Systemic mixed infection in a brown caiman (*Caiman crocodilus fuscus*) caused by *Mycobacterium szulgai* and *M. chelonae:* a case report

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ABSTRACT: A five-year old female brown caiman (*Caiman crocodilus fuscus*) was admitted to a veterinary clinic because of anorexia and lethargy. Chronic deterioration of the patient's condition together with the formation of slushy stools coloured from brown to red was observed during the previous eight weeks. Physical examination showed significant apathy and cachexia. Radiographic examinations of chest and abdomen revealed no pathological findings. Initial blood tests revealed decreased hematocrit and low levels of haemoglobin. Despite treatment with enrofloxacin and intensive supportive therapy with amino acids, vitamins and mineral matter, the animal died 14 days after admission to the clinic. *Post mortem* examination revealed splenomegaly with a total destruction of inner organ structure together with multiple granulomas in liver and lungs. Ziehl-Neelsen staining of tissue samples from liver, lungs and spleen revealed numerous acid-fast bacilli consistent with *Mycobacterium* spp. Identification of isolates was carried out using PCR restriction analysis (PRA) of the *hsp65* gene and DNA sequencing of the *16S rRNA* gene. Two different mycobacterial isolates obtained from separate samples of liver, lungs and spleen were identified as *M. chelonae* and *M. szulgai*. This is the first report of mixed infection caused by *M. chelonae* and *M. szulgai* in a reptile.

Keywords: potentially pathogenic mycobacteria; mycobacteriosis; granulomatous disease; reptiles

Bacteriosis affects animals across many taxa. Many bacteria are either part of the normal flora in reptiles, or may be found in healthy reptiles without causing disease. Very few bacteria have been implicated in reptile diseases as primary causative pathogens. This group comprises *Chlamidiales*, potentially pathogenic mycobacteria (PPM), *Salmonella*, *Dermabacter* and *Mycoplasma* (Pasmans et al., 2008). Although not commonly diagnosed in reptiles, a number of unpublished clinical cases from alligators suffering from pneumonia have reported acid-fast organisms consistent with the presence of *Mycobacterium* spp. (Nevarez, 2006).

Mycobacterial infections in reptiles are often associated with the development of histiocytic granulomatous inflammation with systemic mycobacterial spread resulting in necrosis (Soldati et al., 2004). Mycobacteria can cause systemic illness accompanied by nonspecific signs like anorexia, lethargy and wasting. No successful treatment of infection with *Mycobacterium* spp. has been reported in reptiles (Pare et al., 2006).

PPM are widespread in the environment, particularly in aquatic reservoirs. Mycobacterial infections have been reported in a wide variety of reptiles, including snakes, turtles, lizards and crocodiles.

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PPM cause a wide variety of lesions in reptiles, usually chronic, including granulomatous lessions, involving the lungs, liver, spleen and central nervous system (Johnson-Delaney, 2006).

Mycobacterial isolates from lesions include members of the *M. avium* complex, *M. marinum*, *M. chelonae*, *M. intracellulare* and others (Pare et al., 2006; Pasmans et al., 2008; Pavlik and Falkinham, 2009). Only a few cases of mycobacterial infections in crocodilians have been reported, and these mostly from zoos and other collections. *M. marinum* and *M. fortuitum* have been isolated from spectacled caimans (*Caiman crocodiles*). Infection in crocodiles usually proceeds via the oral-intestinal route and spreads from intestines to all organs. Cases of generalized mycobacteriosis in *C. johnsoni* and *C. niloticus*, as well as granulomatous mycobacterial dermatitis in *C. porosus* have been reported (Huchzermeyer, 2003).

This report describes the first published case of mixed infection occurring in a brown caiman (*C. crocodilus fuscus*) caused by *M. chelonae* and *M. szulgai*.

Case history

A five-year-old female brown caiman with body weight 3.20 kg was admitted to a clinic for observation because of anorexia and lethargy. The caiman had inhabited a spacious aquarium with gravel bedding for the previous four years. The aquarium was equipped with an outer biological filter and a circulating system for to warm water to between 26 and 27°C. In the natural geographic range of the brown caiman, the diet of this crocodile usually includes snails, crustaceans, fish and other aquatic organisms (Grenard, 1991), but the female was fed with laboratory rats or mice. Chronic deterioration of the patient's condition together with the formation of slushy faeces coloured from brown to red had been observed during the previous right weeks.

