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## Occurrence of pentastomida (*Porocephalus crotali*) and nematode (*Kalicephalus sinensis*) in an Indian rat snake in Puducherry

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### Abstract

The postmortem examination of an Indian rat snake (*Ptyas mucosa*) revealed the presence of nymphal stage of a pentastomida (*Porocephalus crotali*) in subcutaneous tissue near abdomen and an adult pentastomida in a lung. The parasite was identified based on morphological features as pentastomida worm. The worm's body was cylindrical, annulated and mouth was centrally located, surrounded by 4 hooks and its egg had two shell membranes. Another nematode worm (*Kalicephalus sinensis*) was found in the stomach of the same snake. The spicules were thin, bent dorsally and were of equal size. The oral opening was dorsoventrally narrowed with 3 pairs of circumoral papillae and had a well-developed buccal capsule. All these parasites were probably recorded for the first time in Puducherry in rat snakes.

**Keywords:** *Porocephalus crotali*, *Kalicephalus sinensis*, Indian rat snake

### Introduction

The oriental rat snake (*Ptyas mucosus*) also known as Indian rat snake is more prevalent in Southeast Asia. The Pentastomida (*Porocephalus crotali*) is generally encountered in lung, trachea and nasal passage of snake and its nymphal stage occurs in viscera of different rodents. The eggs from lungs are swallowed by snake and passed in faeces. Worms were cylindrical and the anterior end had four hooks arranged in the form of an arc or trapeze with a keyhole shaped mouth in the middle. The tail end was tapering and bifurcate. Females with length of averaging 8 cm and width 0.4 cm. The internal organs were fully occupying the whole of the abdomen (Bino sundar et al., 2015) [1]. This snake also inhabits different nematodes in GI tract including *Kalicephalus sinensis*. Strongylid nematodes of the genus belonging to the family Diaphanocephalidae occur in the stomach and intestine of reptiles and lizards. (Junker et al., 2009) [4] have reported and identified *Kalicephalus colubri colubri* in a captive mole snake (*Pseudaspis cana*) in South Africa. (Junker et al., 2009) [4] observed the worms in oesophagus and stomach and most of the worms were found to be attached to the mucosa. This case reports the occurrence of a pentastomida (*P. crotali*) and a nematode (*K. sinensis*) is reported from a rat snake (*Ptyas mucosus*) of Puducherry, India.

### Case report

A dead snake was referred to the Department of Veterinary Pathology, Rajiv Gandhi Institute of Veterinary Education and Research, Puducherry for Necropsy. On examination of the respiratory tract, long elongated worms with a transparent cuticle were present and upon dissection of stomach and intestine, a large number of round worms were observed. These worms were collected in normal saline and transported to the Department of Veterinary Parasitology for identification.

### Processing of parasites

The collected nematode worms were subjected to dehydration in ascending grades of alcohol (70, 90% and absolute alcohol each for 3 min), cleared in lactophenol and mounted in DPX. The worms were then examined under 4x and 10x magnification in a light microscope. Based on morphological characters, the recovered helminths were identified (Schad 1962; Santoro et al. 2013) [7, 8].

### Gross description of worm

A mature pentastomida revealing annulated, transparent cuticle and internal organs were

completely occupied at the abdomen. The abdominal part of the worm is fully filled with tubular structure and they were examined under the microscope, which revealed the presence of eggs in genital tract. The worm was identified as female (Fig 1) as the posterior part was filled with the eggs. The nymph of pentastomida had whitish fluid filled soft tegument and have hooked legs and a penetrating mouth hooks (Fig 2). The nymphs (Fig 2) are quadripeds and contain 4, doubly hooked legs. At their anterior ends, they had a penetration apparatus and at their posterior ends, a bifurcate tail (Brookins, M. *et al* 2009)<sup>[2]</sup> was present.

On gross examination, the worms were cylindrical and had an elongate body. Most of the worms were found to be embedded in the mucosa of the stomach and intestines, whereas some were found to be free inside the lumen of stomach and intestine. The body of nematode worms were short, cylindrical and elongate body. Head is small and compressed laterally, not tilted.



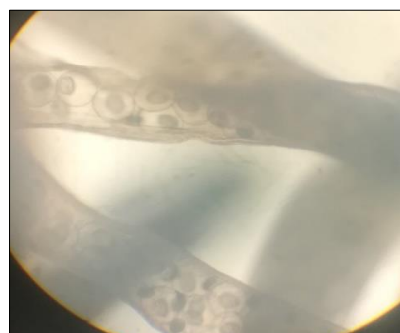
**Fig 1:** Mature Pentastomida revealing annulated



**Fig 2:** Nymph of Pentastomida (*P. crotali*) transparent cuticle

#### Microscopic description of pentastomida worm (*Porocephalus crotali*)

Under microscopy, the tail end was tapering and bifurcate. The abdomen was fully occupied with the internal organs. The intestinal content of snake contains the pentastomida eggs (Fig 3), composed of two shelled membranes of which the outer one is thin and the inner membrane is thick and a fluid-filled space between the two membrane (Fig 4).



