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PNEUMONIA IN OTTOMAN VIPERS (*VIPERA XANTHENA XANTHENA*) ASSOCIATED WITH A PARAINFLUENZA 2-LIKE VIRUS

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ABSTRACT: A paramyxovirus related to parainfluenza 2 (PI_2) virus was recovered from the lungs of two dead ottoman vipers from a zoological collection. Snakes of other species in the collection were unaffected. Histologic examination of the vipers' lungs revealed interstitial pneumonia, and degeneration and hyperplasia of bronchial and atrial epithelia. Scattered vacuoles, some of which contain eosinophilic inclusion bodies, were seen in the cytoplasm of several cells of affected epithelial tissues. The virus recovered from pulmonary tissues of the snakes replicated optimally at 30 C in a variety of cell cultures and hemagglutinated chicken erythrocytes. Viral hemagglutination was inhibited by PI_2 virus antiserum, but not by antisera to PI_1, PI_3, respiratory syncytial, and canine distemper viruses. Indirect immunofluorescence with PI_2 antiserum specifically stained inclusions in the epithelial cells of respiratory tissues and infected cell cultures.

Key words: Ottoman viper, *Vipera xanthena xanthena*, pneumonia, parainfluenza 2-like virus, virus isolation.

INTRODUCTION

Isolation of a paramyxovirus from tropical American pit vipers (fer-de-lance, *Bothrops atrox*) from a snake farm in Zurich, Switzerland was reported by Lunger and Clark (1978) and by Clark et al. (1979). Infection of the vipers by this virus was associated with respiratory signs, lethargy, and eventual death (Lunger and Clark, 1978). They proposed that the virus be named fer-de-lance virus. It was unrelated, by complement fixation, to types A, B, and C influenza viruses, 10 various parainfluenza viruses, mumps virus, measles virus, respiratory syncytial virus, canine distemper virus, and rinderpest virus (Clark et al., 1979).

A virus, similar to fer-de-lance virus, associated with interstitial pneumonia, gliosis, neuronal degeneration, and demyelination in a rock rattlesnake (*Crotalus lepidus*) was reported by Jacobson et al. (1980). They have since identified infections by similar viruses in members of several genera of the family Viperidae held in zoological collections (Jacobson et al., 1981). Their serological studies indicated that snakes of other families experienced subclinical infections of these viruses.

In the present report we describe fatal respiratory disease in Ottoman vipers, associated with infection by a paramyxovirus related to parainfluenza 2 virus.

MATERIALS AND METHODS

Case history

Two Ottoman vipers were obtained for breeding purposes 1 mo prior to their death from a private collector in Florida. The snakes had been in the Florida collection for 2 yr since their purchase from another private collector.

The snakes were placed in housing units with a potential breeding mate. The units had newspaper as a substrate, and a basking lamp providing a temperature gradient of 25 C to 29 C. They had access to water in a dish and were fed white mice.

The snakes were found dead with an abundant amount of clear mucus in their mouths. Gross lesions were unremarkable. The lungs appeared congested and the air sacs contained frothy serous exudate. The gastrointestinal tract was empty. The only bacterium cultured from the lungs and air sacs was *Providencia rettgeri*.

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Cell cultures

The cells used were propagated as described previously (Potgieter and Mare, 1974a). Fathead minnow skin, CRFK feline kidney, BHK-21 hamster kidney, viper heart, and Vero monkey kidney cells were obtained from the American Type Culture Collection (Rockville, Maryland 20852, USA). Swine testicular (ST) cells were obtained from the National Animal Disease Center (Ames, Iowa 50010, USA). Primary bovine turbinate cells (BTU) were prepared as described previously (Potgieter et al., 1980).

Embryonated eggs

Fertile eggs from specific-pathogen-free hens were used.

Antisera

Equine antisera to PI_1 (Sendai), PI_2 (SV_5), PI_3 (HA-1), and respiratory syncytial viruses were purchased from Flow Laboratories, Inc. (McClean, Virginia 22102, USA). Antiserum to canine distemper virus was prepared in rabbits as described previously (Potgieter and Mare, 1974b). Canine distemper virus (Bu strain) used for rabbit inoculation was obtained from M. J. G. Appel (Baker Institute, Cornell University, Ithaca, New York 14850, USA). Canine antisera (no. 789 and no. 698) to PI_2 (SV_5) virus were obtained from David A. Bemis (Department of Microbiology, University of Tennessee, Knoxville, Tennessee 37916, USA).

