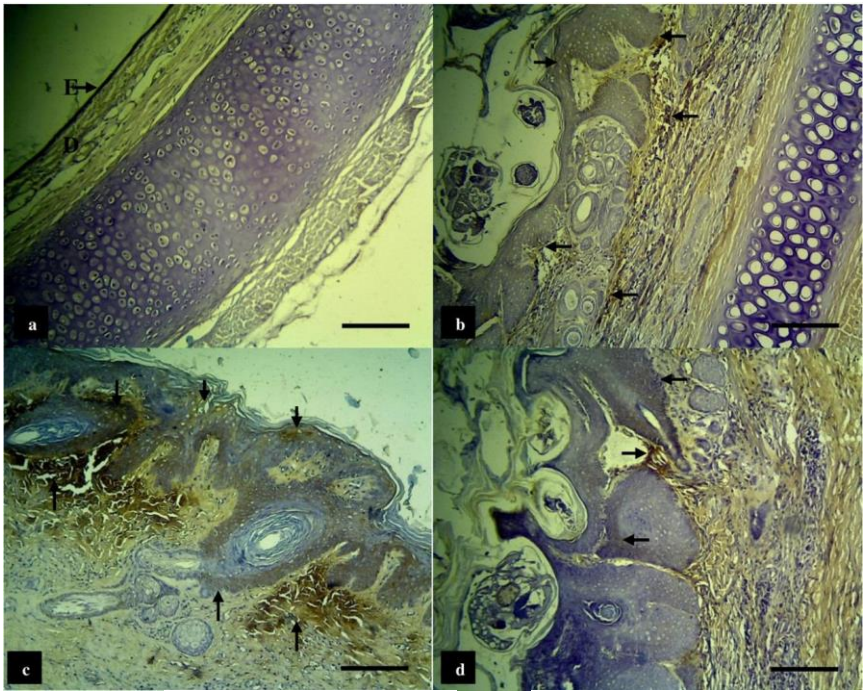

Fig. 3. Histology of rabbit skin stained withanti TNF-α antibody: (a) control, (b-d) *S.scabiei* infected skin.Arrow pertains tocells that expressed TNF-α (bar=100µm).