Protozoa from Polluted Waters; Potential Human Pathogens

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The health risk to divers working in polluted waters to potential protozoan pathogens has not been adequately assessed. The most likely pathogens to be encountered are amphizoic protozoa rather than obligate parasites. Recent isolation of small free-living pathogenic amebae from esturaine and oceanic waters suggests these organisms may pose a low level threat to the health of divers.

INTRODUCTION

Although the nature and diversity of protozoa in variously polluted waters has and is being studied, the existence of potential human pathogens in these waters has not been adequately assessed. There currently does not exist a sufficient basis for determining the degree of exposure to protozoan pathogens by individuals working in polluted waters. Some exposure to potential protozoan pathogens in polluted waters is likely, but the risk is probably low for divers using an air supply and wearing a diving suit.

On the basis of current knowledge, the most likely health risk to divers is from amphizoic protozoa. Amphizoic protozoa are those that are capable of being either endozoic (symbiotic or parasitic) or exozoic (free-living).[1] But exposure to obligate parasitic species in polluted waters cannot be dismissed.

Amphizoic amoebae have been isolated from polluted waters and are known to be causative agents of human disease, or on the basis of virulence testing mice, potentially hazardous to man. Thus far, these pathogenic organisms have been identified as small free-living amoebae[2] and specifically two genera, *Naegleria* and *Acanthamoeba.*

BACKGROUND

There have been several recent reviews concerning these two genera.[2,3,4,5] It is necessary to provide some background for those not familiar with these organisms before addressing the potential health risk posed.

Free-living Pathogens

The life cycle of these amoebae is relatively simple. There is an active amoeboid form (vegetative trophozoite) which feeds primarily upon bacteria, but also upon fungi. Both types of amoebae form a cyst which is resistant to dessication. *Naegleria* trophozoites will also transform to a nonfeeding, swimming, biflagellated form under certain conditions. The only known pathogenic *Naegleria* species, *N. fowleri,* cannot be distinguished on a morphological basis from some nonpathogenic *Naegleria* species. Either the trophozoite or the flagellate form of *N. fowleri* is infective.

The rapidly fulminating human disease caused by *N. fowleri,* primary amoebic encephalitis (PAM), is contracted by the entry of the amoebae through the nose. It has been speculated that the depletion of mucous in the nasopharynx subsequent to long periods in water permits entry to the olfactory nerve which the amoebae follows to the brain. With the exception of a single human case having systemic involvement, infections have been restricted to the central nervous system. A recent environmental isolate of *N. fowleri* from a cooling lake receiving thermal waters produced both brain and lung infections in mice.[6] Based on this observation Willaert and Stevens[6] suggest that *N. fowleri* may cause subacute and acute respiratory infections in humans. Only a few human cases have been successfully treated with antibiotics.[3,6,7]

Reported infections of *Acanthamoeba* have not been restricted to the central nervous system.[7] There are reported cases of eye infections and involvement of the pulmonary system. In a case of transient diarrhea, *Acanthamoeba* were isolated from stools, but it is unclear whether the amoeba was the causative agent. Infection of the central nervous system by *Acanthamoeba* is pathologically different from PAM and is clinically designated as granulomatous amebic encephalitis (GAE).[8] Invasion of the body may be through the nasal route, by inhalation, ingestion, or from a wound. Rather than a single species being involved as is the appartent case with PAM, several different species of *Acanthamoeba* have been implicated in reported infections.

Human Cases and Water Activity

There have been other reports of infections by other genera of amphizoic amoebae, but these have been of animals other than man.[1] The total number of known human cases involving *Naegleria* and *Acanthamoeba* is not large, totaling about 124.[3,5,7,8] Most cases have been associated with activity in water. When PAM was first recognized, in Australia, there appeared to be a specific association with saltwater tidal pools, but since these first cases, the vast majority have been contracted in warm or thermally polluted freshwater. The apparent association with saltwater does not appear to be typical.[5] There have been environmental isolates of *N. fowleri* from polluted waters (other than thermally polluted), but cases of PAM have not been reported from the isolation area.[9] Only from Australia, again, has an apparent association of some cases of GAE with saltwater been reported.[3] As with *Naegleria* infections, the majority of cases have not been associated with saltwater.

