HISTOPATHOLOGICAL INVESTIGATION OF CRYPTOCOCCOSIS 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 cryptococcosis in animals at Dhaka Zoo to ascertain animal health, welfare and public health safety standard. One hundred and two opportunistic tissue samples were collected and preserved in 10% neutral buffered formalin at necropsy for 36 animals of 25 different species from Dhaka Zoo during the study period. Twenty five among the study animals were found suffering from granulomatous diseases, of them nine cases were identified cryptococcosis first ever in Bangladesh. Clinical history, nodular lesions on necropsy findings, granulomatous reactions with fungal spores & both Langhang’s & foreign body giant cells on histopathology and characteristic spores with wide gelatinous band around endospores on special staining revealed cryptococcosis in eight rhesus macaques (Macaca mulatta) and one greater kudu (Tragelaphus strepsiceros). Present study provides evidence of existing cryptococcosis 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: Cryptococcosis, Granuloma, Immunosuppresion, Dhaka Zoo

INTRODUCTION

Zoo is popularly called living laboratory and knowledge generation center for wildlife implying both in-situ and ex-situ (WAZA, 2005 and Ahasan and Azam, 2007). Genetic, species, subspecies and population diversity shaped zoos an environmental hot spot and sourcing of disease producing microorganisms that predispose cross infection of closely
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related animals. Around 150 emerging and re-emerging infectious diseases are originating from or harbored in wildlife round the globe (OIE, 2000 and 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. The worst infectious diseases considered are mycobacteriosis and mycosis; both the groups possess seriously zoonotic, curving the animal health, welfare and public health standard (WAZA, 2003) of zoos in calling it modern zoo. Even then, zoos are inseparable from studying biodiversity and environment, public health and knowledge generation center (WAZA, 2005) for mankind. It is also limited with different social conflicts, health-hazards, management, behavioral menace and so forth (Salem et al., 2001).

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 and 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).

Cryptococcus gattii has recently emerged as a primary pathogen of humans, wild and domesticated animals, life-threatening diseases of the pulmonary and central nervous systems and can be diagnosed by histopathology (Sarah et al., 2007; MacDougall and Fyfe, 2006). The causative agent of cryptococcosis is gelatinous capsulated saprophytic ubiquitous yeast with two varieties Cryptococcus neoformans and Cryptococcus gattii. The former is cosmopolite found in temperate climate regions, frequently isolated from bird feces. On the other hand, the variety gattii frequently reported in tropical areas and isolated mainly from vegetable tissues (Montenegro and Ocorrencia, 1998). Cryptococcosis was reported in all domestic and wild animals and zoo collections including human, while dogs are reported highly susceptible (Kaufman and Blumer, 1978; Montenegro and Ocorrencia, 1998; MacDougall and Fyfe, 2006 and Sarah et al., 2007).

However, these diseases were not investigated in Dhaka zoo except apprehension out of necropsy findings. Therefore, the present study was undertaken to investigate the prevalence of cryptococcosis and to investigate the clinico-pathological changes of cryptococcosis 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. A total of 102 opportunistic formalin-fixed (10% neutral buffered formalin) tissue specimens collected from 36 animals of 25 different species at necropsy of Dhaka zoo captivity during the study
period were investigated clinico-histopathologically and by special staining. The study comprised of eight rhesus macaques (*Macaca mulatta*), four spotted deers (*Cervus axis*), two samber deers (*Cervus unicolor*), two golden pheasant (*Chrysolophus pictus*), while guineapig (*Cavia porcellus*), wildebeest (*Connochaetes taurinus*), striped hyena (*Hyaena 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 (*Spiorotis 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 of 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. Clinical signs were recorded individually during entire study period.

At necropsy, tissue changes were noted; 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) and periodic acid Schiff (PAS) (McManus, J. F. A., Stain technique 23 : 99 – 108, 1948 AIFP modification) 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 (Differential 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 found suffering from granulomatous diseases (about 70%) (Graph 1). Cryptococcosis was noticed in eight rhesus macaques and one greater kudu; first ever identified in Bangladesh. Clinically, affected animals showed frequent coughing and less prominent anorexia, emaciation with occasional weakness followed by death. Necropsy unearthed findings of tiny yellow-white minute to large nodulations (7–18 mm diameter), cavitations, caseations, and suppurations and blackish to greenish discolorations of the affected organs (Fig. 1 and 2).

Routine histopathology depicted granulomatous reactions; limited calcification and with or without encapsulation (Fig. 3). Multifocal to diffuse and severe form of granuloma with formation of both Langhang’s and foreign body giant cells infrequently in same focus along with fungal spores (Fig. 3 and 4) were pertinent.
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Clinical history noted in this study was almost similar to other works; and those were with unnoticeable degree of variation. Yellow white tiny to large nodules on visceral organs at necropsy was typical to findings of other researches (Lisle et al., 2002). Infrequently, non-correspondance to granulomatous reaction with nodular lesions was also observed.

Most literature suggest the presence of foreign body giant cells in case of mycosis with the exception of findings by Jones et al. (1997) that suggests formation of both Langhan’s and foreign body giant cells in case of blastomycosis while present investigation showed presence of both type of giant cells with huge number of Langhan’s type giant cells than foreign body.
This study identified cryptococcal infection in rhesus macaques which is similar with other findings (Jones et al., 1997; Sarah et al., 2007; MacDougall and Fyfe, 2006 and Larsson, 2000). Cryptococcal endospores were identified through H&E and PAS with the presence of organisms in tissues and in macrophages as ovoid to spherical, thick-walled, yeast-like bodies which are surrounded by wide, gelatinous capsule seen as blank band similar with findings of Jones et al. (1997).
CONCLUSIONS

Noticeably zoonotic cryptococcosis 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 cryptococcal organisms. The organism possesses serious public health consequences and therefore, prevention and control of these diseases in Dhaka zoo should brought under notice to ensure health safety standard.

REFERENCES


