Revista Colombiana de Entomología 43 (1): 51-54 (Enero - Junio 2017)

Sarcoptes scabiei (Acari: Sarcoptidae) infestation in rabbits (*Oryctolagus cuniculus*): A case study

Infección con *Sarcoptes scabiei* (Acari: Sarcoptidae) en conejos (*Oryctolagus cuniculus*): estudio de caso

MUHAMMAD SOHAIL SAJID[1,2], MUHAMMAD AHSAN NAEEM[3], ASMA KAUSAR[4], MUHAMMAD JAWAD-UL-HASSAN[1] and MUHAMMAD KASHIF SALEEMI[5]

Abstract: Sarcoptic mange was suspected in three of five European albino rabbits (*Oryctolagus cuniculus*) kept for experimental purposes. Gross examination revealed multifocal areas of alopecia around the eyes, nostrils and lips. Skin snips were processed using PCR for the molecular identification of the suspected mites. Histopathology of the skin snips showed erupted epidermis and stratum corneum with an infiltration of inflammatory cells. Skin scraping examination revealed the presence of adult mites as well as eggs. Microscopic taxonomy identified the adult mites as *Sarcoptes* (*S.*) *scabiei* (Acari: Sarcoptidae). The results of PCR indicated a 311 bp band from all the three cases, which confirmed the *S. scabiei* infestation in rabbits. *Sarcoptes scabiei* may be a public health concernthrough direct transmission from rabbits infested with *S. scabiei* through handling. To the best of our knowledge, this is the first report of *S. scabiei* infection in rabbits from Pakistan.

Key words: Mite infestation. Histopathology. PCR. Public health.

Resumen: A partir de una sospecha de sarna sarcóptica en tres de cinco conejos albinos europeos (*Oryctolagus cuniculus*), mantenidos con fines experimentales, se hizo un examen general que reveló áreas multifocales de alopecia alrededor de los ojos, las fosas nasales y los labios. Se procesaron cortes de piel y un análisis de PCR con el objeto de identificar los ácaros. La histopatología de los cortes de piel mostró epidermis erupcionada y estrato córneo con una infiltración de células inflamatorias. El raspado o frotis de piel reveló la presencia de ácaros tanto en adultos como en huevos. Al examen microscópico, los ácaros adultos fueron identificados como *Sarcoptes* (*S.*) *scabiei* (Acari: Sarcoptidae). El análisis de muestras de los tres casos por PCR reveló una banda de 311 pb confirmando la infestación de *S. scabiei* en los conejos. La infección en conejos con *S. scabiei* puede representar un problema de salud pública de transmisión indirecta debida a la manipulación de conejos infestados. Hasta la presente, este es el primer reporte de infección por *S. scabiei* en conejos de Pakistán.

Palabras clave: Ácaros infeccción. Hispotalogía. PCR. Salud pública.

Introduction

Sarcoptes scabiei (Acari: Sarcoptidae), commonly known as itch mite, is an ectoparasite that burrows into the skin and causes a disease commonly known as scabies in humans and mange in animals. In mammals, such as wild and domesticated dogs and cats, wild boars, ruminants, wombats, koalas, great apes (Pence and Ueckermann 2002), as well as laboratory animals, including rabbits (Suckow *et al*. 2002) are affected. The genus *Sarcoptes* is a part of the larger family of mites collectively known as "scab mites" comprising of one species, *S. scabiei* with further identification by the variety name indicating the host species e.g *S. scabiei* var. *hominius* in humans and *S. scabiei* var. *cuniculi* for rabbits. All the life cycle stages of *S. scabiei* are found on host, and the entire life cycle takes approximately two months (Suckow *et al*. 2002). The impregnated *S. scabiei* female about twice the size of the male (Jofre *et al*. 2009), oviposits into the tunnels made in the stratum corneum of the skin, causing intense itchy skin rashes, hypersensitivity and inflammation. The six-legged larvae hatch with-in three to 10 days and move about on the skin in search of hair follicles, moult into a nymphal stage, and then mature into adult mites (Soulsby 1982). The adult mites live three to four weeks in the host's skin. Mites feed on lymph and sloughed epithelial cells (Hofing and Kraus 1994).