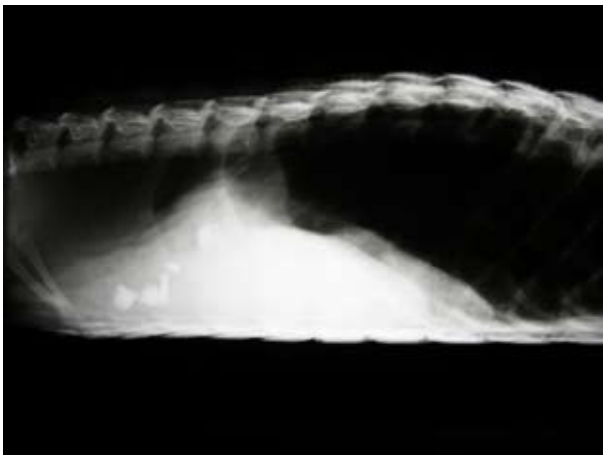

Figure 1. Radiography (LL) of the female brown caiman did not reveal any pathological findings

Physical examination showed significant apathy, cachexia, clean skin with no sign of mechanical damage, clean mouth and nostrils and shiny eyes. Radiographic examinations of the chest and abdomen revealed no pathologic findings (Figure 1). Blood samples from the ventral tail vein were collected on Day 1, 10 and 14 after admission to the clinic. Blood samples were analysed for haematological and biochemical profiles (Table 1). Decreased hematocrit and low levels of haemoglobin were observed. On the day of admission, leukocytosis was detected, which later shifted to leukopenia.

Figure 2. Female brown caiman before the necropsy

Table 1. Haematological and biochemical profiles of the brown caiman's blood

Tested parameter	Haematological and biochemical profiles			
	Day 1	Day 10	Day 14	References*
Total protein (g/l)	78.10	16.32	35.60	50.10
Glucose (mmol/l)	1.54	1.59	0.31	5.67
Urea (mmol/l)	0.26	0.31	0.22	1.14
Uric acid (μmol/l)	470.15	109.15	434.11	NA
ALP (μkat/l)	0.24	0.05	0.46	0.33
ALT (μkat/l)	0.23	0.68	1.02	NA
AST (μkat/l)	4.05	4.23	6.46	NA
Creatinkinase (μkat/l)	6.50	103.60	84.90	NA
TAG (mmol/l)	0.25	NA	NA	NA
Cholesterol (mmol/l)	0.49	NA	NA	3.56
Calcium (mmol/l)	2.80	2.39	2.78	2.52
Phosphorus (mmol/l)	1.69	1.38	1.14	1.74
Sodium (mmol/l)	142.00	142.00	134.00	NA
Potassium (mmol/l)	3.62	3.10	3.16	NA
Haemoglobin (g/l)	35.00	NA	32.00	120.00
Haematocrit (l/l)	0.14	NA	0.11	0.27
Erythrocytes (10^6/l)	0.47	0.25	0.47	0.69
Leucocytes (10^3/l)	30.00	10.00	4.50	16.40
Heterophils (10^3/l)	14.10	0.00	0.77	5.00 (%)
Eosinophils (10^3/l)	0.00	0.00	0.00	21.00 (%)
Basophils (10^3/l)	0.00	0.00	0.00	0
Monocytes (10^3/l)	0.30	0.40	0.05	5.00 (%)
Azurophils (10^3/l)	10.80	2.00	0.09	9.00 (%)
Lymphocytes (10^3/l)	4.80	7.60	3.30	60.00 (%)

ALP = alkaline phosphatase; ALT = alanine transaminase; AST = aspartate aminotransferase; TAG = triacylglycerole; NA = not available

*Troiano and Althaus, 1993; Tourn et al., 1994; Troiano et al., 1996

A 14-day course of non-specific treatment with enrofloxacin (10 mg/kg b.w., *s.c.* q24h, Enroxil 5% inj., Krka, Sloveniia), amino acids (2 ml, *i.m.* q24h, Heparemin, Biotica, Slovakia), parenteral nutrition (6 ml of Duphalyte inj., *s.c.* q24h, Fort Dodge Animal Health, Spain), mineral matter (2 ml of 20% Calcium gluconicum inj., *i.m.* q24h, Biotica, Slovakia) and B-group vitamins (2 ml of B-neuron inj., *i.m.* q24h, Vétoquinol, France) per day was prescribed. The animal was kept in a heated water pond located at the clinic.

