Predominance of *Ancylostoma duodenale* Species among Hookworm Infected Patients from a Tertiary Care Hospital in Varanasi, India

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Despite considerable advances in chemotherapy and control, hookworms rank amongst the most widespread of soil-transmitted intestinal helminths especially in the tropics and sub-tropics.  

*Necator americanus* (*N. americanus*) and *Ancylostoma duodenale* (*A. duodenale*) are two most common species causing infection in humans. Epidemiological distribution reveals that *A. duodenale* predominates in the Middle-East, North America, India and southern Europe, while *N. americanus* predominates in the Americas, Sub-Saharan Africa, Southeast Asia, China, and Indonesia. Knowledge of the predominant species in a particular population is essential for a long-term success in chemotherapy owing to the differences in life histories and drug response rate of the species.

The problem with identification of hookworm species is that though the presence of hookworm ova in stool is diagnostic of infection, eggs of one species are morphologically indistinguishable from the other. While there are established morphological differences between adult *A. duodenale* and *N. americanus*, adult worms are rarely seen except via endoscopy, surgery or autopsy. Alternatively, species identification can also be done by using subtle morphological characteristics to differentiate the infective, third-stage filariform (L₃) larvae, reared from eggs in coprocultures. This study was performed to determine the species prevalence of hookworm infections among patients attending a tertiary care hospital in Varanasi based on coproculture.

The study was conducted in the Department of Microbiology, Institute of Medical Sciences, Varanasi. A total of 16 cases of hookworm infection were studied among patients who had presented with complaints of abdominal discomfort, indigestion, fatigue and bloating and for whom clinicians had requested for a stool examination. None had received any form of anthelmintic therapy in the 6 months preceding the study. Stool samples were examined visually followed by formol-ether concentration and microscopy of saline and iodine mount. Identification of hookworm species was done by culture based on Harada-Mori technique. Briefly, two thick smears of stool specimens containing hookworm ova, were made in the middle of two separate filter papers (12x1 cm). The free end of one of the filter papers was dipped in 5 mL distilled water in a 15-mL centrifuge tube covered loosely with moist cotton wool to permit aeration. The tubes were kept vertically and incubated at room temperature until hatching was seen. The cultures were examined daily with aseptic measures till the formation of mature filariform larva. Morphologic details of the larvae were studied using 40× objective lens.

Filariform larvae of *A. duodenale* and *N. americanus* were characterized by a blunt head and tail and absence of gap between the oesophagus and intestine in case of *A. duodenale*. Morphological differences in cuticle, buccal capsule, junction between oesophagus and intestine were studied to differentiate the species.
The development of larva and morphological features has been shown in Figs. 1 and 2.

![Fig 1: Stages of development of hookworm larva](image1)

A: Hookworm egg with 6 blastomeres, B: Hookworm egg with many blastomeres, C: Encysted hookworm larva, D: Rhabditiform larva

![Fig 2: Filariform larva of hookworm species](image2)

A: Filariform larva of hookworm showing blunt mouth end and acutely tapered posterior end, B: Larve of A. duodenale showing thin cuticular wall and no gap between esophagus and intestine, C: Larve of N.americanus showing thistle funnel shaped esophageal end and gap between esophagus and intestine.

The 16 nonrepetitive cases comprised of 7 male and 9 female patients. All the patients except 4 belonged to the adult age group ranging from 25 to 45 years. *A. duodenale* filariform larvae were identified in 12 cases, while *N. americanus* filariform larvae were detected in 2 cases and filariform larvae of both the species were recovered from another 2 cases. The average time required for formation of mature filariform larvae in majority of the cases was 10 days with a minimum period of 3 days and maximum of 12 days in some cases. Majority (10/16 cases) of the patients belonged to Balia district and had agriculture as their primary occupation. Majority (14/16 cases) were in the habit of open field defecation and did not have proper sanitary facility. They admitted to the habit of walking in the fields barefooted. After detection, all the patients were treated with both albendazole (400 mg single dose) and few were also treated with iron and folic acid for anemia.

Hookworm infection is widely prevalent in India. Some reports suggest that *N. americanus* is predominant in South India and *A. duodenale* is predominant in North India. However, in more recent times, movement of infected persons has blurred the geographic differences in distribution of the two species in the country. Among the two major species, *A. duodenale* is primarily a human pathogen and *N. americanus*, the only species in the genus, infects both man and non-primates. There have been few important issues on the epidemiological distribution of hookworm species as discussed further.

Hookworms inhabit human intestine for approximately 1–3 years in case of *A. duodenale* and 3–10 years for *N. americanus*. Within this life span, intestinal blood loss as a result of their sucking habits begins before production of eggs and remains lifelong. Owing to less amount of blood loss in *Necator* spp as compared to *Ancylostoma*, it is usually believed to be more adapted to ‘human parasitism.’ The predominance of *A. duodenale* as revealed in this study is notable as the burden of iron deficiency anemia in a community is decided by the predominant species of hookworm prevalent in the area. Besides, the two species also differ in other aspects like transmission by breast milk from infected mothers and oral route of entry into the human body.
along with arrested development in adverse situations in *A. duodenale*. ⁹

Rapid reinfection rates and treatment failures have already been attributed to species difference. ⁵ While drugs like ivermectin at certain dosages are totally ineffective on *Necator* spp, susceptibility and dosage regimens against pyrantel and other widely used anthelminthics also vary based on the species. ¹⁰ Based on our finding, we proposed that though treatment might be sufficient for eradication of worms, special attention should be made to anemia in hookworm-infected patients as expected by the predominance of *A. duodenale* in this region. Therefore, correct identification is important to ensure effective control measures.

In conclusion, epidemiological assessment of public health importance of hookworm infections should encompass the species of infecting hookworm for a target-specific therapy and control. Besides treatment, the predominance of *A. duodenale* in our area urges for special attention that should be paid to the transmission dynamics of the parasite.

**Conflict of Interest:** None

**References**


