



Morphological, molecular and histopathological identification of *Sarcoptes scabiei* infection in a goat herd at Goa, India

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ABSTRACT

Mange is caused by infestation with mites of different genera which significantly impacts the farm economy by affecting growth and weight gain in young animals. This study was aimed to investigate a severe mange outbreak in a goat herd in North Goa District, Goa, and to characterize the causative pathogen. The disease was diagnosed by clinical signs, gross and histopathologic lesions, microscopical detection of mites, and confirmed by PCR and *16s rRNA* sequencing, and the genetic characterization of the *S. scabiei* isolate. The animals showed pruritus, erythema, papule formation, alopecia and scab formation. Lesions were mainly distributed on the head around the eyes, lips, nostrils, ears, face, and neck. The KOH digestion method showed the different developmental stages of *S. scabiei*. Histopathological changes like acanthosis, micropustule formation around degenerating mite, acantholysis, infiltration of inflammatory cells and presence of parasite stages and eggs in the tunnels in the stratum corneum were observed. Treatment using Ivermectin, Chlorpheniramine maleate, Deltamethrin and oral vitamin supplements led to the complete recovery of the affected animals. The phylogenetic study based on *16s rRNA* and *ITS* genes revealed that the Goan isolate was phylogenetically close to wild animal and livestock isolates from Egypt, Australia, China and Europe. The study provides pertinent information on the *S. scabiei* prevalence in the goat population in Goa and shows the potential risks of spread of this zoonotic pathogen and emphasizes the need for the development of preventive measures against infestation in animals in the hot humid coastal region.

Keywords: Goa, Goat, India, Phylogenetic analysis, *Sarcoptic scabiei*

Mange in animals is caused by around 50 species of ectoparasitic mites. The sarcoptic mange caused by the parasite *Sarcoptes scabiei* has been reported in a wide range of domestic and free-ranging wild animals (Escobar *et al.* 2022). *S. scabiei* are obligate parasites, where the entire life cycle stages are present in the superficial layers of the host skin, and the parasite feed on the cellular material and intercellular fluid (Fernando *et al.* 2024). Clinical signs and lesions of mange are caused by host immune responses to the parasite (Naesborg-Nielsen *et al.* 2022) and secondary bacterial infection can lead to a higher morbidity. This disease is of serious public health concern as inter-species transmission can occur and is recognized as a neglected tropical disease (WHO 2017). The mites get transmitted by direct and indirect contact, and the host, environmental, and anthropogenic factors influence the environmental transmission of *S. scabiei* (Ringwaldt *et al.* 2023). The warm humid weather favors their long survival outside the host (Chandler and Fuller 2019), and the host density and use of shared environment are the key factors affecting transmission (Browne *et al.* 2022). The mite infestation imposes serious economic constraints on

goat farming as it affects the weight gain in kids. There are only a few studies on *S. scabiei* infestation in goats from India (De and Dey 2010, Borah *et al.* 2015, Jadhav *et al.* 2020, Salvi *et al.* 2022), and not been reported in coastal areas and phylogenetic analysis of the goat isolate from India has not been done. The present study was carried out to characterize the pathologic changes and to genetically characterize the mites associated with a mange outbreak in a goat herd in the North Goa district.

MATERIALS AND METHODS

Herd history: A severe outbreak of mange infestation occurred in a goat farm (15.5489°N and 74.0020°E) in the west coastal region of Goa state in October 2022. The affected farm was visited, animals and shed premises were examined, and data regarding breed, age, housing, method of farming, and weather conditions were recorded.

Sample collection and disease diagnosis: The samples were collected by following the guidelines of the Institutional Animal Ethics Committee (IAEC, (2118/GO/RBi-S/R/-L/21/CPCSEA). Skin scraping samples were collected from 5 severely affected animals. Skin biopsy samples were collected under local analgesia using subcutaneous lignocaine hydrochloride injection (20 mg/ml). The samples were transported over ice to the laboratory.

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The skin tissue samples were processed for histopathology and examined under light microscopy. Skin scrapings were treated with 10% potassium hydroxide (KOH) and examined under a light microscope for detection of mites or fungal hyphae (OIE 2022). The species identification was carried out by morphological criteria as per the Laboratory manual of parasitology (Soulsby 1982). DNA samples were isolated from skin tissues using *DNeasy* Blood and tissue kit (Qiagen) and were screened for *Sarcoptes* species targeting mitochondrial *16S rRNA* gene using SSUDF and SSUDR primers (Angelone-Alasaad *et al.* 2015). The PCR products were sequenced and NCBI BLAST analysis was carried out to assess the sequence identity with published sequences.

