Incidence of *Chirodiscoides caviae* in Laboratory Rats-Screening, Identification and Treatment

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Summary
This is a report on the incidence and treatment of the guinea pig fur mite *Chirodiscoides caviae*, which was so far considered as host specific, in a conventional colony of laboratory rats. *Chirodiscoides caviae* infestation in laboratory rats was accidentally observed during the screening of *Syphacia obvelata* by the peri-anal cellophane tape test (CTT). The organism was identified by comparing the morphology described by various researchers and was differentially diagnosed from other common mites of rat, *Radfordia ensifera and Notoedres muris*. The adult male mites (n=15) were of 330.2±13.3 µm long and the females (n=15) 495.5±25.2 µm. Later on, the entire rat colony consisting of Wistar, Sprague Dawley and Spontaneously Hypertensive Rats (SHR) and the mice colony of Balb/c and Swiss Albino were randomly sampled and screened for the presence of the mite by the cellophane tape technique. All the rat strains were found positive for *C. caviae* infestation, which was more concentrated towards the posterior region of the body and, collectively, the screening results of *C. caviae* revealed that the posterio-dorsal and peri-anal regions are most suitable for sampling-suggesting that, the infestation pattern of *C. caviae* in rats has similarities to that of guinea pigs. Interestingly the mice colony was found free from the infestation.
The Cellophane tape test was found to be an easier method than fur examination by hair plucking and equally accurate for screening of fur mite in a colony of laboratory rats. No clinical symptoms were observed in any of the animals in the colony, which possessed infestation. The facility strictly practised physical separation of animals by species, which pointed to the only possibility of cross infestation being through indirect contact between guinea pigs and laboratory rats and thereby questioning previous reports on the mode of transmission of *C. caviae*. The entire colony was effectively treated with 0.2% Ivermectin spray followed by 1% spray in an interval of 2 weeks. This report is the first one, which demonstrates the guinea pig fur mite in laboratory rats. It also questions the so far documented "host specificity" and "direct contact" mode of transmission and demonstrates indirect contact as a possible mode of transmission.

Introduction
Ectoparasitic infestation in experimental animals is considered to be a substantial problem in laboratory animal management. The presence of certain mites in research animals will interfere with the research as they influence the results (*Nicklas et al., 1999*). So the identification of ectoparasites up to species level in laboratory animals is recommended by FELASA (*Kraft et al., 1994; Nicklas et al., 2002*).
Chirodiscoides caviae Hirst (*C. caviae*) is the common fur mite of guinea pig. They are pelage (i.e. fur) -inhabiting parasites that feed on the scales from hair shaft and usually produce, no clinical manifestations in the host. The life cycle of this mite has not been studied extensively according

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to Wagner *et al.* (*1972*), Flynn (*1973*) and Owen (*1992*). They are considered to be host specific (*Hirst, 1917 ; Tenquist and Charleston, 2001*) and transmitted though direct contact (*Besch-Williford and Franklin, 2007*).

The guinea pig colony of our facility was screened and found 72 % positive for *C. caviae* and was under treatment during the period of this report. A quarterly screening programme for endo and ecto parasites of the rat colony was underway simultaneously when the *C. caviae* were noticed.

This is an accidental incidence report on the identification and treatment of *C. caviae* from the fur samples of conventionally reared laboratory rats. This highlights the necessity of screening laboratory rats for the presence of *C. caviae*. The present incidence report raises a question on the host specificity and mode of transmission of the mite as previously documented.

Materials and Methods

Animal colony

The Wistar and Sprague Dawley colony of the Sree Chitra Tirunal Institute for Medical Sciences and Technology (SCTIMST), Bio Medical Technology Wing (BMT Wing), Thiruvananthapuram, Kerala, India is a random bred, open colony which was established thirty years back with breeding nucleus procured from the National Institute for Nutrition (NIN), National Centre for Laboratory Animal Science (NCLAS), Hyderabad, India. The Spontaneously Hypertensive Rat (SHR) colony is an inbred closed colony founded from Animal Resource Center, Murdoch, Australia three years back. The facility also houses an open colony of random bred Hartley guinea pigs, New Zealand white rabbits, BALB/c and Swiss Albino mice which was established twenty four years back from NIN, NCLAS, Hyderabad, India.

