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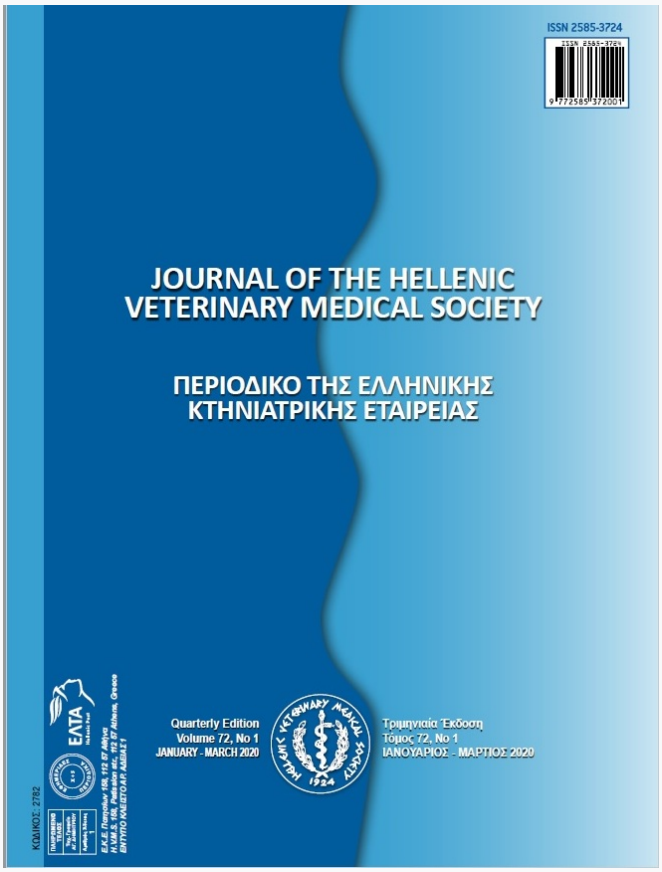

Sarcoptes scabiei dermatitis in adult sheep: an immunohistochemical study of 34 chronic cases with extensive lesions

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Sarcoptes scabiei dermatitis in adult sheep: an immunohistochemical study of 34 chronic cases with extensive lesions

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ABSTRACT: Ovine sarcoptic mange is a contagious ectoparasitic skin disease, seen in many countries with sheep production. Although several studies concerning dermatopathology have been published, the local cutaneous immune response to *Sarcoptes scabiei* has not been studied by immunohistochemistry. The present study aims to evaluate immunohistochemically the adaptive cellular immune response in chronic natural cases with extensive gross lesions. Facial and foot skin biopsies of 32 ewes and 2 rams were obtained, and moreover from the scrotal scabietic lesions of the 2 rams. Each biopsy was bisected and processed for paraffin and cryostat sections. Mites were not observed in the vast majority of skin histology sections. Epidermal hyperplasia and chronic inflammation were the main histopathologic features. The dermal inflammatory infiltrate was mixed, dominated by eosinophils and lymphocytes equally. Tissue sections immunostained with a panel of monoclonal antibodies showed among lymphocytes an almost exclusively T-cell population (CD3+), while CD79a + cells were sparse. T-helper cells (CD4+) were predominant versus T-cytotoxic cells (CD8+) in 4:1 to 5:1 ratios. The mixed inflammatory infiltrate combined with the immunohistochemical findings suggest both a type-I and type-IV hypersensitivity reactions during the chronic course of the disease. Moreover, all these chronic cases in adult sheep are recorded into the hypersensitivity form of sarcoptic mange ("classical or ordinary" scabies) and no cases of the hyperkeratotic form of the disease ("Norwegian or crusted" scabies) were found.

Keywords: adult sheep; *Sarcoptes scabiei;* extensive lesions; immunohistochemistry; adaptive cellular immune response

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INTRODUCTION

Sarcoptic mange or scabies is a parasitic skin disease of humans, domestic and wild mammals caused by *Sarcoptes scabiei* mite. It is characterized by a complex immunopathology with innate and adaptive immune mechanisms (Pence et al., 2002; Bhat et al., 2017). In general, the distribution and severity of the cutaneous lesions as well as the disease outcome vary among host species or among individuals of the same species, due to regional differences in the cutaneous microarchitecture and lipids, the different level of immune response and/or different stages of the disease (Salvadori et al., 2016; Arlian and Morgan, 2017; Bhat et al., 2017). According to histopathological features and cellular immune response in dermatopathological lesions, two main forms of the disease have been described in human and many animal species: a) the hypersensitivity form, and b) the hyperkeratotic form (Pence and Ueckermann, 2002; Bhat et al., 2017; Mauldin and Peters-Kennedy, 2016).