The patient died 14 days after admission to the clinic (Figure 2). Examination of stomach, intestine and vent content revealed no parasitic infestation. *Post mortem* examination discovered splenomegaly with a total destruction of inner organ structure (Figure 3) together with multiple granulomas in liver and lungs (Figures 4 and 5). Granulomatous inflammation was confirmed by histopathological examination of liver, lungs and spleen. No morphological lesions were observed in the heart and kidneys.

Ziehl-Neelsen staining of tissue samples revealed numerous acid-fast bacilli consistent with *Mycobacterium* spp. (Figure 6). Tissue samples (liver, lungs, spleen, heart and kidney) were homogenized and decontaminated according to a procedure described previously (Fischer et al., 2000;

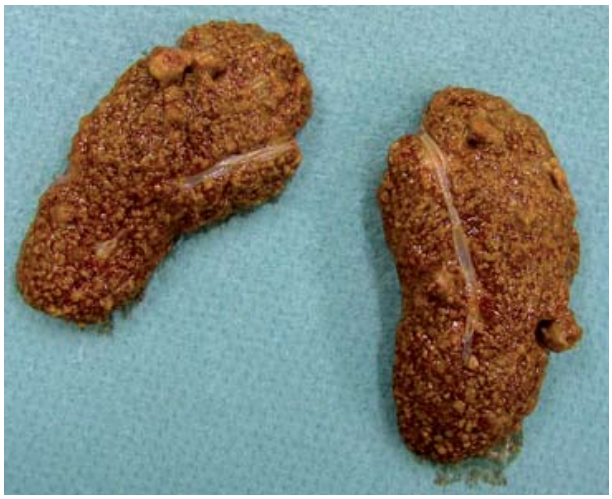

Figure 3. Splenomegaly with a total destruction of inner organ structure together with multiple granulomas in the spleen

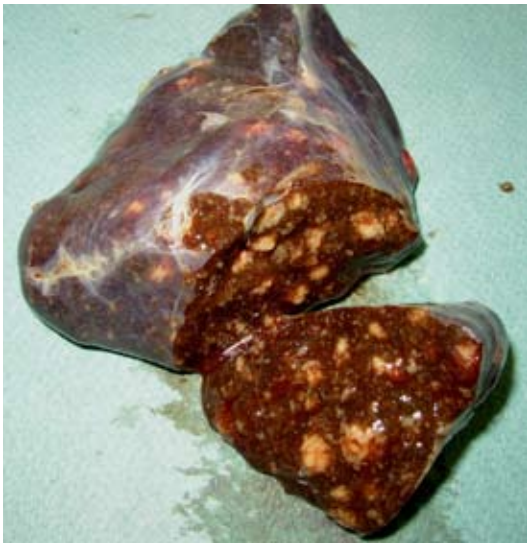

Figure 4. Multiple granulomas in the liver

Matlova et al., 2003). For isolation of mycobacteria, Stonebrink and Herrold egg yolk media were used. Samples were grown in the presence or absence of Mycobactin J (Allied Monitor, Fayette, USA) at 25°C and 37°C. After Ziehl-Neelsen staining, all microscopically positive isolates were subjected to polymerase chain reaction (PCR) and sequence analysis. Identification of isolates was carried out using PCR restriction analysis (PRA) of the *hsp65* gene and DNA sequencing of the *16S rRNA* gene according to previously described methods (Telenti et al., 1993; Harmsen et al., 2003). Two different mycobacterial isolates obtained by sub-culture of mixed primary cultures originating from separate samples of liver, lungs and spleen were identified as *M. chelonae* and *M. szulgai*. It can be concluded that mixed mycobacterial infection was disseminated in the animal body.