Phylogenetic analysis: PCR amplification and sequencing of *16S rRNA* and *ITS-2* genes (Li *et al.* 2018) were carried out. The obtained sequences along with the sequences retrieved from GenBank, distributed in different continents and host ranges, were aligned in clustalW and subjected to phylogenetic analysis by Maximum Likelihood (ML) method of MEGA X (Tamura and Nei 1993) to infer evolutionary relatedness. The pairwise nucleotide sequence distance matrix were calculated with Megalign program of DNASTAR software. The nucleotide sequences of *16S rRNA* and *ITS* genes from this study were deposited in GenBank.

Treatment: All the affected animals were treated with a single dose of Ivermectin injection at 200 µg/kg body weight subcutaneously and Pheniramine maleate 1ml (22.75 mg/ml) intramuscularly once daily for 5 days. The animals were given 2 treatments of Deltamethrin (Butox) spray at an interval of 8 days and oral vitamin liquid supplement for 15 days in feed.

RESULTS AND DISCUSSION

Herd history: Goa is a small state located on the west coast of India between the latitudes 14°53'54" N and 15°40'00" N and longitudes 73°40'33" E and 74°20'13" E. The state has a warm and humid climate for most of the year, where the maximum and minimum temperatures varies from 26.0-38.5°C, and 16.0-28.0°C respectively during the year. A relative humidity of 61% (minimum) to 69% (maximum) was reported during the month of study. In the affected farm, the herd of around 100 goats were maintained in partitions in a single large shed and a total of 82 goats in the herd were showing mild to severe skin lesions. The animals were raised entirely by intensive farming without grazing and were fed with concentrate feed and locally available grass or tree fodder. The goat farm was located near a dairy farm in the same premises and no mange infestation was reported in cattle maintained in the farm. Sarcoptic mange is highly contagious and the spread of infestation occurs through the transmission of mites from an infested animal to uninfected ones by close contact as reported by other workers (Currier *et al.* 2011). The lifecycle of the parasite is completed in 10-15 days within the host, and the mite can survive outside the

host for a long period in warm humid weather conditions (Chandler and Fuller 2019). The warm humid weather of the coastal area is highly favorable for the survival of mites in the environment, which might be the reason for the fast spread of infestation among the goats.

Disease diagnosis and lesions: KOH digestion and microscopic examination revealed the presence of egg, larvae and adult stages of mites (Fig. 1a, 1b, 1c, 1d, 1e) and were identified as *Sarcoptes* genus based on structural details. No fungal hyphae was observed in the sample. The skin samples from all five animals were positive for *Sarcoptes* mites microscopically and by PCR. The *16S rRNA* gene sequences from 2 samples were submitted to NCBI with accession numbers PP697631 and PP697632. The NCBI BLAST analysis showed 100% identity with *Sarcoptes scabiei* *16S rRNA* gene sequences which confirmed the Sarcoptic mange outbreak in the herd.

Lesions in the initial stage of infestation were localized alopecia, the presence of small raised papules, and erythema. There was a regional distribution of lesions,

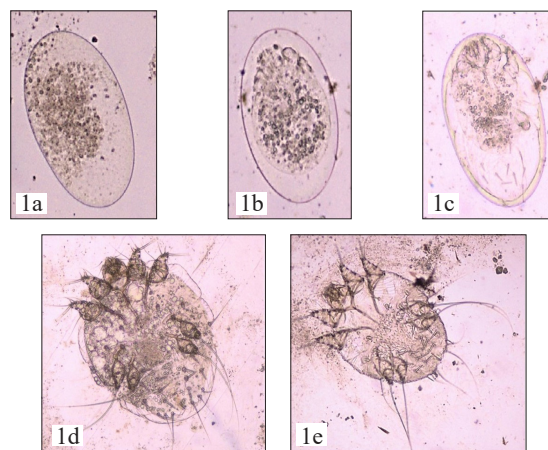


Fig. 1. Image of egg, larvae within the egg, immature and adult stages of *Sarcoptes* mites skin scrapings under light microscopy 10X (Fig. 1a, 1b, 1c, 1d, 1e).