The hypersensitivity form of sarcoptic mange (also known as "classical or ordinary" scabies) is an intensely pruritic dermatitis, typically seen in almost all infected human and animals (Bhat et al., 2017). No or very few mites have been observed in skin sections along with a mixed inflammatory infiltrate consisting of eosinophils and T-lymphocytes. Macrophages, neutrophils, mast cells, as well as a few B-cells and plasmocytes have also been observed (Pence and Ueckermann, 2002; Mauldin and Peters-Kennedy, 2016). In humans, dogs and pigs CD4+ cells have been demonstrated as the most prevalent T-lymphocyte subpopulation in inflammatory skin lesions compared to CD8+ cells (Arlian et al., 1997; Gallardo et al., 2002; Liu et al., 2014). This effective cell-mediated immune response limits the spread of mites, preventing overwhelming infestation and widespread lesions in human or animal hosts (Salvadori et al., 2016; Arlian and Morgan, 2017; Bhat et al., 2017).

The hyperkeratotic form of sarcoptic mange (also known as "Norwegian or crusted scabies") has been reported both in human and veterinary literature. As far as domestic mammals, it has been diagnosed in pigs, dogs and cats (Goyena et al., 2013; Mauldin and Peters-Kennedy 2016). Some cases have been reported in wild mammals as well (Pence and Ueckermann, 2002). Norwegian scabies tends to occur in undernourished or immune-compromised individuals (Pence and Ueckermann, 2002). A high number of mites are seen in skin sections with the dermal inflammatory infiltrate dominated by lymphocytes than eosinophils (Pence and Ueckermann, 2002; Mauldin and Peters-Kennedy, 2016).

Immunohistochemistry applied in skin biopsies obtained from human and pig with Norwegian scabies, has shown a higher number of CD8+ T cells compared to CD4+ cells, and absence of B cells in the dermis (Gallardo et al., 2002; Walton et al., 2008; Liu et al., 2014). The impaired cell-mediated immunity allows *Sarcoptes scabiei* mites to multiply in extremely high numbers within widespread lesions (Arlian and Morgan, 2017; Bhat et al., 2017).

Ovine sarcoptic mange is a disease, prevalent in many Mediterranean and Middle East countries (Fthenakis et al., 2000; Hidalgo-Arguello et al., 2001; Rahbari et al., 2009). *Sarcoptes scabiei* tends to affect the non-woolly body regions (Abu-Samra et al., 1981; Rahbari et al., 2009). The infestation usually begins near the mouth (lips, nostrils) and spreads to the ear pinnae, head, legs and other non-woolly areas, such as scrotum, mammary gland and perineum. Skin lesions include pustules, alopecia, severe scaling and thick crusts, as well as fissures and less frequently excoriations due to intense pruritus (Hidalgo-Arguello et al., 2001; Rahbari et al., 2009; Rodriguez-Cadenas et al., 2010). Generalized lesions have only been observed in the more hairy desert sheep of the Sudan (Abu-Samra et al., 1981). Sarcoptic mange may affect both lambs and adult sheep leading to growth retardation, reduced milk yield and adverse reproductive effects (Fthenakis et al., 2000; Fthenakis et al., 2001).

There has been so far no immunohistochemical study on ovine sarcoptic mange and its immunopathology. Thus, the aim of this study was to evaluate the adaptive cellular immune response in chronic cases of ovine scabies.

MATERIALS AND METHODS

Animals & study design

A total of 44 sheep were included in the study. Thirty four (34) adult sheep (32 crossbreed Karagouniko ewes and 2 Karagouniko rams), aged 1.5 to 5 years old, with chronic dermatitis due to *Sarcoptes scabiei* used. In addition, 10 clinical healthy crossbreed Karagouniko ewes used as controls.

All affected animals (Figures 1, 2) were selected from 5 naturally infested flocks in the Prefecture of Thessaly, Greece. Inclusion criteria included the pres-

ence of *Sarcoptes scabiei* mites in skin scrapings, at least 3 months duration of skin disease and no use of acaricide treatment (either topical or systemic) over the past 6 months.

Figure 1. An ewe with severe chronic lesions all over the head extending to ventral part of the upper neck. The main features are alopecia and skin thickening with deep cracks. The arrow indicates the biopsy site