Rearing Conditions

The physical plant consists of a single storey building having a roof height of 3.6 meters and a width of 1.8 meters with a two corridor system. The facility is situated away from other facilities of the campus. The entire animal facility is wild rodent and pest proofed. All the animals were bred and reared under conventional conditions. Rats were housed in open type polypropylene cages with a wire grill top. They were housed in groups by following the floor space recommendations proposed in "Guide for the Care and Use of Laboratory Animals" (*NRC, 1996*) which is in unison with the recommendations of Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA) Guidelines, Ministry of Environment and Forest, Government of India. Autoclaved paddy husk was used as bedding material for the entire colony of rats. The animals were fed with *ad libitum* standard rat and mice pelleted feed (Amrut Laboratory Animal Feeds, India) and UV-sterilized drinking water. A 12/12 h lighting schedule was provided with an intensity not exceeding 325 Lux at 1 meter height. The temperature of 22±2°C and relative humidity of 40-70% with 12 air exchanges per hour were provided in all the animal rooms. The animals of the colony were frequently transferred between the above mentioned environmentally controlled rooms and rooms under natural climatic conditions by investigators for their specific experiments. Cage changes were done every alternate day. The animal handlers changed gloves between animal rooms. Cages and related equipments were sanitized in a stainless steel station using an alkaline detergent with a fresh water rinse every alternate day and a hot water wash at 180.5°F (82.5°C) once a week at a common cage washing room.

Ectoparasites screening

The mites were accidentally detected during the screening of Syphacia eggs using the peri-anal cellophane tape test (CTT) in rats as described by Dix *et al.* (*2004*) and Iijima *et al.* (*2000*) under a low power (x 10) of microscope. Subsequently the incidence of mites in the entire rats and mice colony was investigated.

Sample collection

The age group and sample size taken were, as per the recommendations of FELASA (*Kraft et al., 1994; Nicklas et al., 2002*). Ten representative individuals, each from separate cages of each strain were sampled randomly. The samples were screened for mites using CTT and confirmed with fur examination by hair plucking.

Cellophane Tape Test (CTT)

A 25 x 150 mm cellophane tape was pressed against the area of pelt at posterio-dorsal, peri-anal and dorsal neck region and then affixed to a microscope slide. The entire slide was examined thoroughly and systematically under x 10 magnification. The observed mites were re-examined under x 40 magnification for identifying up to species level.

Fur examination by hair plucking

Hair samples from the above said regions were collected by plucking using a clean forceps. These hair samples were placed on a microscope slide, one to two drops of 10% potassium hydroxide added and a cover slip placed over it. This was then examined under x 10 magnification and confirmed under x 40 magnification.

Identification of C. caviae

The mites were identified by comparing the morphology published by Owen (*1972*), Flynn (*1973*), Wagner and Manning (*1976*), Georgi (*1985*), Harkness *et al.* (*1984*) and Besch-Williford and Franklin (*2007*). The animal with the presence of any of the three developmental stages *viz.* adult, nymph or egg in any of the three sites of diagnosis was assigned as positive. Photographs of adults, nymphal stages and eggs were obtained using Axiostar plus microscope (Zeiss, Gottingen, Germany) with attached camera (Canon Powershot, A 640, China).

Treatment

Subsequent to the Identification stage, the entire animal colony was sprayed with diluted Ivermectin (Mectin, Alembic, Vadodara, India) at the rate of 0.2 mg/ml (two to three bursts of 1.5-2.0 ml/animal) on rump, sides and back. Re-treatment was performed with 5-6 undiluted Ivermectin drops (10 mg/ml) for each animal after 2 weeks. The entire rat colony was screened for mite after seven months after re-treatment using CTT.

Results

The guinea pig fur mite, *C. caviae*, was identified from all the strains of laboratory rats of the facility (Fig1). All the developmental stages viz, adult, nymph and egg were found in all rat strains. Interestingly, the entire mice colony was totally free from *C. caviae* infestation. The adult male mites (n=15) measured of 330.2±13.3 µm and the females (n=15) 495.5±25.2 µm. The organisms were identified as per the morphological features, as noted above, and differentially diagnosed from the common rat mites, *Radfordia ensifera* and *Notoedres muris*. The CTT was found to be an easier and equally accurate method when compared to fur examination by hair plucking for screening of fur mite in a colony of laboratory rats. Collectively the screening results of C. caviae revealed that the posterio-dorsal and peri-anal regions are suitable for sampling (Table 1). No clinical symptoms were observed in any of the animals in the colony.

The treatment adopted was found to be effective as none of the animals in the colony showed infestation when screened seven months after re-treatment.

