Mycoplasma-associated polyarthritis in farmed crocodiles *(Crocodylus niloticus)* in Zimbabwe

K. MOHAN[1], C.M. FOGGIN[2], P. MUVAVARIRWA[1], J. HONYWILL[2] and A. PAWANDIWA[1]

ABSTRACT

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Outbreaks of polyarthritis in farmed crocodiles *(Crocodylus niloticus)* on five farms in Zimbabwe are described. Cases were reported only among the rearing stock aged 1–3 years. No breeding stock suffered. Morbidity was about 10% and the mortality even lower. All the sick animals consistently displayed swollen limb joints as well as progressive lameness and paresis. The synovial structures in subacute cases contained mycoplasmas and excess turbid mucus which, at a later stage of the disease, became yellowish, inspissated and sterile. Cellular changes in the joint capsule included oedema, necrosis of the superficial layers of membrane, lymphocytic infiltration and fibrosis. Evidence of pneumonia was observed only at necropsies.

Fifteen isolates of *Mycoplasma* were cultured from the clinical specimens collected from the four sick and three dead crocodiles. The affected joints of all these animals yielded *Mycoplasma* in pure culture, but the culture from lungs yielded post-mortem invaders also. The sick animals were treated with a single intramuscular injection of long-acting tetracycline(10 mg/kg), and oxytetracycline mixed in feed at 550 mg/kg was fed for 10 d. The treatment appeared to be effective in ameliorating the clinical signs, but in some cases inflammatory swelling persisted.

All 15 the isolates conformed to the characteristics of the genus *Mycoplasma*, and were serologically indistinguishable in growth-inhibition (GI) tests. Although these isolates shared the main biochemical characteristics of *Mycoplasma capricolum*, they differed serologically. Also goats were refractory to experimental infection with crocodile strains. In crocodile yearlings, however, the disease was reproduced with an isolate from one of the affected farms. The source of infection remained elusive. The farmers suspected poultry meat fed to the crocodiles to be the source. However, GI tests failed to identify the isolates as one of the pathogenic glucose-metabolizing avian mycoplasmas.

This appears to be a first report of isolation of *Mycoplasma* from crocodiles and also of its association in disease.

Keywords: *Mycoplasma*, polyarthritis, crocodiles, Zimbabwe

INTRODUCTION

Crocodile ranching in Zimbabwe began in 1965, with a few farms having a stock of 50–100 animals. There are now 40 such farms throughout the country, with a total of about 70 000 animals. The 1994 export earnings from this industry are expected to be 40 million Zimbabwe dollars. Disease has proved to be one of the major impediments to the rearing of crocodiles in captivity. Besides nutritional disorders (particularly calcium deficiency), a number of infectious diseases have been recorded, notably pox, adenovirus, *Aeromonas* and enterobacterial infections (Foggin 1987; 1992). Septicaemic pasteurellosis has also been recorded (Mohan,

[1] Faculty of Veterinary Science,University of Zimbabwe, P.O. Box M.P. 167, Harare, Zimbabwe

[2] Veterinary Research Laboratory, P.O. Box CY551, Harare, Zimbabwe

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Sadza, Madsen, Hill & Pawandiwa 1994). There is, however, no published report of isolation of mycoplasma from crocodiles in health or disease.

In this paper we report on outbreaks of mycoplasma-associated exudative polyarthritis in farmed crocodiles *(Crocodylus niloticus)* in Zimbabwe. Specifics of the disease, phenotypic characteristics of the isolates, results of experimental infection in goats and crocodiles and measures to contain the outbreaks, are described.

MATERIALS AND METHODS

The outbreaks were reported from five farms located in different parts of Zimbabwe. Synovial aspirates from three randomly selected sick and four dead animals received from these farms were cultured for aerobic bacteria following standard techniques (Carter & Chengappa 1991; Cowan & Steel 1993). Heart blood and lungs from the dead animals were also cultured. In addition, all the samples were examined for mycoplasmas as described by Mohan, Obwolo & Hill (1992). Polyclonal antisera raised in rabbits (Senterfit 1983) against three mycoplasma isolates from crocodiles (strains 145, 149 and 266/93) were used for growth-inhibition (GI) testing (Cottew 1983). All 15 mycoplasma isolates from the crocodiles were subjected to the GI test with antiserum raised in rabbits against *Mycoplasma capricolum, M. gallisepticum, M. pullorum, M. synoviae* and *M. iowae* (reference strains). Overnight culture in mycoplasma broth (Mohan *et al.* 1992) was employed to obtain thin-sectioned electron microphotographs of the strain 266/93, as described by Ellis & Smith (1987). Formol-saline-fixed specimens of lung tissues and synovial membrane embedded in paraffin wax were sectioned (4 μm) and stained with haemotoxylin and eosin (HE).

