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Source: Journal of Wildlife Diseases, 19(4) : 349-352

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-19.4.349

Journal of Wildlife Diseases, 19(4), 1983, pp. 349–352

CHRONIC BACTERIAL PNEUMONIA IN FREE-RANGING EASTERN BOX TURTLES (*TERRAPENE CAROLINA CAROLINA*)

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ABSTRACT: Chronic bacterial pneumonia was diagnosed in two free-ranging Eastern box turtles. Mucoid exudation into the upper respiratory tract and bilateral, caseating pneumonia were seen grossly. Microscopically, chronic, active inflammation with pseudomembrane formation occurred in the nasal sinuses and lungs while caseating granuloma-like structures were also observed in alveoli and associated infundibulae. Since viral, protozoal or fungal agents could neither be demonstrated in tissue sections by light or electronmicroscopy, nor could they be isolated in vitro, the gram-negative bacteria, seen in large numbers within the lesions and easily cultured in vitro, were considered the etiologic agents of this disease.

INTRODUCTION

Bacterial pneumonia resulting from infection by gram-negative bacteria, such as *Pseudomonas* sp. and members of the family Enterobacteriaceae, is a common disease entity in captive chelonians (Keymer, 1978; Jacobson, 1981). Recently Barker and Goltz (1981) detailed the clinicopathology of acute, subacute and chronic forms of bacterial pneumonia in reptiles. Both airborne and embolic routes of infection were described. The present report will describe the clinicopathological features of chronic bacterial pneumonia in two free-ranging Eastern box turtles.

METHODS AND MATERIALS

In October 1981, two moribund male Eastern box turtles were discovered half buried in a dry creek bed about 360 m apart on the grounds of Treehouse Wildlife Center in Macoupin County, 9.3 km southeast of Brighton, Illinois. Both animals were severely emaciated and depressed, moving only with extensive prodding. Copious, bilateral, cream-colored discharge obstructed the external nares and exuded from the swollen, inflamed conjunctivae. Marked respiratory distress, characterized by stridorous, open-mouth breathing was apparent. Both turtles died within 35 min of discovery or 20 min after admission.

Necropsy was performed within 30 min of death and tissues were obtained from all major organ systems, fixed in buffered 10% formalin, routinely processed, embedded in paraffin, section at 7 μm and stained with H&E. Sections of lung were also stained with Brown and Brenn gram stain, Gomori's methenamine silver stain, periodic acid-Schiff and Ziehl-Neelsen acid fast stain (Veterinary Services Lab., Ralston Purina Company, St. Louis, Missouri 63164, USA).

Swabs of lung tissue were plated on blood agar, Mackonkey's agar, Steffen-Hentges agar (S-H) and Sabouraud's agar and incubated aerobically at 37 C. Isolates were identified by standard microbiological methods. (Veterinary Services Lab., Ralston Purina Company, St. Louis, Missouri 63164, USA).

Five grams of lung tissue were aseptically collected from one turtle and stored at −70 C for 63 days. The tissue was thawed, ground and diluted in sufficient sterile saline to produce a 10% suspension, which was clarified by centrifugation. The supernatant was passed through at 45 μm filter to remove bacterial contaminants and 0.15 ml was inoculated via the chorioallantoic membrane (CAM) route into each of 10 7-day-old embryonated chicken eggs. After 2, 4, 6, 8 and 10 days of incubation at 33 C, two eggs each were examined for the presence of plaques on the CAM (Veterinary Services Lab., Ralston Purina Company, St. Louis, Missouri 63164, USA).

Formalized lung tissue was sectioned into 3–5 mm blocks and post-fixed in 2% glutaraldehyde for subsequent electron microscopy (EM Section, Department of Pathology, St. Louis University School of Medicine, St. Louis, Missouri 63164, USA).

RESULTS

A mixture of gram-negative bacteria including *Morganella morganii*, *Acinetobacter calcoaceticus*, *Serratia marcescens* and *Pseudomonas* sp. were isolated from both turtles. *Morganella* was by far the most prominent isolate on culture plates. No fungal growth was observed on Sabouraud's agar. No evidence of CAM plaque formation was noted on the embryonated chicken eggs after 10 days of incubation. Electron microscopy failed to reveal the presence of viral particles, but large numbers of coliform bacteria were present within necrotic debris as well as macrophages and giant cells.

