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Report of Spores of *Henneguya salminicola* (Myxozoa) in Human Stool Specimens: Possible Source of Confusion with Human Spermatozoa

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The spores of *Henneguya salminicola*, a common tissue parasite of salmonid fishes in the northern hemisphere, were observed in stool specimens from two different patients with diarrhea. The spores' superficial resemblance to human spermatozoa resulted, in one instance, in an incorrect report, leading to suspicion of sexual abuse. *H. salminicola* spores and human spermatozoa can be differentiated on the basis of size, morphology, and staining characteristics. Laboratory personnel who perform microscopic examinations of stool specimens for ova and parasites should be aware that spores of *H. salminicola* may be seen from time to time.

Laboratory testing of human stool specimens for parasites is based largely on identification of microscopic parasitic forms. Frequently, elements are encountered which cause confusion and occasionally result in incorrect identification. Such elements include oil droplets, polymorphonuclear leukocytes, and ingested material such as pollen grains, vegetable cells, and microscopic organisms. Sometimes, organisms are seen which are of no significance in human specimens but are parasitic in other hosts.

On two separate occasions in our laboratory, human stool specimens were found to contain spores of the fish parasite *Henneguya salminicola* Ward, 1919 (Myxozoa). In the first instance, these organisms caused considerable confusion due to their superficial resemblance to human spermatozoa. Laboratorians should be aware that these spores may be ingested along with the flesh of salmon and subsequently passed, undigested, in the feces.

CASE REPORTS

Patient 1. A 1-year-old boy presented to his physician with acute nonbloody diarrhea. Stool specimens were cultured for bacterial pathogens and examined for ova and parasites. Viral studies were not performed. Cultures were negative for *Salmonella* species, *Shigella* species, *Yersinia enterocolitica*, *Aeromonas* species, *Campylobacter* species, and *Escherichia coli* O157:H7. No conventional ova or parasites were identified; however, the stool specimen was found to contain spores of the myxozoan *H. salminicola*. These organisms were initially mistaken for human spermatozoa. The presence of spermatozoa in the stool specimen was reported to the family physician, and on the basis of this report, a preliminary investigation into the possibility of sexual abuse was begun.

The child's illness resolved and was thought to be viral in origin. A second stool specimen for ova and parasites was collected and examined 1 week later; no *H. salminicola* spores were observed in that specimen.

Patient 2. A 61-year-old male, who was otherwise healthy, presented with occasional bouts of slightly bloody diarrhea. At the time he consulted his family physician, about 1 month after the onset of symptoms, his illness was resolving. Stool cultures for bacterial pathogens were negative for *Salmonella* species, *Shigella* species, *Yersinia enterocolitica*, *Aeromonas* species, and *Campylobacter* species. Viral studies were not performed. A stool specimen subjected to ova and parasite examination was found to contain spores of *H. salminicola* and the protozoan *Blastocystis hominis* in small numbers.

The patient recovered without treatment in the ensuing 2 to 3 weeks. The cause of his illness was not established, and no follow-up stool specimens were collected.

MATERIALS AND METHODS

Specimen processing. Stool specimens were received in sodium acetate-acetic acid-formalin. Fecal material was concentrated by the formalin-ethyl acetate sedimentation method (1). Wet mounts of the fecal concentrate were examined by bright-field light microscopy.

The concentrate was also examined by electron microscopy. The sample was brought up in an aqueous solution and then drawn down onto a 1-μm-pore-size Nucleopore filter by vacuum filtration. The sample was then coated with gold, using an Edwards 306A sputter coater, and then photographed while being examined under a JEOL scanning electron microscope (model 35C).

Fecal smears for stained preparations were prepared in the following manner. Unconcentrated fecal material was washed twice by suspension in 0.85% NaCl followed by centrifugation for 10 min at 500 × *g*. The supernatant was decanted after each centrifugation. Smears were prepared from the washed sediment and allowed to air dry.

A modified iron hematoxylin staining method was performed on smears of all specimens (4). Trichrome staining was performed on the first fecal specimen from the patient described in first case report (5). A smear from the patient described in the second case report was stained by a modified trichrome method developed for the staining of microsporidia (9).

Identification of spores of *H. salminicola* was confirmed by direct comparison with samples of *H. salminicola* from the flesh of sockeye salmon (*Oncorhynchus nerka*) from Henderson Lake, British Columbia, Canada.

