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Histopathological identification of Coccidioidomycosis in animals at Dhaka Zoo

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Abstract

Dhaka Zoo with 2000 animals of 184 species and five million visitors a year is important from a public health point of view. This study was conducted to investigate coccidioidomycosis in captive animals at Dhaka Zoo. One hundred and two tissue samples were collected and preserved in 10% neutral buffered formalin at necropsy of 36 animals of 25 species. Twenty five animals were suffering from granulomatous diseases, of which ten were identified as coccidioidomycosis. Clinical history, nodular lesions at necropsy, granulomatous lesions on histopathology and characteristic spores on special staining confirmed coccidioidomycosis in six rhesus macaques (*Macaca mulatta*), one horse (*Equus caballus)*, one common langur (*Presbytis entellus*), one beisa oryx (*Oryx beisa beisa*) and one reticulated python (*Python molurus*). It is suggested that coccidioidomycosis in captive animals threatens human and animal health. (*Bangl. vet*. 2013. Vol. 30, No. 2, 54 – 61)

Introduction

Zoos are educational centres for wildlife, but can be sources of infectious diseases and cause infection in closely related animals (OIE, 2000; Lisle *et al.,* 2002; WAZA, 2005; Ahasan and Azam, 2007). About 600 million visitors go to zoos each year (Dollinger, 2006) which makes them a matter of public health concern (O'Reilly *et al*., 1995; OIE, 2000; Tribe, 2004; WAZA, 2005). The worst infectious diseases include mycobacteriosis and mycosis; both groups are zoonotic (WAZA, 2003). The role of zoos has been questioned (Salem *et al*., 2001). Zoos can be a potential source of plague, tuberculosis, herpes virus B, rabies, Marburg virus, fungus and parasitic worms, with recent threat of West Nile and hanta viruses (Renquist and Whitney, 1978; Gary *et al*., 2003). Dhaka zoo has coccidioidomycosis and other bacterial diseases diagnosed from clinical and necropsy records (Rahman and Ahasan, 2006; 2007).

Coccidioidomycosis was first recognized as a human disease in Argentina, and is also a disease of animals (Giltner, 1918). Since then, it has been reported in both wild animals in captivity and free range and domestic animals; non-domestic species

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include several species of non-human primates, aardvark (*Orycteropus afer*), badger (*Meles meles*), cheetah (*Acinonyx jubatus*), coyote (*Canis latrans*), kit fox (*Vulpes macrotis mutica*), tiger (*Panthera tigris*) and reptiles (Pappagianis, 1988). It is highly infectious but not contagious (Kohn *et al.,* 1992; Jones *et al.,* 1996 and Greene, 2006). The causative agents of coccidioidomycosis are two morphologically identical species within a single genus, *Coccidioides immitis* and *C. posadasii*. Both are dimorphic ascomycetes with a free-living, soil-inhabiting saprophytic phase, which forms fungal mycelia of branching septate hyphae, and produce infectious arthrospores that survive in saline soil and in sea water; and a parasitic tissue-dwelling phase (Fisher *et al.*, 2001). The parasitic or tissue form is characterized by sporangia, containing endospores. Increased numbers of infections are reported in the autumn and winter, after crop harvesting, on animal burrows and when strong winds disturb the soil, especially on construction and archaeological sites or fossil beds, and disseminate arthroconidia (Maddy and Crecelius, 1960; Pappagianis, 1988; Rippon, 1988; Cox and Magee, 1998; Johnson *et al.*, 2003). Confirmation of diagnosis may require histopathology (Heinritz *et al.*, 2005). Sporangia and/or arthrospores are the pathognomonic histopathological findings (Maddy, 1957; 1965; Maddy and Crecelius, 1960; Rapley and Long, 1974; Rippon, 1988; Connor *et al*., 1997).

The disease was found in Dhaka Zoo from necropsy findings. The present study was undertaken to investigate the prevalence of coccidioidomycosis and the clinico-pathological changes in animals at Dhaka Zoo.

Materials and Methods

A total of 102 formalin-fixed tissue specimens from 36 zoo animals of 25 species were obtained at necropsy and examined histopathologically. The study included eight rhesus macaques (*Macaca mulatta*), four spotted deer (*Cervus axis/Axis axis*), two sambar deer (*Cervus unicolor*), and two golden pheasant (*Chrysolophus pictus*). Samples were also taken from guinea pig (*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 langur (*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*). The animals could be classified as non-human primates (n = 10), carnivores (n = 4), herbivores (n = 16), reptiles (n = 1) and birds (n = 5).

Recorded clinical history before necropsy was collected from the Zoo register. At necropsy, tissue changes were photographed and lesions tissues were preserved in 10% neutral buffered formalin. Formalin-fixed samples were processed for paraffin embedding, sectioning and staining with routine haematoxylin and eosin staining (Luna, 1968), periodic acid Schiff (PAS) for fungus according to Mallory (1968).