which was seen more on the face around the eyes, ear pinna, around nostrils and lips, dorsal side of the neck, and, on either side of the shoulder. Lesions were in the form of skin wrinkling, thickening, scab formation, and deposition of dander around the crusts. (Fig. 2a, 2b). In a few animals lesions were also seen on either side of the abdomen, hip, and around the tail. In a very few severely affected animals there was intense pruritus, erythema, and extensive skin thickening with deep cracks seen on the earlobe, hock joint, and around the anus (Fig. 2c). Many animals appeared emaciated (Fig. 2d) and showed generalized alopecia. The major lesions seen in the present outbreak were similar to earlier reports in goats (De and Dey 2010, Giadinis *et al.* 2011, Jadhav *et al.* 2020). In the present outbreak, no difference in clinical manifestation was observed among male and female animals, similar to that reported among male and female sheep (Iqbal *et al.* 2015). As in the present outbreak, regional distribution of lesions in the head and



Fig. 2. Alopecia, thickening and dander deposition around the eyes and over the ear and on hind quarters (2a, 2b) skin showing wrinkling, thickening, cracks in the skin, scab formation around the tail. (2c). A severely emaciated animal showing alopecia (2d).

thorax was reported earlier in sheep and goat (Abu-Samra *et al.* 1981, Rahbari *et al.* 2009, Jadhav *et al.* 2020). Here the animals were maintained by intensive farming and were stall-fed which could have contributed to the spread of infection easily in the face and neck region due to close contact of animals during feeding or fighting.

Histopathological examination of the skin showed an accumulation of exudate comprising neutrophils, serum, and keratin fragments over the highly damaged *stratum corneum* (Fig. 3a). Adult mites, mite segments, and eggs were observed in the tunnels in the keratin layers of the *stratum corneum* (Fig. 3b). The epidermis showed acanthosis and micropustule formation around degenerating mites and infiltration of inflammatory cells. Large necrotic foci comprising dead inflammatory and epithelial cells was observed in the *stratum corneum* and there was severe infiltration of neutrophils in the below layers (Fig. 3c). The adjacent area of *stratum spinosum* showed acantholysis and degenerative changes. (Fig. 3d). The dermis showed extensive infiltration of inflammatory cells and congestion of capillaries. Microscopic lesions observed in the skin, including the presence of parasite stages and eggs in the tunnels in the *stratum corneum*, were similar to earlier reports in goat and sheep (Sengupta *et al.* 2008, Rahbari *et al.* 2009).

Phylogenetic analysis: We analyzed the genotypes of the *S. scabiei* isolate from the present outbreak based on *ITS-2* and *16S rRNA* sequences. The sequences of *16S rRNA* and *ITS* were submitted to NCBI GenBank under the accession numbers PP974324 and PP974325 respectively. As the *16S rRNA* gene is more conserved, it is useful in studying the genetic changes based on host adaptation and geographical location (Amer *et al.* 2014). The alignment of the *16S rRNA* sequence from the present study with those from GenBank showed that 10.86% of nucleotides

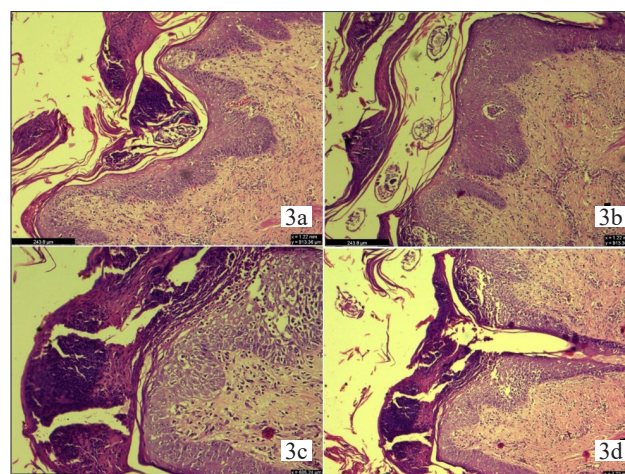


Fig. 3. Accumulation of neutrophils, serum, and keratin fragments over the highly damaged stratum corneum, (3a) and presence of adult mites, mite segments, and eggs in the tunnels in the keratin layers (3b) under Light microscopy 10X. Large necrotic foci comprising dead cells in the stratum corneum, infiltration of neutrophils in the below layers acantholysis (3c) under 20X, and degenerative changes in the adjacent stratum spinosum (3d) under 10X.