RESULTS

Organisms resembling human spermatozoa were observed in fecal concentrates at a magnification of ×400. They were about 40 μm in total length, with a teardrop-shaped spore body

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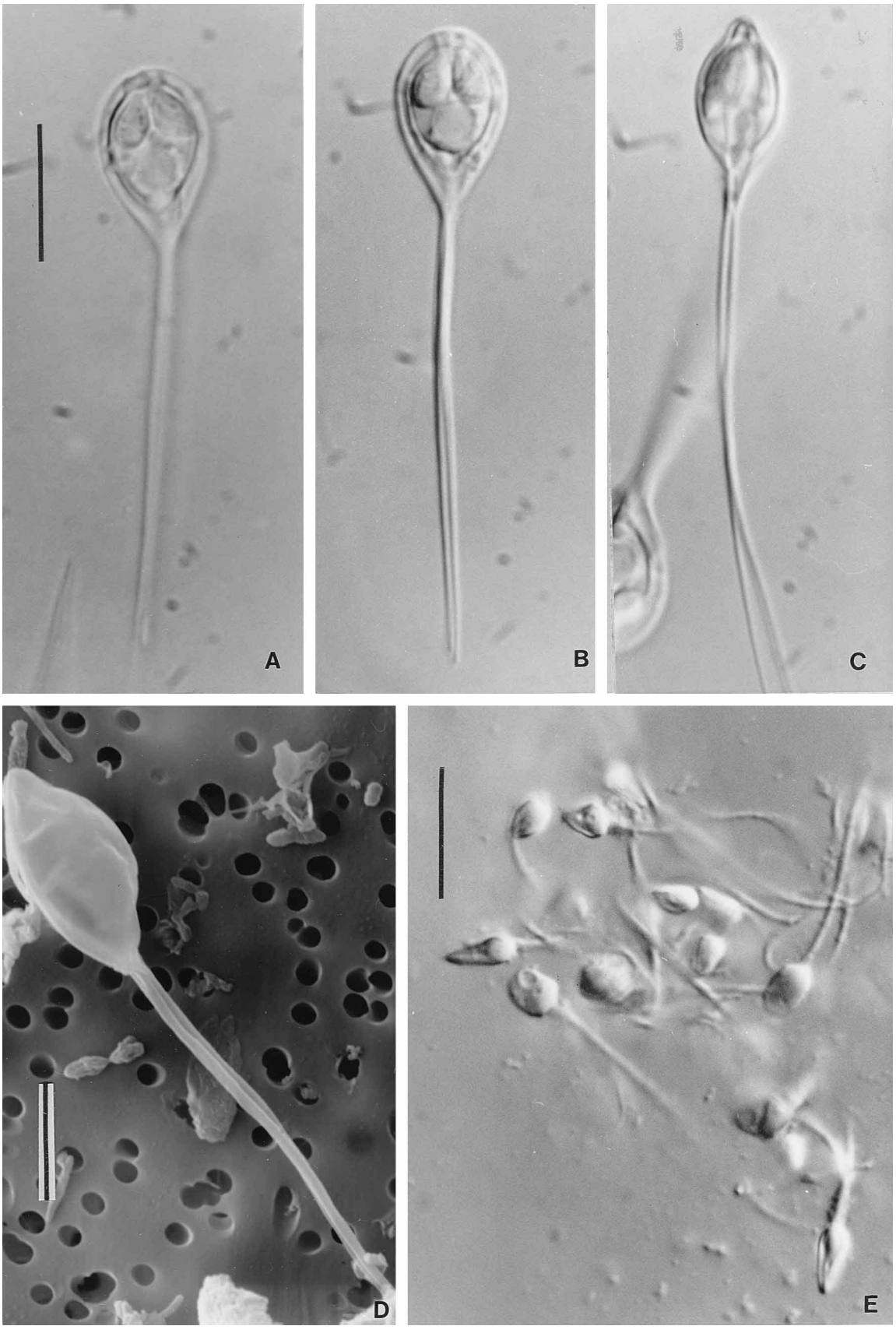

FIG. 1. (A to C) Spores of *H. salminicola* from a human stool specimen (A) and from sockeye salmon (dorsal view [B] and lateral view [C]), as viewed under a bright-field microscope. (D) Scanning electron micrograph of a spore of *H. salminicola* from a human stool specimen. (E) Normal human spermatozoa. Bars: panels A to C and E, 10 μm; panel D, 5 μm.

of approximately 10 μm in length which tapered gradually to one or two caudal filaments (Fig. 1A). When viewed laterally, some of the spores were lemon shaped while others, having collapsed, appeared concave, or cup shaped. Most of the spores had two pyriform polar capsules located in the anterior region of the spore body.

