Neonatal Viral Infections of Pups: Canine Herpesvirus and Minute Virus of Canines (Canine Parvovirus-1)  

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Introduction
Experience has taught many breeders who had accepted neonatal pup death rates of 15 - 25% that simple management could greatly reduce mortality. Examination of bitches for general and reproductive health before and after whelping, supplemental or tube feeding of pups that fail to suckle and providing warmth, which is vital to pups during the first 2 weeks of life since temperature regulation is poor are important factors. Supplemental radiant heat to raise the environmental temperature to ~85ºF, and a relative humidity of ~60% during the first week of life, especially if pups are orphaned, has reduced mortality rates in several kennels from ~25% to <10%. More than 75% of pup deaths occur prior to the 3d week of life, the vast majority occurring during the first week due to physiologic, congenital/genetic, behavioral (bitch), environmental conditions or bacterial septicemias. Unfortunately, there is a discouraging lack of knowledge of the true causes of most neonatal illnesses or death and little research is being done on this important subject.

Infectious diseases are believed to comprise only a very small portion of pup deaths up to the time of weaning; however, two viral infections have been described that affect pups during the first 2 - 5 weeks of life: Canine herpesvirus is widely recognized; minute virus of canines (CPV-1) has only recently been recognized as a pathogen. Canine adenovirus-1, distemper and canine coronavirus, as well as several bacterial infections, also may cause puppy deaths.

Canine Herpesvirus

Etiology
Canine herpesvirus is a typical alpha-herpesvirus. The virus is sensitive to lipid solvents and is readily inactivated at temperatures above 40ºC - the 1/2-life at 37ºC is <5 hours. It also is unstable at pH <~5 and >~8.0. CHV is stable at 40ºC and -70ºC, but is readily inactivated at ~200ºC unless stabilizing solutions are added. It also is rapidly inactivated by common disinfectants. Only one serotype is recognized although differences in cytopathic effects of certain isolates have been reported. Weak antigenic relationships have been shown with several other herpesviruses, but the significance is unclear. The virus grows only in canine cells and growth is best in primary or secondary kidney or testicular cells, although growth also occurs in several canine cell lines. Growth is optimal at 34 - 35ºC, with diminished virus yields above 36ºC. In cell cultures, most isolates produce typical clusters of rounded cells that detach, leaving clear "plaques", especially under agarose, methylcellulose or antibody-containing overlay media. Certain isolates have been reported to form syncytia (giant cells). All produce type-A intranuclear inclusions in infected cells. Analysis of the genomic organization of CHV reveals a closer relationship between CHV and feline herpesvirus, equine herpesvirus-1, pseudorabies virus and varicella-zoster virus than with other herpesviruses.

Epidemiology
The virus appears to be present worldwide in domestic and wild dogs. The virus has been found only in canines. Serosurveys are limited, but seropositive rates of >30% are common in field dogs. Some kennels have antibody prevalence rates as high as 100% without the development of disease in pups (see below). Transmission is by direct contact with infectious body fluids, since CHV is unstable in the environment. As with other a-herpesviruses, CHV becomes latent after a primary infection and is shed periodically, primarily in nasal or, rarely, in genital secretions.

Clinical Signs and Pathogenesis
The disease is generally asymptomatic in dogs infected when older than 1 - 2 weeks of age at the time of exposure. Disease caused by CHV is generally fatal in neonatal pups who lack immunity derived from their dams. Neonatal pups may be infected during passage through an infected dam's birth canal or, more commonly, by contact with oronasal secretions of the
dam or other dogs in a kennel. Infected littermates, or neighboring dogs who are shedding virus, also serve as sources of infection. Deaths of 1 to 4 week old pups are most common. Pups rarely die if they are 2 - 3 weeks old at the time of exposure. The duration of illness in newborn pups is 1 to 3 days. Signs consist of anorexia, dyspnea, pain upon abdominal palpation, incoordination and, often, soft, yellow-green feces. There may be a serous, or hemorrhagic nasal discharge. Petechia are common on the mucous membranes. Rectal temperatures are not elevated. Thrombocytopenia has been reported in dying pups.

CHV also may cause occasional in utero infections that result in the death of fetuses or pups shortly after birth. The virus also has been isolated rarely from dogs with vaginitis, conjunctivitis and respiratory illness. Asymptomatically infected dogs, or dams who suffered in utero infections, remain latently infected and virus may be excreted for about 1 week in nasal secretions or in genital secretions, and, thereafter, at unpredictable intervals over periods of several months, or years. Latent virus may be provoked by stress (movement to new quarters, introduction of new dogs) or, experimentally, by immunosuppressive drugs (corticosteroids) or antilymphocyte serum. Latent virus, demonstrated by the polymerase chain reaction, persists in the trigeminal ganglia, but other sites such as lumbo-sacral ganglia, tonsils, and parotid salivary gland have been identified. Once the virus enters a kennel, it generally spreads and causes asymptomatic infections - except in pregnant dams or very young pups from susceptible bitches, where infections of the fetus or newborn may occur. Recrudescence of latent virus favors spread of the virus among dogs, as well as the development of immunity which is transferred to pups via the placenta and colostrum.

