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Original Article

Molecular and Pathological Studies on *Sarcoptes scabiei* in Sheep in Ismailia Province, Egypt.

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Abstract:

Scabies is one of the important neglected tropical skin diseases which caused by the parasitic mite *Sarcoptes scabiei*. Scabies is usually detected in the developing countries. This study was designed to investigate the molecular characterization and pathological alterations induced by mite infestation in sheep at Ismailia province, Egypt. A total of 760 sheep aged from 3 months to 3 years were examined during the period from October 2017 to March 2019. Among them, 80 (10.5%) were infested with one species of mites *Sarcoptes scabiei*. Molecularly, the second internal transcribed spacer (ITS-2) of nuclear ribosomal DNA (rDNA) was as a genetic marker. TheITS-2 primer was amplified from individual mites by polymerase chain reaction (PCR) revealing the diagnostic specific band at 480 bp, nucleotides sequencing proved the species. Grossly, alopecia with rough leathery and corrugation of skin, erythema, crusts and pruritus were observed. The associated histological lesions were hyperkeratosis with crusting and thickening of the epidermis, acanthosis and vesiculation.

Key words: *Sarcoptes scabiei*, Alopecia, Crusts, Pruritus, Hyperkeratosis, Acanthosis, *ITS2*, PCR.

Introduction

Mange is a severe contagious disease and a major global health problem affecting humans and other mammals (Currier *et al.,* 2011). Mites are the common cause of skin diseases in sheep. They can cause hypersensitivity disorders in animals. They may also cause life threatening anemia in young and/or weakened animals (Araujo *et al.,* 1998), they are microscopic ectoparasites which can cause mild to chronic skin disease known as "Mange" in several hosts including domestic, farm and wild animals. It is an important emerging disease of wildlife and a well-recognized threat to the health and sometimes the existence of endangered or isolated wildlife populations throughout the world (Pence and Ueckermann, 2002).

Mange is characterized by a loss of hairs with itching and scabby eruptions in the affected animals, humans might be also affected (Currier *et al.,* 2011). Skin lesions usually begin with erythematous areas and develop to papules with crusts formation (Osman *et al.,* 2006).

Clinical signs observed in animals in acute infestations consisted of intense pruritus, erythematous eruptions, alopecia, and seborrhea (Burkhart *et al.,* 2000). Sometimes, erythematous and alopecic areas as well as an intense pruritus appear with severe hair loss and hyperkeratosis (Rentería-Solís *et al.,* 2014). Meanwhile, Pence *et al.* (1983) described that crusted plaques, alopecia, or absent hair and with/without crusts have been found in canines.

The infestation of scabies in four wild raccoon dogs, Histologically, epidermal hyperplasia showed, in the papillary dermis with acanthosis accompanying marked rete ridge formation, hyperkeratosis that was predominantly parakeratotic in focal areas, and subcorneally formed tunnels in which mites were evident. Some epidermal tunnels were covered with flattened parakeratotic cells (Eo *et al.,* 2008). In the superficial dermis, infiltrates of cells in a perivascular pattern were detected. A marked acanthosis and hyperkeratosis, predominantly keratotic as well as multifocal mild inflammatory infiltrate, predominantly composed of neutrophils and occasional eosinophils, and superficial bacterial colonies were seen (Teodoro *et al.,* 2018). Subcorneal pustules associated with a discrete lymphoplasmacytic infiltrate were observed in the skin and the superficial dermis of one of the canids (Teodoro *et al.,* 2018).