**Fig 3:** Eggs of Pentastomida inside the uterus under 4x magnification



**Fig 4:** Eggs of Pentastomida in the intestinal content of snake under 10x magnification

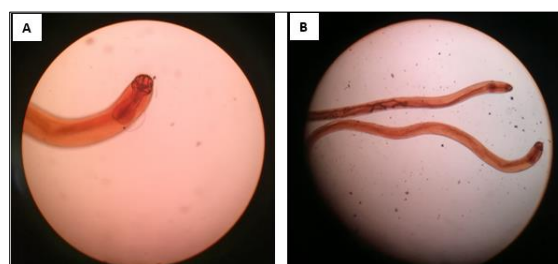
#### Microscopic description of nematode (*Kalicephalus sinensis*)

Under microscopy, the nematode had a short, well-developed esophageal bulb and 2 bent spicules. The cuticle on the posterior part of the head is slightly arched, with angular corners and the oral opening was dorsoventrally narrowed with 3 pairs of circumoral papillae and each papillae led by 3 parenchymatous bands, rounded face, well developed buccal capsule and the anterior chitinoid ridge was curved and narrow (Fig 5A). Esophagus was stout, with a well-developed esophageal bulb, nerve ring anteriorly and ventrally excretory pore was located (Fig 5B).

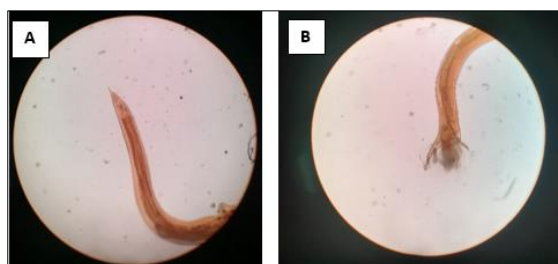
Body of male was much smaller than that of females. Bursa symmetrical. Ventral ray short and situated more to the anterior than other rays. Dorsal ray short and a pairs of stout branches situated at the base of the dorsal ray. The main branch divided into 2 branches, and each branch divided into subdivided inner branches and comparatively long outer branches. Genital cone with 2 rounded papillae on dorsal lip of cloaca. Two spicules thin, bent 1 or 2 times at each 1/3 level, equal in size, with short, spatulate tips. Gubernaculum present (Fig 6B).

Female: Tail short, tapered, and conical-shaped, with a pointed, small mucron. Vulva slightly projected but not on all of the specimens (Fig 6A). Amphidelphic disposition of uterus. (choe *et al.*, 2016)<sup>[3]</sup>.

Microscopic description of the eggs: The egg of *Kalicephalus sinensis* resemblance of a strongyle egg.



**Fig 5:** Anterior part of *K. sinensis* under 10x magnification



**Fig 6:** Posterior part of *K. sinensis* under 10x magnification. A- Female; B- Male

## Discussion

*Porocephalus crotali* is a parasites of oriental rat snakes. Adults live inside the respiratory tract of snakes and larval nymphs live in the viscera of mammals. During their long evolutionary history, adult and larval nymphs have become very adapted to this internal habitat (Layne 1967; Riley and Henderson 1999) [5, 6].

In present observation, a mature female pentastomida worm revealing annulated, transparent cuticle and internal organs were completely occupied at the abdomen. The abdominal part of the worm is fully filled with tubular structure and were examined under the microscope, revealed the presence of eggs in genital tract. The worm was identified as female as the posterior part is filled with the eggs. The tail end was tapering and bifurcate.

Life cycle, eggs ingested by mammals which hatch in the small intestine, primarily larva penetrates in the gut wall and migrates to the liver and the lungs of the mammal, finally encyst in tissues as encapsulated form, also encyst in viscera, mesenteries, and walls of the thoracic and abdominal body cavities. Larvae then moult to become nymph. Snakes ingest the mammalian intermediate host containing seventh instar infective nymphs that encysts and penetrate the gut wall, migrating through the viscera and directly to the lungs. In definitive host, the nymphs develop into adults within the lung cavities. Adults with mature eggs are expelled from the trachea and eliminated from the definitive host by oral expulsion. The adults may also be swallowed, resulting in shedding of eggs in the faeces in which another intermediate mammal host will ingest to begin the life cycle again.

Human visceral pentastomiasis also caused by *Porocephalus crotali*. The majority of cases have been reported from Africa, Malaysia, and the Middle East. It is an unusual parasitic zoonosis, in which humans may accidentally serve as intermediate hosts. Infection occurs when eggs are ingested through the respiratory secretions or feces of the parasite's definitive host. The nymphs hatch and penetrate the host gut where the adults become encapsulated in the viscera. Symptoms are highly dependent on affected organs and include abdominal pain, chronic cough, and night sweats. Heavy infections may cause death, however, most human infections are asymptomatic. Prevention of visceral pentastomiasis from *P. crotali* by the avoiding the consumption of snake meat and washing the hands (Tappe and Buttner., 2009) [9]. A case report of occurrence of pentastomida was reported earlier in Madras Veterinary College.

*K. sinensis* was first described from Chinese snakes of the genus *Elaphe*, and our specimens have been found only from oriental snake species in the genus *Elaphe* (Schad GA *et al.*, 1962) [7] however, the size of the spicules was much more indicative of *K. sinensis* (choe *et al.*, 2016) [3].

On observing the morphological characters of *Kalichephalus* worms revealed a buccal capsule with two lateral valves and an obliquely truncated anterior end. Oesophagus was well developed and was short, thick and muscularised ending in a rounded bulb. The tail end of female worms was blunt and in males, a well-developed trilobed bursa was observed with short and equal spicules. Typical strongyle type eggs were found in the uterus of female worms. All these gross and microscopic characteristics were found to be in accordance with (Ublaker and Dailey., 1966) [10]. The prevalence of pentastomida (*Porocephalus crotali*) and a nematode

(*Kalichephalus sinensis*) in an Indian rat snake may be probably first time recorded in Puducherry.

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