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Degenerative Encephalopathy in a Coastal Mountain Kingsnake (Lampropeltis zonata multifasciata) due to Adenoviral-like Infection

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ABSTRACT: In March 2000, an approximately 30-yr-old, male coastal mountain kingsnake (Lampropeltis zonata multifasciata) presented with disequilibrium and unresponsiveness to stimuli that ultimately lead to euthanasia. Histologically, there were foci of gliosis primarily within the caudal cerebrum, brainstem, and cervical spinal cord. Several glial cells and endothelial cells contained magenta, intranuclear inclusion bodies. Electron microscopy of the inclusions revealed paracrystalline arrays of 79–82 nm, viral-like particles. DNA in situ hybridization of sections of formalin-fixed brain using a mixture of two digoxigenin-end-labeled, adenovirus specific, oligonucleotide probes at low and high stringency was positive for adenovirus.

Key words: Adenovirus, DNA in situ hybridization, encephalopathy, Lampropeltis zonata multifasciata, snake

In January 1996, an approximately 30-year-old, male coastal mountain kingsnake (Lampropeltis zonata multifasciata) housed at a southern Californian zoological park (34°39′ N, 118°14′ W) was observed having mild head and body tremors that for the most part resolved spontaneously in 3 mo. Over the next 4 yr, the tremors continued sporadically and were most evident when the snake was handled. In February 2000, the tremors became progressively worse during feeding resulting in difficulty ingesting food. In March 2000, the snake was euthanized with an intracardiac injection of pentobarbital sodium (Pentosol Injection, Med-Pharmex, Inc., Pomona, California, USA). On the day of euthanasia, the snake was curled upside-down and was nonresponsive to touch. At necropsy, the kingsnake was thin and the kidneys appeared small.

Sections of brain, skin, heart, oral mucosa, fat body, kidney, liver, teeth, eyes, calvaria, cervical vertebrae, spinal cord, skeletal muscle, gall bladder, pancreas, epididymis, lung, stomach, trachea, and esophagus were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at 5.0 μm, and stained with hematoxylin and eosin (HE). For transmission electron microscopy, sections of formalin-fixed cerebrum were transferred into modified Karnovsky’s fixative, washed with sodium cacodylate, and post-fixed in unbuffered 2% osmium tetroxide reduced by 2.5% potassium ferrocyanide. Sections of cerebrum were then dehydrated through a graded ethanol series, soaked in propylene oxide, infiltrated with epoxy resin (Spurr’s Low Viscosity Resin, Ted Pella, Redding, California), sectioned at 70 nm, stained with 6% methanolic uranyl acetate, counterstained with lead citrate, and examined with a transmission electron microscope (EM 10C transmission electron microscope, Carl Zeiss, Thornwood, New York, USA).

For DNA in situ hybridization, replicate sections of formalin fixed, paraffin embedded brain were deparaffinized in three changes of xylene for 5 min each. The sections were then rehydrated through graded alcohol solutions to 1× Automation Buffer, Biomeda Corp., Foster City, California). The tissue specimens were digested for 10 min in pepsin solution at 37 C. Pepsin activity subsequently was destroyed by heating the slides at 105 C for 8 min. The tissue sections were rinsed five times in 1× Automation Buffer. The sections were then treated with 100% formamide for 5 min at 105 C. The probe solution was then added and the charged
microscope slides (ProbeOn Plus slides, Fisher Scientific, Pittsburgh, Pennsylvania, USA) were incubated for 5 min at 105°C and then lowered to 32°C for an additional 1 hr of incubation. The probe solution was composed of a cocktail of oligonucleotide probes FN-23 and FN-96. These probes detect an adenovirus structural capsid protein and had previously been used to detect reptilian adenovirus in paraffin-embedded tissue sections (Ramis et al., 2000; Perkins et al., 2001). Probe FN-23 is probably responsible for most of the reactivity, but the combination of probes is routinely used for diagnostic work. Following hybridization, the sections were rinsed for 2 min in buffer with Triton X-100 and 1% sheep serum. Sites of hybridization were detected using affinity cytochemistry. Antidigoxigenin antibody (Digoxigenin Detection Kit, Roche Diagnostics, GmBH, Mannheim, Germany) conjugated to alkaline phosphatase was applied (500:1 dilution) and incubated for 1 hr at 37°C. Following incubation, the tissue sections were thoroughly rinsed in buffer and the chromagen solution was applied (nitroblue tetrazolium dye solution) for 1 hr at 37°C. The slides were then rinsed in distilled water, counterstained in 1% fast green dye for 5 min, rinsed, dehydrated, covered slipped, and examined microscopically. The presence of DNA hybrids was detected by the deposition of blue-black pigment (formazan). Both avian and reptilian positive and negative control tissues were used to validate the hybridization procedure.