The sick animals on all five the farms were each treated with a single intramuscular injection of long-acting tetracycline (10 mg/kg), followed daily by oxytetracycline mixed in feed at 550 mg/kg and fed *ad lib.* for 10 d.

Experimental infection was attempted in eight goats aged 6–8 weeks. Three received 5 mℓ of a 48-h culture of the strain 149 (10^9 CFU/mℓ) intratracheally, while the other three were given 10 mℓ of a similar culture of strain 266/93 intranasally. The two controls were given sterile broth.

FIG. 1 Polyarthiritis, natural case. Note swelling in the right elbow and both metacarpal joints

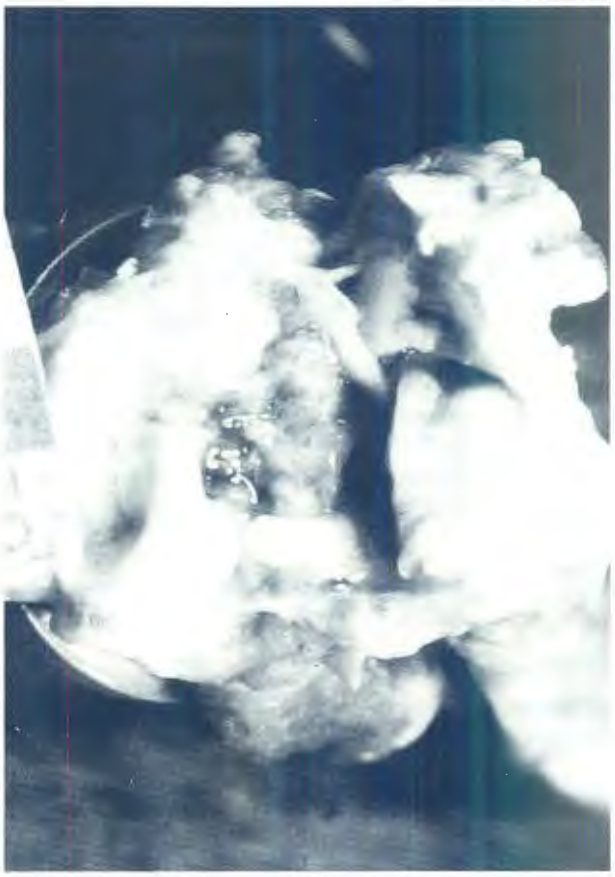

FIG. 2 Dissected kneejoint, natural case. Note turbid mucus exudate in the acute stage of the disease

In addition, eight apparently healthy crocodiles aged 12–15 months, reared in the Veterinary Research Laboratory, were infected with strain 266/93 (10^9 CFU/mℓ) grown in broth supplemented with crocodile instead of pig serum. Three were given 2 mℓ intraperitoneally (IPR), three, 2 mℓ intrapleurally (IPL), and two, 0,5 mℓ intra-articularly (IA) in the knee joints. A single control received 0,5 mℓ of sterile broth IA. All the goats and crocodiles were observed up to 8 weeks post-infection (PI).

RESULTS

On all the farms, only the rearing stock aged 1–3 years suffered, while the breeding stock remained unaffected. The morbidity rate was approximately 10% and the mortality rate, lower. The sick animals consistently displayed swollen joints (Fig. 1) of the hind or fore limbs or both, as well as progressive lameness and paresis. At necropsy a profuse turbid mucoid exudate containing mycoplasmas was present in the synovial structures in subacute cases (Fig. 2), which at a later stage of the disease became yellowish, inspissated and bacteriologically sterile (Fig. 3). There was oedema of the surrounding tissue. Evidence of pneumonia was observed post mortem.

From the clinical specimens, 15 isolates of *Mycoplasma* sp. were cultured; 13 were from the affected joints and two from the lungs. No other bacteria could be cultured from the joints, but the lungs also had post-mortem invaders.

