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# PREVALENCE WATERBORNE *ENTAMOEBA HISTOLYTICA* FROM FRESHWATER BODIES IN SOLAPUR, MAHARASHTRA STATE, INDIA.

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**ABSTRACT**
Waterborne disease interactions in the water environment. It is important to place into context the zoonotic component of waterborne disease. Approximately 4 billion cases of diarrhoea occur each year, leading to nearly 2 million deaths. Intestinal worms infect more than a billion people worldwide. The percentage of these illnesses that are caused by zoonotic pathogens is difficult to determine due to a lack of data, but it is thought to be significant. Waterborne zoonotic pathogens cause both gastrointestinal diseases such as diarrhoea and other illnesses such as leptospirosis and hepatitis. Present study includes studying the prevalence *of Entamoeba histolytica* from freshwater water bodies in Solapur, Maharashtra, India, During the February 2011 to January 2012. Total 360 water samples were collected (each lake 120 water samples for 3 lakes), of which 53 positive samples of *E. histolytica* were found out of 120 samples and the prevalence is 44.16%.

**KEY WORDS:** *Entamoeba histolytica*, gastrointestinal diseases, *Waterborne protozoans.*

## INTRODUCTION

Protozoa are unicellular microscopic eukaryotes that have a relatively complex internal structure and carry out complex metabolic activities. Some protozoa have structure for propulsion or other types of movement. The protozoa are considered to be a sub kingdom of the kingdom Protista, although in the classical system they were placed in the kingdom Animalia. More than 65,000 species have been described, most of which are free living organisms. The different phyla are distinguished from one another by such features as structure, means of locomotion, and formation of spores, although the locomotory organelles are the primary distinguishing feature. There are three main locomotory organelles found in the different classes of protozoa, and they are pseudopodia, cilia, and flagella. Some waterborne zoonoses are well known, information is limited on others, and it is likely that some, possibly many, remain unrecognized. Several waterborne zoonoses, such as cryptosporidiosis and giardiasis, occur regularly in a variety of countries; others, such as leptospirosis, occur more frequently in tropical countries. High quality information on many aspects of waterborne zoonoses is not available. There is also a need to better integrate the disciplines of human and animal health to anticipate emerging waterborne zoonoses and develop appropriate management responses to prevent them. The phenomenon of “emergence” and “re-emergence”

Protozoan pathogens, including microsporidia, amoebae, ciliates, flagellates, and apicomplexans, originating in human or animal feces have been found in surface waters worldwide. Many have been found infrequently or in low numbers or have been identified only by general morphological features that are not precise. The zoonotic protozoa that are emerging or are of renewed interest because their spread is associated with water and include several species of microsporidia, the amoeba *Entamoeba histolytica*, *Giardia* spp. *T. gondii*, and *Cryptosporidium* spp. Although *Cyptospora*spp. is known to be a waterborne threat and has been detected in washings from vegetables contaminated with irrigation water, humans are the only confirmed hosts for this species. The ability to conduct epidemiological studies relating these organisms to human infections, animal sources, and water will now provide a basis for planning, prevention and control strategies. In Maharashtra following researchers have observed the waterborne protozoa in freshwater by Gupta (2011), *Cryptosporidium*, *Giardia*, *Cyclospora*, *Toxoplasma*, and human infective microsporidia (e.g., *Encephalito zoonintestinalis*, *Encephalit zoonhellem*, *Encephalito zooncuniculi*, and *Enterocyto zoonbieneusi*)are human enteric parasites in which transmission is associated with water (Wolfe, 1992).

There are five critical elements in the transmission of zoonotic Protozoans throughwater:
1. Source of the infectious agent
2. Specific water-related modes of transmission
3. Attributes of the organism that allow it to survive and possibly multiply and to move into and within the aquatic environment.
4. Infectious dose and virulence factors of the organism.
5. Host susceptibility factors.

