한국가축위생학회지 제35권 제1호 (2012)
Korean J Vet Serv, 2012, 35(1), 47-52
ISSN 1225-6552
Available online at http://kosves.re.kr

Korean Journal of
Veterinary Service

<Original Article>

Escherichia coli septicemia concurrent with mycotic infection in captive salt water crocodiles in Bangladesh

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(Received 25 August 2011; revised 9 March 2012; accepted 19 March 2012)

Abstract

Crocodile farms are getting popular in Bangladesh in an economic point of view. In one of the farms, some crocodiles were found sick and three of them died between May and July in 2006. This investigation was performed to diagnose the cause of the death. Routine postmortem examination was conducted. Samples were collected in 10% neutral buffered formalin for histopathology and in falcon tube for microbiological study. Additional swabs were collected in nutrient broth. Histopathological and microbiological studies were conducted using routine procedures. In addition Giemsa, Gram and PAS stains were performed to detect the organism in tissues. Grossly, esophagus, trachea, lungs, liver, spleen, heart and kidney were congested. Intestine, rectum and colon were hemorrhagic. Clay colored material was found in colo-rectum. Purulent exudates in lungs and thick and cloudy pericardial fluid in pericardial sac were found. Histologically, multifocal granulomatous inflammation was evident in lung, liver, kidney, intestine and colon with bacterial colonies, fungal spores and hyphae. These bacteria were appeared as Gram negative. Fungal hyphae and spores were detected in liver, lungs and colon by using PAS stain. Bacteriologically, *E. coli* were isolated from lungs exudates, pericardial fluids and intestinal fluids. Therefore, it can be concluded that 3 crocodiles died due to *E. coli* septicemia concurrent with mycotic infection.

Key words : Captive salt water crocodile, *E. coli* septicemia, Fatal, Mycotic infection

INTRODUCTION

Captive crocodile farms are getting popular worldwide in an economic point of view. Crocodile farms in Bangladesh were facing troubles due to high mortality. There are large numbers of agents that may cause mortality in captive crocodiles. *Mucor amphibiorum* a fungus was first reported as a cause of death in captive anurans in Europe (Frank et al, 1974). In natural and experimental infections it produces a disseminated mycosis (Frank et al, 1974; Frank, 1976). *M. amphibiorum* existed in tissues in a unique spherical from, which Frank et al (1974) called a sphaerule. Fatal mycotic pulmonary disease caused by *Beauveria bassiana* occurred in captive American alligators at the Oklahoma City Zoo (Fromtling et al, 1979). The entomopathogenic and fungus, *B. bassiana* was isolated from pulmonary lesions of a dead America alligator. Septate, branching hyphae and fungal spores were seen in stained histological sections of pleura and lung. Dissemination to other viscera had not occurred. Systemic mycotic disease of captive crocodile hatchling (*Crocodylus porosus*) also caused by *Paecilomyces lilacinus* (Maslen et al,

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1988). In one of the crocodile farms in Bangladesh, crocodiles were found sick and three of them died during the months of May to July, 2006. This paper gives a comprehensive description of the pathological changes associated with *E. coli* septicemia concurrent with mycotic infections in captive salt water crocodiles.

MATERIALS AND METHODS

Reptiles Farm Limited is the only crocodile farm in Bangladesh. These crocodiles were imported from Malaysia on December in 2004. Sixty crocodiles were female and 14 were male. These crocodiles were very aggressive in nature. In reptiles farm, the number of pond was 14 and the temperature of water was 29～33°C.

Clinical history

The affected crocodiles were gradually became emaciated, food intake was less, gasping, dyspnea and were reluctant to move. Necrotic muscle around the left stifle joint and right hip joint, superficial to deep biting wound in neck region, tail region, semisolid mucoso-nasal discharge and loud respiratory sound were observed. The wounds smelled putrid odor and were washed with savlon and povidon iodine and treated with oxytetetracycline, gentamicin, ivermectin, metronidazole, ceftriaxone, vitamin B and vitamin C. The affected crocodiles became moribund and died.

Necropsy

Routine necropsy was done and lesions were recorded. Tissue samples from two crocodiles were collected by the veterinarian of the Reptiles Farm Limited for histopathological diagnosis. On the third occasion, we did the post mortem examination. Carcasses were opened along the mid ventral line, viscera removed and examined grossly. Tissue samples from esophagus, trachea, lungs, liver, spleen, kidney, stomach, small intestine, colon and heart were collected in 10% neutral buffered formalin for histopathological examination and in falcon tube for microbiological study. Lung exudates, pericardial exudates and intestinal swabs were collected aseptically in nutrient broth.