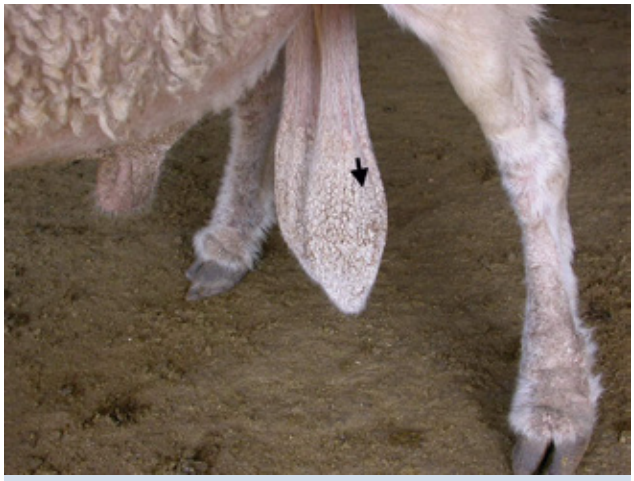

Figure 2. A ram with cutaneous chronic lesions of sarcoptic mange, affecting the feet as well as the prepuce and scrotum. The arrow indicates the biopsy site

The study was completed in 2 phases: a) the 27 cases with sarcoptic mange and the 10 healthy sheep (controls) were selected, examined and evaluated during a doctoral thesis study; b) the next 7 cases with extensive lesions of chronic sarcoptic mange were selected and examined during a 6 month post-doctoral survey.

Tissue Samples

Two (2) skin punch biopsies (8mm), from the face and front feet of affected animals and controls were obtained under local anesthesia with lidocaine. In addition, a biopsy was also obtained from the scabietic scrotum of the two affected rams. Each biopsy was bisected, with one half fixed in 10% buffered formalin for 24 hours, dehydrated in a graded series of ethanol and xylenes, and embedded in paraffin. The other half was put in optimal cutting temperature compound (Tissue-Tek O.C.T compound, Polysciences Inc, USA), immersed in isopentane, cooled to its freezing point in liquid nitrogen and stored at -80^{o}C, until cryosectioning.

A submandibular lymph node of a clinically healthy lamb, sampled within 30 minutes after slaughter in a local slaughterhouse and treated as described above, served as positive control during immunohistochemistry.

Histopathology

The paraffin embedded skin biopsies were sectioned at 5-μm and tissue sections were stained with hematoxylin-eosin (H-E) according to a standard protocol.

Immunohistochemistry (IHC)

Immunohistochemical staining was applied on paraffin and cryostat sections, using a panel of monoclonal antibodies (mouse anti-sheep or mouse anti-human with known cross reactivity to sheep): CD3 as pan T-cell marker; CD4 for helper T-lymphocytes; CD8 for cytotoxic T-lymphocytes; CD79a for both B-lymphocytes and plasmocytes, according to Perez et al., 2005; Gulbahar et al., 2006; Vismarra et al., 2015.

Specific technical details of monoclonal antibodies used are presented in Table-1.

i) Paraffin sections

Immunostaining for CD3 and CD79a was carried out in paraffin-embedded 5-μm tissue sections, which were placed in a 60^{0}C oven for 30 minutes and rehydrated by sequential immersion in xylene, graded concentrations of ethanol, and distilled water.

Antigen retrieval for CD3 antibody was performed by heating the sections in Trilogy solution (Cell Marque) for 20min at 850W microwave oven (the Trilogy solution was preheated for 5 min at 450W, before mmersing the sections). Antigen retrieval for CD79a antibody was performed by heating the sections in Target Retrieval Solution-pH 9 (DAKO) for 20min at 850W microwave oven (the Target Retriev-

al Solution-pH 9 was preheated for 5 min at 450W, before mmersing the sections). Sections were washed with distilled water and washing buffer (Tris-buffered NaCl Solution with Tween 20 (TBST), pH 7.6). Endogenous peroxidase activity was blocked by 3% hydrogen peroxide (H_2O_2) for 10 minutes at room temperature. Subsequently, the sections were washed with buffer and incubated with the primary antibodies at room temperature (Table-1). Then, the sections were washed with the buffer and incubated with the Envision polymer (EnVision™ Detection system, K5007, Dako) for 45 minutes. The signal was developed in 3,3'-diaminobenzidine (DAB) solution for 5 minutes and finally counterstained with Mayer's hematoxylin. After detection, sections were dehydrated with graded ethanol and xylenes, and were finally coverslipped.

ii) Cryostat sections

Immunostaining for CD4 and CD8 was carried out in 5-μm cryostat sections, air-dried at room temperature for 20 minutes. The sections were washed with buffer and incubated with the primary antibodies at room temperature (Table-1). Following incubation with the primary antibody, the steps described above were followed without any other modification.

For all immunohistochemical reactions, histological sections of a healthy lamb lymphnode, treated as described above, were used as positive and negative controls. Each primary antibody was replaced by non-immunogenic mouse serum (Mouse Gamma Globulin, 015-000-002; Dianova, Germany) as control for nonspecific binding of the secondary antibody.