Sarcoptic mange in rabbits is described as an uncommon disease (Scott *et al*. 2001); however, it has been reported from Israel (Eshar 2010), America (Radi 2004) and India (Soundararajan and Iyue 2005). Wild animals have been reported to transmit sarcoptic mange from dog to dog, dog to rabbits, rabbits to rabbits and rabbit to dogs experimentally as well as naturally (Arlian *et al*. 1984). In Pakistan, European albino rabbits (*Oryctolagus cuniculus*) are kept as pets, research laboratory animals and food animals. Rabbit farming is gaining currency in peri-urban and rural areas of Pakistan due to palatable meat quality and the faster rate of reproduction. The present report describes the first study of sarcoptic mange in rabbits from Pakistan diagnosed through clinical signs, gross lesions, taxonomic identification through optical microscopy, histopathology, and polymerase chain reaction (PCR).

Materials and methods

Anamnesis. Three of the five European albino land-race experimental rabbits kept in the Animal Facility of the Faculty of Veterinary Science, University of Agriculture, Faisalabad were reported as scratching and eating less than normal. All the three suspected rabbits were male, albino and approximately six weeks of age. The history revealed that the rabbits were purchased from the local market in Faisalabad for experimental purposes a few days earlier.

[1] Department of Parasitology. [2] US. Pakistan Center for Advanced Studies in Agriculture and Food Security (USPCAS-AFS). Department of Parasitology, Faculty of Veterinary Science, University of Agriculture, Faisalabad, Pakistan. Cell # +92-333-650-8667, *drsohailuaf@uaf.edu.pk*, corresponding author. [3] State Key Laboratory of Agricultural Microbiology, College of Animal Science and Technology, Huazhong Agricultural University, Wuhan, China, *muhammadahsannaeem@gmail.com*. [4] Veterinary Research Institute, Lahore, Pakistan, *asmakausar_22@yahoo.com*. [5] Department of Pathology, Faculty of Veterinary Science, University of Agriculture, Faisalabad, Pakistan, *saleemi74@hotmail.com*.

Clinical examination. The behavior of infested rabbits was-not comfortable due to continuous scratching of their affected areas. The gross lesions were observed and microscopic examination of skin scrapping was performed.

Skin scrapping examination. Skin scrapping was collected from the affected areas using the protocol described by Centers for Disease Control and Prevention (https://www.cdc.gov/dpdx/scabies/dx.html) and Iqbal *et al.* (2006) as per the ethical guidelines given in the International Guiding Principles for Biomedical Research Involving Animals published by Council for International Organization of Medical Sciences and the International Council for Laboratory Animal Science (Greene, 2012). Briefly, the affected skin was moisturized with the mineral oil and scrapped until oozing of the blood with a sharp, clean and sterilized blade from the area of 2.5 cm^2. The scrapping was collected in a McCartney sample collection bottle containing 10 % KOH as macerating agent. The areas were aseptically dressed. Direct smear method was used for microscopic examination of the skin scrapping. Taxonomic identification of the adult mites was done using the keys described by Flynn (1973) and Suckow *et al*. (2002).

Histopathology of the skin snips. For histological examination, multiple tissue specimens of skin (skin biopsy) were collected as per the protocol described by Iqbal *et al*. (2006). Briefly, the areas were disinfected using gauze dipped in alcohol. A disposable needle was used to pull an area of skin. A disposable scalpel blade was used to cut a piece of skin pulled up by the point of needle. The collected skin specimens were fixed in 10 % neutral-buffered formalin, embedded in paraffin wax, sectioned at 5µm thickness, and stained with hematoxylin and eosin for microscopic examination under oil immersion lens.

PCR analysis of the skin snips. The collected skin snips from the affected areas of the three rabbits were also processed through polymerase chain reaction (PCR) for confirmatory diagnosis of *Sarcoptes* spp. To this end, DNA was extracted from the rabbit skin tissue sample (n = 3) using GeneJET Genomic DNA purification kit (Thermo Scientific, USA), according to the manufacturer's recommended protocol and stored at -20 °C till further use. The *S. scabiei* was detected by PCR using specific actin gene primers: (SsF) 5' CAA CCA TCC TTC TTG GGT ATG 3' and (Ss R) 5' CCA GCT TCG TCG TAT TCT TGT 3' (Mounsey *et al*. 2012). The PCR reaction mixture was set up by taking 5 µL of the genomic DNA, 12.5 µL of 2 x PCR master mix, 1 µL of each primers (10 pmol) and balanced with nuclease free water for 25 µL of total PCR reaction volume. The PCR reaction was performed in C1000 Thermal Cycler (Bio-Rad Laboratories, USA) with the following cycling parameters: initial denaturation at 95 °C for 1 minute followed by 39 cycles of 94 °C for 15s, 52 °C for 30s, and 72 °C for 30 s followed by a final extension at 72 °C for 10 minutes. PCR products were separated on 2 % agarose gel and visualized under UV by Molecular Imager® Gel Doc™ XR+ with Image Lab™ software (Bio Rad, USA).

