Progress. Agric. 24(1 & 2): 123 – 129, 2013 ISSN 1017-8139

HISTOPATHOLOGICAL INVESTIGATION OF CRYPTOCOCCOSIS IN ANIMALS AT DHAKA ZOO

S. A. Ahasan[1, 2], E. H. Chowdhury[1], M. A. H. Khan[1], R. Parvin[1, 3], S. U. Azam[2, 4], G. Mohiuddin[2], J. Uddin[2], M. M. Rahman[2], L. Akter[2] and A. Khair[5]
Department of Pathology, Bangladesh Agricultural University
Mymensingh-2202, Bangladesh

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

[1]Department of Pathology and [5]Department of Medicine; Bangladesh Agricultural University, Mymensingh-2202, Bangladesh
[2]Department of Livestock Services, Bangladesh; Krishi Khamar Sarak, Farmgate, Dhaka-1215, Bangladesh
[3]Institute of Virology, University of Leipzig, An den Tierkliniken 29, 04103 Leipzig, Germany
[4]Monash University, Gippsland Campus, Victoria-3842, Australia
Corresponding author (Email: ahasan67@gmail.com)

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 possesses 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 gatii frequently reported in tropical areas and isolated mainly from vegetable tissues (Montenegro and Ocorrencia, 1998). Crypotococcosis 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 collcted 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/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 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.

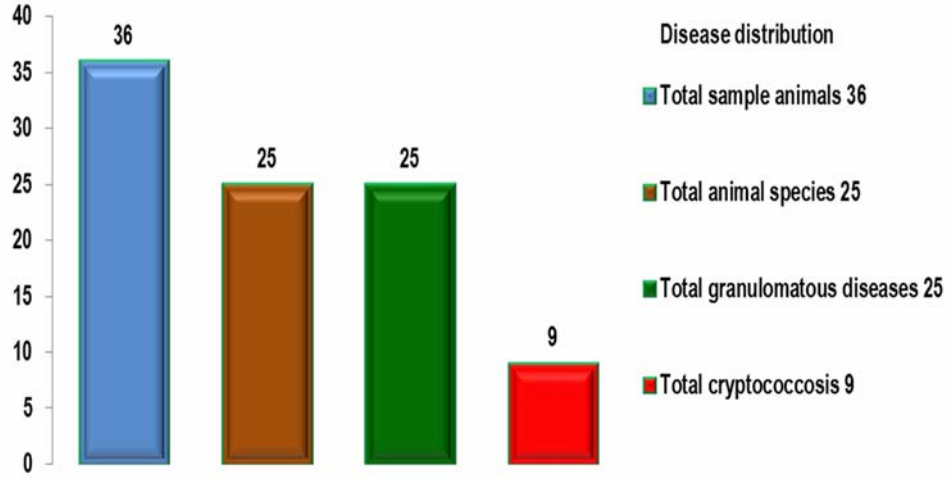
At necropsy, tissue changes were noted; photographed and lesioned tissues of all vital organs with special attention to nodular lesions were preserved in 10% neutral buffered formalin. Formalin fixed samples were processed for paraffin embedding, sectioning and staining as routine hematoxylin and eosin 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 (Diffrential Interference Contrast – DIC) (Olympus, Nizol FC, E-5000, 8.4V, 0.9A, CE N 150) respectively.

RESULTS AND DICUSSION

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.



Graph 1: Distribution of diseases in animals studied

Fig. 1: Lung (parietal view) of rhesus macaque, numerous nodules

Fig. 2: Liver of rhesus macaque, larger nodules and greenish discoloration

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.