Table 1. Technical details of the monoclonal antibodies used

antigen	*clone*	*Isotype*	*specificity*	*source*	*sections*	*dilution*	*incubation time*
CD3	F7.2.38	IgG1	mouse anti-human	**Dako-Agilent Technologies; Santa Clara, California, USA**	paraffin	1/200	30 min
CD4	GC50A1	IgM	mouse anti-sheep	**VMRD, Inc; Pullman, Washington, USA**	cryostat	1/50	4 hours
CD8	CACT80C	IgG1	mouse anti-sheep	**VMRD, Inc; Pullman, Washington, USA**	cryostat	1/200	60 min
CD79a	HM57	IgG1	mouse anti-human	**Dako-Agilent Technologies; Santa Clara, California, USA**	paraffin	1/50	3 hours

Interpretation of the results

Positive cell counting for markers of T-lymphocytes subpopulations (CD3+, CD4+, CD8+), B-lymphocytes and plasmocytes (CD79a+) in the epidermis and dermis was performed under a light microscope. The tissue slides were evaluated by two independent veterinary pathologists (DD and DT). The examiners assessed the expression pattern and evaluated the localization of the immunollabelling cells. The positive cells were determined by a semiquantitative morphometric protocol according to Walton et al. 2008 and modified as follows: - *(none);* + *(sparse/very few);* ++ *(few);* +++ *(some);* ++++ *(many);* +++++ *(abundant).*

For each skin biopsy the CD4:CD8 ratio was estimated as a ratio of integers (in a range of 1:1 to 6:1) by comparing the densities of dermal lymphocytic subpopulations into IHC sections.

Statistical analysis

Student's t-test was used to calculate and compare the mean of CD4:CD8 ratios separately on facial and foot skin between scabietic and control sheep. A p-value equal to or less than 0.05 was considered significant. Statistical analysis was performed using SPSS 16.0 software for Windows (SPSS Inc.)

A digital representative image of each tissue slide was captured (Figures 3-9), using the NIKON ECLIPSE E-200 microscope equipped with Fi1-L2 Digital System (NIKON, Japan).

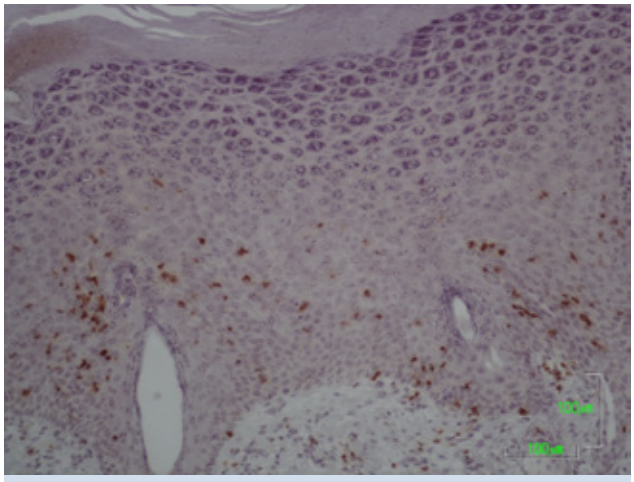

Figure 3. Paraffin section of facial skin from a ewe with sarcoptic mange: exocytosis of CD3+ T-lymphocytes in hyperplastic epidermis. IHC, EnVision™ detection system

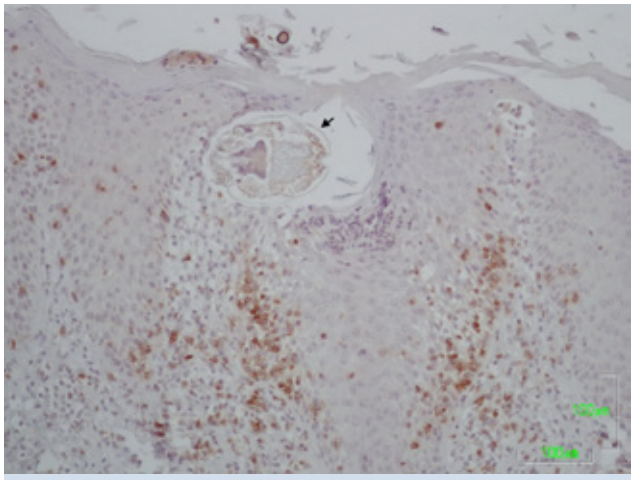

Figure 4. Paraffin section of foot skin from a ewe with sarcoptic mange: infiltration of CD3+ T-lymphocytes in upper dermis (dermoepidermal area) and exocytosis toward a *Sarcoptes scabiei* mite (arrow), burrowing in epidermis. IHC, EnVision™ detection system