**Amoebiasis**

Amoebiasis is a parasitic protozoan disease caused by the protozoan *Entamoeba histolytica*. This disease is prevalent worldwide but is most prevalent and severe in tropical countries where prevalence rate may exceed 40% under conditions of crowding, poor sanitation and poor nutrition. Amoebiasis is the third leading cause of death from parasitic diseases worldwide, with its greatest impact on the people of developing countries. WHO estimates that approximately 50 million people worldwide suffer from invasive amoebic infection annually, resulting in 40,000 to 100,000 deaths annually (Haque, 2007). Transmission is usually by ingestion of infective cysts which contaminated with food and water. The cysts remain viable for 12 days in moist environment and 30 days in water. It is characterized in humans by mild to recurrent diarrhea, stools streaked with blood and mucus. Hepatic amoebiasis is characterized by fever, hepatomegaly, moderate colitis, pain and localized tenderness. Amoebiasis disease can be prevented by strict sanitation and personal hygiene, protective clothing and gloves. In addition, fecal contamination of feed and water should be prevented, and fecal screening and adequate cooking to destroy the cysts need to be done.

**Solapur District**

Solapur district, Maharashtra State, India, is bounded by 17º05' North latitudes to 18º 32' North latitudes and 74º 42' East of 76º 15' East longitudes (Table 1 and Figure 1). Total geographical area of Solapur district is 14895² K.m. divided into eleven tahsils. The Population is 32.4 lakhs in eleven tahsils of District (Censes 2001). It is bounded from the North by Osmanabad district and Ahmednagar district, on North-East by Satara district and at the South and East it has common boundary of Karanataka state. The temperature is high in summer season. Rainfall varies from East to West between ranges of 200 to 600 millimeters. Solapur is an important city in Maharashtra most well-known for its textile industry, and bed sheets in particular.

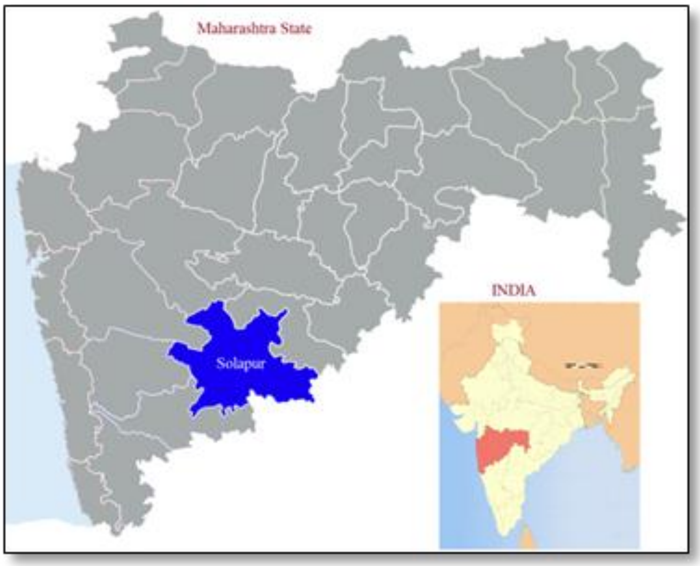



**Figure 1. Map of Solapur shows in blue colour.**

The Latitude and Longitude of the Lakes present in Solapur are shown in Table-1 and Figure-1, 2 and 3.

**Table 1. Lake Latitude / Longitude of Lakes in Solapur**

| Site | N Latitude | E Longitude |
|---|---|---|
| Shiddeshwar lake | 1740.431 | 7554.271 |
| Sambhaji lake | 1738.370 | 7554.370 |
| Ekruk Lake (Hipparga lake) | 1744.784 | 7554.982 |

**Sambhaji Lake (Figure 2)**

The original name of Sambhaji Lake (KambarTalav) was QAMAR lake. This was named after daughter's name of Aurangzeb-the Mughal Emperor. But now this is known as Kambar Talav. It is also known as Sambhaji Lake. The Sambhaji Lake is a well-known attraction in the city where you will see locals flocking in the evening and weekends for a fresh breath of air or just a long walk along the banks.