At necropsy the nasal vestibule and cavity, trachea and extrapulmonary bronchi contained varying amounts of creamy-yellow, tenacious exudate. The lungs were expanded and turgid

Received for publication 20 January 1982.

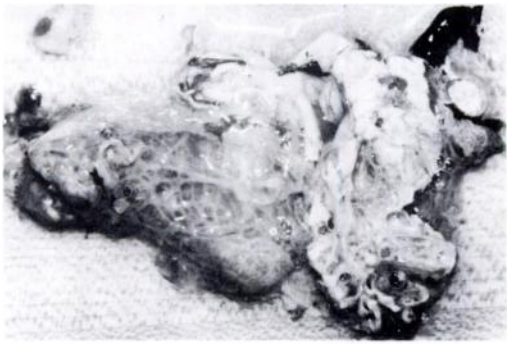

FIGURE 1. Longitudinal gross section of lung of an Eastern box turtle. Note thickened infundibular septae, and caseous exudate filling infundibula and mesobronchi in distal half of lung. ×1.7.

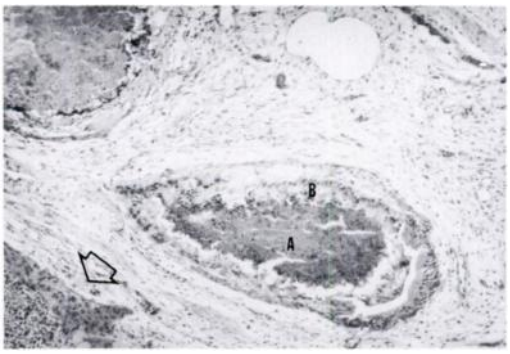

FIGURE 2. Section of distal portion of lung of an Eastern box turtle. Squamous metaplasia is seen in bullae in lower left (arrow). Septal connective tissue is edematous and infundibulum in center contains central necrotic debris (A) surrounded by a confluent layer of giant cells (B). H&E. ×85.

with pronounced, edematous infundibular septae. In the distal portions of the lungs, variably sized, firm, yellowish-white, caseous masses filled most infundibula, mesobronchi and their associated bullae (Fig. 1). A thick, yellow, mucoid exudate filled many of these same anatomical areas in the proximal portion of the lungs. No lesions were noted in other organs, except a small, solitary, yellow nodule in the mucosa of the small intestine of one turtle. Carcasses of both animals were devoid of fat deposits and severe generalized muscular atrophy was obvious.

Histologically the conjunctivae, nasal vestibule and cavity were markedly inflamed. A layer of multinucleate giant cells and macrophages replaced the epithelium over extensive areas of mucosa. A mixture of necrotic cellular debris, heterophils, macrophages, inflammatory edema and colonies of gram-negative bacilli overlaid the giant cells. Large numbers of heterophils and monocytes were emigrating from hyperemic blood vessels into and through the submucosa and lamina propria immediately below these areas. Occasionally, areas of normal epithelium or stratified squamous metaplastic epithelium free from inflammation were noted.

In the trachea and extrapulmonary bronchi, changes included small, scattered foci of loss of cilia and goblet cell hyperplasia with extensive mucus extrusion, squamous metaplasia or mucosal necrosis and inflammation. The latter areas were devoid of the normal pseudostratified epithelium and coated by a thick layer of necrotic inflammatory debris with admixed gram-negative bacilli. The debris was surrounded to varying extents by multinucleate giant cells and macrophages. Large numbers of mixed inflammatory cells diffusely infiltrated the lamina propria and submucosa.

Lesions were relatively few and mild in the cranial portions of the lungs. In the mesobronci and their bullae, variably sized focal areas of epithelial necrosis and desquamation with luminal exudation of heterophils, macrophages, inflammatory edema and some fribrin were noted. In associated infundibula, focal epithelial necrosis, inflammatory exudation and infiltration of macrophages and the formation of small numbers of multinucleate giant cells occurred. Mucous cuboidal metaplasia or squamous metaplasia of epithelium was noted immediately bordering some of these foci.