Host-associated populations of *S. scabiei* are taxonomically divided into morphologically indistinguishable varieties with a high degree of host specificity and a low degree of cross infectivity. The varieties are named based on their host species:*S. scabiei* Var. *hominis*, *S. scabiei* Var. *canis*. Historically, genetic research on scabies has been extremely limited (Walton *et al.,* 2004). This is primarily might be due to the difficulty in obtaining sufficient quantities of the mite and usable amounts of genetic material. The taxonomic status of mites of the genus *Sarcoptes* was clarified by Zahler *et al.* (1999) utilizing the second internal transcribed spacer (ITS-2) of the rRNA gene, and described the phenotypic characters, and investigated them in 23 isolates from different host species (pig, cattle, dog, fox, raccon and lynx). Phenotypic differences among isolates were observed. Concerning the genotypic difference between distinct groups, they observed that there was no correlation with the different host species or even with the geographic origin. These results supported the co-specificity of the mites investigated

and proved the concept that the genus *Sarcoptes* consists of a single heterogeneous species.

Material and Methods

1. Study area and animals

A total of 760 sheep, aged from 3 months to 3 years screened for mites infestation during the period from October 2017 to March 2019 from different farms in Ismailia province (Kasassin, Elaabtal, Kantara, AbuAtwa, Sarabiom and Abuswear). The suspected animals (110/760) were examined following the standard procedures of skin scrapings, 80 suffered from Sarcoptic mange.

2. Examining and Sampling from the animals

Sheep were clinically examined to evaluate the health condition of the animals and to exclude any other disease affecting the health condition**.**

After clinical examination, suspected sheep were selected for parasitological investigation. Profound skin scrapings from the peripheral or the edges of lesions which were obtained from different areas of the body such as head, face, neck, ears, tail and trunk (scraping area varied from 1 to 2 .5 cm^2) area of affected lesions, until the skin was bleeding slightly.

3. Parasitological preparation and identification

Scraped samples were treated with 5 ml of 10% of potassium hydroxide to dissolve tissue materials and heated for 5-10 min. Thereafter, samples were centrifuged at 1500 rpm for 4-5 min, and then the sediment was spread on a glass slide, microscopically examined under 10x magnification.

Permanent preparation of mite specimens

Mite specimens were mounted on glass slides from 70% alcohol after rinsing in water using Berlese medium (Hoyer´s) which is prepared according to (Baker & Wharton, 1959). Species identification of mites was determined morphologically according to Mellanby (1985).

4. Molecular identification

DNA extraction and PCR analysis:

A total of 17 specimens were utilized in this study, previously observed by light microscope, DNA was extracted from these specimens using the Genomic DNA Purification kit (Applied biotechnology). PCR amplification of the ITS-2 was done using primers RIB-18 and RIB-3 as described by Zahler *et al.* (1999). The PCR was done following the cycling condition: initial denaturation at 95ºC for 5 minutes followed by 10 cycles of 92ºC for 1min, 48ºC for 1 minute and 72ºC for 90 seconds. This step was followed by additional 32 cycles of 92ºC for 1 minute, 54ºC for 35 seconds and 72ºC for 90 seconds, this was followed by a final extension at 72ºC for 7 minutes. The amplification products from ITS-2 were separated on 1.6% agarose gel containing 0.4 µg/ml of ethidium bromide (Bio-Rad Laboratoies Inc., Hercules, CA) at 90 volts for 40-60 minutes, The PCR products were sent for sequencing. Sequences were amplified using primers the upstream primer RIB-18 5’ -GGG CTG CAG TAT CCG ATG GCT TCG T-3’. and RIB-3 5 ’- CGG GAT CCT TC (A,G) CTC GCC G(C,T)T ACT- 3’.

DNA Sequence analysis:

PCR products were sequenced directly using Big Dye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) and an ABI 3130 Genetic Analyzer (Applied Biosystems). Sequences were assembled using the ChromasPro. The accuracy of data was confirmed by bi-directional sequencing. The obtained sequences were

aligned with each other and reference sequences of each gene using ClustalX to confirm the identification of *S. scabiei*. Maximum Parsimony analysis of taxa methods and in the MEGA X10.1 software was used to assess the phylogenetic relationship among different populations of *S. scabie* (Kumar *et al.,* 2018)

5. Histopathological Preparations:

The skin of slaughtered sheep infested with *Sarcoptes scabiei* was collected and immediately fixed in 10% formalin, washed several times in 70% ethanol and then fixed in a mixture of 70% ethyl alcohol 95% and glycerin 5%. The specimens were then dehydrated in ascending grades of ethyl alcohol, cleared in xylene for two days, then washed in benzene for 10 minutes and embedded in three changes of pure paraffin wax. Serial transverse sections of skin, 5 microns-thick sections were cut and mounted on clean glass slides, stained in haematoxylin and eosin, cleared in xylene and mounted in Canada balsam (Bancroft and Bamble, 2008).