The isolates were very rapid growers, recording a colony size of 100–300 μm diameter (Fig. 4) within 48 h at 37 °C under CO_2. Under similar conditions of incubation the growth reached about 6×10^{12} CFU/mℓ in broth containing pig or horse serum and almost one log higher in broth supplemented with crocodile serum. Broth cultures remained viable for over 3 weeks at 4 °C.

The strains were facultative anaerobes; they produced greenish discoloration on blood agar and grew well at temperatures between 25 and 42 °C, but most rapidly at 37 °C.

All the isolates were sensitive to digitonin (Cottew 1983), and were serum-dependent, filtrable (450-nm-pore filter) and maintained a "fried-egg" colonial morphology throughout ten serial passages on the medium, free of inhibitors. The electron-microphotographic appearance was that of the Mollicutes (Fig. 5). All the isolates metabolized glucose and mannose actively, reduced

FIG. 3 Dissected metacarpal joint, natural case. Inspissated thick exudate in advanced stage of the disease

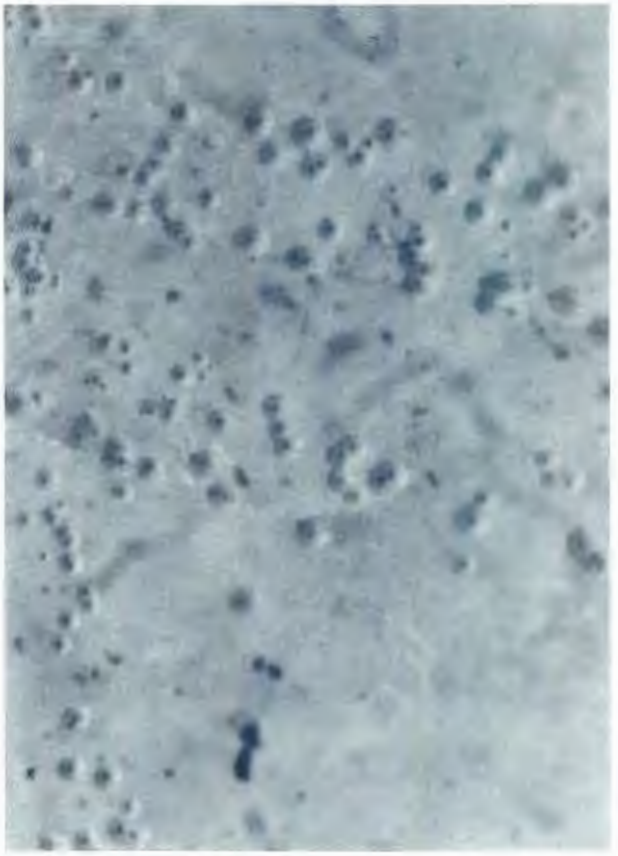

FIG. 4 Direct agar culture from a natural case. Typical "fried-egg"-type colonies; 48-h growth. X400

tetrazolium both aerobically and anaerobically, and produced phosphatase, but none of them hydrolysed arginine or aesculin (Rose & Tully 1983) or produced film and spots (Cottew 1983). The results of casein and serum digestion remained equivocal after several repeat tests. The results of physiological and GI tests (Senterfit 1983; Wallace 1983) with antiserum to crocodile strains confirmed that the 15 strains were indistinguishable. However, none of these strains reacted with avian mycoplasmas or *M. capricolum* antisera in GI tests.

Histopathological examination of the joint capsule and the surrounding tissues in subacute cases showed inflammatory oedema, necrosis of the superficial layers of the synovial membrane, fibrin deposition, lymphocytic infiltration and fibrosis. The lungs showed extensive areas of consolidation and evidence of oedema. The alveoli were filled with a mixture of polymorph, mononuclear cells and erythrocytes, with slight thickening of the interlobular septa. The treatment appeared to be effective in ameliorating the clinical signs, but in some cases, inflammatory swelling persisted.

Goats proved refractory to experimental infection, but the disease was reproduced in crocodiles. The three IPR-infected crocodiles developed progressive lameness with swollen joints (Fig. 6) 7–10 d PI, but only one among the IPL-infected animals was similarly affected. *Mycoplasma* was reisolated from the affected joints of these animals. The IA-infected animals and the control developed lameness and slight swelling which subsided within a week, but no mycoplasmas could be cultured from the joints. One animal in the IPR group was euthanased 15 d PI for necropsy. Its affected joints were found to contain much turbid mucus similar to the exudation seen in the natural cases, but the lung appeared normal and did not yield mycoplasmas on culture. No lateral transmission took place from the experimentally infected animals to healthy in-contacts. All the animals recovered clinically within 6–8 weeks from the date symptoms were first seen.