The kingsnake had microscopic lesions confined to the brain that were centered primarily within the cerebrum. Histologically, several glial cells, endothelial cells, and few epithelial cells lining the lateral ventricles and in the choroid plexuses contained single, magenta to eosinophilic, intranuclear inclusion bodies (Figs. 1, 2). Individual endothelial and glial cells with intranuclear inclusion bodies were degenerate and necrotic. There were increased numbers of glial cells forming small nodular aggregates within the gray and white matter. Glial nodules centered primarily around neurons and small caliber blood vessels. The gray matter was multifocally vacuolated. Clear space surrounded several small arteries and capillaries within the neuropil. There were very low numbers of heterophils scattered throughout the neuropil. Multifocally, axons were swollen, dilated, and had spheroid formation. There were also multifocal, variably sized, (<50 μm), round, eosinophilic, globular bodies within...
the neuropil. Overall, similar but milder changes were noted within the brain stem and cervical spinal cord. Only a small section of cerebellum was examined microscopically and it was histologically normal.

Transmission electron microscopy of the cerebrum revealed many, 79–82 nm, nonenveloped, hexagonal outlined, viral-like particles arranged in paracrystalline arrays in few endothelial cells and glial cells (Fig. 3). The particles had electron lucent cores and electron dense outer rims (Fig. 4). The size, shape, and formation of paracrystalline arrays are characteristics of Adenoviridae (Cheville, 1994). The inclusions appeared intracytoplasmic in cells that had an absence of a recognizable nucleus. There was also similar viral-like arrays extracellular admixed with cellular debris. Endothelial cells with arrays had swollen mitochondria and vacuolated endoplasmic reticulum. Axons adjacent to affected blood vessels were swollen with dilatation of myelin sheaths. There was clear space and fibrillar material within the Virchow–Robin space of few blood vessels.

Following DNA in situ hybridization, adenoviral nucleic acid was demonstrated within nuclei of glial cells and fewer endothelial cells (Fig. 5). Chromagen deposition was observed within nuclei, but also exhibited diffusion into the cytoplasm of some cells. Chromagen deposition was intense under conditions of lower stringency, but was somewhat diminished under conditions of high stringency. These observations indicated that adenovirus infection was present in both glial and endothelial cells.

