Pathology of mycotic gastritis in a wild Indian freshwater/marsh crocodile (Mugger; *Crocodylus palustris*): a case report

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ABSTRACT: There is no report on systemic mycotic disease in wild crocodilians so far. This report describes possibly the first ever case of deep gastric mycosis in a wild Indian crocodile. A carcass of an adult female, broad snouted Indian marsh crocodile was brought for necropsy. Externally, all visible mucous membranes, eyes, cloacal opening and joints were normal. On opening the carcass, all visceral organs were found in normal position and appeared grossly normal. The stomach was empty except for the presence of a few small wooden and bony pieces, and several dark blackish patches of raised plaques on the gastric mucosa. Other areas of gastric mucosa showed diffuse congestion with petechial haemorrhages and oedema. Microscopically, gastric mucosa in plaque areas appeared extensively thickened and fibrosed with moderate infiltration of mononuclear cells. Gastric glands were atrophied due to massive fibrosis which appeared to have completely replaced the mucosal architecture in the affected area. In the superficial gastric mucosa and also deep in the fibrosed tissue, spherical to oval, variable sized, thick walled bodies, several of them budding and often forming small chains, sprouting and even germinating, giving rise to branched hyphae, were conspicuously observed. These fungal bodies were found to be PAS-positive. Species identification of the mould could not be done. The present case appears to be the first report of invasive mycotic gastritis in a wild Indian crocodile (*Crocodylus palustris*), most probably caused by *Candida albicans*.

Keywords: *Candida albicans*; crocodile; *Crocodylus palustris*; fungal; gastritis; invasive; mycotic; wildlife

Mycotic diseases, especially deep mycoses in wild reptiles and amphibians are most likely to be undiagnosed on account of the clinically silent and slow nature of the disease, and are usually incidental findings at necropsy, often only after histopathology has been performed (Pare and Jacobson, 2007). There is no report on systemic mycotic disease in wild crocodilians so far; and very few studies on diseases in captive reptiles and amphibians. An investigation into normal cutaneous and gastrointestinal mycobiota in reptiles indicated the presence of more than 50 genera of fungi including *Penicillium* spp., *Aspergillus* spp., *Paecilomyces lilacinus*, Chrysosporia, Zygomycetes, *Scopulariopsis* spp., *Cladosporium* spp. and Fusaria, with the first two genera being isolated from as many as 78% and 69% of animals, respectively (Pare et al., 2003). Being

poikilotherms, reptiles and amphibians are more susceptible to acute and chronic stress, particularly in the captive environmental settings characterised by overcrowding, inappropriate housing, humidity, temperature, excessive light or noise and inadequate or unsuitable nutrition, and are thus frequently predisposed to infections by opportunistic pathogens like fungi (Pare and Jacobson, 2007; Ladds, 2009). Mycotic diseases described in captive crocodilians, mostly in captive crocodile hatchlings included dermatomycoses and pneumomycoses, with two fungal pathogens *Paecilomyces lilacinus* and *Fusarium* having been implicated repeatedly in crocodilian mycotic pneumonias (Maslen et al., 1988; Ladds and Sims, 1990; Ladds et al., 1995; Huchzermeyer, 2002; Ladds, 2003, 2009; Pare and Jacobson, 2007). Among deep mycoses, fungal in-

fections of lungs are more common in crocodilians and tortoises than reptiles and gastrointestinal mycoses are quite rare in spite of the omnipresence of fungal spores in the gut, with *Candida* spp. being implicated most frequently (Pare and Jacobson, 2007). The present report describes possibly the first ever case of deep gastric mycosis in a wild Indian crocodile (Mugger; *Crocodylus palustris*).

Case description

A carcass of an adult female, broad snouted Indian marsh crocodile, locally called 'mugger' (*Crocodylus palustris*), weighing 41 kg and measuring 1.98 m in length was brought for postmortem examination (Figure 1-SM, see Supporting Material). The crocodile was found dead on the bank of Ganges River, near the village of Kacchala in the Badaun district of Uttar Pradesh and was submitted for postmortem examination by the Director of Forest Department, Badaun, U.P. Detailed necropsy was performed and observed gross changes were recorded. Representative tissue pieces from organs were collected in 10% neutral buffered formalin solution for histopathological examination, and without any preservative in the vials in ice for microbial isolation studies. Formalin fixed tissue were dehydrated in increasing grades of alcohol, cleared in xylene and embedded in paraffin blocks to obtain 4–5 μm thick sections. The tissue sections were subjected to haematoxylin and eosin (H&E) staining for microscopic study. Duplicate sections were subjected to special staining such as periodic acid Schiff (PAS) staining whenever required.