DISCUSSION

Frequent occurrence of PPM in the environment combined with a low prevalence of mycobacteriosis in poikilotherms suggests that these species may possess an innate resistance to these organisms. Mycobacteriosis in reptiles results in sporadic granulomatous disease (Girling and Fraser, 2007;

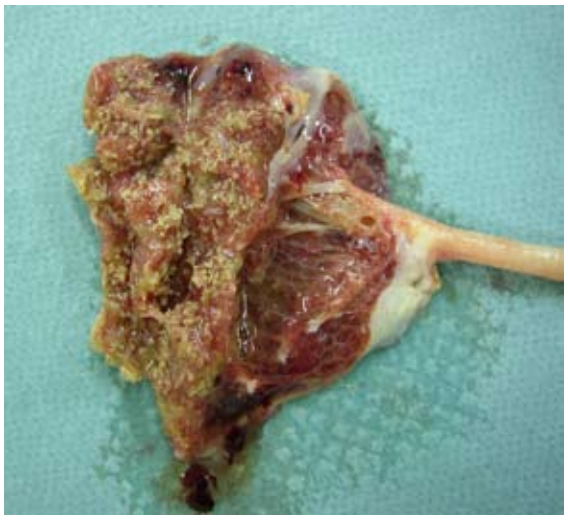

Figure 5. Granulomatous inflammation of the lung tissue

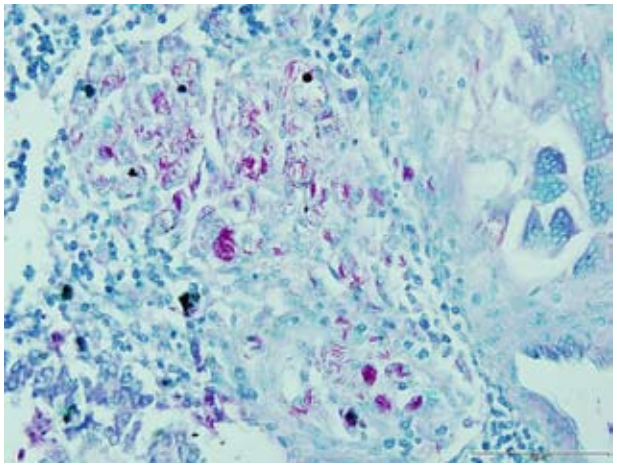

Figure 6. Acid-fast bacilli consistent with *Mycobacterium* spp. revealed by Ziehl-Neelsen staining of the liver

Pasmans et al., 2008). Differences in the distribution of mycobacteria in reptile tissues and the character of the inflammatory response may be influenced by host factors or the species of mycobacteria (Stacy and Pessier, 2007). In most cases of reptile mycobacteriosis, treatment is not recommended because of the chronic nature and often advanced stage of the disease, long-term and expensive nature of potential treatment regimens, and the risk of spread to other animals, including humans.

The few reported cases of mycobacterial disease in reptiles have mostly indicated single organ involvement such as the lung, liver, spleen or epidermis (Maslow et al., 2002; Soldati et al., 2004; Noyes et al., 2007; Pavlik and Falkinham, 2009). Cases of farmed crocodiles with granulomatous lesions in the lungs, trachea and intestines have been reported (Youngprapakorn et al., 1994). The lack of reports of mycobacteriosis in reptiles may be due to the special stains and bacterial isolation techniques needed for the identification of disease.

On the basis of microbiological and molecular methods, it was found that in the brown caiman with granulomas which we examined, two species of mycobacteria were present: *M. chelonae* and *M. szulgai*. Several cases of *M. chelonae* infection in reptiles are present in the literature (Greer et al., 2003; Soldati et al., 2004), but to the best of our knowledge, this is the first case of *M. szulgai* infection in a reptile. Non-specific signs, including anorexia and chronic weight loss observed in the brown caiman were described previously in reptiles systemically infected with PPM (Jacobson, 2007).

Generally PPM may be introduced into an aquarium together with aquarium plants, sand, water or through the contaminated surface of various aquarium appliances (Goslee and Wolinsky, 1976; Horvathova et al., 1997; Kiesch, 2000; Pavlik and Falkinham, 2009). Another possible source of PPM may be infected food (pork meat, fish; Huchzermeyer, 2003).

From a pathogenic perspective, it is also important to note that *M. szulgai* did not occur in the afflicted reptile independently, but was found together with *M. chelonae* which is known to be pathogenic for reptiles. It may, therefore, be assumed that *M. szulgai* was not the main cause of disease in the reptile, but that it appeared with the weakening of the organism by *M. chelonae* or simply through accompanying other PPM "contamination".

To better prevent and possibly treat reptiles with mycobacterial infections, it is important that the scientific community continues to gather information on the species of identified PPM found in reptiles and the pathologic effects that each strain has on every organ in the body. It could be pointed out that both these PPM species can infect immunosuppressed humans. Due to these facts, good hygiene and preventative measures should be implemented in the terrariums of reptiles infected with PPM.

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