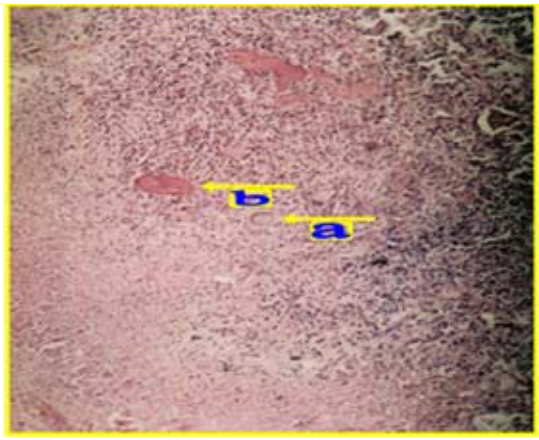


Fig. 3: Lung of rhesus macaque, granuloma and formation of Langhan's giant cells (a), congestion (b), fungal spores, multifocal to diffuse, severe, multifocal granulomatous mycotic pneumonia, H&E, × 82.5

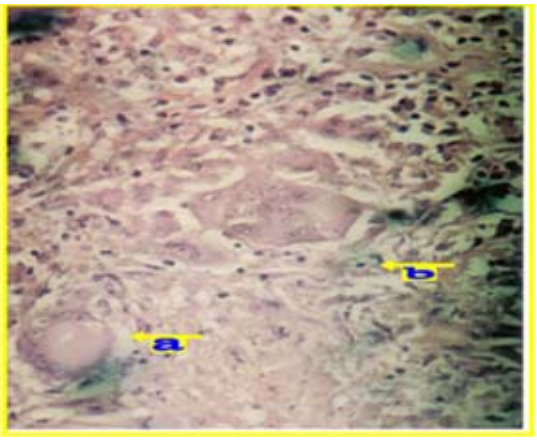


Fig. 4: Liver of rhesus macaque, formation of Langhang's giant cells (a), wide band capsule arround endospores (b), chronic mycotic hepatitis, Cryptococcosis, H&E, × 330.

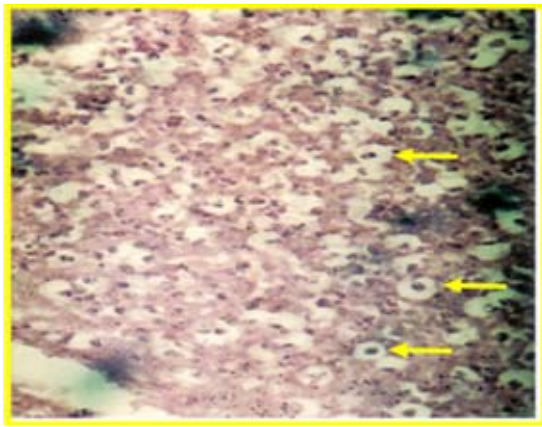


Fig. 5: Liver of rhesus macaque, organisms as ovoid to spherical, thick-walled, yeast like bodies surrounded by wide gelatinous capsule seen as blank band, mycotic hepatitis, cryptococcosis, H&E, × 330.

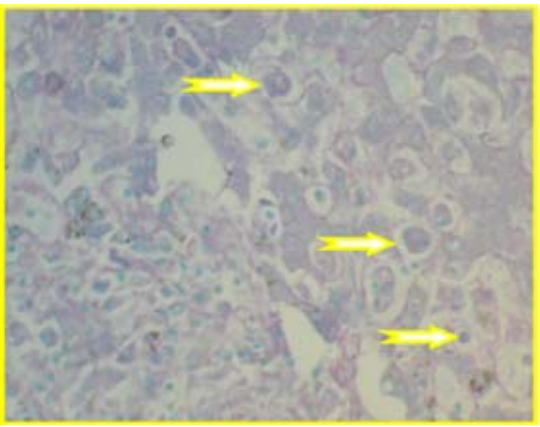


Fig. 6: Liver of rhesus macaque, organisms as ovoid to spherical, thick-walled, yeastlike bodies surrounded by wide gelatinous capsule seen as blank band, Cryptococcosis, PAS, ×200

This study identified cryptococcosis 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 was identified through H&E and PAS with the presence of organisms in tissues and in macrophages as ovoid to spherical, thick-walled, yeastlike bodies which are surrounded by wide, gelatinous capsule seen as blank band similar with findings of Jone *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

Ahasan, S. A. and Azam, S. U. 2007. Contribution of Zoological Gardens towards conservation of Wildlife and Biodiversity - Bangladesh perspective. *Zoos' Print Magazine*, 22: 13-16.

Dollinger, P. 2006. Marketing the conservation role of zoos' and aquariums. *Zoos' Print Magazine,* 21**:** 17.