Photomicrograph was taken using Olympus PM-C 35 camera and Digital Camera Mounted Photomicrographic device (Differential Interference Contrast–DIC; Olympus, Nizol FC, E-5000, 8.4V, 0.9A, CE N 150).

Results and Discussion

Twenty five out of 36 investigated animals were suffering from granulomatous diseases (70%; Graph-1) while 10 were suffering from coccidioidomycosis: six rhesus macaques, one horse, one common langur, one beisa oryx and one reticulated python. Recorded history showed very weak condition, anorexia and emaciation with occasional coughing. Necropsy findings showed minute to large nodulation, cavitation, caseation, and suppuration and black to green discolouration of the organs (Fig. 1 and 2).

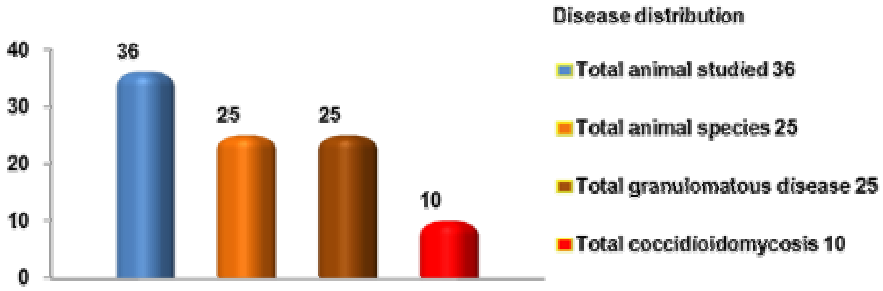

Fig. 1. Distribution of diseases in animals studied

Routine histopathology depicted typical granulomatous reactions with multifocal to diffuse and severe granuloma with formation of Langhans and foreign body giant cells, infrequently in same field of view (Fig. 3). Limited calcification with or without encapsulation (Fig. 3) were also found.

Coccidioides organisms are spherical yeast cells with a double contoured wall having a refractile central mass without budding (Fig. 4 and 6), spherules full of endospores, spherule septation (Fig. 5) and individual spores. The organisms were obtained in liver, spleen, lung, heart and kidney of all the affected animals, while pathognomonic arthrospores were found occasionally in some tissues in association with spherules and spores observed by PAS staining.

The disease has been reported in deer, tigers, snakes, squirrels, bison, gorillas, monkeys, dogs, cats, sheep, horses, lions, cheetah and reptiles, with a large variety of other zoo animals (Posada, 1892; Maddy, 1957; Maddy and Crecelius, 1960; Ajello, 1970a,b; Rapley and Long, 1974; Pappagianis *et al*, 1979, 1994; Pappagianis and Zimmer, 1992; Pappagianis 1980, 1988, 1993; David and Whitney, 1987; Rippon, 1988; Reed *et al.*, 1994; Cox and Magee, 1998; Jones *et al.*, 1996; Connor *et al.*, 1997; Adaska, 1999; Fisher *et al.*, 2001; DiCaudo and Connolly, 2001; Johnson *et al.*, 2003; Butkiewicz *et al.*, 2005; Heinritz *et al.*, 2005; Greene, 2006; Morrow *et al.*, 2007). Most cases of coccidioidomycosis reported in non-domestic species have occurred in zoos (Pappagianis, 1988). Coccidioidomycosis is primarily a respiratory disease of animals

and man with the mycelial form of the fungus entering the body via the respiratory tract, from which dissemination is thought to occur (Giltner, 1918). Recorded clinical history in the respiratory form of the disease includes coughing.

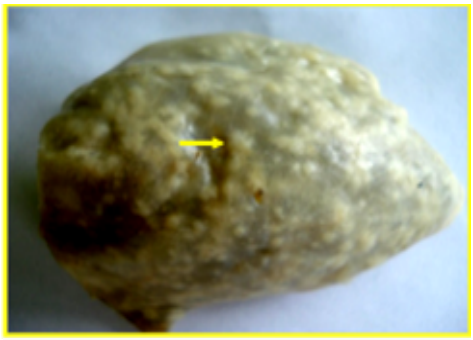

Fig. 1. Lung of rhesus macaque, presence of numerous tiny nodules

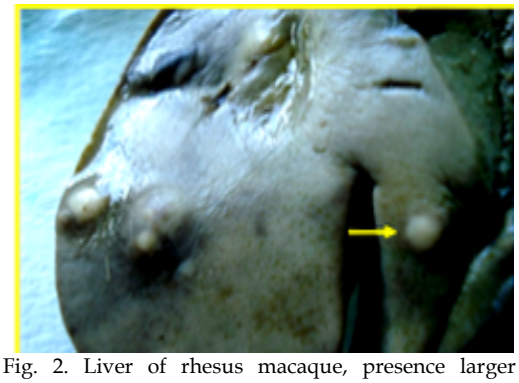

Fig. 2. Liver of rhesus macaque, presence larger nodules and greenish discoloration