Histopathology

Specimens of esophagus, trachea, lungs, liver, spleen, kidney, stomach, small intestine, colon, heart tissues were fixed, processed and embedded in paraffin. Sections were stained with hematoxylin and eosin (H&E) (Luna, 1968).

Special staining

Giemsa stain: Suspected paraffin embedded thin tissue sections were stained with Giemsa solution for 30 minutes to detect extra or intracellular organisms.

Gram stain: Suspected tissue sections were stained with crystal violet and safranin as per standard procedure (Luna, 1968).

PAS (Periodic Acid Schiff) stain: Suspected tissue sections were stained with schiff reagent and counterstained with light green as per the standard procedure (Luna, 1968).

Bacteriological examination

Culture of bacteria: The broth containing swabs in the test tubes were incubated at 37°C for 24 hours for the growth of bacteria. Then they were plated on different culture media viz, McConkey's agar, Eosin-Methylene Blue (EMB) agar, Salmonella-Shigella (SS) agar, blood agar and brilliant green agar (BGA) following standard procedure for the isolation of specific bacterial colonies (Merchant and Packer, 1967; Buxton and Fraser, 1977).

Staining of bacteria: Modified Gram stain was used for the staining of isolated bacteria as described by Merchant and Packer (1967) to study their morphology.

Biochemical tests: The isolated bacteria were subjected to different biochemical test. Fermentation of different sugars viz, lactose, sucrose and maltose were performed for identification of the organisms following the procedures described by Merchant and Packer (1967).

RESULTS

Necropsy

Esophagus, trachea, lungs, liver, spleen, heart and kidney were found congested. Intestine, rectum and colon were hemorrhagic. The clay colored materials were found mainly in the colo-rectum. Purulent exudates in lungs and thick and cloudy pericardial fluid in pericardial sac were found. Urate crystals deposited in kidney and cloaca. Marked distention and narrowing of the lumen of rectum was found. Clay coloured materials was also found in all parts of the intestine. Stomach was hemorrhagic and contained plastic bag. Colon was hard in consistency and tough to cut. Peritoneum was found attached to the abdominal wall.

Histopathological findings

Lung: Severe congestion, small to very large multi-focal granulomatous inflammation (arrow) which mostly consisted with proliferation of the macrophages and fibrous connective tissue (Fig. 1). Granulomatous lesion consistent with fungal spore (arrow) and hyphae (Fig. 2). Bacterial emboli (arrow) also found in the lungs alveoli.

Liver: Multi-focal to diffuse infiltration of mononuclear inflammatory cells in the liver parenchyma with stasis of bile and necrotic lesions consistent with bacteria. A huge number of fungal spore (arrow) and hyphae in the liver parenchyma (Fig. 3).

Kidney: Degenerative changes (arrow) in the tubular epithelium (Fig. 4). Aggregation of bacteria and protein cast in kidney parenchyma.

Small intestine: Loss of mucosal epithelium, lamina propria was severely affected that consisted with focal to diffuse granulomatous (arrow) lesions (Fig. 5). Formation of cystic spaces that was consistent with bacteria in muscular coat. The lesions also extended in the muscular coat.

Colon: Focal to diffuse severe granulomatous colitis (arrow) consistent with giant cell and bacteria (Fig. 6).

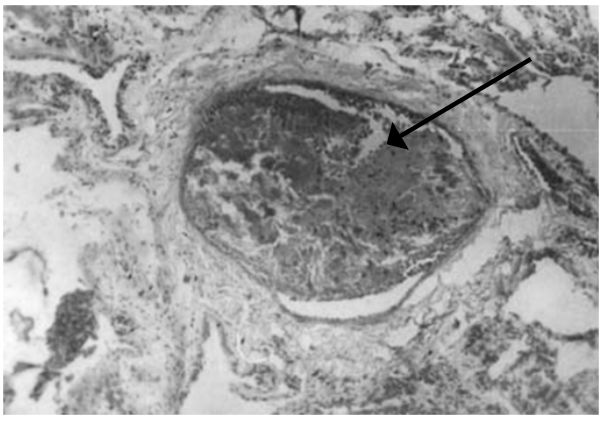

Fig. 1. Lung; crocodile. Bacterial emboli found in the blood vessel. The lesions are consistent with severe congestion, different types of bacilli and coccoid bacteria (H&E stain, ×82.5).