Gary, W., Thomas D. L., Kurt, V. and Peter B. 2003. Mycobacterial diseases in wildlife; wildlife damage management, Internet center for USDA National Wildlife Research Center, *Staff Publication, University of Nebraska, Lincon.*

Jones, T. C., Hunt, R. D. and King, N. W. 1997. Diseases caused by fungi in *Veterinary Pathology,* 6th edn., Williams and Wilkins. 8: 506-522.

Kaufman, L. and Blumer, S. 1978. Cryptococcosis, the awakening giant in: The black and white yeasts. *International Health Organization Science Publishing,* 356.

Larsson, C. E. 2000. Criptococose. ***In****: Simpósio Brasileiro Sobre Micoses Animais, Porto Alegre* (Brasil), 7: 72.

Lisle, D. G. W., Bengis, R. G., Schmitt, S. M. and O'Brien, D. J. 2002. Tuberculosis in freeranging wildlife: detection, diagnosis and management. *Revue Scientifique at Technique des Office International des Epizooties,* 21: 317-334.

Luna, L. 1968. Manual of histologic staining methods of the *Armed Forces Institute of Pathology. New York.*

MacDougall L. and Fyfe, M. 2006. Emergence of Cryptococcus gattii in a novel environment provides clues to its incubation period. *J Clin Microbiol.* 44: 1851-62.

Mallory, F. B. 1968: Pathological Technique, New York, *Hafner Publishing Co.* pp. 275. AFIP modification.

Montenegro, N., and Ocorrência D. V. D. 1998. *Cryptococcus neoformans* na Cidade de São Paulo (Brasil): *isolamento em fezes de pombo e em bosques de eucalipto. São Paulo (Brasil)*: 89.

Office International des Epizootic (OIE) 2000. Zoonoses transmissible from non-human primates. Chapter 2.10.1. *In International animal health code, 9th Ed. OIF, Paris,* 285-290.

O'Reilly, L. M., and Daborn, C. J. 1995. The epidemiology of *Mycobacterium bovis* infections in animals and man. *A review, Tubercle and Lung Disease, Supplement 1, August*. 76: 1-46.

Rahaman, A. Z. and Ahasan, S. A. 2007. Mortality in Dhaka Zoo due to microbial agents. *Bangladesh J Microbiol.,* 24: 154-156.

Rahman, A. Z. and Ahasan, S. A. 2006. Spotted deer (*Cervus axis)* herd in Dhaka Zoo: case report. *Zoos' Print Magazine,* 21-23.

Renquist, D. M. and Whitney, R. A. 1978. Tuberculosis in non-human primates - an overview. In Proc. Symposium on mycobacterial infections in zoo animals (R.J. Montali, ed.), 6-8 October 1976, Front Royal, Virginia. *Smithsonian Institution Press*, Washington, DC, 9-16.

Salem, D. J., Rowan and Andrew, N. 2001. The State of the Animals: Humane Society Press, Gaithersburg, Maryland, Chapter 9: Hancocks, David, "Is There a Place in the World For Zoos?" 137-144; Farinato, Richard. *Another View of Zoos*, pp. 145-147.

Sarah, E. K., Yat, C., Sunny, M., Paxton, J. B., Huiming, C., Adrian, O., Hingston, J. W., Kronstad and Karen, H. B. 2007. Characterization of Environmental Sources of the Human and Animal Pathogen *Cryptococcus gattii* in British Columbia, Canada, and the Pacific Northwest of the United States. *Applied and Environmental Microbiology,* 73**:** 1433-1443.

Tribe, A. 2004. "Zoo Tourism", in Wildlife Tourism: Impacts, management and planning. Higginbottom, Karen (ed.). *Common Ground Publishing*, Altona, Victoria, 35-56.

World Association of Zoos and Aquaria (WAZA), 2003. Code of ethics and animal welfare. *Proceedings of the 58th Annual Meeting, San Joe's, WAZA Executive Office*, Berne, 58: 139-142.

World Association of Zoos and Aquaria (WAZA), 2005. Building a future for wildlife. *The World Zoo and Aquarium Conservation Strategy*, pp. 74.