This coastal mountain kingsnake had...
progressive neurologic disease, which started as mild head tremors that pro-
gressed over a few years to disequilibrium and unresponsiveness to stimuli. Micro-
scopically, there were degenerative lesions within the caudal cerebrum that consisted
primarily of glial foci centered around neurons and small caliber blood vessels.
Degenerate glial cells and endothelial cells contained intranuclear inclusion bodies.
The damaged endothelial cells and glial
cells most likely elicited the glial cell pro-
liferation. The clear space and fibrillar ma-
terial around some of these blood vessels
suggested cerebral edema secondary to
endothelial damage.
Transmission electron microscopy of the
inclusions within the cerebrum revealed
paracrystalline arrays of viral-like particles
that had features consistent with adenovi-
rus. Herpesvirus was another differential
diagnosis based on the histopathologic re-
sults, but herpesviruses are 120–180 nm in
diameter and enveloped (Cheville, 1994),
which were not electron microscopic fea-
tures of the virus in this snake. Because of
the extensive degenerative lesions within
the brain, most of the inclusions appeared
developmentally from
release of the arrays following lysis of the
cells. Arrays noted by electron microscopy
within cells appeared intracytoplasmic.
This is not a characteristic of adenovirus,
which forms intranuclear arrays (Cheville,
1994). Most of the cells with apparent in-
tracytoplasmic arrays either didn’t have a
nucleus in the visualized field or had a
lysed nucleus.
The technique of DNA in situ hybrid-
ization used in this study was previously
used to help positively identify adenovirus
as the cause of hepatitis in a boa constrict-
tor (Boa constrictor) (Ramis et al., 2000)
and a viral enteropathy in a Mojave rattle-
snake (Crotalus scutulatus scutulatus)
(Perkins et al., 2001). DNA in situ hybrid-
ization helped to identify the intranuclear
inclusions in the brain of this kingsnake as
adenoviral-like inclusion bodies.
Viral diseases involving the central ner-
vous system are infrequently reported in
snakes. Pythons and boa constrictors with
inclusion body disease are sometimes af-
flicted with nonsuppurative meningoen-
cephalitis (Schumacher et al., 1994b).
Ophidian paramyxovirus usually causes
pneumonia, but has also been associated
with central nervous system disease in rat-
tlesnakes (Jacobson et al., 1980). A reovi-
rus has been isolated from the brain of a
rattlesnake with neurologic signs but no le-
sions were noted microscopically within
the brain (Vieler et al., 1994). Adenovirus
infection has been previously reported in
the gastrointestinal tract, liver, kidneys,
and heart of snakes (Heldstab and Bestet-
ti, 1984. Jacobson et al., 1985; Schumacher
et al., 1994a; Ramis et al., 2000), but there
have been no reports of adenoviral infec-
tion in the central nervous system of
snakes. Approximately 60% of the DNA
sequence of an adenovirus from a corn
snake (Elaphe guttata) has been cloned
and sequenced (Farkas et al., 2002), and
a complete DNA sequence of a frog ade-
novirus has been reported (Davison et al.,
2000).
Degenerative lesions in the brain with
minimal to no associated inflammation is

FIGURE 5. Adenoviral DNA within the nuclei of
glial cells (arrows). In situ hybridization with FN-23
and FN-96 oligonucleotide probes and fast green
counterstain. Bar = 25 μm.
not a typical presentation for viral infection. Usually, viral infections of the central nervous system elicit a nonsuppurative type of inflammatory response (Jubb and Huxtable, 1993). However, there is a reported case of a rattlesnake with paramyxoviral infection of the central nervous system that had only gliosis and axonal degeneration (Jacobson et al., 1980), which are lesions similar to the degenerative lesions noted in the cerebrum of this kingsnake.

Adenoviral infection can cause different forms of neurological disease in humans and animals. Adenovirus was associated with fatal cerebral edema in infants (Chatterjee et al., 2000) and adenovirus type 7 has been associated with acute encephalopathy in humans (Kim and Gohd, 1983). Reye’s syndrome, a primarily juvenile-onset, neurologic disease linked with salicylates, has been associated with different viral infections including adenovirus (Brown, 1974; Daugherty and Heubi, 1985). Patients can develop encephalopathy characterized by cerebral edema and notable absence of inflammation, which was similar to the lesions noted in this kingsnake. Certain strains of mice infected with mouse adenovirus type-1 can develop fatal hemorrhagic encephalopathy (Guida et al., 1995). This disease causes mainly central nervous system signs like tremors, ataxia, and paralysis. Endothelial cells in the brain and spinal cord are sites of viral infection, leading to vasculature damage (Kaplan et al., 1998). The adenoviral-like infection in this kingsnake also primarily affected endothelial cells causing vascular damage and cerebral edema.

This kingsnake had an atypical presentation for adenoviral infection. The viral infection centered within the brain and caused degeneration and necrosis of individual endothelial cells and glial cells. This elicited the edema and gliosis noted with the brain. Though DNA in situ hybridization on the brain helped to identify the inclusions as adenovirus, virologic cultures are needed for definitive confirmation of the adenoviral etiology.

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LITERATURE CITED


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