Results

All the three suspected rabbits had clinical signs of pruritis and gross lesions of variable-size alopecia around the nostrils, lips and ears. The affected skin area of ears was covered with tannish yellow, scaly crusts (Fig. 1).

Adult mites were clearly identified in the microscopic examination with round body and shorter legs (Fig. 2A) with the presence of mite eggs attached within the hair follicles (Fig. 2B).

Microscopic examination of the sections revealed the erupted epidermis and stratum corneum with infiltration of inflammatory cells (Fig. 3).

The PCR results of all the three PCR reactions from the three suspected rabbits showed the amplified fragment of 311 bp of actin gene of the *S. scabiei* var *cuniculi.* For representation of bands, 3 µL of the reaction mixture was poured in each of the wells 3 and 4 from one of the three positive amplicons for *S. scabiei* var *cuniculi* (Fig. 4).

Discussion

In Pakistan, rabbits are mostly kept as pets, experimental lab animals and food animals. These are an excellent source of economical and good quality meat due to their fast rate of reproduction. Sarcoptic mange is a highly contagious disease of the skin transmitted through direct and indirect contact (Arlian *et al*. 1984).

The present study provides the first report of *S. scabiei* var *cuniculi* infestation in rabbits in Pakistan diagnosed through clinical signs, microscopic examination, histopathology and PCR. The clinical signsof acute sarcoptic mange may include severe pruritus, alopecia and seborrhea in result of hypersensitivity reaction (Davis *et al*. 1991) whereas, crusting and hyperkeratosis occur in chronic infestation (Van-Nesteand Sta-

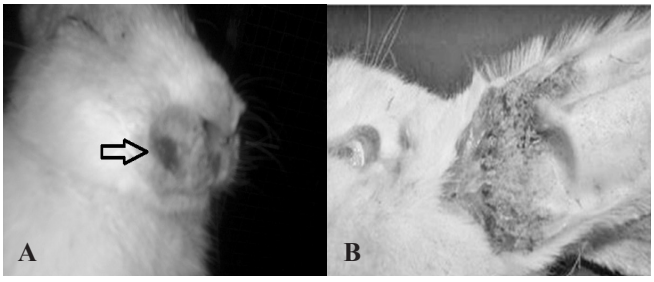

Figure 1. Photographs of gross lesions of sarcoptic mange in a rabbit. **A.** Notice multifocal areas of alopecia around eyes, nose and lips with scratched wound beside nose (arrows). **B.** Alopecia and crusting of exudate on the ventral surface of the pinna.

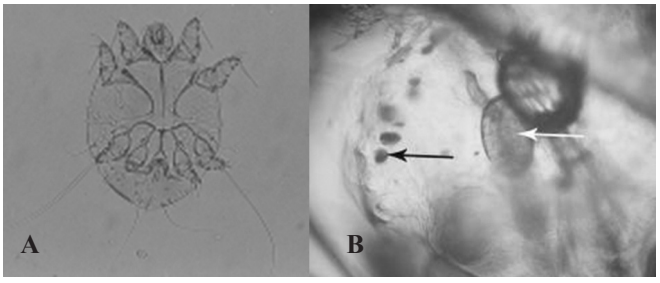

Figure 2. Photomicrographs of sarcoptic mite from the skin scrapping of the rabbit. **A.** Ventral view of the adult mite with rounded body and shorter legs at 10 x magnification. **B.** The egg of mite are visible (arrow) at 100 x magnification.

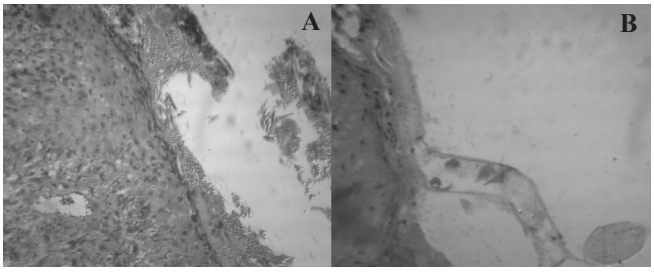

Figure 3. Histological sections of skin scrapping from rabbit with crusted lesion stained with hematoxylin and eosin. **A.** Note the erupted epidermis and dermis with mild inflammatory infiltrate in the dermis. **B.** The mite egg beside the hair follicle.