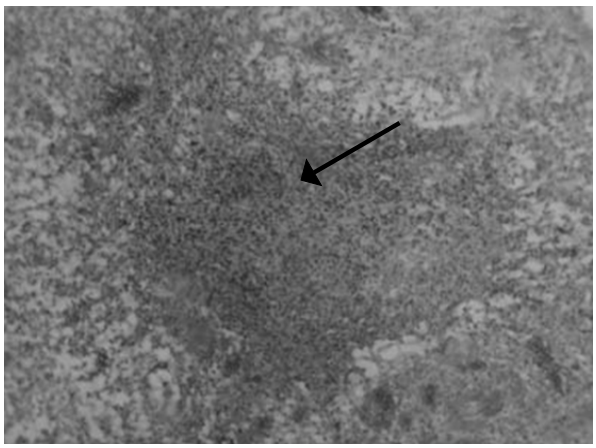

Fig. 2. Lung; crocodile. Granulomatous lesion consistent with fungal spore and hyphae (H&E stain, ×330).

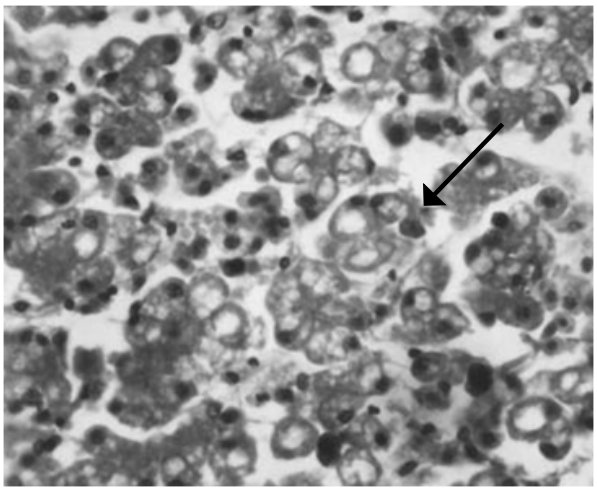

Fig. 3. Liver; crocodile. Huge amount of fungal spores with stasis of bile (H&E stain, ×330).

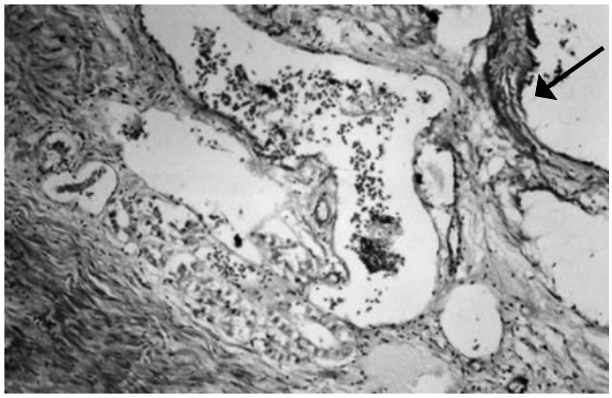

Fig. 4. Kidney; crocodile. Degenerative changes in the tubular epithelium (H&E stain, ×82.5).

Diffuse colitis with fungal hyphae (arrow) and spores was also found.

Isolation and identification of microorganisms

Bacteria were identified in the tissue section of lungs, liver, small intestine and colon by using Giemsa stain. The bacteria was also identified by using bacteriological media that produced smooth circular colonies with dark centers and metallic sheen on EMB agar and pink colonies on MacConkey's agar. The organisms fermented lactose, maltose and sucrose. These bacteria appeared as Gram negative. On the basis of the cultural, morphological and biochemical characters, the organisms were identified as *E. coli*. Fungal hyphae and spores were detected in liver, lungs and colon by using PAS stain.

DISCUSSION

This is the first comprehensive description of the histopathological and bacterialogical examination of *E. coli* septicemia and mycotic infection in captive salt water crocodile in Bangladesh. Previous descriptions have been incomplete and lacked details of the gross pathology, histopathology and bacteriological examination. In this study, histopathological and microbiological studies were conducted using routine procedures. In addition Giemsa stain, Gram stain and PAS stain were performed to detect the organism in tissues. Histologically, in

