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HISTOPATHOLOGICAL IDENTIFICATION OF HISTOPLASMOSIS IN ANIMALS AT DHAKA ZOO

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ABSTRACT

Dhaka Zoo with 2000 animal heads of 184 species from significant genetic diversity and five million visitors' influx round the year is placing it a public health important spot. This study was conducted to investigate histoplasmosis in animals at Dhaka Zoo to ascertain animal health, welfare and public health safety standard. One hundred and two necropsied tissue samples preserved in 10% neutral buffered formalin at necropsy from 36 animals of 25 different species were collected from Dhaka Zoo during 2007 - 2009. Twenty five out of 36 study animals were suffering from granulomatous diseases of mycotic and/or bacterial origin. Among them three animals were found suffering from histoplasmosis. Clinical history, nodular lesions from necropsy findings, granulomatous reactions with fungal spores on histopathology; macrophages laden with histoplasma organisms on histopathology and on special staining were revealed histoplasmosis in six rhesus macaques (*Macaca mulatta*). Present study provides evidence of existing histoplasmosis and similar long standing zoonotic diseases in majority of rest of the animals with health risk that shades health safety standard at Dhaka Zoo.

Key words: Histoplasmosis, Granulomatous, Health safety, Dhaka zoo.

INTRODUCTION

Zoo is popularly called living laboratory and knowledge generation center for wildlife implying both in-situ and ex-situ (WAZA, 2005; Ahasan and Azam, 2007). Genetic, species, subspecies and population diversity shaped zoos an environmental hot spot and sourcing of disease producing microorganisms and predispose cross infection of closely related animals. Around 150 emerging and re-emerging infectious diseases are originating from or harbored in wildlife round the globe (OIE, 2000; Lisle *et al.*, 2002) while 600 million visitor influxes each year in zoos of the whole planet (Dollinger, 2006). Considering the biological diverse range of wildlife, multiple disease susceptibility and huge visitor influx confirms zoos as an important and obligated public health concern arena (O'Reilly and Daborn, 1995; OIE, 2000; Tribe, 2004 and WAZA, 2005). Among others, bacterial and fungal diseases are most prevailing infections in zoo collections (Rahman and Ahasan, 2006 and 2007). The worst infectious diseases considered are mycobacteriosis and mycosis; both the groups posesses seriously zoonotic, curving the animal welfare and public health standard (WAZA, 2003; Salem and Rowan, 2001) of zoos in calling it modern zoo

Since its inception, zoo is a potential source of plague, tuberculosis, herpes virus B (hepatitis), rabies, Marburg virus, fungus and parasitic worm among others (Renquist and Whitney, 1978; Gary *et al.*, 2003). A recent threat has come up with West Nile and hanta viruses (Gary *et al.*, 2003). Dhaka zoo has a prevalence of mycobacteriosis, salmonellosis, colibacillosis, coccidioidomycosis and those are diagnosed only on the basis of clinical history and necropsy records (Rahman and Ahasan, 2006 and 2007).

Histoplasma thrives in soil that is enriched with bat or bird droppings, the disease are also called Ohio River valleys fever (James *et al.*, 2007). Infection with *Histoplasma* is common, but the disease is rare**.** Histopathological findings of histoplasmosis are infiltration of epitheloid cells and macrophages containing yeast-like bodies of organisms in the cytoplasm, irregularly-egg shaped fungi reproduced tiny buds, the centrally spherical shaped and basophilic body is surrounded by a thin cell wall with a false capsular appearance, the organism appeared as an empty red ring; formation of multinucleated giant cells, macrophage and reticulo-endothelial cells laden with yeast form of the organism, clear bordering stained ring encircling the organism (Jones *et al.,* 1997). Histoplasmosis was reported in dogs, cats, and non-human primates, human including many zoo animals (Mackinnon *et al.*, 2000; *Betty et al.,* 2001*;* MedExpert, 2007; James *et al.*, 2007).