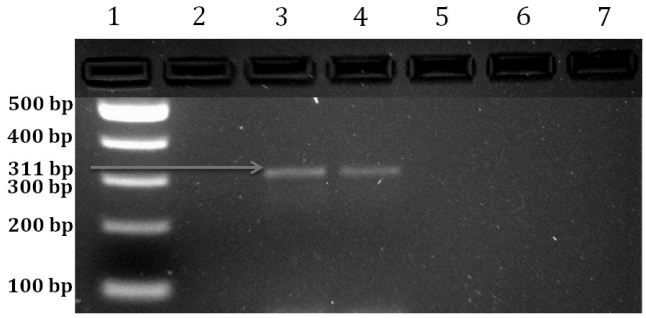

Figure 4. Molecular detection of *Sarcoptes scabiei* from the gDNA of skin snips of three rabbitssuspected for mite infestation using PCR. Lane 1: DNA 100 bp marker/ladder. Lane 2: Negative control, Lanes 3 and 4: 3 µL of the amplicon was poured into each of the wells which showed band of 311 bp confirming *Sarcoptes scabiei* var *cuniculi*. All the three PCR products from three rabbits showed similar bands of 311 bp.

quet 1986). In the present cases, acute infestation was found with the signs of pruritus and alopecia. Amyloidosis, anemia, and leukopenia have been reported in rabbits with sarcoptic mange (Arlian *et al*. 1984), however, we did not find evidence for amyloidosis in the study cases. Generally, *S. scabiei* in rabbits affects the face, nose and external genitalia (Percy and Barthord 2001). In the present cases, affected areas were the face, nose and ears but not the external genitalia.

Diagnosis of sarcoptic mange can be made by identification of the mite by microscopic examination of skin scrapings (Suckow *et al*. 2002) and histopathology of skin lesions. Sarcoptic mite is round in shape with short legs having a long unjointed stalk with a sucker on front pair of legs. The body wall of *Sarcoptic scabiei* is thick and chitinous with large spines on the dorsal body surface (Chitwood and Lichtenfels 1972), the anus is terminal and the dorsum possesses scales, cones and bladelike setae. The size of a female sarcoptic mite is 303 to 450 µm x 250 to 350 µm.

Mite infestation is highly specific, however, occasionally; exposure to animals can cause infestation in humans as their aberrant host (Beck 1965). Recently, a 56-years-old human case of scabies infection with a history of contact with his pet dog has been reported (Bandi and Saikumar 2013). Radi (2004) reported that ear canker caused by *Psoropte scuniculi* infestation in rabbits is relatively abundant than sarcoptic mange caused by *S. scabiei* var. *cuniculi.* The conventional diagnostic tests for mites are having less than 50 % accuracy (Shelley and Currie 2007); hence, confirmatory diagnosis of the species of mite infestation through PCR was necessary. Identification of mites at molecular level using PCR is highly sensitive, reliable and specific method.The amplification of specific fragment can confirm the species of the mite (Naz *et al*. 2013) as confirmed in this report by amplification of specific fragment of the house keeping actin gene of *S. scabiei***.** This report provides the standardized protocol for confirmatory diagnosis of the mite infestation in rabbits which can be helpful for planning future strategies of risk assessment from rabbits as potential reservoirs of infestation.

Acknowledgements

The authors would like to thank Dr. Thomas Nolan, Professor of Parasitology, University of Pennsylvania, Philadelphia, for reviewing the manuscript. Spanish translation of the abstract (resumen) was kindly made by the editorial board of the Revista Colombiana de Entomología. Suggestions of anonymous reviewers of the manuscript are also acknowledged.

Literature cited

ARLIAN, L. G.; REBECCA, A.; RUNYAN, B. S.; STEPHEN, A.; DAYTON, E. M. D.; CINCINNATI, O. H. 1984. Cross infectivity of *Sarcoptes scabiei*. Journal of the American Academy of Dermatology 10: 979-986.

BANDI, K. M.; SAIKUMAR, C. 2013. Sarcoptic mange: A zoonotic ectoparasitic skin disease, Journal of Clinical and Diagnostic Research 7: 156-157.

BECK, A. L., Jr. 1965. Animal scabies affecting man. Archives of Dermatology 91: 54-55.

CHITWOOD, M.; LICHTENFELS, J. R. 1972. Identification of parasitic metazoa in tissue sections. Experimental Parasitology 32: 407-519.

DAVIES, P. R.; MOORE, M. J.; POINTON, A. M. 1991. Sarcoptic mite hypersensitivity and skin lesions in slaughtered pigs. Veterinary Record 128: 516-518.