The methods of entry and the number of reported cases imply that these organisms pose a low-level hazard to divers using an air supply and wearing a suit. The risk increases with prolonged periods in the water, with splash back of polluted water in the face mask, or with the exposure of a wound. The greatest risk would be for divers not protected by a suit or using an air supply.

Presence in Polluted Waters

Another factor affecting the level of risk, would be the degree of exposure to amphizoic amoebae. There is recent evidence that several pathogenic *Acanthamoeba* species not yet implicated in human cases are associated with polluted estuarine and marine environments. Sawyer, *et al.*,[10] have described *A. hatchetti*, pathogenic when tested experimentally, collected from sediment from the Baltimore harbor. During the course of an investigation of the use of starch gel electrophoretic analysis of enzymes for the identification of small amoebae, additional pathogenic *Acanthamoeba* were discovered by this laboratory[11] (and unpublished observations).

Prior to investigating the application of this technique to *Acanthamoeba* species, the method was found effective for separation of pathogenic and nonpathogenic *Naegleria* strains.[12] Since the study of *Acanthamoeba* isolates included strains known to kill experimentally infected mice, strains nonpathogenic to mice, and strains which had not been tested for virulence in mice, all strains used were tested for virulence by intranasal instillation in CFW mice. Six previously not tested environmental isolates collected by Dr. Tom Sawyer (1-1Δ, 2-1Δ, NYB5a, OC3-A, OC3-2) killed experimentally infected mice. Two of these (1-1Δ, and NYB5a) were isolated from a New York dump site 7-8 miles offshore from Long Island, New York, and closed to shellfishing because of high coliform counts. One isolate was from a Philadelphia dump site located 40 miles offshore from the Maryland-Delaware area and also closed to shellfishing because of high coliform counts. The zymograms of propionyl esterase, acid phosphatase, and alkaline phosphatase proved identical to each other for 1-1 Δ and 31-B. Strains 2-1Δ and NYB5a also formed a matching pair. Zymograms of remaining two strains did not match each other or those of the matching pairs. These zymograms are also unique from the other 26 *Acanthamoeba* strains thus far examined, which include both pathogenic and nonpathogenic isolates. These results suggest that the six newly discovered pathogenic strains may include several new species, with two being from dump sites.

Dr. Tom Sawyer has found that *Acanthamoeba* can be readily isolated from active and inactive dump sites, as well as from polluted waters[13] (and personal communication). *Acanthamoeba* are frequently isolated from unpolluted estuarine and oceanic waters. The sites from which *Acanthamoeba* are readily isolated often have high coliform bacterial populations. Because pathogenticy testing of *Acanthamoeba* from acquatic isolations is not routinely performed in mice, the frequency of isolation of the virulent strains is not known. It is also not known whether strains pathogenic to mice are necessarily pathogenic to men.

Obligate Parasites

Two obligate parasites which pose a possible hazard to divers are another amoeba, *Entamoeba histolytica*, and flagellate, *Giardia lamblia*. Both cause intestinal disease and can be distributed by the improper disposal of human feces.[13,15,16] For both organisms, cysts in water or in contaminated food must be ingested to contract the disease. Active trophozoites will not survive the acidity of the stomach. Cysts of *E. histolytica* will survive typical municipal chlorine levels, temperatures of 0 C for several weeks, and temperatures of 30 C for up to 3 days.[15] High bacterial densities will adversely affect the viability of cysts.[14] How well cysts of either organism survive in seawater is not known. It is probable that these two organisms could not tolerate high salinities (Dr. L.S. Diamond, personal communication).

Risk to Divers

Potential protozoan pathgens apparently present a low

level health risk factor for divers in polluted waters. Invasion by these protozoa would probably be effectively blocked by using a mask, an air supply and a suit. Unprotected divers would be at greater risk depending upon the frequency of exposure to these protozoa. The population densities of potential protozoan pathogens in polluted waters has not been assessed. The current sparse data implies that amphizoic amoebae are the potentially pathogenic protozoans most likely to be encountered by divers. Eight different *Acanthamoeba* strains belonging to several different species, isolated from estuarine and oceanic waters, are pathogenic to experimentally infected mice[9,10] (and unpublished observations). Whether these organisms infect humans is unknown, but it would not be surprising. There may be other unknown amphizoic protozoa in polluted waters potentially pathogenic to man. The risk from obligate parasites is probably even lower. Exposure to *E. histolytica* and *G. lamblia probably would be restricted to areas of sewage effluent.*

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