Molecular Diagnosis of Abdominal *Armillifer grandis* Pentastomiasis in the Democratic Republic of Congo

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Pentastomiasis is an emerging snake-borne parasitic zoonosis in the tropics. We describe a molecular and morphological study to diagnose a cluster of asymptomatic abdominal human infections caused by *Armillifer grandis*. The findings may indicate a silent epidemic in a rural area where severe symptomatic ocular cases with the same parasite species have recently surfaced. Molecular diagnostics are of increasing importance when patient material from remote areas cannot be thoroughly examined locally for logistic reasons.

Pentastomiasis, an emerging parasitic zoonosis in rural tropical areas, is caused by nymphs (larval stages) of pentastomes, a group of unique crustacean-related vermiform organisms commonly known as tongue worms (1–3). Adult parasites of the genus *Armillifer* inhabit the respiratory tract of snakes (definitive hosts), and infective parasite ova are excreted by snake feces and secretions. After ingestion of parasite eggs, small mammals serve as intermediate hosts, while humans may become accidental dead-end hosts. After hatching in the gut, larvae migrate to various organs, where they encyst and molt (1). Risk factors for human infections include the consumption or handling of contaminated snakes or their products, such as meat (1, 3). The majority of reported human cases have been due to *Armillifer armillatus* (1), a species endemic to West and Central Africa, especially the Congo region and Nigeria (1, 4, 5). Infections with this species in African immigrants have been documented twice in North America and <10 times in Europe (5). In contrast, *Armillifer grandis*, a less-known species sympatrically distributed in Central Africa, has only rarely been diagnosed (1). Very recently, a series of heavily symptomatic ocular infections with *A. grandis* has emerged in the Sankuru district, a remote region of the Democratic Republic of Congo (DRC) (6). According to current local surgeons' statements, cystic and often calcified lesions of unknown etiology are found in in the abdominal cavity and mesentery of at least a third of patients in this geographic region. We investigated the etiology of these lesions in a small subset of patients and describe here a cluster of abdominal *A. grandis* infections, diagnosed parasitologically, molecularly, and histopathologically.

In two patients from the Sankuru district, three abdominal cysts were found on the visceral serosa during surgery for unrelated abdominal conditions. The patients had surgery in December 2012 in Kole and in April 2014 in Katako Kombe, respectively, and the cysts were resected (Fig. 1). Two formalin-fixed paraffin-embedded cysts from the first patient were necrotic. Only remnants of a cuticular structure and round opening-like elements were visible, typical for pentastomid larvae (Fig. 2). Microscopic examination of an ethanol-preserved specimen from the second patient showed parts of a parasite covered by a translucent membrane, also characteristic for a pentastomid larva. PCR targeting the nuclear pentastomid 18S rRNA gene (small subunit rRNA gene [3, 4]) was conducted after DNA extraction from all specimens. Only the PCR from the ethanol-fixed sample was positive. The 424-bp amplicon was sequenced, and BLAST analysis (www.ncbi.nlm.nih.gov/blast) confirmed 100% identity (366 of 366 nucleotides) with *A. grandis* (GenBank accession number KM023155) and 99% homology (2 nucleotide difference each) with *A. armillatus* (HM756289), *Armillifer agkistrodontis* (FJ607339), and *Armillifer moniliformis* (HM048870).

In humans, larval pentastomiasis is predominantly asymptomatic and found incidentally by radiology, during surgery, or during autopsy. The nymphs are often located in the abdominal or thoracal cavity (1). The eye is infected only rarely (6). Diagnosis is most often established by demonstrating typical C-shaped calcifications on radiological films or by gross pathology and histopathology. Species identification of intact parasites is performed morphologically by counting the body annulations and measuring the size of the larvae (1). Depending on the degree of decay of the nymphs, histopathological analysis may lead to genus-specific diagnoses. Most often, however, only decay-refractory parts of the parasite remain (1, 5), as described here in one patient. In such cases, the diagnosis of pentastomiasis due to an unidentified genus can be made based on cuticular remnants, perioral chitinous hooklets (1, 5), or, as found here, the sclerotized cuticular openings unique to pentastomids. Recently, PCR was used successfully to diagnose a human *A. armillatus* infection from a formalin-fixed paraffin-embedded specimen (4). In that case, the parasite was completely intact and not necrotic and was thus better suited for successful molecular analysis despite formalin fixation. It is known that formalin fixation, which leads to nucleotide cross-