are variable. Phylogenetic tree based on the Maximum Likelihood method of MEGAX showed clustering of sequences into 3 major groups (Supplementary Fig. 1a). Group 1 included PP974324 from Goa along with isolates from humans, dogs and wild animals from Australia, Egypt and Japan, Italy, Spain, Tanzania, Chile and China and it showed 100% identity with most of the isolates in this group. Maximum divergence of 1.1% (Supplementary Fig. 2) was observed with three isolates (AY493412, MF083735 and MF083742) from wild animals of Australia in this group. Most of the human isolates including those from Australia, Panama, Italy and Pakistan formed the group 2 along with a canine isolate from China. Isolates from Australian wild animals formed group 3 with those from Japan, and a canine isolate from China and a human isolate from Pakistan. The estimate of average evolutionary divergence of 16S sequence pairs is 0.005 M. Earlier studies on genetic studies based on *16S rRNA* of *S. scabiei* isolates reported that mites belonging to different clades in phylogenetic studies are genetically isolated (Andriantsoanirina *et al.* 2015). The clustering based on the 16s sequence in the present study showed the separation of human isolates from animals, but there was no clustering based on geographical location. Even though there was segregation of most of the human isolates, there was no differentiation between mites from different hosts of the same country, like isolates from different livestock species from Egypt, as well as animal and human isolates from Australia were grouped in the same cluster. This finding was consistent with previous study, showing no host-based grouping of isolates (Li *et al.* 2018) and contrast to the report showing the clustering of Egyptian *S. scabiei* based on host species (Amer *et al.* 2014). A recent study based on Cytochrome C

oxidase 1 and voltage-sensitive sodium channel genes of *S. scabiei* could distinguish human and animal isolates but it could not distinguish host or geographical specific isolates belonging to animal origin (De *et al.* 2020).

The ribosomal ITS-2 gene is highly conserved and is used to differentiate host species and study the relationships between closely related taxa (Makouloutou *et al.* 2015; Oliverio *et al.* 2009; Wei *et al.* 2006). The ITS sequence of the Goan isolate PP974325 showed an identity varying from 98.2% (KX695125) to 100% (AB778919, AB778913 ON391165, OR676824) with various isolates (Supplementary Fig. 3). Based on ITS sequences the isolates were grouped into three clades on the ML tree (Supplementary Fig. 1b). The clade 1 is formed by wild animal isolates from Japan, Italy and Australia and human and domestic animal isolates from New Zealand, Malaysia, India, Egypt and US. The present isolate PP974325 was grouped in clade 2 along with wild animal isolates from Europe, China, Japan, US, New Zealand and Egyptian livestock isolates with an identity varying from 98.5 to 100%. The clade 3 included human isolate from Pakistan and animal isolates from China and Italy. Similar to the previous reports (Alasaad *et al.* 2009, Amer *et al.* 2014), the ML tree based on the ITS gene showed no clustering by location or host species. For example, the mites from the same host species from Italy and Egypt were not clustered in the same clades.

Treatment: The animals in the herd showed improvement. Skin lesions healed gradually with the complete disappearance of mange lesions like crusts, skin wrinkling, and scabs and the appearance of healthy skin with hairs, which was noticed by the twenty first day post-treatment. The present study showed that the treatment with a single dose of Ivermectin injection, with supportive therapy using vitamin supplements and topical application of Deltamethrin, has helped in complete recovery of affected animals. Secondary bacterial or fungal infection can occur during mange infestation which can lead to increased severity and morbidity in animals. But in the present outbreak the animals were recovered without antibiotic treatment.

The study confirmed the *S. scabiei* infection by detection of mites and mite DNA. The gross and histopathological changes and the detection of parasite stages and eggs in the tunnels in the *stratum corneum* of the affected revealed the extent of the pathogenic effect exerted by the pathogen in affected goats. The intensive form of farming and warm humid climate of the region might have led to the fast spread of infection affecting 80% of the animals in the herd. With the timely treatment, the affected animals in the herd showed gradual improvement. From phylogenetic trees based on *16S rRNA* and ITS genes, it was evident that the Goan isolate was phylogenetically close to wild animal and livestock isolates from Egypt, Australia China and Europe. This study provides pertinent information on the *S. scabiei* prevalence in goat population in Goa located on the west coast of the country and shows the potential risks of spread

of this zoonotic pathogen to people residing near the farm premises. The study also shows the need for preventive measures against *S. scabiei* infestation in animals in the hot humid coastal region.

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