Externally, carcass appeared fresh and all visible mucous membranes, eyes, cloacal opening and joints were normal. On opening the carcass, all visceral organs were found in normal position and appeared grossly normal. Fat deposition was seen in moderate quantities in subcutaneous tissue as well as around visceral organs. Stomach was empty except presence of few small wooden as well as bony pieces, and showed diffuse mucosal congestion with petechial haemorrhages and edema. The gastric mucosa revealed several variable-sized grayish-white fibrinous depositions to black raised plaques (Figure 1). Removal of grayish fibrinous depositions exposed underlying naked haemorrhagic ulcerated lesions. The mucosa appeared thickened and firm in consistency in the areas of raised grey-black plaques with sharply demarcated

irregular edges. The liver appeared slightly swollen, whereas kidneys, lungs and heart did not reveal any abnormality grossly.

Histopathologically, stomach revealed extensive fibrous tissue reaction in the gastric mucosa which appeared to have replaced the mucosal architecture in the affected area, obliterating the normal glandular structures (Figure 2). The gastric glands were severely atrophied due to massive fibrosis and appeared as isolated small islands of clusters or nests of epithelial cells. The fibrous tissue reaction was extensive in the lamina propria and extended up to submucosa, frequently accompanied with aggregations of mononuclear cells comprising primarily of lymphocytes and macrophages (Figure 3). Many apparently septate, sometimes branched fungal hyphae were seen scattered widely in the areas of fibrous tissue reaction in the gastric mucosa (Figures 4 and 5). Some hyphae appeared to be bearing hollow chlamydospore-like bodies (Figure 5 and Figure 2-SM, see Supporting Material). In addition, gastric mucosa also revealed presence of numerous spherical to oval, variable sized, thick-walled and also thin-walled bodies, several of them budding and often forming small chains, sprouting and even germinating, giving rise to branched hyphae (Figures 6, 7, and Figure 3-SM, see Supporting Material). Necrobiotic or microfloral debris attached to the gastric mucosa as well as in the immediate vicinity in the lumen also contained similar thick-walled, bilayered fungal spores and yeast-like bodies which often showed budding in chain or multibudding around single yeast-like cells, and also in vegetative form giving rise to hyphal sprouts. These fungal bodies were found to be PAS-positive (Figure 8). Species identification of the mould could not be done as isolation studies did not result in the growth of any mould. However, the presence of dimorphic morphological characteristics along with structures similar to chlamydospores and blastospores indicated that the organism was most likely *Candida albicans*. Other organs did not reveal any significant lesions.

DISCUSSION AND CONCLUSIONS

A diverse array of fungal agents are found in reptiles and amphibians and these often differ from those causing mycosis on other vertebrates. There are about 100 primary and an additional 200 opportunistic pathogenic fungi in human medi-

Figure 1. Crocodile stomach showing diffuse mucosal congestion and oedema and variable-sized grey to black areas of raised plaques (arrows) on gastric mucosa
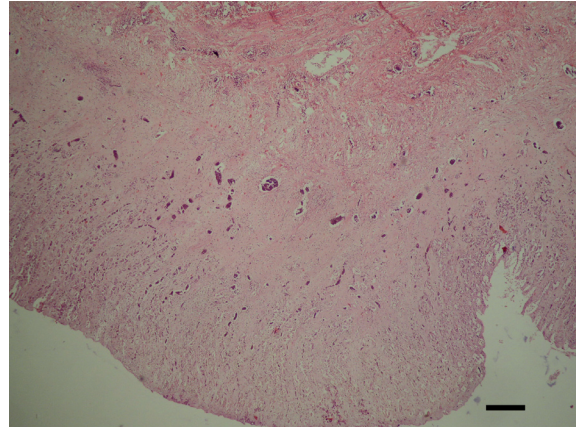

Figure 2. Crocodile stomach: Gastric mucosa showing fibrosis and severe atrophy of glands; H&E ×40
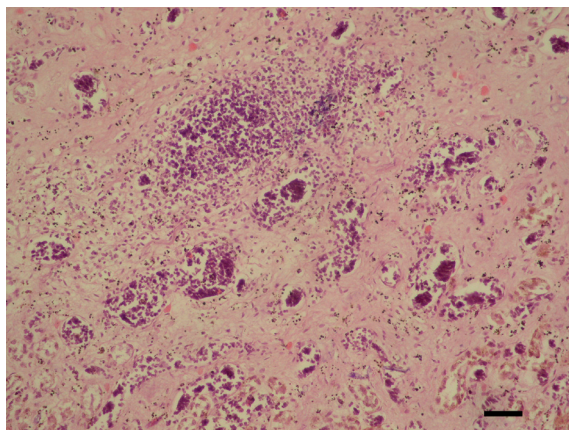