Immunofluorescence

The indirect fluorescent antibody test was done on tissue impression preparations and infected cell cultures as described previously (Potgieter and Aldridge, 1977b).

Hemagglutination (HA) and hemagglutination inhibition (HI) tests

The HA test was done with cell culture fluids in round-bottom polystyrene microtiter plates (Flow Laboratories, Inc., McClean, Virginia 22102, USA). Serial two-fold dilutions of fluids in 0.1 M pH 7.2 phosphate buffered physiological saline (PBS) were mixed with equal volumes (0.05 ml) of 0.75% chicken erythrocytes in PBS. The mixtures were held for 60 min at room temperature and observed for hemagglutination. Appropriate erythrocyte controls were maintained simultaneously. The greatest dilution of material which hemagglutinated erythrocytes was considered to contain one hemagglutinating unit. The number of hemagglutinating units per unit volume of undiluted material was calculated.

The HI test was conducted by mixing equal volumes (0.025 ml) serial two-fold dilutions of antiserum in PBS with four HA units of virus in round bottom microtiter plates which then were incubated for 30 min at 37 C. An equal volume (0.05 ml) of 0.75% chicken erythrocytes was then added to the virus-serum mixtures. Appropriate serum, virus and erythrocyte control tests were done simultaneously. After 1 hr at room temperature the greatest dilution of serum which inhibited hemagglutination was recorded. The reciprocal of this dilution was considered to be the antibody titer of the serum.

Virus sampling

A 10% suspension of lung tissue from two of the Ottoman vipers was prepared in cell culture medium with antibiotics as described previously (Potgieter et al., 1977). Flasks (25 cm^2) containing one of each of the cell culture types were inoculated with the suspension as described before (Potgieter et al., 1977). An inoculated flask containing one of each of the cell types was incubated in a 5% CO_2 atmosphere at room temperature, 30 C, 33 C, and 37 C. The cell cultures were examined for a cytopathic effect (CPE) daily for 14 days. Fluids from cultures with a CPE or collected after 14 days incubation were tested for hemagglutinating activity.

Electron microscopy

Ultrathin sections of infected cell cultures and viper tissues were prepared and examined as described previously (Potgieter et al., 1979; Kocan et al., 1977).

Histologic examination

Lung samples were fixed in neutral buffered 10% formalin, embedded in paraffin, sectioned at 5 μm, and stained with hematoxylin and eosin.

RESULTS

The snakes' oral cavities contained abundant clear mucus. The lung in each snake was mildly to markedly congested and air sacs contained frothy serous exudate.

Lung lesions seen by histologic examination included an interstitial pneumonia with accumulations of lymphocytes, macrophages, scattered heterophils and a few multinucleated cells in the bronchial lamina propria (Fig. 1). Bronchial and atrial epithelia were moderately to markedly hy-

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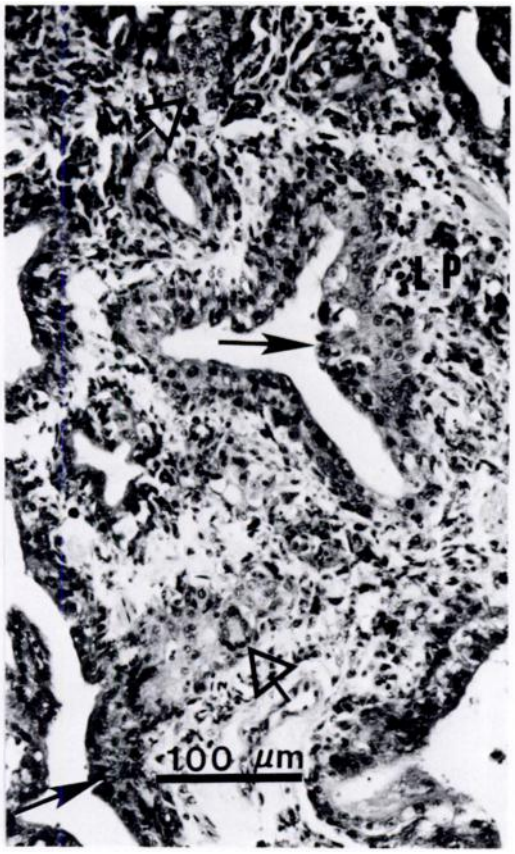


FIGURE 1. Moderate to marked thickening and hyperplasia of the bronchial and atrial epithelia (arrows). Multinucleate giant cells are present in the epithelium (arrowheads). The lamina propria (LP) contains an infiltrate of mononuclear cells and heterophils. H&E stain.