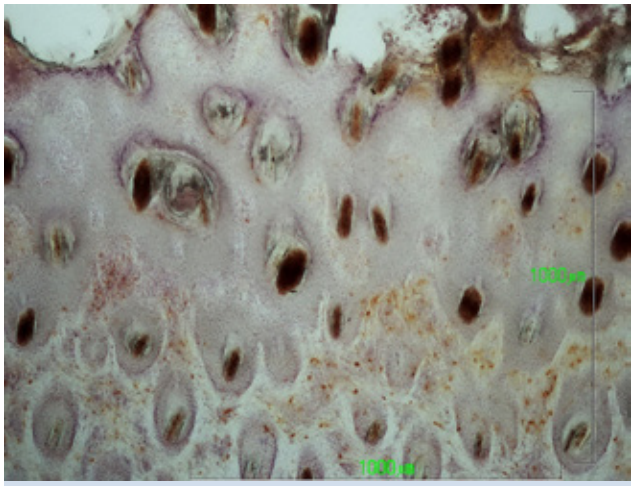

Figure 5. Cryostat section of foot skin from a ewe with sarcoptic mange: infiltration of CD4+ T-lymphocytes in upper dermis. IHC, EnVision™ detection system

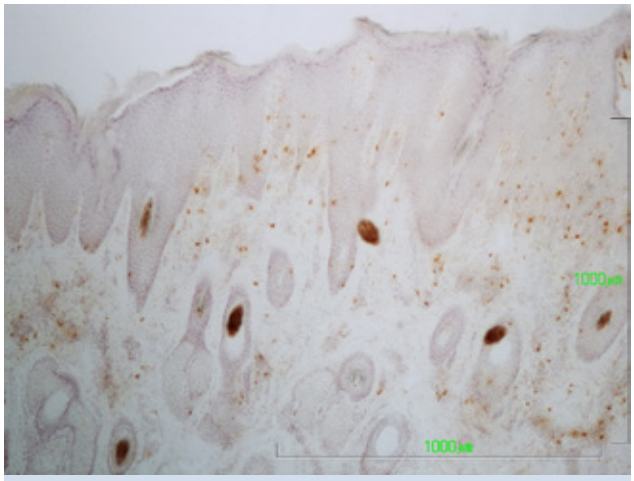

Figure 6. Cryostat section of foot skin from a ewe with sarcoptic mange: mild infiltration of CD8+ T-lymphocytes in upper dermis, as well as focal lymphocytic exocytosis in epidermis. IHC, EnVision™ detection system

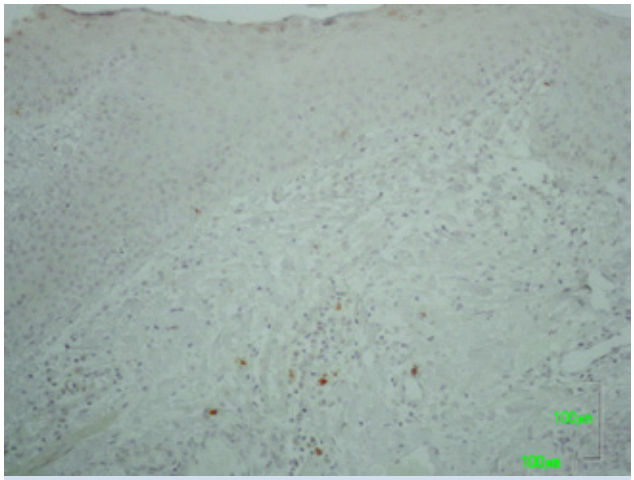

Figure 7. Paraffin section of foot skin from a ewe with sarcoptic mange: there are few CD79a+ cells in the dermis. IHC, EnVision™ detection system.

Figure 8. Paraffin section of scrotum skin from a ram with sarcoptic mange: occurrence of many *Sarcoptes scabiei* mites in thick serocellular crusts. Haematoxylin-Eosin stain

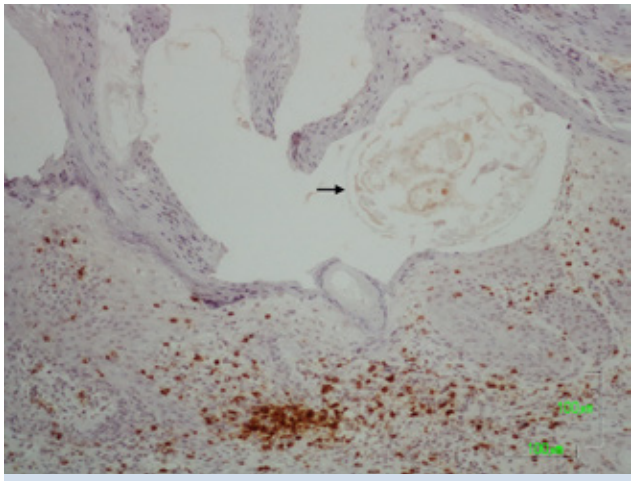

Figure 9. Paraffin section of scrotum skin from a ram with sarcoptic mange: notice the *Sarcoptes scabiei* mite (arrow) burrowing into parakeratotic stratum corneum associated with exocytosis of CD3+ T-lymphocytes in epidermis and severe focal dermoepidermal inflammation. IHC, EnVision™ detection system

