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Evidence of Occurrence of *Ancylostomaduodenale* in Cameroon

Deguy Dor Luogbou Nzu[1, 2, 3, *], Nguemaïm Ngoufo Flore[4, 5], Romuald Issiaka Kamwa Ngassam[1, 5], Laurentine Sumo[1, 5], Palmer Masumbe Netongo[2], Wilfred Fon-Tarkeh MbachamII[2], Louis-Albert TchuemTchuente[1, 5]

[1]Centre for Schistosomiasis & Parasitology, Yaounde, Cameroon

[2]The Biotechnology Centre, University of Yaounde I, Yaounde, Cameroon

[3]Department of Biochemistry, University of Bamenda, Bambili, Cameroon

[4]Department of Biomedical Science, University of Bamenda, Bambili, Cameroon

[5]Department of General Biology, University of Yaounde I, Cameroon

Email address:

luogbounzu@gmail.com (Deguy D. L. N.), ngflorema@yahoo.fr (Nguemaïm N. F.), issiaka73@yahoo.fr (Romuald I. K. N.), sumolaure@yahoo.fr (Laurentine S.), masumben@yahoo.fr (Palmer M. N.), wfmbacham@yahoo.com (W. Fon-Tarkeh M.), tchuemtchuente@schisto.com (Louis-Albert T. T.)

[*]Corresponding author

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Abstract: *Necator americanus* and *Ancylostoma duodenale* are the 2 major species of human hookworms occurring in Sub-Saharan Africa. Up to date, without any hookworm species identification study, *Necator americanus* remains the only species reported in Cameroon. In order to identify hookworm species from two Health Districts in Cameroon, stool samples from ~ 100 hookworm-positive schoolchildren were cultured to the third-stage (L3), filariform larvae, using the Harada-Moritest-tube method. In the Health District of Mouanko, *N. americanus* larvae were recovered from a total of 27 (62.79%) coprocultures while *A. duodenale* larvae were recovered from a total of 24 (55.81%) stool cultures. 44.18% of the hookworm infections were due solely to *N. americanus*, 37.2% solely to *A. duodenale* and 18.6% were mixed infections with both species. In all mixed infections, *N. americanus* appears to be the predominate species. In contrast, in the health district of Loum, *N. americanus* larvae were the only species found in all of the20 hookworm-positive coprocultures. This study reports for the first time the presence of *A. duodenale* in Cameroon. This new data is very important because it emphasizes the need to assess the actual distribution of these two species in Cameroon, for better taken into account in treatment and control strategies.

Keywords: Hookworm, Occurrence, *Necator Americanus*, *Ancylostoma duodenale*, Cameroon

1. Background

Hookworms are parasitic nematodes of the small intestine of mammals, including humans, canids and felids [1]. They have a direct life cycle involving release of eggs via the faeces, and development of a skin-penetrating infective third-stage larva (L3). Some species can also establish after oral ingestion of L3. Human hookworm disease is mainly caused by two species *Necator americanus* and *Ancylostoma duodenale*, which infect approximately 740 million people in areas of rural poverty in the tropics and subtropics [2], causing chronic iron deficiency, severe anemia and failure to thrive [1]. *Necator americanus* and *Ancylostoma duodenale* are sympatric over much of their distribution and people are often simultaneously infected with both species in endemic areas [3, 4]. Despite significant differences in their life histories and the anthelmintic susceptibilities between the species, they have traditionally been considered to be identical for treatment and control strategies [5, 6]. The implementation of a rational design of hookworm control strategies requires knowledge of the infecting species to ensure long term success of treatment. Most studies do not

attempt to speciate hookworm infections and rely on past epidemiological data, which indicate the predominance of one species over the other [7], because the eggs of the 2 species are similar and not readily distinguishable from one another by classical parasitological methods [8]. Hookworms are among the most prevalent soil-transmitted helminth affecting people in Cameroon, the national prevalence based on previous studies [9] is 18%. It is estimated that about 2.6 million people are infected with hookworms [10]. More recently a study carried out in Loum reported a few cases of *Necator americanus* infection [11]. However, in this later study, it is automatically considered that the hookworm species present is *Necator americanus* based on past epidemiological data which indicate that this later species predominate in Sub-Saharan Africa [4]. So, little attention has been given to the identification of hookworm species in a particular population. In this report, we indicate the preliminaries results on species characterization of hookworms occurring in Cameroon.