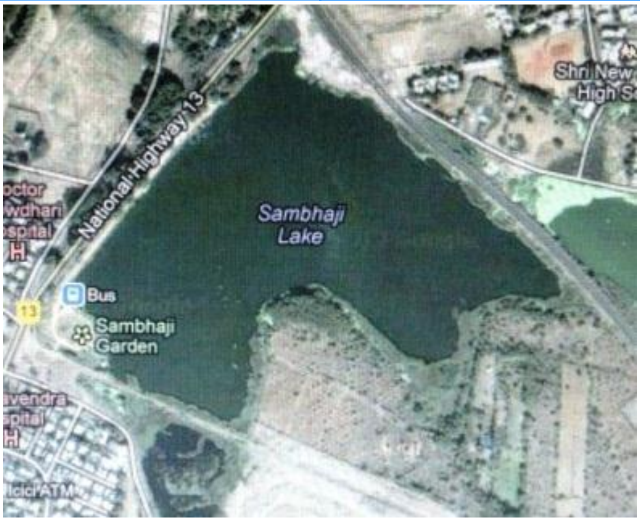
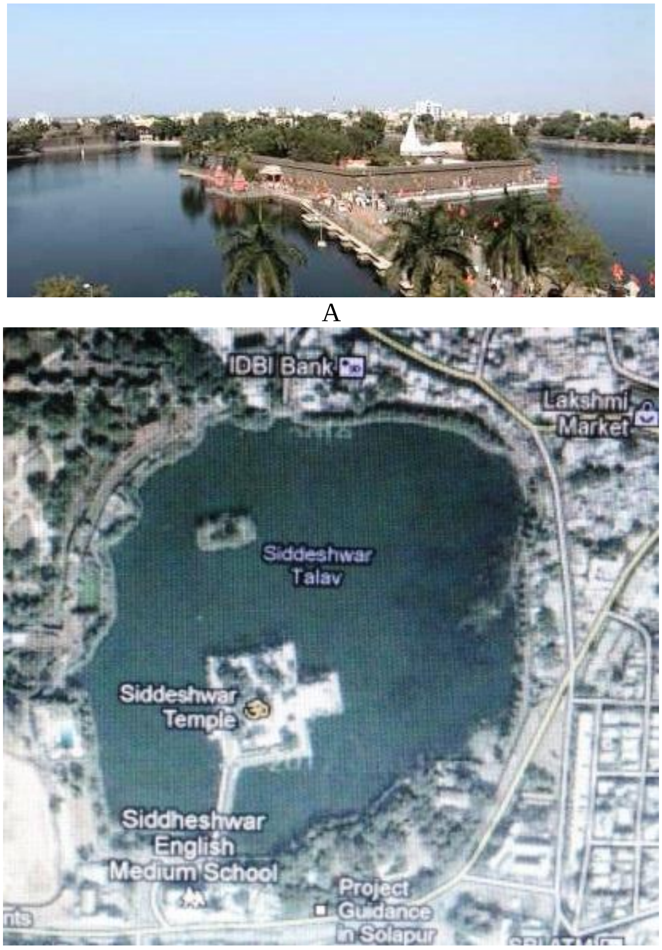



**Figure-2.Sambhaji lake, Solapur, Maharashtra State, India (Source Google Map)**

**Shiddeshwar lake Solapur (Figure 3)**

Siddheshwar Temple is the temple of "Lord Shiddheswara". The temple, situated in the middle of the lake, is picturesque; with a backdrop of the fort looming behind it.




A

B

**Figure- 3.Shiddeshwar lake Solapur, Maharashtra State, India. (Source Google Map) A. Shiddheswar temple B. Over view by satellite image (Google map)**

The temple was built by a yogi, Shri Siddharameshwar, who was a devotee of Sri Mallikarjuna of Srisailam. He was on his way to Srisailam, when he was ordered by his guru to return to Solapur and many Shiva lingams. He duly returned to Solapur and began his work, starting from this temple. In all, he installed 68 Shiva lingams in Solapur, Another feature of this temple is the garden that has a shrine dedicated to Saint Nalatwad. Other shrines in the temple complex are dedicated to Lord Vithoba and Goddess Rukmini.