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However, these diseases were not investigated in Dhaka Zoo by histopathology or molecular means before this study except apprehension from necropsy lesions. Therefore, the present study was undertaken to investigate the prevalence and clinico-pathological changes of histoplasmosis in animals at Dhaka Zoo to apprehend public health safety standard prevailed here.

MATERIALS AND METHODS

The research work was conducted in the Department of Pathology, Bangladesh Agricultural University (BAU), Mymensingh-2202 and Dhaka Zoo, Bangladesh in 2009. A total of 102 necropsied formalin-fixed tissue specimens, almost all nodular lesioned vital organs, from 36 animals of 25 different species at necropsy from Dhaka Zoo captivity were investigated clinico-histopathologically and special staining. Before necropsy, well recorded clinical history was noted down. The study comprised of eight rhesus macaques (*Macaca mulatta*), four spotted deers (*Cervus axis*/*Axis axis*), two samber deers (*Cervus unicolor*), two golden pheasant (*Chrysolophus pictus*), while guineapig (*Cavia porcellus*), wildebeest (*Connochaetes taurinus*), striped hyena (*Hyena hyena*), Indian/Asiatic lion (*Panthera leo persica*), gayal (*Bos frontalis*), American rhea (*Rhea americana*), Australian terrier dog (*Canis lupus familiarizes*), zebra (*Equus zebra hartmannae*), nilgai (*Boselaphus tragocamelus*), horse (*Equus caballus*), barking deer (*Muntiacus muntjak*), ostrich (*Struthio camelus*), crested serpent-eagle (*Spilorins cheela*), common languor (*Presbytis entellus*), fishing cat (*Felis viverrina*), beisa oryx (*Oryx beisa beisa*), reticulated python (*Python molurus*), water buck (*Kobus L. leche*), greater kudu (*Tragelaphus strepsiceros*), and olive baboon (*Papio anubis*) were each single sampled animals. The animal belongs to non-human primates (n=10), carnivores (n=4), herbivores (n=16), reptiles (n=1) and birds (n=5) groups.

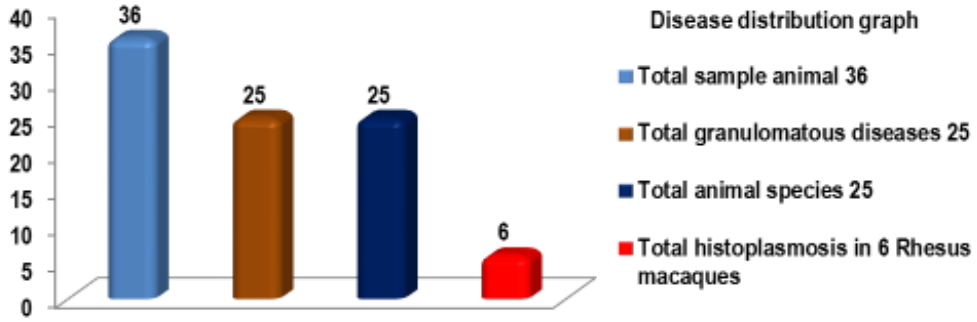
At necropsy, tissue changes were recorded; photographed and lesioned tissues of all vital organs with special attention to nodular lesions were preserved in 10% neutral buffered formalin. Formalin fixed samples were processed for paraffin embedding, sectioning and staining as routine hematoxylin and eosin staining (Luna, 1968), periodic acid Schiff (PAS) for fungus according to standard method of histopathological study (Mallory, 1968).

Photomicrograph was taken at the Department of Pathology and Field Fertility Clinic of the Department of Surgery and Obstetrics, Bangladesh Agricultural University, using photomicrographic camera (Olympus PM-C 35 Model) and Digital Camera Mounted Photomicrographic device (Diffrential Interference Contrast – DIC) (Olympus, Nizol FC, E-5000, 8.4V, 0.9A, CE N 150) respectively.