Figure 3. Crocodile stomach: Mononuclear cell aggregation and extensive fibrosis in the lamina propria of gastric mucosa resulting in atrophy of glands; H&E ×200
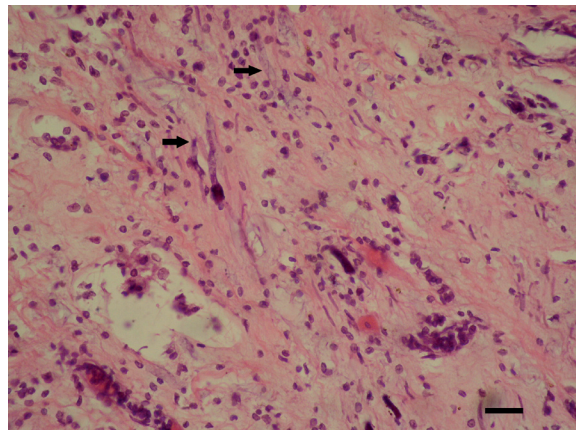

Figure 4. Crocodile stomach: Fibrous lamina propria showing hyphal structures (arrows) and mononuclear cell reaction; H&E ×400
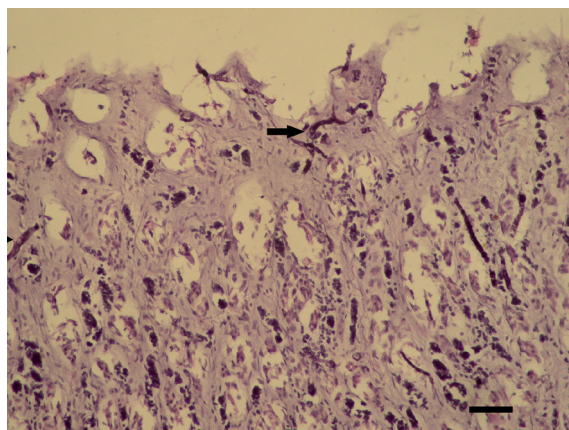

Figure 5. Crocodile stomach: Note many apparently septate and branched fungal hyphae, some bearing hollow chlamydospore-like bodies (arrows), in the gastric mucosal tissue; PAS ×200
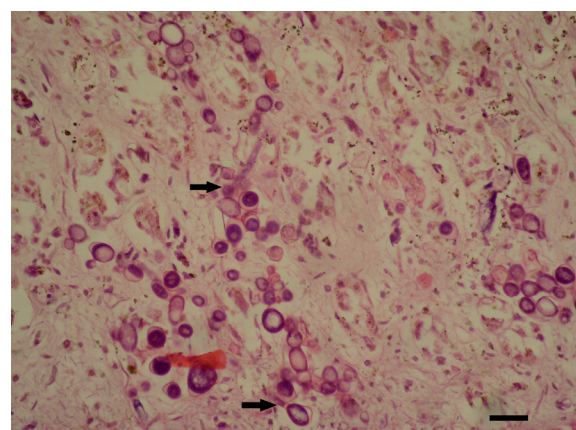

Figure 6. Crocodile stomach: Gastric mucosa exhibiting many variable-sized spherical to oval, thick and thin-walled bodies. Note yeast-like bodies budding and forming chains, some even vegetating giving rise to hyphal structures (arrows); H&E ×400
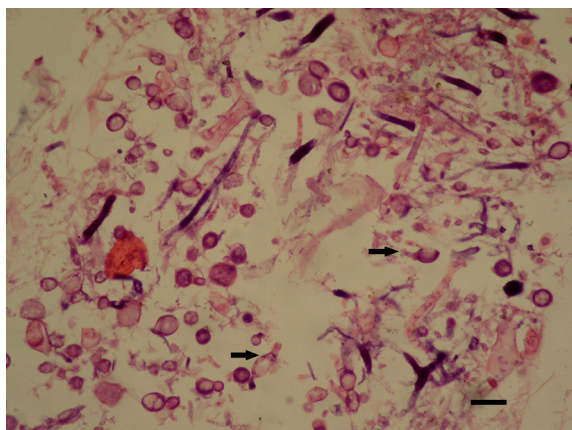
Figure 7. Crocodile stomach: Fibrinonecrobiotic material showing presence of several variable-sized spherical to oval, thick and thin-walled bodies, budding and vegetating (arrows) to give rise to branched and septate hyphae; H&E ×400
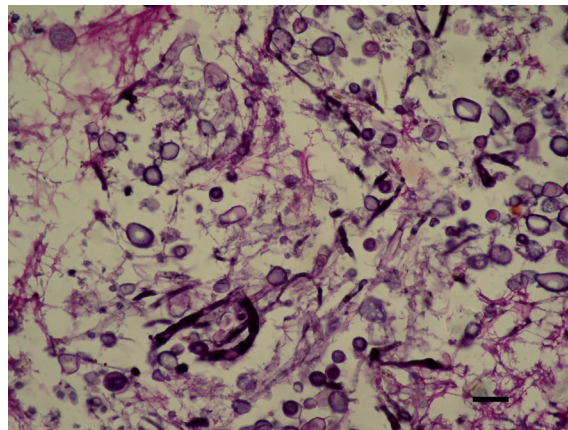