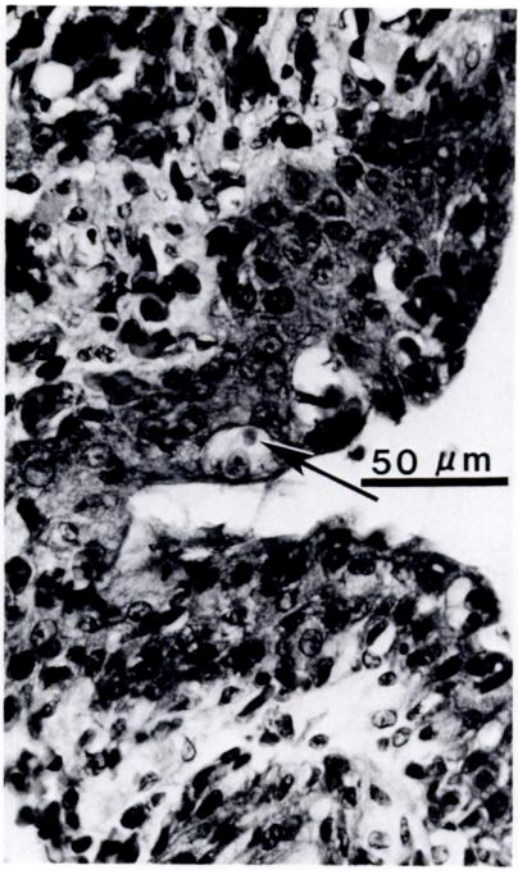


FIGURE 2. Marked hyperplasia of respiratory epithelium in the lung. Numerous vacuoles are evident, some of which contain eosinophilic inclusions (arrow). Degenerating epithelial cells with pyknotic nuclei are present. H&E stain.

perplastic. Several epithelial cells were vacuolated. Some of these vacuoles were large and contained well demarcated eosinophilic inclusion bodies or degenerating cell debris with pyknotic nuclei (Fig. 2). Karyomegaly was seen in some epithelial cells whereas others were degenerating, indicated by an increased eosinophilic staining of the cytoplasm, karyorrhexis, and nuclear pyknosis (Figs. 1, 2). Widely scattered multinucleated giant cells and heterophils were present in these epithelia especially in areas of epithelial degeneration (Fig. 1).

Electron micrographs of epithelial inclusions revealed tubular particles, seen in longitudinal and transverse planes, characteristic of paramyxovirus ribonucleoprotein particles (Fig. 3). The particles were 12 to 18 nm in diameter and had the regular periodicity of ribonucleoprotein helices of paramyxoviruses. Maturing virions were budding from plasma membranes of some cells (Fig. 4).

The virus was grown initially in FHM cells from lung tissue. It produced massive syncytia in these cells after 8 to 10 days incubation at 33 C. The cells degenerated eventually and became detached from the growth surface. Cytopathic changes were seen in FHM, BHK-21, ST, Vero, and BTU

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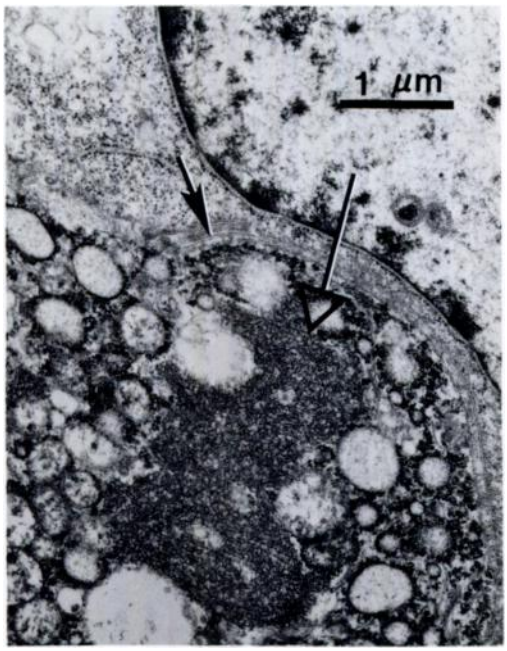


FIGURE 3. Cytoplasmic aggregation of tubular particles (large arrow) in an area of dilated endoplasmic reticulum in a respiratory epithelium cell. The affected area appears to be surrounded by a cytoplasmic membrane (small arrow).