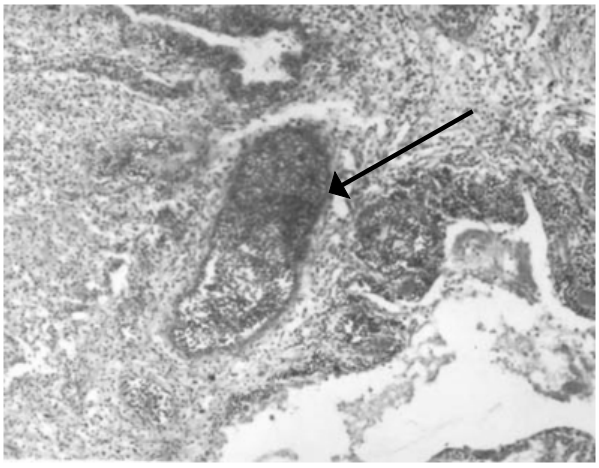

Fig. 5. Small intestine; crocodile. Severe focal to diffuse granulomatous enteritis (H&E stain, ×82.5).

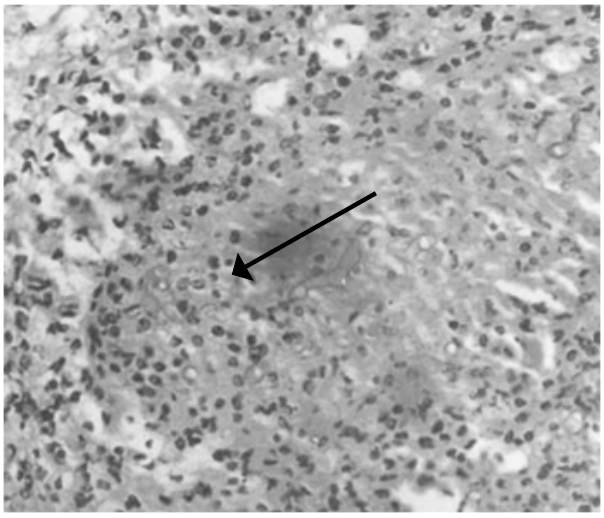

Fig. 6. Colon; crocodile. Granulomatous lesion with formation of giant cell and consistent with bacteria (H&E, ×82.5).

lungs, small to very large multifocal granulomatous lesions containing a huge amount of bacteria and fungal spore; bacterial emboli in the blood vessel indicating septicemia; in liver, multifocal to diffuse chronic necrotizing hepatitis consistent with fungal spores and bacteria were found. Stasis of bile was very common in liver. In kidney, aggregation of bacteria and protein casts in the parenchyma was evident. In intestine, granulomatous necrotizing enteritis consistent with bacteria; in colon, granulomatous lesion with formation of giant cell and consistent with bacteria, fungal hyphae and spores were found which were supported by several authors (Frank et al, 1974; Frank 1976; Munday and Peel, 1983;

Obendorf et al, 1993; Speare et al, 1997; Thomas et al, 2002).

Numerous branched septate within the necrotic debris lining the bronchi and rarely infiltrating into the adjacent stroma in lungs of a captive alligator (*Aligator mississippiensis*) caused by *Fusarium moniliforme* (Frelier et al, 1985). In this study, similar but more prominent lesions were seen in lungs, liver, intestine and colon of captive salt water crocodiles.

The host specificity of *M. amphibiorum* is reasonably wide and may include most anurans and some mammals with lower body temperatures. Platypus has a body temperature of 33°C to 34°C (Grant and Dawson, 1978), Echidnas also have low body temperature (Augee, 1978), and cane toads have a body temperature close to the ambient temperature (Speare et al, 1997) and might be at risk of fungal infection. In Reptiles Farm Limited, the temperature of pond water is 29～33°C and crocodiles also have low body temperature. Both of the cause predispose to fungal septicemia. In this study, bacteriologically *E. coli* were isolated from lungs exudates, pericardial fluids and intestinal fluids which were corresponded with the findings of Huchzermeyer et al(2000). Therefore, it can be assumed that 3 crocodiles died due to *E. coli* septicemia concurrent with mycotic infection.

Crocodiles probably become infected by ingesting an infectious stage in clay (possively the sporangiospore) and contaminated water. Water test revealed numerous pathogenic bacteria like *E. coli*, *Salmonella* and fungus (Lab. Agro, Gazipur). Infected crocodiles can excrete fungus in their feces. Therefore, infected crocodiles could contaminate soil and water and can be a threat to other crocodiles. It can be concluded that quality of water and other environmental factors are important for the captive crocodiles.

ACKNOWLEDGMENTS

This work was supported by Reptiles Farm Limited, Bhaluka, Mymensingh.

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