RESULTS

1.Clinical observation:

The initial lesions were localized accompanied by alopecia and were found on the body parts which had thin skin and less wool. Scabby lesions appeared as erythematous skin with irregular alopecic areas and scab formation Fig (1A&B).

2. Morphological identification:

The collected *Sarcoptes scabiei* were identified by its size, shape and morphology following up the identification key of Mellanby, (1985). Adult scabies mites are roughly round, ventrally flattened and dorsally convex tortoise-like bodies. Adult female scabies mites have four pairs of short legs (two pairs in front and two pairs behind), and its first and second pairs of legs well separated from the third and fourth pairs of legs Fig. (2A, B&C).

3. Molecular identification:

In this study, a total of 17 mite specimens were analyzed based on ITS2 PCR (Zahler *et al.,* 1999) (Fig 3), the specimens sequences had been blasted on the genebank and were identified as *Sarcoptes scabiei* with 99% identity.

Phylogenetic analysis:

The phylogenetic analysis was performed using MEGA X10.1 software and the tree was constructed using maximum parsimony analysis of taxa methods (Fig: 4). The sequenced samples clustered with *Sarcoptes scabiei* species from Iran and Egypt. There are low degree of sequences variation observed with them since they all share the same ancestor.

4. Pathological lesions produced by the mite:

Grossly, alopecia with rough leathery and corrugation of skin, erythema, crusts and pruritus were observed. Poor body condition, sunked eyes, and extensive areas of alopecia were seen at the skin of slaughtered sheep obtained from the abattoir, and red smooth areas appeared with thick, irregular, opaque skin and yellow-brownish crusts that detached easily. These areas were observed on the head, trunk and abdomen.

Histopathological description:

Microscopically, it was characterized by acanthosis, hyperkeratosis, the formation of hyperplastic rete-pegs (Fig 5, A; 5, C), destruction of dermis and epidermis (Fig 5, B), hyperplastic changes in sebaceous glands, sweet gland and hair follicular cells, pyogranuloma in papillary layer and hair follicles and infiltration of neutrophils, eosinophils, lymphocytes and few macrophages (Fig 5, D).

Figure legends

Figure (1): (A) Adult sheep infected with *Sarcoptic scabiei* mites suffer from damaged wool in abdomen area; **(B):** Adult male sheep infested with *Sarcoptic scabiei* mites on the ear and abdomen area.