RESULTS AND DISCUSSION

Twenty five out of 36 investigated animals were suffering from granulomatous (about 70%) diseases (Graph 1) while six rhesus macaques were identified infected with histoplasmosis first ever in Bangladesh (Table 1). Clinically, the disease was found almost asymptomatic except less prominent coughing, anorexia and emaciation; weaker form of occasional weakness followed by death. Infrequently, necropsy unearthed findings of tiny white minute to large nodulation, cavitation, caseation, and suppuration and blackish to greenish discoloration of the organs (Figure 1 and 2). Routine histopathology depicted granulomatous reactions and fungal spores (Figure 3). Multifocal to diffuse and severe form of granuloma with formation of both Langhang's and foreign body giant cells with epitheloid cells and macrophages containing yeast-like, irregular shaped basophilic body in the cytoplasm were noted (Figure 3 to 5). Limited calcification and with or without encapsulation (Figure 3) were also noticed on histologic focuses. Granuloma with larger sized giant cells of both Langhang's and foreign body type in same focus was significant findings on histopathology that was masking the tissue architecture (Figure 5). Centrally spherical shaped and basophilic body is surrounded by a thin cell wall with a false capsular appearance, the organism appeared as an empty red ring, macrophage and reticulo-endothelial cells laden with yeast form of the organism, clear bordering stained ring encircling the organism by PAS were noted too (Figure 6).

Clinical history noted in this study was almost similar to other works without unnoticeable degree of variation. The disease found in this study was almost asymptomatic except less prominent coughing, anorexia and emaciation; weaker form of occasional weakness followed by death. Yellow white tiny to large nodules on visceral organs at necropsy was typical to findings of other researches (Lisle *et al*., 2002). Frequently, moreover, non-correspondence to granulomatous reaction with nodular lesions was observed.



Graph 1. Distribution of diseases in zoo animals

Table 1. Distribution of diseases among animal species

Sample animal	Scientific name of sample animal	Granulomatous reactions	Histoplasmosis
Rhesus macaque - 6	*Macaca mulatta*	+	+
Rhesus macaque - 2	*Macaca mulatta*	+	-
Spotted deer - 3	*Cervus axis/Axis axis*	+	-
Samber deer - 2	*Cervus unicolor*	+	-
Nilgai - 1	*Boselaphus tragocamelus*	+	-
Horse - 1	*Equus caballus*	+	-
Stripped Hyena - 1	*Hyena hyena*	+	-
Gayal - 1	*Bos frontalis*	+	-
Common langur - 1	*Presbytis entellus*	+	-
Beisa oryx - 1	*Oryx beisa beisa*	+	-
Water buck - 1	*Kobus L. leche*	+	-
Greater Kudu - 1	*Tragelaphus strepsiceros*	+	-
Reticulated python - 1	*Python molurus*	+	-
Wilde beest - 1	*Connochaetes taurinus*	+	-
Crested serpent-eagle - 1	*Spilorins cheela*	+	-
Olive baboon -1	*Papio anubis*	+	-

Most literature suggests the presence of foreign body giant cells in case of mycosis with the exception of findings by Jones *et al.* (1997b) that suggests formation of both Langhan's and foreign body giant cells in case of blastomycosis only while present investigation showed presence of both type of giant cells with huge number of Langhan's type giant cells than foreign body.

Histoplasmosis was investigated and revealed in rhesus macaque of Dhaka zoo which is similar to other works (James *et al*., 2007). Macrophage laden with histoplasma organism was significant findings on H&E and PAS in this study which is similar with the findings of Jones *et al.* (1997). In this study, histoplasmosis was not found as individual infection rather concurrent with other granulomatous reactions.