DISCUSSION

The investigations on the five affected farms apparently confirm a *Mycoplasma* sp. as the cause of polyarthritis. The isolation of *Mycoplasma* sp. from the affected joints of all the crocodiles examined, the apparent clinical recovery after treatment with tetracyclines, and the experimental reproduction of the disease in crocodiles (fulfilling the Koch's postulates) gave convincing proof of mycoplasma aetiology. Colonization of the joints after IPR and IPL infection confirmed the predilection site of the isolates, but the failure to reproduce the disease

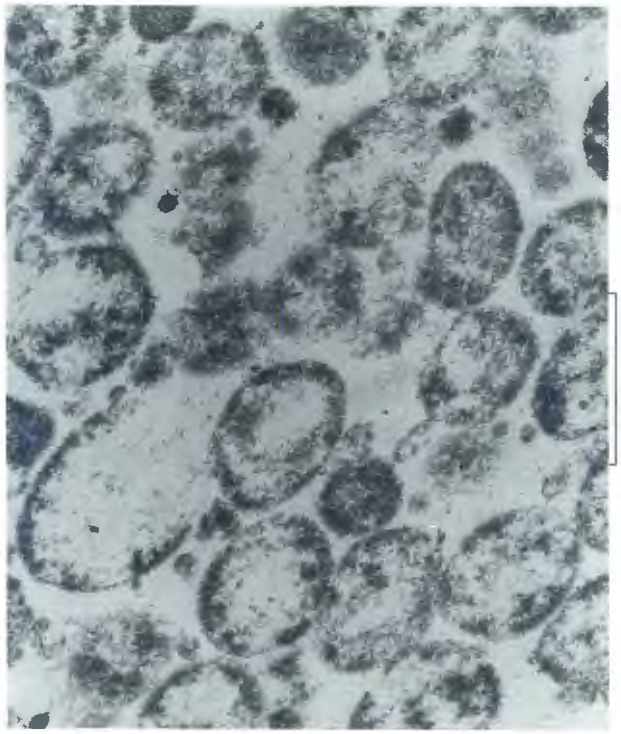

FIG. 5 Electron micrograph of ultrathin section of *Mycoplasma* strain 266/93

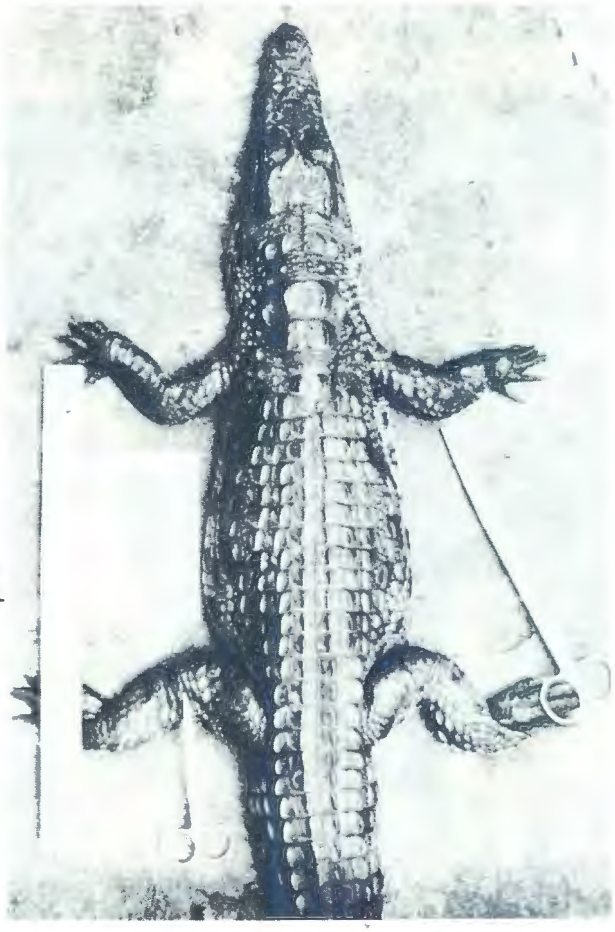

FIG. 6 Polyarthritis, experimental infection. All the joints except the shoulder joints of both front limbs are swollen: 14 d after infection

by intra-articular injection defies objective pathological explanation. However, it should be pointed out that this method of inoculation in crocodiles proved to be technically difficult. Polyarthritis following mycoplasma infection is well known in goats, pigs, poultry and mice (Freundt & Razin 1984). Although there is no publication related to mycoplasmas in polyarthritis in crocodiles, the pathology seen in the joints of these alligators was similar to changes described in other animal species *(vide supra)*.