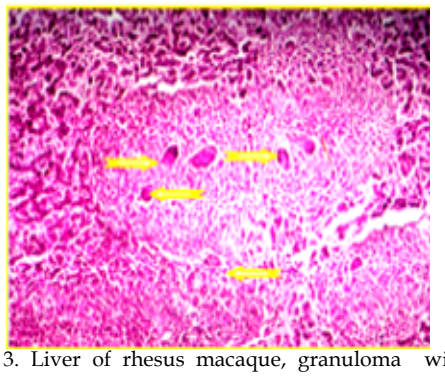

Fig. 3. Liver of rhesus macaque, granuloma with formation of huge number of LH giant cells presence of fungal spores, multifocal, severe, granulomatous mycotic hepatitis, H & E, × 82.5

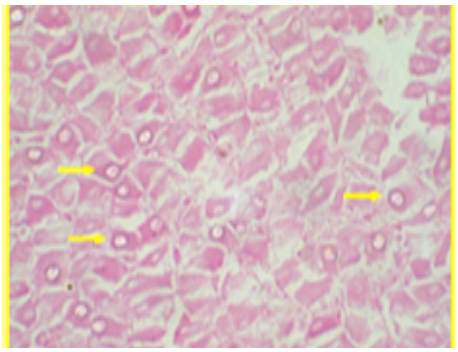

Fig. 4. Liver of rhesus macaque, spores with double-contoured wall (no budded, coccidioidomycosis, PAS × 400

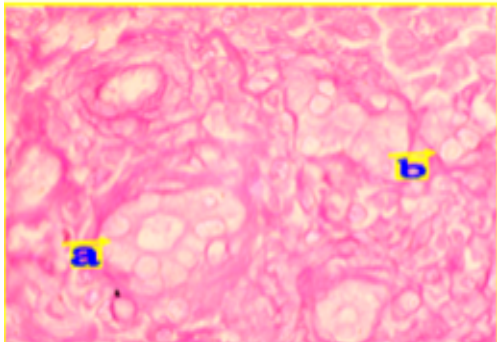

Fig. 5. Liver of rhesus macaque, endosporulated spherules (a), numerous spores and arthrospores (b) of coccidioides of different developmental stages, coccidioidomycosis, PAS × 600

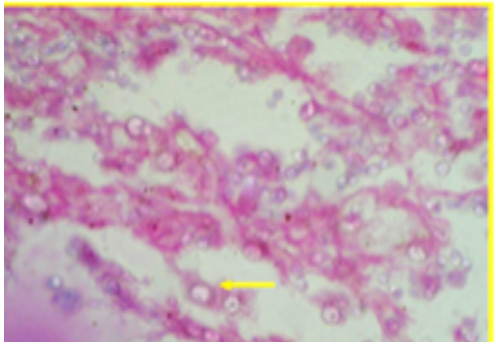

Fig. 6. Digestive tract of reticulated python end spores without budding, demonstrate double-contoured wall, coccidioidomycosis (*Coccidiodis imitis*), PAS × 200

Other clinical history of coccidioidomycosis noted was similar to earlier reports. Yellow white tiny to large nodules on visceral organs have been found at necropsy (Lisle *et al*., 2002), which is different from the present findings.

Foreign body giant cells in cases of mycosis have been reported by Jones *et al.* (1997) who suggested formation of Langhans and foreign body giant cells in case of blastomycosis. The present investigation showed both type of giant cells with a huge number of Langhans type giant cells.

Coccidioidomycosis in rhesus macaques is similar to other reports (Johnson *et al.,* 2003; Heinritz *et al.,* 2005; Greene, 2006; DiCaudo and Connolly, 2001). The disease in a horse at Dhaka zoo was comparable with that in other studies (Karen *et al*., 2003; Jubb *et al*., 1992; Jones and Hunt, 1983). Coccidioidomycosis was revealed in reticulated python, as seen by others (Jones *et al.,* 1996; Heinritz *et al.,* 2005; Pappagianis, 1988; Reed *et al.,* 1996). But the disease in *Beisa oryx* and *Common langur* has not been reported before. Numerous captive wild animals including primates, mountain gorilla, Bengal tiger and lions have been reported with coccidioidomycosis. Fungal conidia, arthrospores and spherules full of endospores without budding surrounded by epithelioid cells mixed with neutrophils and lymphocytes in H&E and PAS are comparable with the work of Jones *et al.* (1997) and Michele and Peggy (2006). Infective arthroconidia and arthrospores were found on PAS: this finding differs from reports by Kohn *et al.* (1992); Jones *et al.* (1996); Greene (2006) who confirm no arthroconidial stages in tissue level.

Conclusions

Coccidioides organism possesses serious public health consequences and therefore, prevention and control of this disease in Dhaka zoo is necessary to improve public health. However, further studies should be focused on typing and molecular characterization of coccidioidomycosis.

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