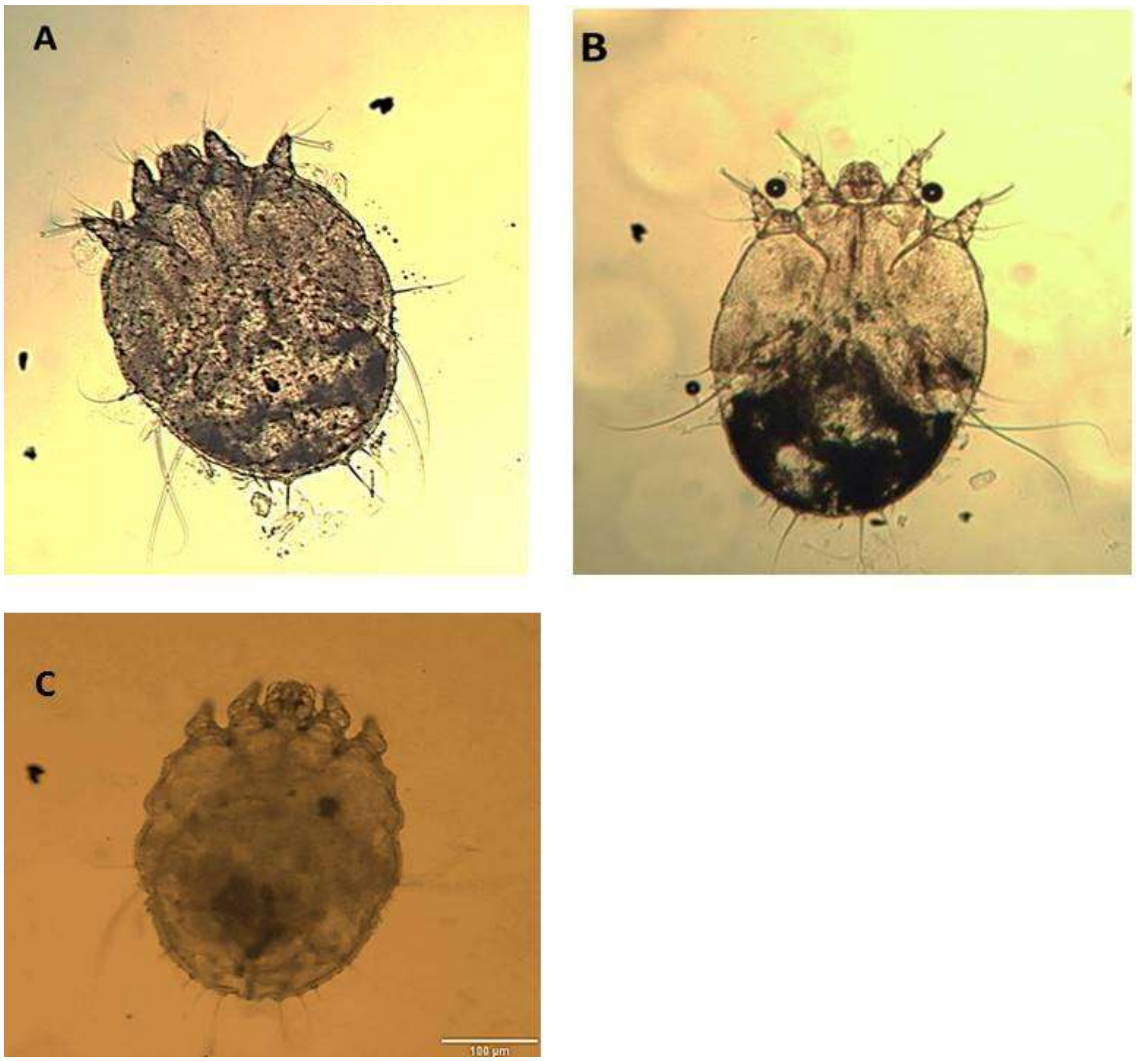

Figure (2): (A, B): Light microscopy (LM) of fresh specimens *Sarcoptes scabiei* adult (A): dorsal view (B): ventral view
(C): (LM) of permanent preparation *Sarcoptes scabiei* adult