**Ekruklake (Figure 4)**
The Ekruk lake, the largest artificial lake in the Bombay Presidency, lies five miles north-east of Solapur. The scheme was prepared in 1863 and sanctioned in 1866. It comprises a reservoir formed by an earthen dam 7,200 feet long and seventy-two feet in greatest height and three canals. The dam is thrown across the valley of the Adhila, a feeder of the Sina, which has a drainage area of 160 square miles above the lake. The lake is sixty feet deep when full, and holds 3,350 millions of cubic feet. The area of water surface is 4,640 acres or 7 square miles. Of the canals one on each bank is at a high level, designed for four months' watering, and the third on the left bank is at a low level, designed for a twelve months discharge. Of the two high level canals the right bank canal is eighteen miles long, discharges sixty cubic feet a second, and commands 565 acres; and the left bank canal is four miles long, discharges twenty-five cubic feet a second, and commands 856 acres. The low level left bank canal is twenty-six miles long, discharges seventy cubic feet a second, and commands 10,601 gross acres. The canals are bridged and regulated throughout and can be lengthened so as to command a larger area. The low level canal flows close past the town of Solapur. The work was begun in 1866, and the dam was closed in December 1869. Some water was supplied to the *kharif* or rain crop of 1871-72. At the end of 1876-77 the work was completed, except the masonry heads to distributaries and the last two miles of the low level canals and the last twelve miles of the high level right bank canal. By the end of 1881-82 all the works connected with the Ekruk lake were completed at a total cost of about 121,262 (Rs. 12,12,620). In 1882-83, of 15,320 acres, the arable area under command, 1,306 acres were watered and paid 524 (Rs. 5,240) for water rates. Besides tillage water, the Ekruk Lake supplies drinking water to the town of Solapur.


**Figure-4.Ekrukh Lake (Hippargalake), Solapur, Maharashtra State, India. (Source Google Map)**

## MATERIALS AND METHODS
### Methodology
During the February 2011 to January 2012**,** estimation of different physic-chemical parameter of the water samples are done as per standard method for the examination of water and microscopic observation on free living ciliates were done after their movements were slowed down with 10% methyl cellulose.

**Field operations**
The study is conduct in different water bodies in Solapur city.

**Sample Preparation**

Surface water samples were collected from identified stations of the lake at a depth of one feet using polythene cans of two litre capacity for a period of one year, from February 2011 to January 2012 at monthly intervals. Transparency and Vertical attenuation coefficient (VAC) was determined by sacchi disc. pH was measured by using pH meter. The chemical analysis was carried out following the methods suggested by Trivedy and Goel (1986) and APHA (1995). For the enumeration of protozoa, each site in a 50mL sample bottle by filtering about 50 liters of water through plankton net. Sample was fixed simultaneously with 20 ml of 1% lugol solution for sedimentation. This sedimented sample was observed under microscope. The identification of Protozoa up to the level of genus was made with the help of literature available. Although considerable work has been done on the limnological studies on some lakes of Maharashtra. The three lakes of Solapur city selected for the present study remains scientifically unexplored.

Standard protocol use for the description of fixation, storage, staining and mounting techniques commonly used to prepare the material for study. Laboratory procedures for Protozoan parasites: Standard collection, Staining, preservation for the proposed work. The following stains are use for protozoa staining namely, Romanowsky Stains, Giemsa stain, Modified Ziehl-Neelsen stain, Phenol-auramine stain, Trichrome Stain, Funguqual (Uvitex 2B or Calcofluor) stain, Iron Haematoxylin stain. The routine analysis of protozoan parasites in water samples relies upon direct microscopic detection after concentration of particulate matter by filtration or centrifugation.

**Spot 1 (South part- Sambhaji Lake)**

**Spot 1 (East part- Sambhaji Lake)**

**Figure- 5. Sampling location and station Sambhajilake, Solapur, Maharashtra State, India Spot 1 and 2**

**Spot 1 (North part- Shiddheswar Lake)**

**Spot 1 (South part- Shiddheswar Lake)**
**Figure- 6. Shiddeshwar lake Solapur, Maharashtra State, India.**

**Identification**
A list of some of the major taxonomic works useful for identification of specimens to the generic level. This is intended as a starting point, not a definitive listing of taxonomic works. Microscopic examination of and recording of the results by using statistical methods.

