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HERPESVIRUS-LIKE INFECTION IN A PAINTED TURTLE (*Chrysemys picta*)

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Abstract: A painted turtle (*Chrysemys picta*) which died in captivity had marked necrosis in the liver and lungs with numerous intranuclear inclusion bodies in hepatocytes and respiratory epithelial cells. Electron microscopy revealed herpesvirus-like particles in cells in affected tissues.

INTRODUCTION

Herpesvirus diseases are reported with high frequency in most classes of vertebrates including fish,^{8,17} birds^{5,11,12} and mammals.^{2,4,6} Herpesviruses have been isolated from green iguana (*Iguana iguana*) cells in tissue culture¹ and from the venom of the Indian cobra, *Naja naja*, and the banded krait, *Bangarus fasciatus*.⁷ In these cases, however, the presence of the virus was not associated with any disease process. Herpesvirus-like infection caused focal necrosis in the venom gland of Siamese cobras¹³ and a herpesvirus-type agent has been described in association with skin lesions in captive green sea turtles (*Chelonia mydas*).¹⁰ Although a review of liver diseases in reptiles by Von Rainer Will states that no known diseases of the liver are caused by viruses,¹⁶ a subsequent report of viral hepatitis in Pacific pond turtles (*Clemmys marmorata*) is available.³ Here we describe a similar disease associated with herpesvirus-like particles in a captive painted turtle, (*Chrysemys picta*).

CASE HISTORY

An adult male Painted turtle was hospitalized at the Metropolitan Toronto

Zoo. A swelling containing an abscess was surgically removed from the side of the head. The turtle was treated with Betadine topically and given injections of chloramphenicol and ascorbic acid post-operatively. Six days later, the turtle died.

At necropsy, performed by zoo veterinarians the same day, findings included pulmonary edema. The liver was friable and greenish-brown in colour and the gall bladder was distended with bile. The spleen was congested. The kidneys were pale. The stomach contained several nematodes of various sizes adhering to the lining. Shell rot lesions were evident on the plastron. The surgical wound on the side of the head was healing.

MATERIALS AND METHODS

Representative samples of liver, lung, intestine, spleen and kidney were fixed in buffered 10% formalin. Following dehydration through graded alcohol and embedding in paraffin, 5 μ m sections were cut and stained with hematoxylin and eosin.

For electron microscopic examination, small cubes of formalin-fixed tissue were washed twice in water then placed into

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glutaraldehyde for two hours. The tissues were then transferred to Sorenson's buffer with 2% sucrose and subsequently embedded in epon. Thin sections were cut on an ultramicrotome, stained with 5% uranyl acetate and 0.4% lead citrate, and examined on a Philips electron microscope, model 200.

RESULTS

At histological examination, the relevant findings were in the liver and lung. Numerous randomly scattered microfoci of coagulation necrosis were throughout the liver (Fig. 1). Inflammatory response to these foci was minimal, consisting only of a very few infiltrating granulocytes. Hepatocytes around these foci and, occasionally, those in more normal areas, contained large intranuclear eosinophilic inclusion bodies (Fig. 1). In affected nuclei, marked margination of chromatin occurred and

a distinct clear halo was around the inclusion.

In the lung, peribronchial accumulations of mononuclear cells and granulocytes, often with granulocytic invasion of the mucosa, were evident. The lumina were usually filled with accumulated granulocytes, red blood cells and sloughed epithelial cells (Fig. 2). Metaplastic epithelial cells lining infundibula were vacuolated and degenerate, often piled three or four deep, and frequently intranuclear eosinophilic inclusion bodies were seen. In the pulmonary infundibula there was an accumulation of sloughed, inclusion-bearing epithelial cells, granulocytes and fibrin. Occasionally, foci of necrosis were in infundibular septa.

In the kidney, degeneration had occurred in randomly scattered tubules which contained mineralized debris. There were often small accumulations of

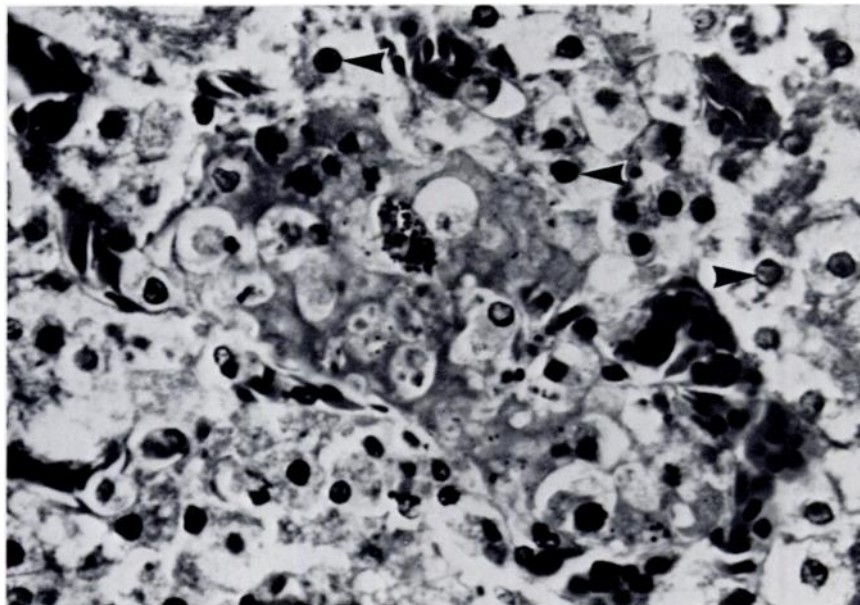


FIGURE 1. Small focus of necrosis in the liver with adjacent hepatocytes containing large dense intranuclear inclusions (arrows). H&E \times 560.

