THE PREVALENCE OF *BLASTOCYSTIS SP.* IN VARIOUS ANIMAL HOSTS IN CAPTIVITY AT KARACHI ZOO.

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Abstract

Blastocystis sp. is an anaerobic enteric protozoan harboring the intestinal tract of a wide variety of animals, including humans which can be symptomatic or asymptomatic. A total number of 350 fecal samples of animals from two different zoological gardens i.e. Karachi Zoological Garden and The Wildlife Experience Centre were investigated for the presence of *Blastocystis sp.* Out of 350 fecal samples 280 (80%) indicated the presence of the parasite while others were free of the species. However, the 250 fecal samples from Karachi Zoo demonstrated, 210 (84%) infested animals while the 100 samples from The Wildlife Experience Centre showed 70 (70%) animals infested with *Blastocystis sp.* The infected organisms pose threat to the human begins in contact.

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Introduction

Blastocystis is an unusual anaerobic enteric protozoan parasite of humans and many animals (Stenzel and Borcham, 1994). It has a cosmopolitan distribution and is often the most commonly isolated organism in parasitological surveys (Aguiar *et al*., 2007).

The taxonomic classification of *Blastocystis sp*. has proven challenging and was only recently unambiguously placed within the stramenopiles despite the application of modern molecular phylogenetic approaches (Arisue *et al*., 2002). A recent phylogenetic study showed that, based on the genetic distance between homologous genes, *Blastocystis spp.* from humans and animals can be potentially divided into 12 or more species (Noel *et al*., 2005). Other studies have confirmed that *Blastocystis* genotypes are prevalent throughout the animal kingdom, with a number of genotypes comprising isolates from both humans and animals (Yoshikawa *et al*., 2003).

Blastocystis is not a host-specific organism, transmission from human to human and animal-to-human. It is needed to be studied in order to have a better understanding on the reservoir hosts and origin of *Blastocystis* infection among animals.

Materials and Methods

Blastocystis cells are generally identified microscopically via direct examination of stool, fecal concentration techniques, or in vitro culture of fecal material.

Study Area: 350 fresh feces were collected from Karachi Zoological Garden and The Wildlife Experience Centre (TWEC) with the permission of concerned authorities.

Animals examined: Animals were classified according to the phyla. The concentration of *Blastocystis sp.* present in the fecal samples from two different zoological gardens was compared. Animals examined from Karachi Zoological Garden included 17 species of mammals namely; Agile wallaby (*Macropus agilis)*, Asian elephant *(Elephas maximus)*, Bactrian camel *(Camelus bactrianus)*, Bengal tiger *(Panthera tigris tigris)*, Black buck *(Antilope cervicapra)*, Cougar *(Puma concolor)*, Chital *(Axis axis)*, Fishing cat *(Prionailurus viverrinus)*, Golden jackal *(Canis aureus)*, Indian porcupine *(Hystrix indica)*, Jungle cat *(Felis chaus)*, Miniature horse *(Equus ferus caballus)*, Nilgai *(Boselaphus tragocamelus)*, Rhesus monkey *(Macaca mulatta)*, Striped hyena *(Hyaena hyaena)*, White lion *(Panthera leo krugeri)*, and Zebra *(Equus quagga)*. six species of birds namely; Emu *(Dromaius novaehollandiae)*, Green-winged macaw *(Ara chloropterus)*, Ostrich *(Struthio camelus)*, Patagonian parrot *(Cyanoliseus patagonus)*, Indian peafowl *(Pavo cristatus)*, White cockatoo *(Cacatua alba)* and two species of reptiles namely; Spur-thighed tortoise *(Testudo graeca)*, Afghan tortoise *(Testudo horsfieldii)*.The animals examined from TWEC included three species of mammals namely; Dog (*Canis lupus familiaris*), Bactrian deer *(Cervus elaphus bactrianus)* and Anglo-Nubian *(Capra aegagrus hircus)* while bird species included Helmeted guinea fowl (*Numida meleagris*), Blue-and-gold macaw *(Ara ararauna)*, Patagonian parrot *(Cyanoliseus patagonus)*, Indian peafowl *(Pavo cristatus)*, domesticated fowl (*Gallus gallus domesticus)*, Congo African grey parrot *(Psittacus erithacus)* and Common pheasant (*Phasianus colchicus).*

Centrifuge-sedimentation technique: Out of a total of 350 samples, in 280 of them became possible to identify *Blastocystis sp* using the centrifuge-sedimentation technique. *Blastocystis sp.* was found in wet amount of the fecal sediment from stools collected, while some samples were also prepared through direct filtering method.

One drop of the sediment was transferred to a glass microscope slide, added to another drop of iodine solution (pure), covered with a cover-glass and examined in a light microscope.

Microscopy of direct fecal smear and culture from stool: Direct fecal smears stained with iodine were observed using light microscope to confirm the presence of Blastocystis cells. Prepared smears from cultures were observed under 10x, 40×, and 100x magnification of a bright field microscope to check for the presence of the characteristic vacuolar cells of *Blastocystis*.

