Successful medical management of neonatal canine herpesvirus: a case report

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Abstract
A 25 day old litter of eight Labrador Retrievers was presented for evaluation due to acute onset of abnormal vocalization and dyspnea in two of the puppies. The two puppies showing clinical signs were hypothermic, tachypneic, and had abdominal pain. Despite supportive care and presumptive therapy for bacterial sepsis, the two puppies died. Postmortem examination revealed lesions characteristic of canine herpesvirus infection (CHV). This diagnosis was confirmed by CHV-specific polymerase chain reaction. Two additional puppies showed clinical signs. Specific antiviral therapy was then undertaken with the remaining six puppies, using exogenous warming and acyclovir suspension administered orally. The treated puppies all survived, were successfully weaned, and have uneventful health histories through their current age (26 months).

Introduction
Exposure of a naive bitch to CHV during the last three weeks of gestation can result in either late term abortion or neonatal death within the first few weeks of life, because inadequate maternal antibodies exist to allow passive immunity to be acquired by the neonates. Transmission of CHV occurs subsequent to contact with infectious vaginal fluids or oronasal secretions. Signs in the neonate include anorexia (poor weight gain), dyspnea, abdominal pain, incoordination, diarrhea, serous to hemorrhagic nasal discharge and petechiation of the mucous membranes. The mortality rate in litters infected in utero or during birth is commonly 100%, with deaths occurring during the first few days to a week of life. Puppies born to a naïve bitch may come in contact with CHV from another dog shedding the organism. Older (> three to four weeks of age) puppies exposed to herpes virus may have an inapparent infection but later central nervous signs including blindness and deafness have been observed. The recently infected bitch generally has no clinical signs. Subsequent litters of the bitch are usually normal, having acquired maternal antibodies.3,4,6
Materials and Methods
Two 25-day-old female Labrador Retriever puppies were examined because of acute dyspnea and vocalization. These puppies were two of a litter of eight whelped without incident and showing normal weight gain and behavior until the previous 24 hours. Four days previously, the litter was vaccinated for Bordetella bronchisepticaa (intranasally) and dewormed with pyrantel pamoateb (5.0 mg/kg, PO) according to routine preventative health care practice. The litter nursed from birth, and the dam was normal.

Physical examination of both puppies revealed hypothermia (rectal temperature 36.3 C and 36.0 C respectively), dyspnea, a markedly tense abdomen and continuous vocalization. Despite supportive care (exogenous warming, supplemental feeding with artificial bitch's milk, subcutaneous lactated ringers solution, and ceftiofur sodium d (2.5 mg/kg, SC, q12h) the puppies died within 12 hours and then underwent postmortem examination. A culture of the peritoneal cavity was performed for aerobic bacteria. Tissue samples were collected for microscopic evaluation and included kidney, liver, lung, thymus, mesenteric lymph node and spleen.

Because of gross findings of hemorrhagic foci in the lungs and kidneys, renal tissue was submitted for CHV-specific polymerase chain reaction (PCR). A 0.1 mg piece of renal tissue was finely minced with a sterile blade and then genomic DNA was extracted using a kit e following manufacturer's instructions. The PCR reactions were performed as described previously for the glycoprotein B gene of CHV-1. Amplification of a 120 base pair fragment was performed using primers CHV-F and CHV-R in a thermal cycler. The reaction was performed using canine genomic DNA as a negative control. Protocols for minimizing contamination including performing PCR in a hood dedicated to PCR in a separate room from all other upstream and downstream applications, dedicated pipettors and other equipment, and plugged pipette tips were performed. The products were visualized in a 2.5% agarose gel stained with ethidium bromide by UV transillumination. To confirm that the product was amplified from CHV-1, the amplicon was sequenced directly using PCR primers after purification with a kit. Big dye terminator cycle DNA sequencing was performed. The electropherogram was analyzed with Chromas and the sequence compared to published CHV using the BLAST algorithm from the National Center for Biotechnology Information.

Poor weight gain and vocalization were reported in two additional puppies. The primary differentials included neonatal bacterial sepsis and CHV infection. Because of the lack of response to therapy for bacterial infection in the two deceased puppies, the remaining six puppies were treated with acyclovir suspensioni at 20 mg/kg, PO, q6h for 7 days, and exogenous heat adequate to raise the rectal temperature to just above 37.7 C.

Results
Gross postmortem evaluation of the deceased puppies found foci of hemorrhage in the lungs and kidneys. Histopathologic evaluation revealed severe nephrosis (proximal renal tubular epithelial necrosis and renal pelvic epithelial apoptosis with swollen vesicular nuclei), diffuse severe hepatic congestion and inflammation with vacuolar hepatopathy (neutrophilic, histiocytic and lymphocytic infiltration with apoptosis), acute severe
necrotizing bronchointerstitial pneumonia with pulmonary edema and hemorrhage (terminal bronchiolar hemorrhage and edema with fibrin accumulation, macrophage infiltration, necrosis and alveolar disruption), and thymic, lymph node and splenic lympholysis (severe diffuse cortical lympholysis with sinus congestion). Intranuclear inclusion bodies were suspected in macrophages from the lung of one puppy (not shown). Aerobic culture of the abdomen was negative for bacterial growth. A CHV-specific PCR confirmed the presence of CHV in the renal tissue (data not shown).