**Taxonomic Aids and Keys to Species**
Identification of many parasite species may require consultation of original descriptions in the primary literature. However, for most common groups, identification to genus and often to species can be done through the synthetic keys. Voucher specimens are deposited in the permanent collection of a recognized museum, for future reference and use for researchers.

**Statistical analysis**
Detection of prevalence, abundance and frequency is measure by Standard statistical analysis methods.

**Spot 1.(South part- Ekrukh Lake)**

**Spot 2 (North part- . Ekrukh Lake)**
**Figure- 7. Ekrukh Lake (Hippargalake), Solapur, Maharashtra State, India.  Spot 1 and 2**

**RESULTS AND DISCUSSION**

**Prevalence of *E. histolytica* (waterborne protozoa) from water bodies of Solapur city shown in table 2.**

**Taxonomic Classification**

***Entamoeba histolytica***

| Domain: | Eukaryota |
|---|---|
| Kingdom | Protista |
| Subkingdom | Protozoa |
| Phylum | Sarcomastigophora |
| Subphylum | Sarcodina |
| Class | Lobosea |
| Order | Amoebida |
| Family | Endamoebidae |
| Genus | *Entamoeba* |
| Species | *histolytica* |

**Table 2. Percentage of Prevalence of *Entamoeba histolytica* (%) of zoonotic waterborne Protozoa in Sambhaji Lake during the Period February 2011 to January 2012**

| Sr. No | Month | Sample Tested | | | Positive samples of *E. histolytica* | | | % of Positive samples | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Sambhaji Lake | Shiddheswar Lake | Ekrukh Lake | Sambhaji Lake | Shiddheswar Lake | Ekrukh Lake | Sambhaji Lake | Shiddheswar Lake | Ekrukh Lake |
| **1** | Feb | 10 | 10 | 10 | 5 | 2 | 1 | 50 | 20 | 10 |
| **2** | March | 10 | 10 | 10 | 3 | 2 | 0 | 30 | 20 | 00 |
| **3** | April | 10 | 10 | 10 | 2 | 1 | 0 | 20 | 10 | 00 |
| **4** | May | 10 | 10 | 10 | 0 | 0 | 0 | 00 | 00 | 00 |
| **5** | June | 10 | 10 | 10 | 0 | 0 | 0 | 00 | 00 | 00 |
| **6** | July | 10 | 10 | 10 | 4 | 1 | 2 | 40 | 10 | 20 |
| **7** | Aug | 10 | 10 | 10 | 6 | 2 | 3 | 60 | 20 | 30 |
| **8** | Sept | 10 | 10 | 10 | 6 | 4 | 3 | 60 | 40 | 30 |
| **9** | Oct | 10 | 10 | 10 | 8 | 3 | 2 | 80 | 30 | 20 |
| **10** | Nov | 10 | 10 | 10 | 7 | 4 | 4 | 70 | 40 | 40 |
| **11** | Dec | 10 | 10 | 10 | 6 | 2 | 2 | 60 | 20 | 20 |
| **12** | Jan | 10 | 10 | 10 | 6 | 1 | 1 | 60 | 10 | 10 |
| Total | | **120** | 120 | **120** | 53 | 22 | 18 | 44.16 | 18.33 | 15.00 |