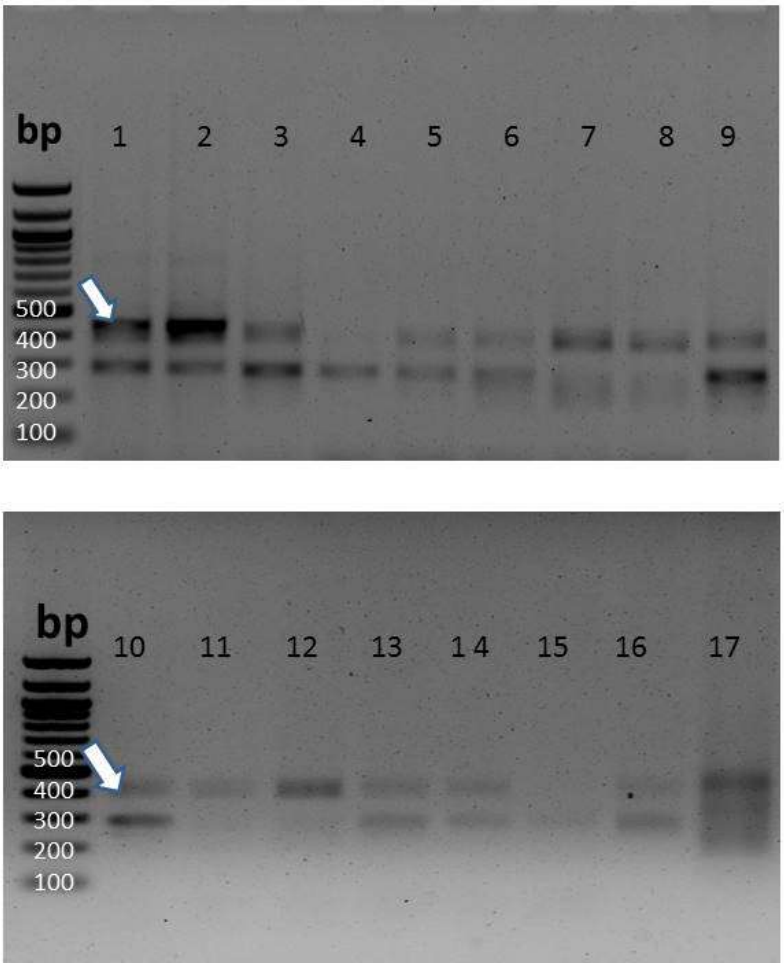

Figure (3) Analysis of ITS-2 PCR products of *Sarcoptes scabiei* by agarose gel electrophoresis. Left Lane represents 100 bp (base pair) DNA ladder plus marker, **lanes from (1to17)** represent PCR product for DNA extraction samples of *Sarcoptic scapiei* isolated from sheep with product size 480 bp.

Figure (4): Phylogenetic analysis of the present samples sequence with other mite species sequences from the gene bank.

Maximum Parsimony analysis of taxa

The evolutionary history was inferred using the Maximum Parsimony method. The most parsimonious tree with length = 1060 is shown. The consistency index is 0.807183 (0.764977), the retention index is 0.588710 (0.588710), and the composite index is 0.475197 (0.450349) for all sites and parsimony-informative sites (in parentheses). The MP tree was obtained using the Subtree-Pruning-Regrafting (SPR) algorithm (pg. 126 in ref. [1]) with search level 0 in which the initial trees were obtained by the random addition of sequences (10 replicates). This analysis involved 9 nucleotide sequences. There were a total of 467 positions in the final dataset. Evolutionary analyses were conducted in MEGA X.