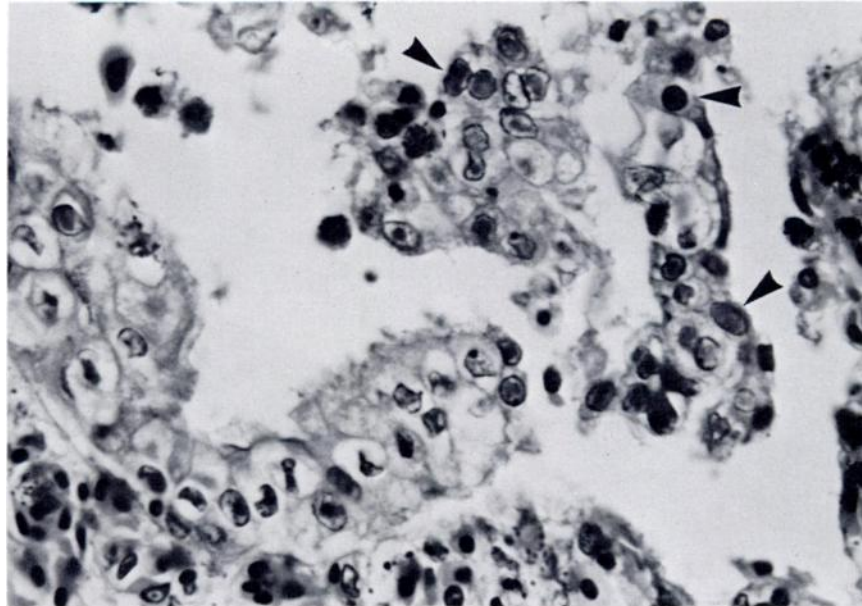


FIGURE 2. Bronchial epithelium, with many cells bearing intranuclear inclusions (arrows), sloughing into the lumen. Scattered heterophils are present in the submucosa and among epithelial cells. H&E \times 560.

granulocytes and mononuclear cells in interstitial tissue of affected areas.

Electron microscopic examination revealed numerous viral particles (Fig. 3) in nuclei and cytoplasm of hepatocytes and bronchial epithelial cells. As is often the case with formalin fixed tissues,^{4,6} autolysis and fixation artifact impaired ultrastructural cellular detail. The virus particles, however, were in generally good condition. Most particles were naked, but an occasional enveloped virus could be found. The hexagonal capsids measured 85 to 115 nm in diameter. Both empty capsids and complete particles were seen.

DISCUSSION

The histological lesions of the case described here are entirely compatible with a virus-induced disease. Intranuclear inclusion bodies are highly suggestive of a

virus infection, although non-viral intranuclear inclusions do occur.¹⁴ In this case, however, virus particles were demonstrated in the affected tissue. The nephrosis may be due to renal constipation resulting from inadequate water intake during the concurrent illness.

The virus particles can be presumptively placed into the herpesvirus group based on their size, structure and shape.¹⁵ Adding further support to this classification is the necrotizing pattern of the lesions which is typical of many herpesvirus-induced diseases in other species.^{2,4,5,6,8,9,12} The disease in this turtle also resembles that described in Pacific pond turtles.³

Unfortunately, only formalin-fixed tissue was received in this case so that the diagnosis of herpesvirus infection could not be confirmed by animal inoculation or virus isolation. One can only wait for another case of this disease to

occur in order to pursue the problem further. Herpesvirus infection, however, should be considered in the differential diagnosis of chelonian mortality.

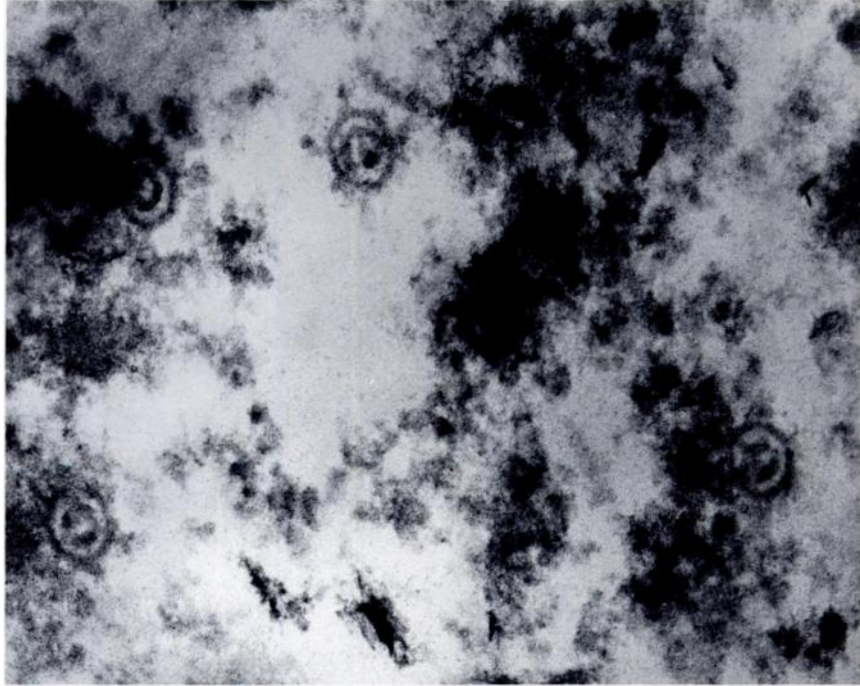


FIGURE 3. Electron micrograph of herpesvirus-like particles in a hepatocyte. $\times 18,400$.

Acknowledgements

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