ESHAR, D. 2010. Prevalence of sarcoptic mange in pet rabbits (*Oryctolagus cuniculus*) in Israel. Israel Journal of Veterinary Medicine 65: 140-141.

FLYNN, R. J. 1973. Parasites of laboratory animals. Iowa state University Press, Ames Iowa. p. 448-463.

GREENE, M. (Ed.). 2012. International guiding principles for biomedical research involving animals. Council for International Organization of Medical Sciences and the International Council for Laboratory Animal Science. Accessed at http://www.cioms.ch/publications/guidelines/1985_texts_of_guidelines.html.

HOFING, U. R.; KRAUS, A. L. 1994. Arthropod and helminth parasites. pp. 231-257. In: Manning, P. J.; Ringer, D. H.; Newcomer, C. E. (Eds.). The biology of the laboratory rabbit.2nd ed. Academic Press, Inc., San, Diego, California. https://www.cdc.gov/dpdx/scabies/dx.html.

IQBAL, Z.; SAJID, M. S.; JABBAR, A.; ABBAS, R. Z.; KHAN, M. N. 2006. Techniques in Parasitology. Higher Education Commission, Islamabad, Pakistan.

JOFRE, L.; NAOMI, I.; NEIRA, P.; SAAVEDRA, T.; DÍAZ, C. 2009. Acarosis and related zoonosis. Revista Chilena de Infectología 26: 248-257.

MOUNSEY, K. E.; WILLIS, C.; BURGESS, S. T. G.; HOLT, D. C.; MCCARTHY, J.; FISCHER, K. 2012. Quantitative PCR-based genome size estimation of the astigmatid mites *Sarcoptes scabiei*, *Psoroptes ovis* and *Dermatophagoides pteronyssinus*. Parasites & Vectors 5: 3.

NAZ, S.; RIZVI, D. A.; JAVAID, A.; ISMAIL, M.; CHAUHDRY, F. R. 2013. Validation of PCR assay for identification of *Sarcoptes scabiei* var. *hominis*. Iranian Journal of Parasitology 8: 437-440.

PENCE, D. B.; UECKERMANN, E. 2002. Sarcoptic mange in wildlife. Scientific and Technical Review of the World Organisation for Animal Health 21: 385-398.

PERCY, D. H.; BARTHOLD, S. W. 2001. Pathology of laboratory rodents and rabbits. Iowa State University Press, Ames, Iowa.

RADI, Z. A. 2004. Outbreak of sarcoptic mange and malasseziasis in rabbits (*Oryctolagus cuniculus*). Comparative Medicine 54: 434-437.

SCOTT, D. W.; MILLER, W. H .; GRIFFIN, C. E. 2001. Dermatoses of pet rodents, rabbits and ferrets. pp. 1415-1458. In: Scott, D. W.; Miller, W. H.; Griffin, C. E. (Eds). Muller and Kirk's small animal dermatology. W.B. Saunders, Philadelphia, PA.

SHELLEY, F. W.; CURRIE, B. J. 2007. Problems in diagnosing scabies, a global disease in human and animal populations. Clinical Microbiology Reviews 20: 268-279.

SOULSBY, E. J. L. 1982. Helminths, Arthropods and Protozoa of the Domesticated Animals. Baillaire, Tindall, London, UK.

SOUNDARARAJAN, C.; IYUE, M. 2005. Incidence of mange infestation in rabbits. Journal of Veterinary Parasitology 19: 2.

SUCKOW, M. A.; BRAMMER, D. W.; RUSH, H. G.; CHRISP, C. E. 2002. Biology and diseases of rabbits. pp: 349-350. In: Fox, J. G.; Anderson, L. C.; Loew, F. M.; Quimby, F. W. (Eds.). Laboratory animal medicine. 2nd ed. Academic Press, Inc San Diego, California.

VAN NESTE, D. J.; STAQUET, M. J. 1986. Similar epidermal changes in hyperkeratotic scabies of humans and pigs. American Journal of Dermatopathology 8: 267-273.

Received: 01-Dec-2015 • Accepted: 28-Apr-2017

Suggested citation:

SAJID, M. S.; NAEEM, M. A.; KAUSAR, A.; JAWAD-UL-HASSAN, M.; SALEEMI, M. K. 2017. *Sarcoptes scabiei* (Acari: Sarcoptidae) infestation in rabbits (*Oryctolagus cuniculus*): A case study. Revista Colombiana de Entomología 43 (1): 51-54. Enero-Junio 2017. ISSN 0120-0488.