Discussion

This is a novel report of *C. caviae* from a laboratory rat colony. The mite was diagnosed by reference to the literature and differentiating its character from common mites reported in rats. A similar protocol was followed by Peper (*1994*) for identifying *Dermatophagoides farinae* Hughes, one of the common house dust mites, from a fuzzy rat breeding colony. The mouth parts of *C. caviae* were compressed and striations were observed at the sternal region which was modified as a hair clasping organ. The first two pairs of legs are modified for clasping hair but the third and fourth pair are more elongate and less

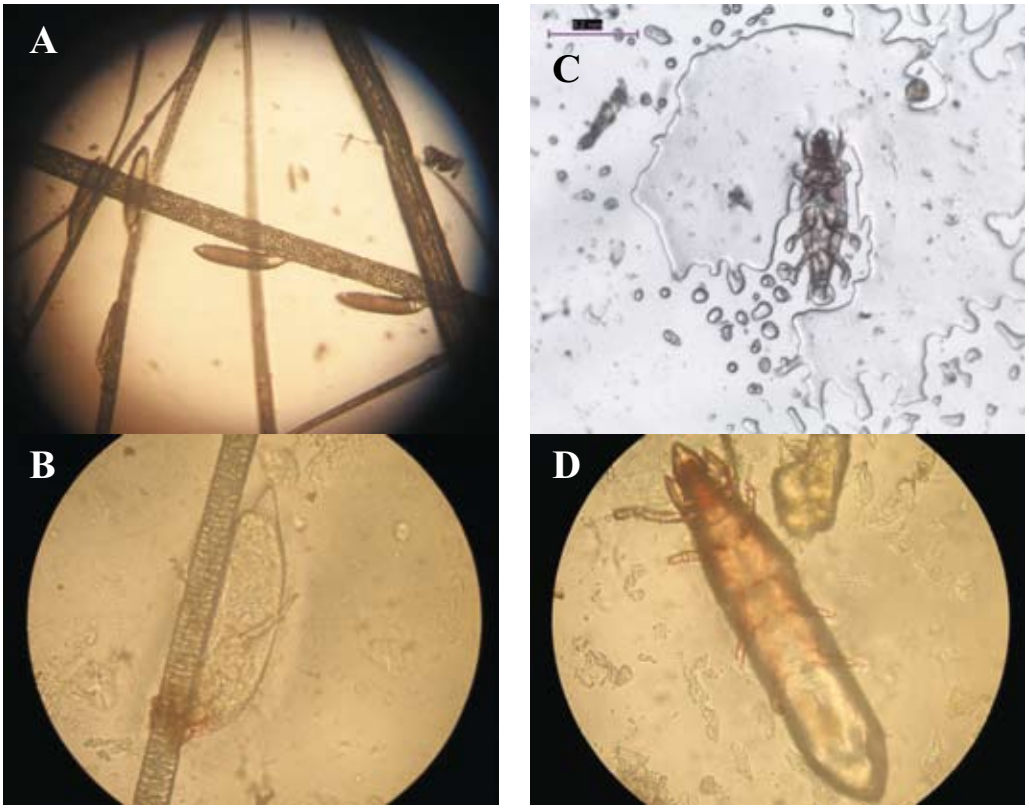

Figure 1. Different developmental stages of C. caviae found in Laboratory rats (A) eggs attached to the hair shaft (x 40); (B) Male deutonymph showing scales on the dorsal aspect of the body and a tail like process (x 40); (C) Adult male (x 10); (D) Adult female (x 40). A, B and D are size independent.

modified. Both the sexes had an elongated and heavily chitinized body. Nymphal stages were characterized by a series of scales running along the dorsal surface and the male deutonymph by the presence of a tail-like projection. The eggs were long and slender and attached by one end to the hair. These findings were in accordance with Owen (*1972*) and Flynn (*1973*).

Radfordia ensifera is the common pelage (i.e. fur) -inhabiting mite of rats, present in wild and laboratory rats throughout the world (*Bakar et al., 1956*). They are under sub order Prostigmata having claws on the tarsi of 2nd pair of legs with paired and equal 2nd pair of legs (*Flynn, 1973; Hsu, 1979*).

The second mite to be considered for differential diagnosis was *Notoedres muris*, the rat ear mange mite which belongs to the suborder Astigmata (*Flynn, 1973*). They are characterized by a roundish body with short legs and there are suckers in the first and second pairs of legs. Heavy infestation of this mite in rats may be fatal (*Georgi, 1985*).

Luyon and Salibay (*2007*) reported the presence of *C. caviae* in 13.2% of wild rats they have studied in Philippines. We were not able to find any report of this organism being in laboratory rats. Indeed, the pelage inhabiting guinea pig fur mite was concluded to be host-specific by Hirst (*1917*) and Tenquist and Charleston (*2001*). In the present report,

Table 1. Results of Chirodiscoides caviae screening in different strains of Laboratory Rats.