Results and Discussion

From the total number of 250 samples analyzed from the Karachi Zoological Garden, 210 (84%) tested positive. Among the 17 mammalian species 170 fecal samples were collected. Prevalence were calculated as,

$$\text{Prevalence} = \frac{\text{No. of animal examined}}{\textit{Blastocystis sp.}} \times 100$$

140 (82%) were positive for *Blastocystis sp.*, while from the 6 avian species 60 fecal samples were collected, 50 (83%) were positive, whereas from 2 reptilian species 20 fecal samples were taken and all (100%) were positive to *Blastocystis sp.* Green-winged macaw *Ara chloropterus* and Chital *Axis axis* show high prevalence (100%) of *Blastocystis Sp*, while Agile wallaby *Macropus agilis* show low prevalence rate (7.69) of *Blastocystis sp* among all animals (Table 1).

100 fecal samples of avian and mammalian species were collected from TWEC. Seventy (70%) tested positive for *Blastocystis sp.* 7 avian species fecal sample were examine out of which 40 (57%) were positive for the *Blastocystis sp.* All 30 mammalian fecal samples of 3 mammalian species were positive for *Blastocystis sp.* The Bacterian deer *Cervus elaphus bactrianus* show high prevalence rate (60%) while Anglo-Nubian *Capra aegagrus hircus* have low prevalence (7.6) of *Blastocystis sp* in captive animals of TEWC(Table 2)

Blastocystis has been reported in many parasite surveys of animals in zoological gardens, especially in non-human primates while researches of *Blastocystis* in domestic animals have also revealed high frequency of infection (Abe *et al*., 2003). Additionally, people with close animal contact were found to have a higher prevalence of *Blastocystis* infection (Salim *et al.*, 1999) and some zoo primate keepers have been found to be infected with STs or ST alleles that are otherwise rare in humans but common in the monkeys (Stensvold *et al.*,2012). *Blastocystis sp* show low occurrence (2.1%) in animals of zoo in Malaysia (Lim *et al.*,2008). Although the occurrence is low, the zoonatic potential of *Blastocystis sp* should not be underestimated as Rajah *et al*.,(1999) reported the possibility of these protozoa being transmitted to humans or other animals as shown in their study where animal handlers at the zoo were reported to be infected with *Blastocystis sp.*

During the present study *Blastocystis* appeared to be a polymorphic protozoan, with high prevalence in captive animals is due to poor hygiene, consumption of contaminated food and water.

Conclusion

290 of the 350 collected samples were positive for the *Blastocystis sp.* which is a threatening sign for the animals inhabiting zoo regarding the pathogenic role of *Blastocystis sp.* Further molecular studies are essential to be conducted in order to have a better and clear understanding on the reservoir hosts and origin of Blastocystis infection among animals.

Water contamination has been identified as a source of transmission, so water resources used for animals should be screened for *Blastocystis sp*

Table 1: Prevalence of *Blastocystis sp*. in different animals at Karachi Zoological Garden.

S. No.	Host	No. of Animals in Karachi Zoological Garden	No. of animals Examined	Infected Animals	*Blastocystis sp.*	Prevalence (%)
1.	*Testudo horsfieldii*	30	17	15	25	68%
2.	*Testudo graeca*	15	10	07	12	83.3%
3.	*Dromaius* novaehollandiae	02	02	02	03	66.6%
4.	*Ara chloropterus*	05	02	02	02	100%
5.	*Struthio camelus*	04	04	-	-	-
6.	*Cyanoliseus patagonus*	06	03	03	11	27.3%
7.	*Pavo cristatus*	05	05	04	09	55.5%
8.	*Cacatua alba*	04	02	02	02	100%
9.	*Macropus agilis*	01	01	01	13	7.69%
10.	*Elephas maximus*	02	02	02	06	33.3%
11.	*Camelus bactrianus*	02	02	02	06	33.3%
12.	*Panthera tigris tigris*	03	02	-	-	-
13.	*Antilope cervicapra*	10	05	-	-	-
14.	*Puma concolor*	02	02	02	07	28.6%
15.	*Axis axis*	12	10	08	10	100%
16.	*Prionailurus viverrinus*	03	03	03	06	50%
17.	*Canis aureus*	05	04	03	08	50%
18.	*Hystrix indica*	02	02	02	16	12.5%
19.	*Felis chaus*	02	02	-	-	-
20.	*Equus ferus caballus*	01	01	01	08	12.5%
21.	*Boselaphus tragocamelus*	04	02	02	10	20%
22.	*Macaca mulata*	04	04	04	08	22.2%
23.	*Hyaena hyaena*	02	02	02	10	20%
24.	*Panthera leo krugeri*	02	02	02	06	33.3%
25.	*Equus quagga*	02	02	02	16	12.5%

Table 2: Prevalence of *Blastocystis sp*. in different animals at Wildlife Experience Centre

S. No.	Host	No. of Animals in Karachi Zoological Garden	No. of animals Examined	Infected Animals	*Blastocystis sp.*	Prevalence (%)
1.	*Ara ararauna*	04	04	02	25	16%
2.	*Phasianus colchicus*	02	02	02	25	8.6%
3.	*Psittacus erithacu*	04	04	-	-	-
4.	*Gallus gallus domesticus*	06	06	06	12	50%
5.	*Numida meleagris*	03	03	-	-	-
6.	*Pavo cristatus*	04	04	02	10	40%
7.	*Cyanoliseus patagonus*	01	01	-	-	-
8.	*Capra aegagrus hircus*	02	02	02	26	7.6%
9.	*Cervus elaphus bactrianus*	06	06	04	10	60%
10.	*Canis lupus familiaris*	04	04	03	19	21%

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