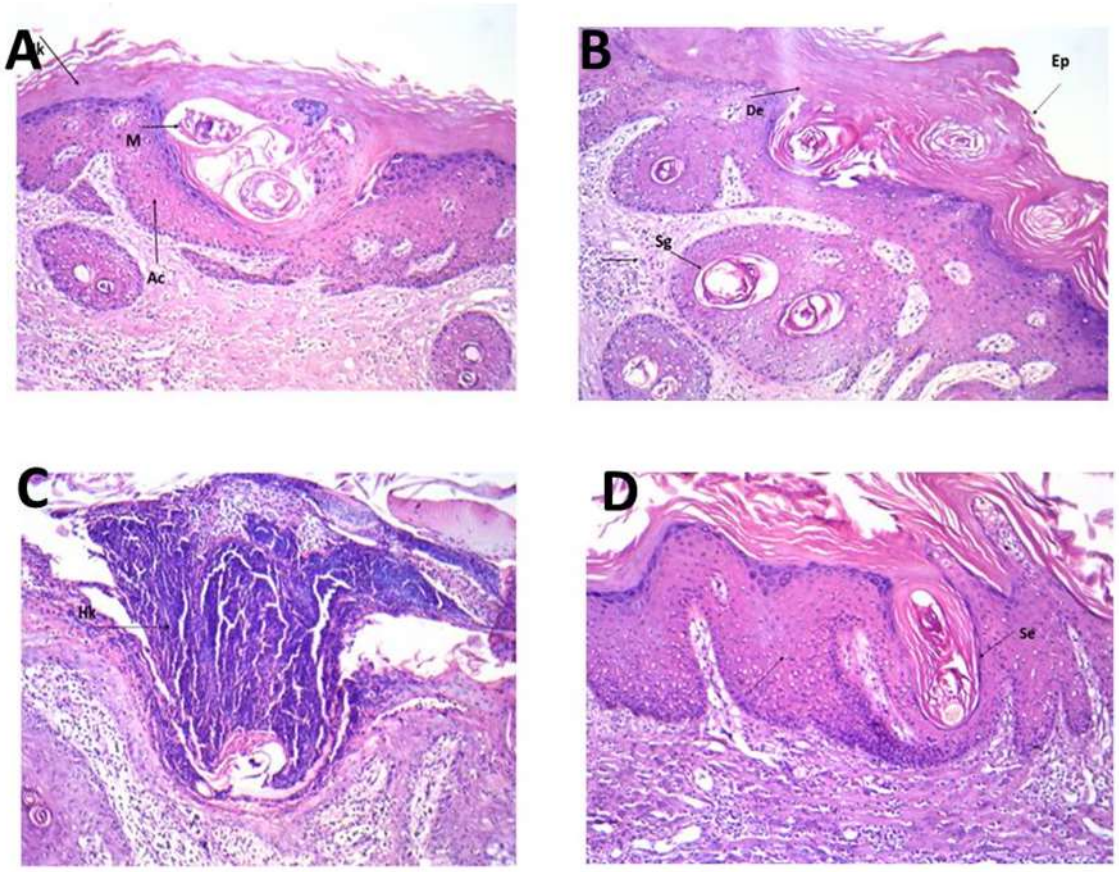

Figure (5) (A): Sarcoptic mange in sheep. Histological aspect of the skin with *Sarcoptes scabiei*, in which are evidenced idiosoma (body of mite) (M) associated with predominantly orthokeratotic hyperkeratosis (Hk) and acanthosis (Ac). X100; (**B)**: Destruction of dermis (De) and epidermis (Ep), hyperplastic changes in sebaceous glands (Sg) and infiltration of neutrophils, eosinophils, lymphocytes and few macrophages (arrow). X10; (**C**): Epidermal covering lined by hyperplastic stratified squamous epithelium showing mild papillomatosis, moderate acanthosis, marked hyperkeratosis (Hk) with focal hypergranulosis and parakeratosis. X100; (**D**): Microscopic figure characterized by acanthosis (arrow), showing changes in sweet gland (Se). X100

Discussion

The present study showed that infestations of *Sarcoptes scabiei* in sheep was potentially significant. The result demonstrated that the induced lesions of the mite in host tissues produced irritations which led to itching and scratching. The resulting inflammation of the skin is accompanied by an exudate which becamecoagulative and formed crusts on the surface and is further characterized by an excessive keratinization and proliferation of connective tissues.Progressively, the skin became much thickened and wrinkled.

In some animals, deaths occurred possibly due to the malnutrition, as the affected animals spent less time feeding because of the intense pruritus, and the severity of the lesions which can lead to ulcers formation, myiasis, secondary bacterial infections, toxemia (Mauldin & Peters-Kennedy, 2016).