All six treated puppies survived and were successfully weaned between five and six weeks of age. All were placed in private homes and had uneventful subsequent health histories and normal behavior. Physical examinations including complete fundic evaluations were performed at 12-14 months of age and were normal in all. Two of the dogs have become active guide dogs for the blind and one is an assistance dog for the hearing impaired. All remain physically normal at 26 months of age.

Discussion

Canine herpesvirus is a widely recognized cause for fading puppy syndrome resulting in neonatal death. Premortem diagnosis of CHV infection in neonates can be challenging. Pathognomonic changes occurring in the kidneys include multifocal petechial hemorrhages. Intranuclear inclusion bodies can be difficult to find. Diagnosis by virus isolation or CHV-specific PCR is confirmatory. Treatment has been reported to be unrewarding and rare, with recovery suspected to be associated with residual cardiac and neurologic damage. Treatment with immune serum from affected dams is reported to be ineffective in infected puppies. Vaccine development is hampered by the poor immunogenicity of other herpesviral vaccines developed for other species, such as feline and bovine rhinotracheitis.

The epidemiology of this outbreak suggests that the puppies were born from a naïve bitch and exposed to CHV from another carrier dog at day 14-17 after birth. Two of eight (25%) succumbed to the disease, two of the remaining six showed early clinical signs after which antiviral therapy was initiated.

Acyclovir is an antiviral agent with activity against a variety of viruses including herpes simplex. Acyclovir is preferentially taken up by susceptible viruses and converted into the active triphosphate form, inhibiting viral DNA replication. Acyclovir is poorly absorbed after oral administration and is primarily hepatically metabolized. Acyclovir can increase the toxicity of nephrotoxic drugs. The half-life in humans is approximately three hours. Its use in veterinary medicine is not well established and it should be used with caution and only in situations where indicated. The safety and effectiveness in humans less than two weeks of age is not established. The dose was extrapolated from that for humans.

Incompletely developed immune systems and inadequate thermoregulation during the first days of life make neonates vulnerable to systemic infection (bacterial and viral). Adequate ingestion of colostrum must occur promptly post partum for puppies to acquire passive immunity. The transmission of protective immunity (placental or colostral
antibodies) between a bitch and her puppies depends upon the prior existence of adequate serum maternal antibodies.

The umbilicus of neonates should be treated with tincture of iodine immediately after birth to reduce contamination and prevent ascent of environmental bacteria into the peritoneal cavity (omphalitis-peritonitis). Neonatal bacterial peritonitis with septicemia can cause rapid deterioration of the puppy resulting in death if not recognized and treated promptly. Factors shown to predispose a puppy to septicemia include endometritis in the bitch, a prolonged delivery/dystocia, feeding of replacement formulas, the use of ampicillin, stress, low birth weight (< 350gms), and chilling with body temperature < 35.5 C.8 The bacterial organisms most frequently associated with septicemia are E.coli, Streptococci, Staphylococci, and Klebsiella spp.. Commonly, a decrease in weight gain, failure to suckle, hematuria, persistent diarrhea, unusual vocalization, abdominal distention and pain, and sloughing of the extremities indicate septicemia may be present. Prompt therapy with broad spectrum, bactericidal antibiotics, optimal nutrition via supported nursing, tube feeding or bottle-feeding, maintenance of body temperature, and appropriate fluid replacement are indicated. The third generation antibiotic ceftiofur sodium is an appropriate choice for neonatal septicemia as it alters normal intestinal flora minimally and is usually effective against the causative organisms.10 Failure to respond to antibiotic therapy for neonatal septicemia should prompt consideration of CHV infection.

This case report emphasizes the importance of isolating naïve bitches and their offspring from dogs shedding CHV during late gestation and through six weeks post partum. Specific antiviral therapy, if instituted in a timely manner, may reduce mortality from CHV.

References


Endnotes

Progard-KC, Intervet, Millsboro, DL 19966
Nemex Tabs, Pfizer, New York, NY 10017
Esbilac, Pet Ag Inc, Hampshire, IL 60140
Naxcel, Pfizer, New York, NY 10017
Dneasy Tissue Kit, Qiagen, Germantown, MD 20874
MJ Research, Boston MA 02451
Microcon, Millipore, Bedford, MA 01730
ABI Prism, Foster City, CA 94404
Zvirax, GlaxoSmithKline, Research Triangle Park, NC 27709