Group	No of animals screened	Wistar*			Sprague Dawley*			SHR*		
		PD	PA	DN	PD	PA	DN	PD	PA	DN
Weanlings	10	2	1	0	3	2	0	0	0	0
10-14 weeks (Young adults)	10	7	7	1	8	8	0	7	7	2
>6 months (Retired breeders)	10	10	8	1	8	7	1	6	6	1

* Number of positive cases diagnosed using CTT.
PD= Posterio dorsal region PA= Peri anal region DN- Dorsal neck region

however, laboratory rats were also found to be potential hosts for *C. caviae*, in contrast to the above findings. An incidence rate of 52.8% to 100% *C. caviae* infestation was reported in guinea pigs in India by Deoras and Patel (*1960*). The guinea pigs in the present study had 72% incidence rate of *C. caviae*. Although Besch-Williford and Franklin (*2007*) reported that direct contact is the only mode of transmission, we found a cross infection between guinea pigs and rats probably through animal house equipments and personnel, since species separation is strictly practised in our facility. Even though the animal handlers changed their gloves between each of the holding rooms, the shoes, aprons and trolleys used were the same in all rooms. In addition to this, a common facility was used for cleaning the cages of all species. Apart from direct contact, which was documented till now as the only mode of transmission, indirect contact is postulated as the possible route of infestation reported here.

In the present case study even if there was infestation in all the strains, none of them showed any clinical manifestations. This finding was in strong agreement with the findings of Harkness *et al.* (*1984*) and North (*2001*) about *C. caviae* in guinea pigs. All the developmental stages of the mite were found attached to the hair shaft of rats, which is similar to the infestation pattern observed in guinea pigs by Wagner *et al.* (*1972*) and Harkness *et al.* (*1984*). The infestation was heavy at the posterior parts of the body, similar to that in guinea pigs (*Deoras and Patel, 1960; Hirst, 1922 and Flynn, 1973*). The area to be sampled for diagnosing the organism in both rats and guinea pigs are posterior aspects of the body, preferably the perianal region and posterodorsal region.

The mice colony which was reared under similar environmental conditions to that of rats was totally free from the infestation of *C. caviae*. The absence of the organism in mice clearly suggests the establishment of laboratory rats as a host of *C. caviae*.

The CTT gave the same results as that of fur examination by hair plucking in the diagnosis. Iijima *et al.* (*2000*) used and recommended CTT for the diagnosis of fur mites in mice. But Besch-Williford and Franklin (*2007*) reported that it is not very reliable for detection and mites collected by this method probably cannot be speciated. Anyway in this report, CTT was found to be an easier tool, when compared with the hair plucking method,

for screening a large colony of laboratory rodents quickly. It is a faster and accurate tool in fur mite identification to species level.

Treatment adopted in this study was a modification of the treatment reported by Hirsjarvi and Phyala (*1995*) for guinea pigs. Depending on the weight of the rats the total dose was reduced in the present study. Ivermectin spray was avoided in pregnant and weanling rats in our study as it was found to be toxic by Skoepts *et al.* (*1996*). Ivermectin treatment did not affect the general health and body weight and was not found stressful for the animals, as reported by Hirsjarvi and Phyala (*1995*) and Davis *et al.* (*1999*). The treatment was easy to perform and effective. Both the findings were in strong agreement with Le Blanc *et al.* (*1993*).

This study throws light into the possibility of intercepting *C. caviae* infestation in laboratory rats. In conventional facilities with semi-rigid barrier capabilities, precautions must be followed to avoid inter-species cross contamination of the fur mite. This can be done by assigning separate personnel to attend different species permanently. If such a staff positioning is not viable, glove and shoe cover/footwear changes between rooms and using separate sets of cleaning equipment and trolleys in rooms of different species should be instigated. Along with these management precautions, quarantine, quarterly screening and treatment can effectively prevent the infestation of *C. caviae* in laboratory rats. Since this study was done after incidence, data on the effectiveness of the chosen treatment regime is also presented.

We thus arrived at a conclusion that, laboratory rat colonies could be screened for *C. caviae* infestation, particularly in semi – rigid barrier facilities and conventional facilities that also houses guinea pigs. Incoming rats of unknown parasitic status should be screened as well. *C. caviae* can no longer be considered as a host-specific organism restricted to guinea pigs and can get transmitted by indirect contact also. All developmental stages in the life cycle of *C. caviae* were identified from laboratory rats which suggests that it is not short lived in laboratory rats. Mice under similar conditions of management were free of infestation and this suggests us to perform an experimental study regarding host preferences of *C. caviae* in this species. CTT is recommended to be an easier and accurate tool for fur mite diagnosis in laboratory rats and guinea pigs. Treatment for *C. caviae* infestation in rats in our report is also found to be extremely successful and may be adopted in similar cases.

Acknowledgements

We thank Dr. K. Mohandas, Director, and Dr.G.S.Bhuvaneshwar, Head, Bio Medical Technology Wing, Sree Chitra Tirunal Institute for Medical Sciences and Technology, Thiruvananthapuram, Kerala, India for supporting this work, Dr. Sabareeswaran.A, Mr. Sreekanth.S.L and Ms. Mary Ayola Lopez for photographic assistance and to our animal handlers for their assistance in screening and treatment.

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