OBSERVATIONS ON A FIELD OUTBREAK OF POX VIRUS INFECTION IN YOUNG NILE CROCODILES *(CROCODYLUS NILOTICUS)*

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ABSTRACT

A field outbreak of pox virus infection in juvenile Nile crocodiles *(Crocodylus niloticus)*, in which high morbidity and negligible mortality occurred, is described. Histopathological examination of the skin lesions revealed numerous large intracytoplasmic inclusions in the dermis and a very mild dermal inflammatory reaction. Scanning electron microscopical examination of the skin revealed the presence of large numbers of virus particles in the inclusions. Skin lesions persisted for 5 to 6 months.

Key words: Nile crocodile, *Crocodylus niloticus*, pox, stress

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Outbreaks of crocodile pox have been reported from North America[5] and southern Africa[13] and the disease is now stated to be present on many crocodile farms in Zimbabwe and elsewhere in Africa (Foggin C M 1989, personal communication). There are fears that the disease could have serious consequences, not only by causing mortality, but also by reducing the economic value of the survivors as a result of permanent skin damage.

This report describes various aspects of an outbreak of pox infection in hatchling Nile crocodiles *(Crocodylus niloticus)* on a crocodile farm in the Transvaal Lowveld.

A group of approximately 1 500 7-month-old crocodiles, which had hatched on the farm, were housed in a closed-environment unit, situated at least one kilometre away from other crocodile houses and the ponds housing the brood crocodiles. Water for the crocodiles was pumped from wells in a river bed and was treated with chlorine at a rate of 5 mg ℓ^{-1}. In the closed-environment house, the young crocodiles had access to shallow water as well as to dry areas. Air and water temperatures in the unit were maintained at 35°C and 32°C respectively. The crocodiles were fed daily on a mixture of minced red meat, bone meal, carcass meal and a vitamin and electrolyte premix (Soluble vitamins & Electrolytes, Salisbury S.A. Veterinary (Pty) Ltd).

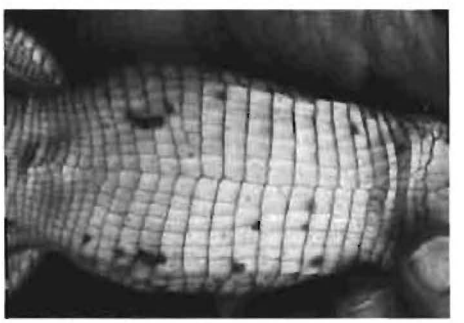

Fig. 1: **Pox lesions on the ventral body surface of a crocodile**

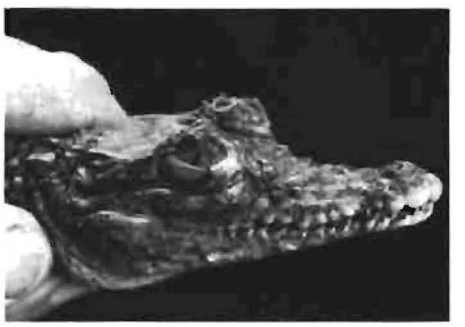

Fig. 2: **Pox lesions on gingivae and eyelids of a 7-month-old crocodile**

The affected crocodiles had survived an outbreak of salmonellosis which occurred 2 to 3 weeks prior to the development of multiple crusty lesions on the skin around the mouths and eyes and on the tail tips. During the outbreak, antibiotic therapy was administered over a period of several weeks, initially by intramuscular injections every 48 h for 10 d, followed by prolonged medication in the feed. This treatment was followed by vaccination of all the crocodiles with an inactivated calf paratyphoid vaccine (Inactivated polyvalent calf-paratyphoid vaccine, Veterinary Research Institute, Onderstepoort). During this entire period, the crocodiles were frequently handled and subjected to the additional stress of daily pond scrubbing and disinfection (Adcodyne, Adcock Ingram).

The skin lesions appeared as dark brown, crusty pox-like lesions up to 3mm in diameter, with a sharply outlined central depression. The lesions were situated between the scales and occurred over the entire body (Fig. 1), but were concentrated mainly on the ventral and lateral surfaces of the body and tail, the upper and lower surfaces of the limbs, and around the jaws and eyes (Fig. 2). Occasionally lesions were evenly spaced in a straight line (Fig. 3). Secondarily infected crusts, which tended to develop into small ulcerated moist areas, were noted in particular on the gingivae around the teeth, on the eyelids and on the feet. The lesions in the mandibular area were often confluent, with an accompanying loss of pigmentation.