RESULTS

a) Dermatitis due to *Sarcoptes scabiei*

In all affected ewes and rams, skin biopsy sections from the face and feet showed similar histopathological features. No significant differences between the prevalence of mites on the two different body sides were observed. In the vast majority of sections mites could not be observed, while in a small percentage of biopsies 1-2 mites were found per section. The main histopathological lesions of all facial and feet biopsies were epidermal hyperplasia, orthokeratotic hyperkeratosis, patchy hypergranulosis, acanthosis and pronounced rete ridge formation In a few sections in which mite (s) were present, focal parakeratotic hyperkeratosis was observed around the parasitic burrow, as well as spongiosis, especially beneath or at the sides of the burrow. Moreover, multifocal crusting was observed in some sections. The epidermis was characterized by exocytosis of eosinophils and neutrophils, forming micropustules in some fields, as well as T-lymphocytes (CD3+), found either scattered (Figure-3) or centered beneath the sites containing a mite (Figure-4). In particular, T-cells were cytotoxic CD8+cells, since CD4+ and CD79a+ cells were completely absent throughout the epidermis.

In the dermis, histopathology revealed a mixed inflammatory infiltrate, with high numbers of eosinophils and lymphocytes and lower one of histiocytes. The distribution pattern of inflammatory infiltrate was mainly dermoepidermal to deep perivascular and/or diffuse; and rarely focal periadnexal, especially when the parasitic burrows were located at the infundibulum of hair follicles. The immunohistochemical staining revealed that the predominant lymphocytes in dermal infiltrates were T-lymphocytes (CD3+), with the main subpopulation being CD4+ (Figure-5) as opposed to CD8+ cells (Figure-6). In each anatomical region, the CD4+:CD8+ ratio ranged from 4:1 to 5:1. B-lymphocytes and plasmocytes (CD79a+) cells were sporadically seen (Figure-7). The semi-quantitative evaluation of lymphocytic subpopulations and CD4+:CD8+ ratio are presented in Table-2.

The 2 biopsies from scabietic scrotum showed a relatively larger number of mites in hyperplastic epidermis in comparison to facial and foot biopsies of the same individual. Each skin section of scrotum contained 4-7 mites at multiple levels within stratum corneum. Severe orthokeratotic hyperkeratosis along with extensive parakeratosis and severe crusting and spongiosis were observed (Figure-8). Exocytosis of lymphocytes, eosinophils and neutrophils was seen in epidermis. Immunohistochemistry revealed a mild epidermal exocytosis and severe focal dermoepidermal infiltration of CD3+ cells (Figure-9). Moreover, the dermal CD4+:CD8+ ratio was estimated to 4:1 approximately.

B) Healthy skin (controls)

The skin of healthy sheep (controls) showed a normal histological structure, as it was expected. No evidence of epidermal hyperplasia was seen. Eosinophils and neutrophils were not observed. Very few lymphocytes were seen around blood vessels at the upper dermis. Semi-quantitative evaluation of lymphocyte subpopulations in the dermis and CD4+:CD8+ ratios are presented in Table-2.

Table 2. Semi-quantitative evaluation of the dermal lymphocyte subpopulations of facial and foot skin (scabietic vs. healthy skin)

Lymphocytes	Facial skin with scabies (n=34)	Healthy facial skin (n=10)	Front foot skin with scabies (n=34)	Healthy front foot skin (n=10)
CD3+	+++++	+	+++++	+
CD4+	++++	+	++++	+
CD8+	++	+	++	+
CD79a+	- to +	-	- to +	-
CD4:CD8 (mean)	**4,7 : 1**	**1,2 : 1**	**4,4 : 1**	**1,5 : 1**

- (none); + (sparse/very few); ++ (few); +++ (some); ++++ (many); +++++ (abundant)

DISCUSSION

The current study presents histopathological and immunohistochemical findings, concerning cases of sarcoptic mange in adult sheep with chronic extensive lesions.

As far as the histopathology of sarcoptic mange in domestic and wild mammals, the main features on the basis of which the distinction between the two forms of the disease is made are the number of mites throughout the hyperplastic epidermis, as well as the dominant cell type infiltrating dermis (Pence and Ueckermann, 2002; Mauldin and Peters-Kennedy, 2016). Thus, the histopathological lesions of all facial and feet biopsies of our ovine chronic cases are compatible with the "classical (hypersensitivity) form" of the disease, because very few mites were observed in stratum corneum, while the dermal inflammatory infiltrate was mixed, consisting mainly of eosinophils and lymphocytes (Pence and Ueckermann, 2002; Mauldin and Peters-Kennedy, 2016).