Currently, the molecular identification based on DNA sequences was applied to overcome the fact that the morphological identification is insufficient for the accurate detection of the species (Abouelhassan *et al.,* 2019). The sequencing analysis based on the amplification of the ITS-2 rDNA as well as the phylogenetic analysis proveded that the examined mite species were *Sarcoptes scabiei.* This result was in concordance with those of Zahler *et al.* (1999) and Gu and Yang (2008). The former reported a very little genetic variation among sarcoptic mites collected from different hosts and geographic locations, while Gu and Yang (2008) could not differentiate sarcoptes mites among different hosts in China. Although Berrilli *et al.* (2002) detected some genetic variability between individual mites. The sequence variations were randomly distributed in different hosts from several locations, thus, resulting in an indistinct geographic or host-specific clustering.

in Egypt *Sarcoptes scabiei* was recorded by Yassin (2011) in Egyptian buffaloes at Giza governorate, in addition to Mazyad *et al.,* (2001) who reported the presence of sarcoptes mites in man and sheep in North Sinai, The present study revealed that there is no genetic variation in the sarcoptic mites collected from different sheep farms in the same geographic area and there is little genetic variation comparing with the other sequences from the genebank of the collected mite species from different geographic locations in Egypt. Furthermore, the present findings went parallel with those Amer *et al.* (2014) who used ITS2 sequence analysis for the sarcoptes mites derived from different hosts (water buffalo, cattle, sheep, and rabbits) from farms at K afr El Sheikh, Egypt. He reported very little genetic variation in sarcoptic mites from different hosts species and geographic locations with ITS2 sequence analysis.

Herein, histopathological alterations appeared consisted of hyperkeratosis with crusting and thickening of the epidermis, acanthosis, vesiculation and mites in the stratum corneum. Dermal affections were intradermal proliferation of connective tissue, edema in the papillary layer and severe degenerative and necrotic changes of the hair follicles. Those findings corresponded to the classic description of sarcoptic mange in dogs (Morris, 1996; Teodoro *et al.,* 2018). In addition, the resulted characteristic pruritic ectoparasitism was the appearance of hyperplasia and infiltration of eosinophils

(Morris, 1996; Teodoro *et al.,* 2018). The occurrence of numerous mast cells in the affected dermis was consistent with the pathogenesis of sarcoptic mange, which is largely associated with hypersensitivity to the mites (Skerratt, 2003). Those features agreed with the effect of sarcoptic mange in domestic and wild mammals (Nimmervoll *et al.,* 2013). Interestingly, the cellular response was also clear (Eo *et al.,* 2008, Nimmervoll *et al.,* 2013 and Teodoro *et al.,* 2018).

Conclusion:

Mange is a contagious and debilitating skin disease of sheep and a notable problem in Ismailia province. It could be concluded that the molecular identification of the mite species, based on DNA sequences, is an alternative and demanded tool for the accurate identification of mite species to overcome the difficulties associated with the morphological identification, Further studies are required to choose good genetic marker to help in identification of mite species.

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الملخص العربي

دراسات جزيئية وباثولوجية على حلم الجرب فى الأغنام المصابة فى محافظة الإسماعيلية بمصر

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الجرب من اهم الامراض الطفيلية التي تصيب الاغنام في مصر و هو واحد من امراض الجلد الاستوائية المهملة فى البلدان النامية، صممت هذه الدراسة لتقصي الخصائص الجزيئية والتغيرات المرضية التي يسببها الحلم في الاغنام بحافظة الاسماعيلية بمصر و قد تم فحص ٧٦٠ خروفا تتراوح اعمارهم من ٣ اشهر الى ٣ سنوات خلال الفترة من اكتوبر ٢٠١٧ الي مارس ٢٠١٩ وتبين اصابة عدد٨٠ خروفا بنسبة ١٠.٥% بنوع واحد من حلم الجرب

وقد تم التعرف علي النوع ميكروسكوبيا و عند فحص الحمض النووي للعينات عن طريق تفاعل انزيم البلمرة المتسلسل با ستخدام الجين الداخلي المنسوخ

وقد لو حظ تغيرات في الجلد المصاب بالطفيل مثل ظهور تصلب وقشور واختفاء للشعرمع التغيرات المرضية التي وجدت باستخدام الميكروسكوب .