In the central lung area, necrobiotic and inflammatory changes became progressively more severe and chronic. They occurred along broad expanses of the mesobronchi and into their associated bullae and infundibula, many of which were filled with an amorphous, eosinophilic coagulum. A layer of macrophages and multinucleate giant cells was forming between the denuded mucosal surface and the coagulum. In many infundibula this layer had completely surrounded the coagulum. Infrequently there were areas of normal epithelium or mucous cuboidal or stratified squamous epithelium bordering inflammatory foci.

The most severe lesions were encountered in the distal portions of the lungs. Mesobronchial, bullar and infundibular surfaces were almost

completely denuded of epithelium; areas of normal or squamous epithelium were found only occasionally. The layer of giant cells had succeeded in completely walling off the coagulum in most bullae and infundibula (Fig. 2). An uninterrupted layer of giant cells and macrophages was present between the inflammatory debris and the denuded mesobronchial mucosa.

The septal connective tissue in the distal portions of the lungs was edematous and contained a very mild and diffuse scattering of mixed inflammatory cells (Fig. 2). Immediately adjacent to occasional infundibula in the distal portions of the lung the septum exhibited severe necrosis, intense mixed inflammation, vascular hyperemia and perivascular heterophilic cuffing. Multinucleate giant cells were forming along the edge of this area. Focal areas of mesothelial hypertrophy and hyperplasia occurred on the visceral pleura. Subpleural heterophilic and macrophage aggregations were common.

Special stains revealed large numbers of gram-negative bacilli colonizing the inflammatory exudate or coagulum as well as packed within macrophages and giant cells. No fungi, acid-fast bacilli or protozoan parasites were found in lung sections.

The small intestine nodule noted on gross examination was a granuloma containing an unidentified parasite. No other lesions were observed in the organs of these turtles.

DISCUSSION

The microscopic picture described in these turtles is that of chronic bacterial pneumonia. As the lesions appeared to be arising in and disseminating through the respiratory tree and no lesions suggestive of embolism or septicemia were found in the interstitium or elsewhere in the body, the route of infection seemed to be aerogenous.

The pathogenesis of these lesions probably began with a loss of cilia and goblet cell hyperplasia and was followed by acute inflammatory exudation and epithelial necrosis resulting in the formation of an inflammatory coagulum or caseous mass. Eventually the coagulum was walled off by macrophages and giant cells producing a granuloma.

Areas of squamous metaplasia were frequently encountered immediately adjacent to the above areas or as an isolated change.

Clinical-pathologic correlations of bacterial pneumonia in reptiles has been described by Barker and Goltz (1981). These authors characterized aerogenous subacute to chronic bacterial pneumonia as the accumulation of large amounts of necrotic debris, heterophils and bacteria in the infundibula. Respiratory epithelial necrosis, especially at the blind end of the infundibula was followed by a granulomatous response of macrophages and "occasionally giant cells" which formed "abscesses or granuloma-like structures." Further it was noted that "squamous metaplasia of bronchial and infundibular epithelium" occurred in chronic areas.

Morphologically the lesions described in the present report differ little, if any, from these. The bacteria isolated from these turtles are reported to be responsible for this type of pneumonia (Frye, 1981).

Lesions of viperid paramyxo-like virus infections such as proliferative interestitial pneumonia with occasional pneumocyte inclusion bodies (Jacobson et al., 1980, 1981) were not seen in these cases. Nor were hepatic necrosis and pneumonia with intranuclear inclusion bodies characteristic of herpes-virus infection in painted turtles (*Chrysemys picta*) (Cox et al., 1980) observed.

Electron microscopy and virus isolation in embryonated eggs failed to reveal any evidence of viral infection. It should be noted, though, that attempts at virus isolation could have been better performed if isolation attempts had been accomplished earlier, if embryonated eggs were inoculated amniotically, or if the incubation temperature had been closer to chelonian body temperature, i.e., 25–27 C. However, embryo mortality would have been expected at this temperature. Ideally a reptilian cell culture system should have been used for isolation attempts.

ACKNOWLEDGMENTS

I wish to thank Mr. Jim Gary for performing microbiological culturing and Mr. Jessie Urhan for assistance in electron microscopy.

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