On the contrary, in the sections of the scrotum of the two rams many mites were seen at multiple levels in the stratum corneum, while intense orthokeratotic hyperkeratosis was observed, as well as parakeratosis. The increased number of mites in parakeratotic stratum corneum is the main feature of the "hyperkeratotic (Norwegian) form" of sarcoptic mange (Pence and Ueckermann, 2002). However, the mixed inflammatory infiltrate in scrotum sections composed mainly by eosinophils and lymphocytes is a diagnostic feature not related to the "Norwegian form" of sarcoptic mange (Pence and Ueckermann, 2002; Mauldin and Peters-Kennedy, 2016). It is worth noting that the Norwegian form of sarcoptic mange has not been recorded in sheep to date, even in cases where more than 1-2 mites have been observed into histopathological sections. For example, during an experimental study of ovine sarcoptic mange the presence of many mites has been recorded in chronic lesions (7-9 weeks post infection) without being considered as Norwegian scabies. In these experimental cases, the inflammatory infiltrate was mixed consisting of lymphocytes, eosinophils, macrophages and a few neutrophils (Ibrahim and Abu-Samra, 1987). On the other hand, the presence of many *Sarcoptes scabiei* mites with the complete absence of eosinophils in inflammatory infiltrate has been recorded in a case of fatal sarcoptic mange in Blue Sheep (*Pseudois nayaur*), suggesting the lack of an appropriate immune response to the parasite or other coping strategies because there has been no abatement of the clinical signs in affected animals over several years (Dagleish et al., 2007).

In addition, according to modern literature, the diagnosis of "Norwegian form" of sarcoptic mange is complex, based both on the histopathological features and on CD4:CD8 lymphocytic ratio in dermal lesions (Bhat et al., 2017). The role of CD4+ and CD8+ lymphocytes as well as all immunohistochemical findings of the present study are discussed below.

Immunohistochemistry has already been applied in some ovine ectoparasitoses. The immunophenotype of lymphocyte subpopulations has been studied in healthy ovine skin (Gorrell et al., 1995; McElroy et al., 1998), as well as in skin lesions induced by the ectoparasites *Lucilia cuprina, Hyalonema anatolicum* and *Psoroptes ovis* (Bowles et al., 1992; Boppana et al., 2005; Van den Broek et al., 2005), or infectious agents, such as orf and sheep pox (Jenkinson et al., 1992; Gulbahar et al., 2006).

Our study demonstrated a significant infiltration of T-cells (CD3+) into ovine scabietic skin, suggesting a local T-cell mediated immune response (Arlian et al., 1997; Salvadori et al, 2016; Bhat et al., 2017). The exocytosis of cytotoxic CD8+ T-lymphocytes throughout epidermis or centered beneath the sites in stratum corneum containing a mite is a characteristic feature, which has also been reported in experimental sarcoptic mange in dogs (Arlian et al., 1997). Also, CD8+ lymphocytes may induce dysregulated keratinocyte apoptosis contributing to the elicitation and progress of epidermal hyperproliferation (Salvadori et al, 2016). The predominance of CD4+ T-lymphocytes in the dermis is a feature similar to that has been observed in humans, pigs and dogs with the hypersensitivity form of sarcoptic mange (Gallardo et al., 2002; Bhat et al., 2017). CD4+:CD8+ ratio in the dermal infiltrate has been evaluated to at least 4:1, as it has also been reported in humans with ordinary scabies (Cabrera et al., 2005) and sheep with *Lucilia cuprina* myiasis (Bowles et al., 1992). It is likely that the ectoparasite-derived antigens in ovine skin cause similar immune responses regardless of the parasite involved.

In general, *Sarcoptes scabiei* mites, as they penetrate and burrow through the epidermis, produce a variety of antigens (both secretory and excretory) evoking a complex immune response (Arlian and Morgan, 2017). The type of immune response is largely dependent on the general immune status of the host (Pence

and Ueckermann, 2002). In humans and animals the classical (hypersensitivity) form of sarcoptic mange elicits a combined type-I and type-IV reactions (Pence and Ueckermann, 2002; Skerratt, 2003; Espinosa et al., 2017; Niedringhaus et al., 2019).

The high number of CD4+ cells (helper T-lymphocytes) in dermal infiltrate in ovine sarcoptic mange suggests the recognition of specific parasitic antigens by these cells, as it has also been reported in bovine skin infestation by lice (Milnes et al., 2007). The interaction of mite antigens with specific CD4+ cells may lead to massive influx of eosinophils observed in sheep psoroptic mange lesions (van den Broek et al., 2005).