During the year Feb. 2011 to January 2012 total 360 water samples were collected (each lake 120 water samples for 3 lakes), of which 53 positive samples of *E. histolytica* were found out of 120 samples and the prevalence is 44.16%.
In Shiddheswar Lake 22 positive samples of *E. histolytica* were found out of 120 samples and the prevalence is 18.33%. In Ekrukh Lake 18 positive samples of *E. histolytica* were found out of 120 samples and the prevalence is 15%. In the month of May and June there was no protozoa recorded from the sample and hence the prevalence reaches to zero. The maximum percentage of prevalence was recorded in the month of October to January which gradually decreases up to April (0.0%). In Ekrukh lake. The organelles of protozoa has functions similar to the organs of higher animals. The plasma membranes enclosing the cytoplasm also cover the projecting locomotory structures such as pseudopodia, cilia and flagella. The ciliates are a group of protozoan's characterized by the presence of hair like organelles called cilia, which are identical in structure to flagella but typically shorter and present in much larger numbers with a different undulating pattern than flagella. Cilia occur in all members of the group and are variously used in swimming, crawling, attachment, feeding, and sensation. Their early appearance as living organism, their adaptability to various habitats and their capacity to remain viable in the encysted condition, probably account for the wide distribution of the protozoa throughout the world. In geographic areas of high prevalence, well-tolerated infections are often not treated to eradicate the parasite because eradication would lower the individual's immunity to the parasite and result in a high likelihood of re-infection. High mortality rate of diarrheal cases has been found related to sanitation, unsafe water and hygiene (WHO, 2012). The collected data compared with the observation of Bhosale et al., (2010), Dhimdhime et al., (2012), Chakraborty *et al*, (1977), Govind (1963), Shashi *et al*., (2008), Smitha (2007). Our findings are resembles with above observed data. Faecal contamination of water in extreme events (droughts, floods) could result in increased prevalence of diseases such as echinococcosis, taeniasis, and toxoplasmosis (Singh *et al*., 2010). Waterborne protozoa caused illnesses are likely to increase with rising temperatures (Greer *et al.*, 2008). The temperature of Solapur is very high in summer (40-45$^0$C). The close relationship between climate, environment and infectious diseases in the developing world is well recognized.

## CONCLUSION

Amoebiasis and other studied protozoan diseases can be prevented by strict sanitation and personal hygiene, protective clothing and gloves. In addition, fecal contamination of feed and water should be prevented, and fecal screening of NHP and adequate cooking to destroy the cysts need to be done. This research can be useful for all the discipline of Protozoology and Life Sciences. Changing global disease situation is very important, as climate change increases the risk of waterborne zoonoses by expanding the host, reservoir, and vector base. The provision of safe food and water and the control of zoonoses and associated outbreaks are important challenges to be faced. Public health infrastructure for food and water safety, disease surveillance, control of vectors and disease reservoirs, and public health outbreak response needs to be strengthened. National, Multinational and collaborative scientific efforts to minimize the waterborne protozoans and spread of these diseases is to be controlled. We aware the peoples to minimize the entry of animal wastes to source waters, by controlling animal movements, proper storage and disposal of farm animal wastes, using procedures that will minimize the survival of zoonotic protozoan pathogens, and limiting transport of these wastes in surface water runoff. But these water bodies only one water body (Ekrukh Lake) was useful for drinking-water. Arrangement of two health education campaign in the research area for students and local peoples. National, surveillance activities can help officials detect outbreaks or epidemics, identifynew and emerging disease threats, and assess temporal trends. Surveillance may consider laboratory-confirmed, physician- or self-reported disease, or disease symptoms and the collection of other information, such as water quality data or information about disease or infection in animal populations. Because zoonotic agents can be transmitted by contaminated drinking-water, recreational water, or food and by direct or indirect contact with infected humans and animals, epidemiological analyses are needed to evaluate waterborne transmission. The timely investigation of an outbreak by a multidisciplinary team (e.g., epidemiologist, engineer, water quality specialist) with appropriate laboratory assistance can provide information about the mode of transmission, the etiologic agent, sources of contamination, and deficiencies in water and wastewater treatment technologies and watershed protection programmes. The dearth of data on the prevalence of these zoonotic protozoa in surface waters is related to the lack of rapid and sensitive methods to recover and detect the encysted stages in the aquatic environment. The present study will provide the updated information of various aspects of waterborne protozoa and their distribution, composition, taxonomic details, symptoms and health consequences. This research work will fill the existing lacuna in the knowledge.

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