Fig. 1: Liver of rhesus macaque, numerous nodules and swollen liver

Fig. 2: Lung of rhesus macaque, numerous nodules and blackish coloration

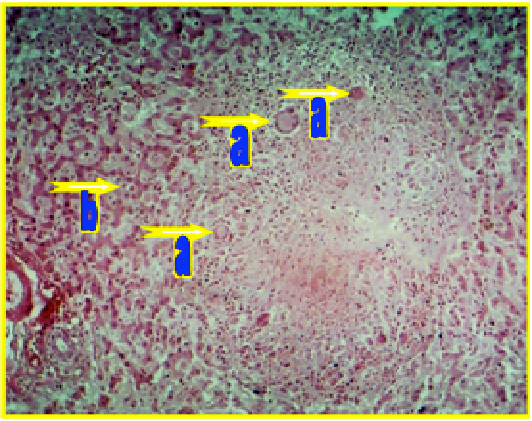


Fig. 3: Spleen of rhesus macaque, formation of granuloma and huge number of giant cells (a), fungal spores (b), multifocal to diffuse, severe, granulomatous mycotic splenitis, H&E, × 82.5

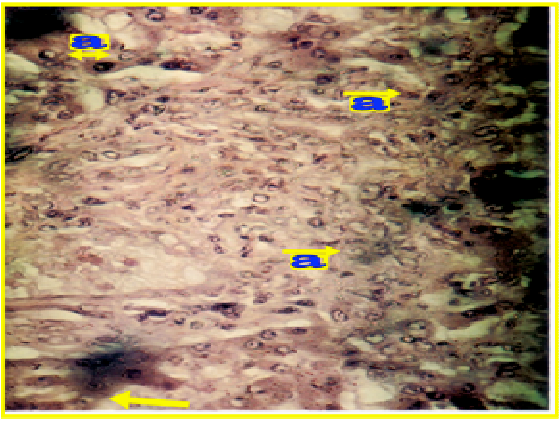


Fig. 4: Liver of rhesus macaque, macrophages laden with histoplasma organisms (a), giant cell, mycotic hepatitis, Histoplasmosis, H&E, × 330.

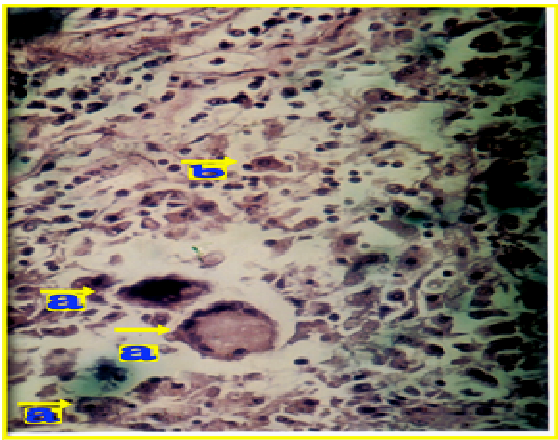


Fig. 5: Lung of rhesus macaque, formation of huge number of LH ad FB giant cells (a), macrophages laden with histoplasma organism (b) severe, chronic mycotic, pneumonia, histoplasmosis ,H&E, × 330

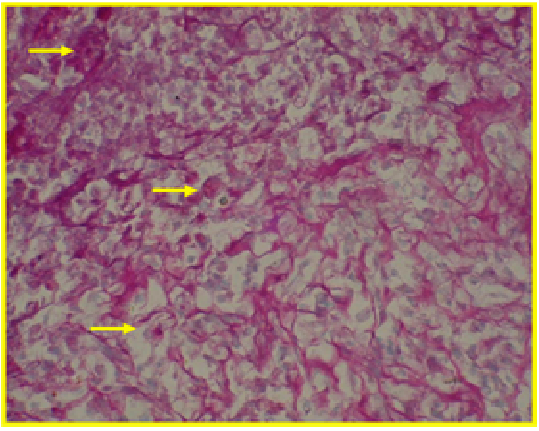


Fig. 6: Liver of rhesus macaque, macrophages laden with yeast form of organisms having clear bordered stained ring, histoplasmosis, PAS, × 400

Noticeably zoonotic histoplasmosis may invade Dhaka zoo through importation of animals from endemic area, dusty storm, having eucalyptus tree and pigeon drooping, bird nesting and immunosuppression. However, further studies should be focused on typing and molecular characterization of histoplasmosis.

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