The lesions were initially seen in the larger crocodiles within the groups and the latter animals also developed more severe lesions than the smaller crocodiles. During the course of the disease, the number of lesions per crocodile increased steadily over a one-month period and the number of crocodiles affected also increased, resulting in an almost 100% morbidity. The appetite of the crocodiles, which had been severely depressed during

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Fig. 3: **Pox lesions on the lateral surface of the tail of a crocodile. Lesions situated in a straight line (arrow) suggest infected bite wounds**

Fig. 4: **Crocodile pox lesion. Note the inclusions in epidermal cells and mild perivascular inflammatory reaction (arrow) HE X 100**

the salmonellosis outbreak, improved steadily. The occasional mortalities which still occurred, were attributed to chronic lesions associated with salmonellosis (a necrotising enteritis and subsequent intestinal occlusion by fibrinous exudate). Although the pox lesions were numerous on many of the crocodiles, no mortalities could be ascribed to this infection.

Crocodiles with severe facial pox lesions were treated topically with gentian violet (Gentian Violet 1%, Tedro). No other treatment was administered. The health of the crocodiles progressively improved over the next few months, while mortalities virtually came to an end.

When the crocodiles were re-examined 6 months later, they were found to be in good condition and no further mortalities had occurred. Approximately 3% of the population had retained a few faintly visible, focal dark areas on the ventral body surface, presumably at the sites of previous pox lesions.

Crocodiles of approximately 7 months of age (n = 6) with multiple skin lesions from the outbreak described above, were submitted to the Veterinary Research Institute, Onderstepoort. All animals but one, were euthanased and skin lesions excised for freezing in liquid nitrogen for future viral isolation attempts, as well as for light microscopical and scanning electron microscopical (SEM) examination. The remaining animal was kept for further observation.

Frozen sections were prepared from unfixed skin lesions and stained with Sudan IV. Further skin specimens were fixed in 10% buffered formalin, and routinely processed, sectioned and stained with haematoxylin and eosin (HE).

Skin lesions from the body, eyelid and lip of one of the crocodiles were fixed in 4% glutaraldehyde buffered with 0,1M Millonings phosphate buffer (MPB) pH7,3[4] for 24 h. After 2 MPB rinses, the tissue was post-fixed in 1% osmium tetroxide in 0,1M MPB. Two further rinses in MPB preceded conventional ethanol dehydration (50, 70, 90, 96 and 3 x 100%). The samples were then critical point dried (CPD), using liquid CO_2 in a Polaron critical point drier bomb (Bio-Rad, Watford, England). After CPD, each lesion was bisected with a feather-cut razor blade and attached with silver paint (cut surface uppermost) to a SEM viewing stub. The samples were sputter-coated with 30nm of gold using a Balzers SCD 020 Sputter Coater (Balzers Union, Union, Liechtenstein), and then viewed in an Hitachi S-2500 scanning electron microscope at 20kV.

The skin lesions persisted for 5 months on the affected animal which had been kept for observation. One month later, however, only small scars remained on the edges of scales adjacent to previous lesions.

The skin lesions were characterised by marked epithelial proliferation. The epithelial cells were enlarged and filled with single large eosinophilic intracytoplasmic inclusions (Fig. 4) which did not stain with Sudan IV. The perivascular tissue in the dermis adjacent to the epithelial lesions, was infiltrated by a small number of lymphocytes.

Inclusions consisted of densely packed virus particles. These had the appearance of rectangular to ovoid discs measuring approximately 285nm x 195nm x 135nm (Fig. 5-7).

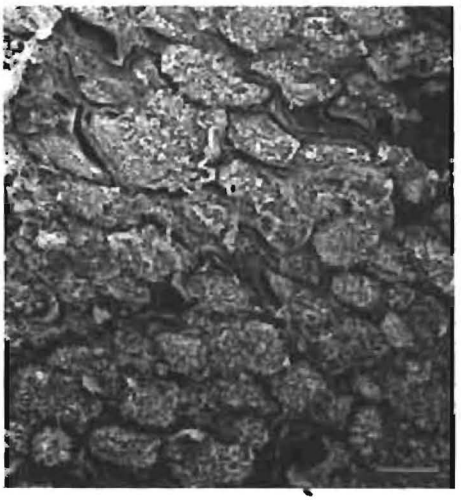

Fig. 5: **Scanning electron micrograph of crocodile pox lesion showing the inclusion bodies (arrow), (bar = 20μm)**