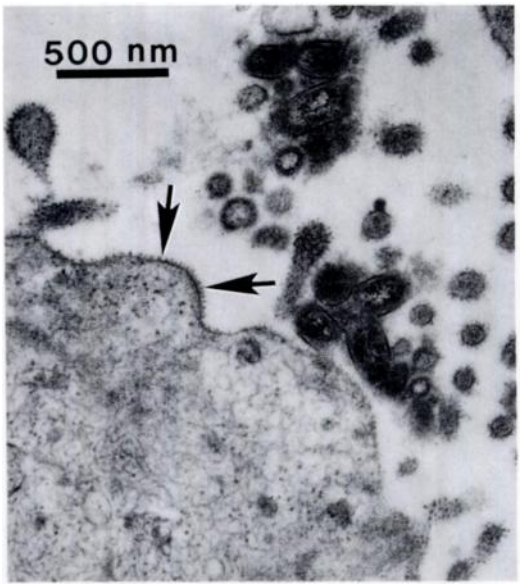


FIGURE 4. Paramyxovirus-like particles can be seen budding from the plasma membrane of a respiratory epithelium cell. Ribonucleoprotein-like tubules are present in the cytoplasm close to where virions are forming at the plasma membrane. Distinct peplomeres are present on the membrane where budding of virions is imminent (arrows). Numerous extracellular virions can be seen.

cells incubated at room temperature, 30 C and 33 C. A CPE developed in BHK-21 cells at 37 C but not in the other cell cultures held at this temperature. Cytopathic changes did not develop in CRFK or viper heart cells. Cytopathic changes developed sooner in cells at 30 C than at any other incubation temperature and hemagglutination titers were higher in fluids from infected cell cultures incubated at this temperature. Hemagglutination titers varied from 4 to 64 units in 25 μl of fluid.

TABLE 1. Hemagglutination inhibition of Ottoman viper virus with various antisera.

Antiserum to virus	Source of antiserum	HI titer
PI_1 (Sendai)	Horse	10
PI_2 (SV_5)	Horse	80
PI_2 (SV_5)	Canine no. 789	320
PI_2 (SV_5)	Canine no. 698	80
PI_3 (HA-1)	Horse	<10
CDV	Rabbit	<10
RSV	Horse	<10

Embryonating hens' eggs, the allantoic cavity of which had been inoculated with the virus and incubated at 30 C, were unaffected. Allantoic fluid harvested 7 days after inoculation did not hemagglutinate chicken erythrocytes.

The virus reacted in the HI test with PI_2 antisera from a horse and two dogs (Table 1). A slight reaction occurred with PI_1 antiserum, but all other antisera used did not inhibit hemagglutination by this virus.

Thin sections of infected BHK-21 cells were examined by electron microscopy. Many cells contained cytoplasmic inclusions consisting of viral-like tubules (Fig. 5).

Cytoplasmic fluorescence was seen in lung tissues and in infected cell cultures when PI_2 antisera were used in the indirect fluorescent antibody test (Fig. 6). Slight

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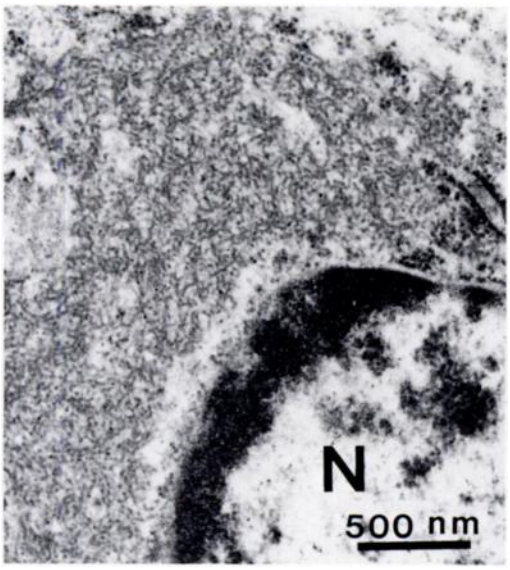


FIGURE 5. Tubular structures resembling paramyxovirus ribonucleoprotein are present in the cytoplasm surrounding the nucleus (N) of an infected BHK-21 cell.