The isolates have yet to be assigned to a species and this must await the completion of the current studies undertaken at the Institut für Mikrobiologie, Hannover, Germany. However, the main phenotypic characteristics of the isolates resembled those of *M. capricolum* which causes a similar disease in goats. This motivated the attempt to infect goats. However, the results of the GI test and the failure to induce infection in goats suggested that the strains from crocodiles were not *M. capricolum*. All the isolates bear very close similarities, to the extent that they could eventually be assigned to a single taxon (new ?).

In this study, crocodile serum was used for the first time to grow mycoplasmas, and good results were obtained. Crocodile serum might support growth of mycoplasmas from other animal species as well.

The source of infection remained elusive. The farmers suspected the poultry carcases fed to the crocodiles to be the source. However, GI tests failed to identify the isolates as one of the pathogenic glucose-metabolizing avian mycoplasmas. There was no contact between the five affected farms, but it is of interest that the pond water on each of these farms had been treated with NaCl to a concentration of 0,5% w/v to tone up the hide quality of the animals.This practice was also suspect in the opinions of some farmers. So far there has been no report that mycoplasmas survive in crystalline NaCl, and these wall-less bacteria are highly susceptible to high or low osmolarity (Freundt & Razin 1984).

As this is the first published report of *Mycoplasma*-associated disease in crocodiles, attempts should be made to elucidate mechanisms of transmission and pathogenesis when similar cases in farmed or wild crocodiles are reported from elsewhere.

REFERENCES

CARTER, G.R. & CHENGAPPA, M.M. 1991. Pasteurella and Francisella, in *Essentials of Veterinary Bacteriology and Mycology*. Philadelphia: Lea & Febiger: 172–173.

COTTEW, G.S. 1983. Recovery and identification of caprine and ovine mycoplasmas, in *Methods in Mycoplasmology*. II, edited by S. Razin & J.G. Tully. Academic Press: New York: 91–104.

COWAN, S.T. & STEEL, K.G. 1993. *Manual for the identification of medical bacteria*. Cambridge University Press: 50–60.

ELLIS, D.S. & SMITH, M.D. 1987. *Laboratory manual for electron microscopy*. London School of Hygiene and Tropical Medicine: 27–28.

FOGGIN, C.M. 1987. Diseases and disease control on crocodile farms in Zimbabwe, in *Wildlife Management: Crocodiles and Alligators*, edited by G.J.W. Webb *et al.* Surrey Beatty & Sons: Harare: 351–362.

FOGGIN, C.M. 1992. Diseases of farmed crocodiles, in *Handbook on crocodile farming*, edited by G.A. Smith & J. Marias. The Crocodilian Study Group of Southern Africa, South Africa: 107–140.

FREUNDT, E.A. & RAZIN, S. 1984. The genus *Mycoplasma*, in *Bergey's manual of systematic bacteriology*. I, edited by N.R. Kreig. Williams & Wilkins: Baltimore: 749–769.

MOHAN, K., OBWOLO, M.J. & HILL, F.W.G. 1992. *Mycoplasma ovipneumoniae* infection in Zimbabwean goats and sheep. *Journal of Comparative Pathology*, 107:73–79.

MOHAN, K., SADZA, M., MADSEN, M., HILL, F.W.G. & PAWANDIWA, A. 1994. Phenotypic characterization of Zimbabwean isolates of *Pasteurella multocida*. *Veterinary Microbiology*, 38:351–357.

ROSE, D.L. & TULLY, J.G. 1983. Detection of beta-glucosidase: Hydrolysis of esculin and arbutin, in *Methods in Mycoplasmology*. II, edited by S. Razin & J.G. Tully. Academic Press: New York: 385–389.

SENTERFIT, L.B. 1983. Preparation of antigens and antisera, in *Methods in Mycoplasmology*. II, edited by S. Razin & J.G. Tully. Academic Press: New York: 401–404.

WALLACE, A.C. (Jr) 1983. Growth inhibition test, in *Methods in Mycoplasmology*. II, edited by S. Razin & J.G. Tully. Academic Press: New York: 405–410.