Morphological characterization of demodex mites and its therapeutic management with neem leaves in canine demodicosis

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Abstract
The present investigation was focused on morphological characterization of demodex mites and its therapeutic management with neem leaves in canine demodicosis conducted in Department of Veterinary Parasitology, Veterinary College, Hassan from April 2016 to March 2017. The skin scrapings were collected from clinical cases suspected for canine demodicosis presented to the Teaching Veterinary Clinical complex, Hassan Veterinary College among which 25 clinical cases were selected and examined. Two species of demodex mites were observed viz., D. canis and D. cornei. The microscopic examination of skin scrapings revealed the presence of mixed infection with D. canis and D. cornei in 4 and only D. canis in 5 dogs. The morphometry of mites revealed that mean total body length (130.1 ± 14 µm) of D. cornei was much less than that of D. canis (210.6 ± 12.6 µm).

Keywords: D. canis, D. cornei, Skin scrapings, Morphometry

1. Introduction
Demodicosis is also called as demodectic mange or red mange or follicular mange caused by Demodex canis [1], D. canis is a normal inhabitant of dog skin. The mite spends its entire life in the hair follicle of dog [1]. Puppies get demodex mites from their mother by direct contact during nursing [1]. Canine demodicosis occurs when there is an altered immune response which allows proliferation of mites, leading to development of clinical signs [2]. D. canis was the first to be identified and named; the two additional Demodex mites may be mutations of D. canis, or separate species [3]. The occurrence of three species of Demodex has been reported from different parts of the world including India [4-8]. Stubby form of the Demodex was described as D. cornei being about one half of the length of the female of D. canis [2-7] described D. injai, a long bodied demodex mite.
Two types of demodicosis are generally seen in dogs. Localised demodicosis starts with one or two hairless spots especially on muzzle, face, leg and around the eyes which needs no treatment. The generalized demodicosis characterized by involving five or more local lesions with patchy areas, erythematous lesions, scales and papules [9]. Affected skin becomes crusted, ulcerated from secondary superficial and deep pyoderma [9]. Diagnosis is made either by demonstrating adult mites or by finding increased ratio of immature stages to adults. There is paucity of information on occurrence of different species of Demodex mites in Karnataka. The present study reports the morphological characterization of Demodex mites and their successful treatment with Neem leaves from Hassan, Karnataka.

2. Materials and Methods
A study on speciation of demodex mites was conducted in Department of Veterinary Parasitology, Veterinary College, Hassan from April 2016 to March 2017. In the present study skin scrapings collected from 25 dogs presented to the Teaching Veterinary Clinical complex, Hassan Veterinary College were selected. The dogs had a history of severe itching, hair loss and erythematous lesion on different parts of the body. Skin scraping samples were presented to Dept. of veterinary Parasitology for confirmative diagnosis. The samples were examined using 10% KOH; individual mites were picked up with fine forceps and mounted in Hoyer’s media for identification of mites as per [24]. A total of 25 mites from each sample were subjected to micrometry.
The length and width of Gnathosoma, Podosoma, Opisthosoma, total length of body and Ratio of length to width were considered for speciation of mites. Differentiation of both the mites (D. cornei and D. canis) was mainly based on their size, inhabitant or location of the mite and morphological difference.

The infected animals were divided into 2 groups. Group I were treated with Amitraz 0.05% twice a week for 4 weeks. Group II were treated with Topical application of Neem leaf paste and orally neem leaves were also given to the 2nd group @ 5 leaves (medium sized) per day for one month. The neem paste was mixed with a pinch of turmeric powder and applied in the direction opposite to the growth of hair follicle.

2.1 Statistical analysis
The measurements of both types of mites viz., D. canis and D. cornei were analyzed using Student’s t-test. Length of total body and opisthosoma as well as podosoma and gnathosoma of both the mites was done. Lengths of total body and opisthosoma of both the mites differed statistically significant (P < 0.01) while podosoma and gnathosoma did not differ significantly (P > 0.05).

3. Results and Discussion
The occurrence of three species of Demodex has been reported from different parts of the world including India [4-10]. Examination of skin scraping from 25 dogs in the present revealed the presence of Demodex mites in nine dogs. All the animals were heavily infected. Microscopic examination revealed the presence of mixed infection with D. canis and D. cornei in 4 dog samples and only D. canis was found in 5 dog samples. In the present study, the mean total body length (130.1 ± 14 µm) of D. cornei was much less than that of D. canis (210.6 ± 12.6 µm). The length of D. canis is agreeable with Chesney (1999) [4], Keith (2003) [10] and Sreedevi et al. (2014) [8]. The mean body length of D. cornei was agreeable with Tamura et al. (2001), [5] Lopez et al. 2011[7] and Chesney (1999) [4].