Cutaneous eosinophilia due to *Sarcoptes scabiei* infestation corresponds to type-I (immediate) hypersensitivity reaction, as it has been commonly observed in dermatitis associated with other ectoparasites (Skerratt, 2003; Nimmervoll et al., 2013). Studies on type-IV (delayed-type) hypersensitivity reactions in humans, pigs, and sheep have shown that the participating lymphocytes are mostly CD4+ T-cells with a fewer CD8+ T-cells (Pyrah and Watt, 1995; Jorundsson et al., 1999). Moreover, T-lymphocytes entered the dermis in sarcoptic mange corresponds to a type-IV (delayed) hypersensitivity response (Skerratt, 2003). The inflammatory dermal infiltrate observed in this study likely suggests a combined type-I and type-IV hypersensitivity reaction. Thus, our cases are considered chronic cases of the hypersensitivity form of sarcoptic mange ("classical or ordinary form").

In human dermatopathology, the predominance of CD8+ T-cells with few CD4+ T-cell in the dermal infiltrate and absence of B-cells are significant immunohistochemical features of "Norwegian scabies" (Gallardo et al., 2002; Walton et al. 2008). As far as the two cases of rams, their scrotal lesions could not be considered as a "hyperkeratotic (Norwegian) form" of sarcoptic mange. The dermal inflammatory infiltrate was composed equally by eosinophils and CD3+ T-lymphocytes whith the predominance of CD4+ T-helper cells. These features are compatible to the hypersensitivity form of sarcoptic mange ("classical or ordinary" scabies). This does not rule out the possibility of "Norwegian scabies" existence in few infected immunocompromised sheep individuals, especially in flocks with endemic sarcoptic mange for very long time even years. A study in pigs has also provided evidence that in herds with long-standing exposure to *Sarcoptes scabiei*, the infection becomes highly over dispersed with large mite populations present only in a few pigs and in specific body areas (Goyena et al., 2013).

The differences in histopathological features of cutaneous inflammation, between different body regions in an individual, are correlated with the different degree of disease severity and chronicity in each region (Van den Broek et al., 2004; Nimmervoll et al., 2013). In ovine psoroptic mange, the histopathological lesions at the advancing margin of an extensive lesion were more severe than those at the initial site of infestation, and this was reflected by the numbers of mites present (Van den Broek et al., 2004). According to the history, the scabietic lesions on the scrotum followed those on the face and legs. Moreover, the regional differences in the cutaneous microarchitecture and lipids (Lyne and Hollis, 1968; Arlian and Morgan, 2017) and the interaction of mite with local microenvironment and microbiome (De Candia et al., 2019) may have an impact on the involved immunopathologic mechanisms and parasitic load.

T-lymphocytes (CD3+ cells) include CD4+, CD8+ and γδ+ subpopulations. The lymphoid system of ruminants contains a large number of γδ+ T cells, in contrast to human, dog and cat. In sheep, they are more prevalent in lambs than in ewes (Hein and Mackay, 1991; Watson et al., 1994). In healthy ovine skin, γδ+ T-cells are the predominant lymphocyte subpopulation in wooled body regions regardless of age (McElroy et al., 1998). Some immunohistochemical studies have tried to investigate their involvement both in innate and adaptive immune mechanisms in ovine skin diseases (Bowles et al. 1992; Jorundsson et al. 1999; Boppana et al., 2005; van den Broek et al., 2005; Gulbahar et al. 2006).

As far ovine sarcoptic mange, there have been no reports on the role of γδ+ T-cells play in its immunopathogenesis. Even if the chronic cases of recent study involve adult sheep, with lesions located only in the non-wooled body regions, the participation of γδ+ T-cells in the inflammatory infiltrate should not be considered negligible, because these cells have not been immunohistochemically investigated. This limitation of our study on the specific role of γδ+ T-cells in immunopathology of ovine sarcoptic mange could be the subject of a future research study. In fact, the evaluation of the nature of γδ+ T-cell cutaneous response in ruminants would require both cell immunophenotyping and investigation of the local cytokines profile (Milnes et al., 2007; Shu et al., 2009).

CONCLUSSION

In adult sheep the dermal inflammatory infiltrate in chronic scabietic lesions is mixed, dominated by eosinophils and lymphocytes equally. The immunophenotypical characterization of lymphocytes subpopulations showed almost exclusively T-cell population (CD3+) with predominant T-helper cells (CD4+) versus T-cytotoxic cells (CD8+) in 4:1 to 5:1 ratios. The mixed inflammatory infiltrate combined with the immunohistochemical features suggest a type-I and type-IV hypersensitivity reaction during the course of the disease. In conclusion, all our chronic cases in adult sheep are recorded into the hypersensitivity form of sarcoptic mange ("classical or ordinary" scabies) and no cases of the hyperkeratotic form of the disease ("Norwegian or crusted" scabies) were found.

CONFLICT OF INTEREST STATEMENT

None of the authors have any conflicts of interest to declare.

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