2. Methods

This study was part of a large-scale trial to test the efficacy of Albendazole (ABZ) for the treatment of STH infection [12]. It was carried out in schoolchildren of Mouanko and Loum, two health districts located in Littoral Region of Cameroon. Consent for participation in the study was sought from the parents or guardians of the children and a clearance from the ministry of health was also available.

Stools from 1485 schoolchildren (boys and girls aged between 5 and 20 years) were individually examined by McMaster method [13] and 153 (10.3%) samples were positive to hookworms. Some highly infected samples (≥ 500 epg of stools) were selected for hookworm culture. The hookworm eggs in about 0, 5 g of fresh, unfixed stool were spread on filter paper and then cultured to the infective, third-stage larvae, using the Harada-Mori test tube method [14]. The cultured larvae were concentrated by centrifugation and stained with Lugol's iodine. The first 10 larvae (if more than 10) were examined under the microscope and identified according to the criteria indicated by Hsieh [15].

3. Results and Discussion

Based on the shape of the filariform larvae's anterior body, intestine and oesophageal bulb junction, and tail-end, we distinguished *N. americanus* from *A. Duodenale* (see Figure 1).

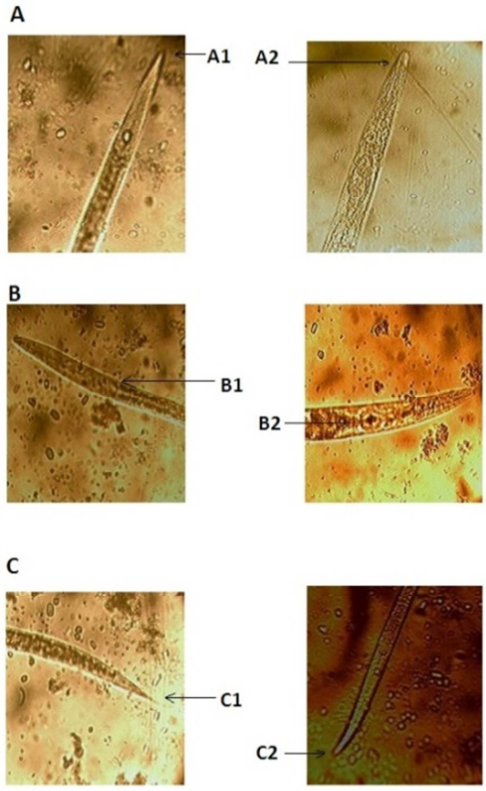

Figure 1. *Distinctive description of N. americanus and A. duodenale from Cameroon.*

Figure legend text.
A: Anterior end of body (not the sheath).
A1: Anterior end of body rounded, like the small end of a hen's egg=*N. americanus*
A2: Anterior end of body blunt=*A. duodenale*
B: Intestine and oesophageal bulb junction.
B1: Anterior portion of intestine as wide as oesophageal bulb=*N. americanus*
B2: Intestine narrower in diameter than oesophageal bulb=*A. duodenale*
C: Tail-end.
C1: Tail-end sharply pointed=*N. americanus*
C2: Tail-end blunted=*A. duodenale*

Table 1 gives the relative prevalences of the 2 hookworm species, *N. americanus* and *A. duodenale*in the study population.