Figure 8. Crocodile stomach: PAS-positive fungal structures including variable-sized spherical to oval, thick and thin-walled bodies, budding and vegetating to give rise to branched and septate hyphae in the fibrinonecrobiotic material; PAS ×400

cine, which include dermatophytes (*Trichophyton*, *Microsporum* and *Epidermophyton* spp.), yeasts (*Cryptococcus neoformans*), dimorphic fungi (*Blastomyces*, *Coccidioides* and *Histoplasma* spp.) and others (Pare and Jacobson, 2007). Fungi like *Aspergillus* spp., *Candida* spp., and others are typically opportunistic and, though part of the common gut microflora, can become pathogenic in immunocompromised situations. In amphibians, mucoromycosis, chromomycosis and chitridiomycosis are more common mycoses (Pare and Jacobson, 2007). The present case of mycotic gastritis in a wild crocodile possibly caused by *Candida* spp. appears to be of opportunistic nature, since the crocodile underwent a long starvation as indicated by the necropsy findings of an empty stomach while the apparently highly polluted water of the Ganges River could also contribute as a predisposing factor.

A diagnosis of deep mycosis essentially relies on demonstrating the presence of fungal hyphae or yeast in histological lesions and isolation of the fungal agent from the clinical material alone is viewed unreliably as fungi are normal part of cutaneous and gut microflora and also present in the environment (Pare and Jacobson, 2007). In the present case, the fungal agent was conspicuously present in the inflamed and fibrosed gastric mucosal tissue and was demonstrable by both H&E and PAS staining methods. Although isolation of the agent could not be done, the morphological features of fungus in tissue sections such as the presence of large to small spherical to oval thick-walled as well

as thin-walled bodies often in budding and chain form, appearing as chlamydospores and/or blastospores, and their germination to give rise to hyphae, apparently septate and/or branched hyphae, suggested that the fungal agent was most probably *Candida albicans*, as in addition to pseudohyphae produced by most *Candida* spp., only *Candida albicans* and *Candida dubliniensis* are also able to produce true septate hyphae (Calderone, 2002). Furthermore, there were many fungal hyphae seen in the present case widely spread in the mucosal lesion of the stomach. Pseudohyphae and hyphae help *Candida albicans* to invade deeper tissues after it colonizes the epithelium (Ernst, 2000; Haynes, 2001). As part of normal gut microflora, *Candida albicans* and *Candida tropicalis* remain in yeast form up to pH 4.5; however, germ tube formation and hyphal transformation occurs at pH 5.6 and above (Dede and Okungbowa, 2009), suggesting that at this pH the fungi become more virulent. It has been found that nitrogen starvation triggers the formation of proteinase and other collagenolytic enzymes by *Candida albicans* facilitating germ tube formation and penetration of mucosal epithelial cells (Kaminishi et al., 1986; Haynes, 2001; Lorenz, 2002; Nishimura, 2002). In the present case, it is likely that the inflamed stomach of the crocodile together with starvation might have led to alteration of the pH to one favourable for *Candida* to become virulent and cause invasive gastric mycosis. The histological features of extensive fibrous tissue reaction in gastric mucosa accompanied with

mononuclear cell infiltration comprising mainly lymphocytes and macrophages is in agreement with the findings of Evans (1980) who noted that hyphae stimulated mononuclear cell reactions mainly of lymphocytes and macrophages in experimental *Candida albicans* infection in mice.

The present case appears to be the first report of invasive mycotic gastritis in a wild Indian crocodile (*Crocodylus palustris*), most probably caused by *Candida albicans*.

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Supporting material

More figures available online at http://vetmed.vri.cz Full paper

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