Mean length of total body, opisthosoma of the mites were different statistically significant (p<0.01) but gnathostoma and podosoma did not differ significantly (p > 0.05) (Table 1). The egg of demodex mites (Fig. 1) was photographed. Canine demodicosis was always attributed to D. canis whereas other two species of demodex mites were also reported. The finding of D. canis mites in (n= 5) out of 25 scrapings is suggestive of D. canis which is the major species responsible for canine demodicosis. The findings were in contrast with Sreedevi et al. (2014) [8] who have reported D. cornei was the major species on clinical examination of dogs from Andra Pradesh. The finding of D. canis as the major species is usually attributed to deep scrapings collected for diagnosis. D. cornei localizes in statum corneum of epidermis [4] whereas D. canis inhabits hair follicle and sebaceous glands. The superficial skin scraping is ideal for the diagnosis of short tailed mite D. cornei, whereas deep skin scrapings and hair plucks are necessary for the diagnosis of D. canis infections.

Table 1: Micrometry of D. canis and D. cornei

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Demodex canis</th>
<th>Demodex cornei</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Length of the Gnathosoma</td>
<td>26.05(18-31) ±0.18a</td>
<td>19.5(15-28) ±0.12</td>
<td>0.141</td>
</tr>
<tr>
<td>Width of the Gnathosoma</td>
<td>22.9(16-36) ±0.10a</td>
<td>21.15(16-28)</td>
<td></td>
</tr>
<tr>
<td>Length of Podosoma</td>
<td>63.6(5-81) ±0.21a</td>
<td>53(39-66) ±0.32</td>
<td>0.053</td>
</tr>
<tr>
<td>Width of podosoma</td>
<td>38(28-46) ±0.11a</td>
<td>36.75(28-40)</td>
<td></td>
</tr>
<tr>
<td>Length of Opisthosoma</td>
<td>124.8(74-167) ±3.11a</td>
<td>55(36-99) ±2.2</td>
<td>0.001</td>
</tr>
<tr>
<td>Width of Opisthosoma</td>
<td>33.95(25-42) ±0.12</td>
<td>34.25(23-40)</td>
<td></td>
</tr>
<tr>
<td>Total length of the body</td>
<td>210.6(144-265)</td>
<td>130.1(93-165) ±14.0</td>
<td>0.001</td>
</tr>
<tr>
<td>Ratio of length to width</td>
<td>5.2-6</td>
<td>3.4-3.8</td>
<td></td>
</tr>
<tr>
<td>Microhabitat</td>
<td>Hair follicle</td>
<td>Epidermis</td>
<td></td>
</tr>
</tbody>
</table>

* --significant (p<0.05) *--non significant (p>0.05) **--highly significant (p<0.01)

Fig 1: Microscopic image of egg of demodex in skin scrapings (100 X)

D. cornei is being identified for the first time from Karnataka. Short body with rounded end of opisthosoma was taken as major morphological character for identification of D. cornei (Fig. 2). In addition to that it also had a belt shaped plate which divided the prosoma and opisthosoma clearly into two halves. The length of prosoma and opisthosoma of D. canis was more or less equal (Fig. 3)

Fig 2: Microscopic image of D. canis in skin scrapings (100x)

Fig 3: Microscopic image of D. cornei in skin scrapings(100 x)
There was a variation in measurements of the length and width of Gnathosoma, Podosoma, Opisthospoma, total length of body and ratio of length to the width of D. cornei (Fig. 2) when compared to Chesney (1999) [41] and Tamura et al. (2001) [42]. These variations in measurements may be attributed to type of collection and preservation of skin scrapings as well as individual variations while taking micrometry readings.

When the animals infected with D. cornei were observed, they were found to have scaly and pruritic skin disease and the disease pattern was in the form of alopecia when D. canis was involved. The lesions in case of D. canis was found to be aggravated in only one animal which was due to secondary bacterial infection in long standing condition. This observation was in agreement with Keith (2003) [9].

The measurements of both types of mites viz., D. canis and D. cornei were analyzed using Student’s t-test. Lengths of total body and opisthosoma of both the mites differed statistically significant (P<0.01) while podosoma and gnathosoma did not differ significantly (P>0.05). The results were in agreement with Sivajothi et al. (2013) [16].

The infected animals divided into 2 groups. Group I treated with Amitraz 0.05%, showed better results and general skin condition of the animals started improving apparently, the results were in agreement with Folz et al. (1984) [9] and Keith (2003) [9]. Kachhawa et al. (2016) [10], Satheesh et al. (2016) [11] and Karen (2011) [9]. Group II were treated with neem with slight modifications in the procedure followed by Preeti makwana. The animals showed better recovery which revealed similarities with the study of Singh et al. (2011) [19], Upadhyay (1992) [20], Fernandes et al. (2015) [21], Marwat et al. (2008) [22], Preeti et al. (2015) [23].

The condition of the animals in both Groups I and II gradually improved and recovered completely after one month of treatment.

4. Conclusion
Two species of demodex mites were observed in the study viz., D. canis and D. cornei. The morphometry of mites revealed that mean total body length of D. cornei was much less than that of D. canis. D. cornei had short opisthosoma and blunted posterior end when compared with D. canis. Demodicosis can be controlled if the immune system is under check. Neem paste acts both as a miticidal and also controls the secondary bacterial infection in combination with turmeric. It is eco friendly and economical. Giving neem leaves orally improves the immunity of the animals.

5. Acknowledgements
Authors are thankful to Dean, Veterinary College, Hassan, KVAFSU, Bidar for providing facilities.

6. References
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