FIG 1 Map of the Democratic Republic of Congo showing locations of Kole and Katako Kombe, Sankuru district, part of the Kasai-Oriental province (outlined in red).

links, may hamper successful nucleic acid amplification. *A. grandis*, originally described by Hett in 1915 in African vipers (7), is the least known and seemingly rarest zoonotic pentastomid species. Until recently, only few reports on human infections with this species existed (8–10). In one of these reports, from the West African Ivory Coast, the parasite was likely misidentified as *A. grandis* (incorrect size and number of annulations and wrong geographic place) (10). However, there may be more true *A. grandis* cases than concluded, as in many local case descriptions based on X rays, the species is often automatically assumed rather than morphologically or molecularly confirmed as *A. armillatus*. It is impossible to discriminate pentastomid species by radiology or ultrasound investigations. In 2013 and 2014, a cluster of severe ocular pentastomiasis cases emerged in Kole, DRC, diagnosed by PCR as being caused by *A. grandis* (6). These symptomatic ocular infections were regarded as a possible "tip of the iceberg" of all pentastomiasis cases, suggesting an unnoticed asymptomatic epidemic in the region (6). This view is likely further supported by our current findings of asymptomatic abdominal cases in the same area. The calcified and encapsulated nature and the serosal localization of the lesions were strong indications for pentastomiasis in the cases described by the surgeons and were proved in the two patients investigated. Other parasitic causes (e.g., ectopic tapeworm or nematode larvae) cannot be excluded but seem less
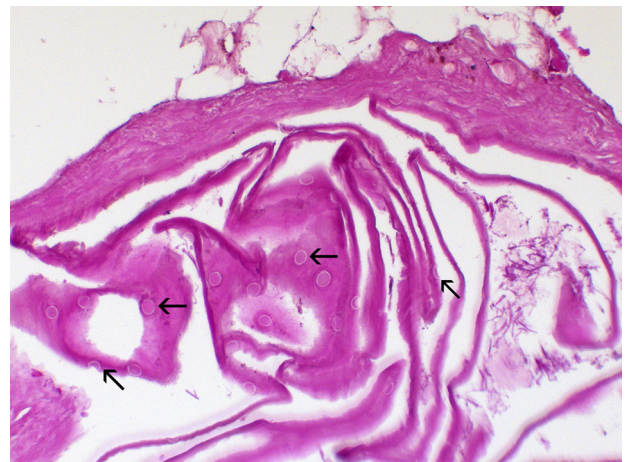

FIG 2 Microscopy of larval pentastomid parasites recovered during abdominal surgery in the Democratic Republic of Congo. Histological section through completely necrotic formalin-fixed, paraffin-embedded pentastomid larva from one patient. Only the highly decay-refractory folded cuticular remnants (red folded membranes in the background) and the abundant round sclerotized openings of the cuticle remain identifiable. The arrows point exemplarily to four of the openings: two openings in transverse section (horizontal arrows) and two openings in oblique section (oblique arrows). The second specimen from the same patient showed similar structures. Periodic acid-Schiff stain, magnification ×200. For comparison of the cuticular openings depicted here, see Fig. 4 in reference 1 and Fig. 2 in reference 5.

likely. None of the patients from which the abdominal cysts were analyzed suffered from concomitant pentastomid eye infection. The local people are known to occasionally consume snake meat, and the two patients discussed here reported eating snake meat in the past. The rise in these snake-borne zoonotic cases likely mirrors a shift from consumption of bushmeat toward more consumption of reptile meat, which is an important source of protein in the region, as populations of large-bodied birds and mammals are increasingly overexploited (6). However, waterborne transmission, a long-assumed route of infection, might also play a role.

Our data of abdominal infections contribute to the emerging picture of a local *A. grandis* epidemic. Molecular diagnostics are of increasing importance when patient material from rural, remote areas cannot be thoroughly examined in the field for logistic reasons. However, necrosis and/or formalin fixation hampers molecular investigations, and specimens might be best preserved in ethanol. Serological prevalence studies, which until now have been hampered by the lack of cooling facilities for patient sera, will follow in the near future in order to estimate the extent of infections in this region.

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