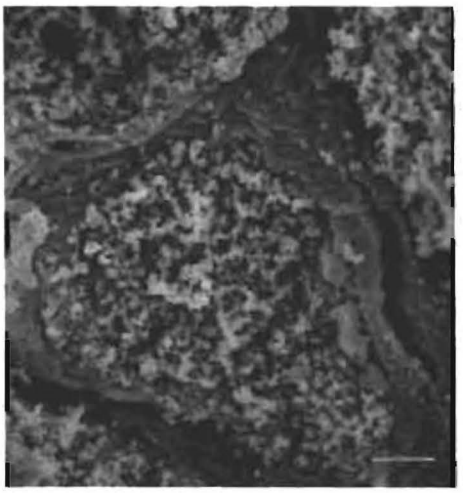

Fig. 6: **Scanning electron micrograph of a single epithelial cell revealing densely packed virus particles within the inclusion body occupying most of the cell (bar = 2 μm)**

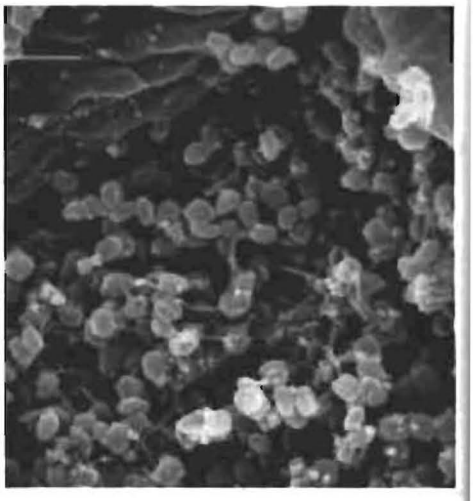

Fig. 7: **High magnification of virus particles in the same cell as shown in Fig. 6 (bar = 0,5 μm)**

Animals infected with crocodile pox in this outbreak, have not yet been slaughtered for commercial use and therefore the effect of the pox lesions on the skins could not be ascertained. However, considering the limited inflam-

matory reaction in the dermis associated with the epidermal lesions, it appears unlikely that the skins would be permanently blemished by scars. The limited dermal inflammatory reaction may furthermore indicate a poor immunological reaction to the infection.

Viral contamination of the environment in a crocodile farm may be very severe as a consequence of the unnaturally dense population of hatchlings. The resulting waterborne infection could then penetrate small skin lesions, including bite wounds as shown in Fig. 3, and consequently cause a large percentage of animals in a pen to become infected.

The epidemiology of crocodile pox infection is as yet unknown. To date, all the reported cases of pox outbreaks have occurred in juvenile crocodiles within one year of hatching[1 3]. In the present outbreak, the animals had been under severe stress. Stress factors were also implicated in the pox outbreak described by Horner[3]. It is likely that wild-caught crocodiles introduced onto most farms as breeding stock, are carriers of the virus. If this is not the case, one would have to postulate the existence of a non-crocodilian virus reservoir. Pox viruses have as yet, however, not been described in any other reptiles or amphibians. While the present outbreak produced minimal mortality, an outbreak in Zimbabwe was associated with heavy losses[1].

Culture of crocodile pox virus, has so far been reported only in crocodile embryo cells (Foggin C M 1989, personal communication). Since crocodile eggs are very expensive and available only for a very short period during the summer, another system or cell line for culturing this virus needs to be found. These difficulties have prevented us up to now from investigating the pathogenesis and epidemiology of the disease. It is also not known, whether recovered animals can act as carriers. Nor is it possible to develop a vaccine to protect animals against this disease without a suitable culture system. The classification of the virus is the subject of a separate study. In HE stained preparations, the inclusion bodies resemble those seen in fowl pox infections. However, the inclusions in the present study did not stain with Sudan IV, which distinguishes this virus from avian pox virus. Furthermore fowl pox lesions are more proliferative and show more marked inflammatory reactions. The recorded measurements of the virus particles in dermal epithelial cells differ only slightly from those of fowlpox virus[6].

REFERENCES

1. Foggin C M 1985 Diseases and disease control on crocodile farms in Zimbabwe. In: Webb G J W, Manolis S C, Whitehead P J (ed.) Wildlife Management: Crocodiles and Alligators. Surrey Beatty Ltd: 351-362
2. Fritzsche K, Gerriets E 1962 Geflügelkrankheiten. Paul Parey Berlin Hamburg, 2nd edn: 182
3. Horner R F 1988 Poxvirus in farmed Nile crocodiles. Veterinary Record 122: 459-462
4. Hayat M A 1970 Principles and techniques of electron microscopy. Van Norstand Reinhold Company Vol 1: 338 and 344
5. Jacobson E R, Popp J A, Shields R P, Gaskin J M 1979 Poxlike skin lesions in captive caimans. Journal of the American Veterinary Medical Association 175: 937-940
6. Tajima M, Ushijima T 1966 Electron microscopy of the avian pox viruses with special reference to the significance of inclusion bodies in viral replication. Japanese Journal of Veterinary Science 28: 107-118