Table 1. *Relative prevalence of Necator americanus and Ancylostoma duodenale in Mouanko and Loum health districts.*

Health districts	Nr. of Sample cultured	Nr. of sample with L3 larvae$^{\alpha}$	*N. americanus*		*A. duodenale*		Mixed infections$^{\varepsilon}$	
			Positif	Prevalence$^{\beta}$ (%)	Positif	Prevalence$^{\gamma}$ (%)	Positif	Prevalence$^{\delta}$ (%)
Mouanko	77	43	19	44.18	16	37.2	8	18.6
Loum	30	20	20	100	0	0	0	0
Total	107	63	39	61.9	16	25.39	8	12.69

Table legend text.
α: Number of sample in which third-stage larvae (L3) was observed.
β: Proportion of samples with only *N. americanus*
γ: Proportion of samples with only *A. duodenale*
δ: Proportion of samples with *N. americanus*and*A. duodenale*
ε: Presence of both species in the same sample.

Of the 43 hookworm-positive coprocultures from Mouanko health district, *N. americanus* filariform larvae were recovered from a total of 27, while *A. duodenale* filariform larvae were recovered from 24. Nineteen of the 43 (44.18%) hookworm-positive stool samples had only *N. americanus* filariform larvae while 16 (37.2%) had only *A. duodenale*larvae. Eight (18.6%) of the coprocultures had double species infection as evidenced by the recovery of filariform larvae of both species (*N. americanus*and *A. duodenale*). However, in all mixed species infections, and despite the low number of larvae examined, *N. americanus* is probably the predominant species in mixed infections. On contrary, in all of the 20 hookworm-positive coprocultures from Loum health district, only *N. americanus* filariform larvae were found.

The small size of the hookworm-positive coprocultures was the main limitation in this study. Since that the probability to detect a species could increase with the size of the sampling. Among the identification criteria indicated by Hsieh [15]**,** solely tree of them were easily found together on a larvae in the present study. A similar observation was made by Urjel et al [16]. This study reveals the presence of *N. americanus* and *A. duodenale* in the health district of Mouanko, whereas only *N. americanus* was found in Loum health district. The result in Loum confirms the assumption in reference [11]. This is the first time that, *A. duodenale* is reported in Cameroon. This finding could be partly explained by the following reasons; first, no studies involving the identification of hookworm species had been undertaken in the past. Secondly, populations living in Mouanko are from different countries including Nigeria and Ghana where *A. duodenale* is already known to occur [17]. Meaning that, this species had settled through the migration of population over time. Thirdly, the health district of Loum has a history of large scale anthelmintic treatment, which is not the case in Mouanko health district. It has been reported that, the efficacy of ABZ was better for *A. duodenale* infections (cure rate: 91.8%) compared to *N. americanus* infections (cure rate: 75.0%) [18]. Most studies showed that *A. duodenale* is more susceptible to anthelmintic treatment than *N. americanus* [19, 20]. Contrary to Oyerinde (1978) [21], which seems to show that, apparently, all infections with *A. duodenale* occurred always in association with *N. americanus*, with the later occurring independent of the former, our study showed that hookworm infection solely due to *A. duodenale,* accounted for 37.2% of all hookworm infections despite the small sample size. Mixed hookworm infection represents 18.6% of the hookworm-positive stool samples. Unfortunately, in this study, it was not possible to clearly indicate if *N. americanus* had the higher numbers of infective larvae; because at most 10 larvae per sample were examined under the microscope. So, the predominance of *N. americanus* on *A. duodenale* in mixed infection is suggestive but fall short from providing conclusive evidence.

4. Conclusion

Finally, although these results were obtained only through the Harada-Mori culture method and the Hsieh [15] identification scheme, it is the first study which reveals for the first time, the presence of *A. duodenale* in Cameroon. Further molecular studies are needed to confirm these results. Epidemiological assessment of the public health significance of hookworm infections should not, as has been the case over the years, be focused only on estimation of the number of hookworm infections, which occur in a given population. It should also include identification of the infecting hookworm species because therapy and control of the disease should be specific and targeted at the infecting hookworm species [17]. So, knowledge of the specific distribution of these 2 hookworm species in Cameroon is important to the evaluation of hookworm infection as a public health problem.

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