The morphology of the spores was compared with that of previously identified specimens of *H. salminicola* (Fig. 1B and C). This comparison confirmed that the organisms in the stool specimens were identical to spores of that myxozoan parasite. Scanning electron microscopy showed that the spores had a smooth outer coat and that the caudal filaments were continuous with the spore body (Fig. 1D). These characteristics are consistent with the identification of *H. salminicola*. Comparison with normal human spermatozoa revealed significant differences in size and structure (Fig. 1E).

The modified iron hematoxylin staining method failed to stain the spores; they were occasionally visible as very faint pink outlines against a bluish background. When the trichrome method was used, the spores stained red. Staining failed, however, to reveal any morphological details not visible in the unstained wet preparation.

The modified trichrome staining method resulted in much brighter staining of the spores and better visualization of the internal structures. The external wall was seen to be 1 μm thick, and the two polar capsules stained red and appeared striped.

DISCUSSION

Myxozoans are common parasites of fish worldwide (6). There are about 1,300 nominal species, with new forms being described each year. Formerly classified as protists, recent morphological and molecular phylogenetic studies have indicated that these parasites are metazoans (10, 12, 13). They parasitize a wide variety of fish tissues, where they often produce pseudocysts which contain hundreds of thousands of small spores. *H. salminicola* is a histozoic species that produces pseudocysts within striated muscle of salmonid fishes throughout the northern hemisphere (7, 8, 11). Spores such as those of *H. salminicola* are chitinized and are known to pass intact through the vertebrate gut (2).

We are not aware of any other reports of spores of *H. salminicola*, or other myxozoans, being observed in human stool specimens. It seems unlikely, however, that their presence in feces is an unusual occurrence since the organisms are present in the flesh of healthy salmon and could be ingested with canned, frozen, or fresh fish. Unfortunately, it was not possible to confirm that either of the patients whose cases are described here ingested salmon prior to specimen collection. The probability of obtaining the spores through ingestion of water or mud is very low because they disperse rapidly in the environment to undetectable densities.

There is no evidence that these parasites are capable of causing disease in humans, and it was not possible to attribute disease to their presence in either case. Both patients had self-limited illnesses, and although stool specimens were not examined for common viruses, the symptoms of the patient in the first case report were consistent with viral enteritis. The cause of illness in the second patient was not established; however, the presence of *B. hominis* in the patient's feces indicated exposure to fecal contamination in some form. The pathogenicity of *B. hominis* is a subject of ongoing debate (14). The patient did not seek medical attention until about 1 month after the onset of illness, and it is possible that an enteric pathogen was present but was not detectable by the time stool specimens were collected.

The initial identification of the spores as human spermatozoa was incorrect. Differentiation of spermatozoa and spores of *H. salminicola* is achieved by careful observation of the morphology and size of the organisms. The head of a normal human spermatozoon measures about 3 to 5 μm in length. When the dorsal surface is presented, it is smoothly rounded at the tip; however, it will appear pointed when viewed from the side. A normal spermatozoon has a single flagellum, or tail, which is typically more than 10 times the length of the head. No internal structures are discernible by light microscopy, and the cell is stained dark blue by iron hematoxylin stain.

In contrast, the spore body of *H. salminicola* is about 10 μm in length. From a dorsal perspective, the spore body is smoothly rounded at the anterior extremity and is very similar in shape to a spermatozoon. However, when it is viewed laterally, it is either lemon shaped, with a ridge running around the midline, or is collapsed and has a cup-shaped appearance. One or two caudal filaments are visible, and they are only about three times as long as the spore body. If internal morphology is visible, two pyriform polar capsules are situated at the anterior end of the spore, and they may take on a striped appearance when stained due to the enclosed coil of polar filaments. Unlike spermatozoa, spores of *H. salminicola* are typically not stained by iron hematoxylin stain.

The difficulties caused by the presence of organisms and objects that resemble human parasites in stool specimens are well documented (3). Free-living amoebae, the ova and larvae of free-living nematodes, and objects such as pollen grains, which can resemble helminth ova, may all occasionally result in the incorrect diagnosis of a parasitic infection. In the case of spores of *H. salminicola*, the resemblance to human spermatozoa has the potential for serious consequences, particularly when the patient is a child. A report of spermatozoa in the feces of a child may lead to a misguided investigation of presumed sexual abuse, as it did in one of the cases reported here. It is hoped that this report will prevent a recurrence of these circumstances.

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