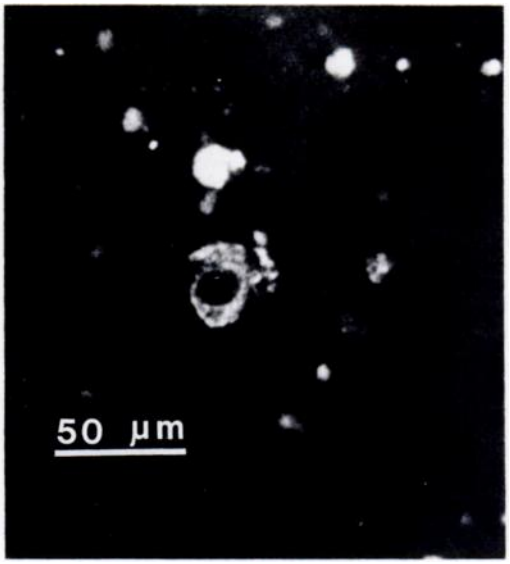


FIGURE 6. Immunofluorescence of a lung tissue touch impression with parainfluenza-2 virus antiserum. Note the cytoplasmic fluorescence.

fluorescence was seen with PI_1 antiserum, but no fluorescence occurred when the other antisera were used.

DISCUSSION

Illness associated with a paramyxo-like viral infection is reported in members of several genera of the family Viperidae (Clark et al., 1979; Jacobson et al., 1981). These genera include *Bothrops*, *Bitis*, *Crotalus*, *Trimeresurus*, and *Vipera*.

In the present report we document paramyxovirus-associated respiratory disease in Ottoman vipers. A virus was recovered in cell cultures which morphologically resembled paramyxoviruses and it agglutinated erythrocytes, as do many members of the Paramyxoviridae. The virus shared antigens with PI_2 virus because it reacted with SV_5 antisera in the HI procedure and in the IFAT. Slight reaction was noted also with PI_1 antiserum. However, there are discrepancies on the nomenclature of the SV_5 group of viruses (Hsiung, 1972). Some antigenic relationships exist between the SV_5 group of viruses and PI_2 virus (Chanock et al., 1961) but Hsiung (1972) indicated that significant differences exist and suggested classifying the former as PI_5. In addition, the International Committee on Taxonomy of Viruses has concluded that the simian and canine paramyxoviruses related to SV_5 are PI_5 viruses (Matthews, 1979).

Fer-de-lance virus differs from the isolate reported here in that the former grew in a greater variety of cell cultures (Clark et al., 1979). Moreover, fer-de-lance virus was antigenically distinct because it did not share antigens with various parainfluenza viruses, various orthomyxoviruses, respiratory syncytial virus or rinderpest virus (Clark et al., 1979).

The paramyxoviruses from various members of Viperidae reported by Jacobson et al. (1981) agglutinated chicken erythrocytes. They were able to detect antibodies to these viruses in various Viperidae and other nonviperid snakes. However, the antigenic relationship of their isolates to fer-de-lance virus or any other

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paramyxoviruses was not determined. They reported minor biological differences between the various isolates which, however, were antigenically homogeneous.

In many respects, the disease in Ottoman vipers reported here resembles that associated with fer-de-lance virus (Clark et al., 1979) and the paramyxovirus-associated disease of Viperidae reported by Jacobson et al. (1981). Lesions in the snakes occurred primarily in the respiratory tract and were characterized by interstitial pneumonia, epithelial hyperplasia, epithelial metaplasia, and epithelial eosinophilic cytoplasmic inclusions. In all instances, members of the Viperidae were affected and the diseases were associated with paramyxovirus-like viral infections. The viruses grew optimally at 30 C in a variety of cell cultures and they hemagglutinated erythrocytes. However, it is evident also that antigenic and biologic variations exist among the various paramyxovirus-like viruses recovered from the viperid snakes. The interrelationships among these various paramyxoviruses of viperid snakes needs to be elucidated.

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