Initial viral replication occurs in the nasal mucosa, the pharynx and tonsils of pups infected when they are less than ~1 week of age. CHV spreads in the body via the blood (in macrophages) to liver, kidneys, lymphatic tissues, lungs and the central nervous system. The incubation period is about 6 - 10 days and most affected pups are 1 to 3 weeks old at the time of onset of illness. Deaths in affected littermates usually occur over a period of a few days to a week. Litter mortality is commonly 100%. Pups exposed when they are older than 2 - 3 weeks of age, like adult dogs, usually have inapparent infections although central nervous signs, including blindness and deafness related to brain damage, have been observed. Pregnant dogs infected at mid-gestation, or later, may abort weak or stillborn pups with no signs in the dam; fetal pups infected during late gestation may appear normal at parturition, but die within a few days of birth. In mature females, primary genital infections are characterized by enlargement of the submucosal lymphoid follicles with variable degrees of vaginal hyperemia and petechial or ecchymotic hemorrhages. Vesicular lesions also have been reported during proestrus, but they regress during estrus. Discomfort appears to be minimal. Similar lesions have been reported over the base of the penis, but reports are scanty. Only one case of a repeated episode of CHV abortion/infected pups in a bitch has been reported - in Japan. Normally, naturally infected bitches that have lost pups with CHV subsequently give birth to normal litters, probably as a consequence of low levels of maternal antibody that protect the pups from clinical disease during the first week of life when they are most susceptible. Canine herpesvirus is not considered a significant cause of respiratory illness; however, this virus has been isolated from the tracheas of dogs with respiratory disease; other agents (Bordetella bronchiseptica, canine distemper, canine parainfluenza virus) are considered the principal cause of respiratory illnesses.

Pathology Characteristic ("pathognomonic")
Pathological changes occur in the kidneys. They consist of petechial or ecchymotic hemorrhages and focal necrosis, giving the kidneys a "speckled" appearance - circumscribed areas of hemorrhage ("red spots") on a pale gray cortex (Fig. 1). Multifocal areas of necrosis and hemorrhage occurs in several organs, including the lung, liver, brain and intestine. Lymph nodes and spleens are enlarged. Meningoencephalitis also is common. Necrosis in the placenta is observed in infected pregnant females. Fetal lesions are similar to those seen in affected puppies. Intranuclear inclusions may be seen in necrotic areas, but they may be difficult to find. Primary genital infections are characterized by lymphofollicular lesions and vaginal hyperemia; severely affected bitches may have ecchymotic submucosal hemorrhages. There appears to be no discomfort or unusual vaginal discharges. Vesicular lesions have been reported during proestrus and they regress during anestrus. Males may have similar lesions over the base of the penis and the prepuce.

Figure 1. Pathological changes in the kidneys: petechial or ecchymotic hemorrhages and focal necrosis, giving the kidneys a "speckled" appearance - circumscribed areas of hemorrhage ("red spots") on a pale gray cortex. - To view this image in full size go to the IVIS website at www.ivis.org. -
Factors that Influence the High Susceptibility of Neonatal Pups
The high susceptibility of neonatal pups to generalized infection has been associated with poor thermoregulation, low body temperatures, and incompletely developed immune systems during the initial 10 days of life. Experimentally infected newborn pups reared at elevated temperatures survived CHV infection; however, artificial temperature elevation of sick pups is not beneficial and cannot be recommended as a "treatment". Some pups who survived experimental infections at elevated temperatures became blind, deaf or suffered brain damage.

Immune Response
Neutralizing antibodies may be detected within 2 - 3 weeks of infection, and they persist for several years. Repeated viral shedding occurs sporadically, primarily in the nasal secretions. Shedding has been observed shortly after introduction of new dogs into a kennel ("threat stress") and, as noted above, immunosuppressive drugs given over a course of several days provoke episodes of recrudescence, where virus is shed for about 1 week. In such cases, there are concurrent increases in neutralizing antibodies. Such intermittent shedding assures the survival of CHV in the dog population and in breeding kennels.

Vaccines
An inactivated, subunit vaccine (Eurican Herpes 205, Merial Animal Health) has been available in Europe since 2003. The vaccine is specifically indicated for bitches during pregnancy. It consists of purified CHV glycoproteins in a mineral oil solvent. It has been shown to have few undesirable effects; nevertheless, transient edema may occur at the injection sites. Reactions usually regress within 1 week. Eurican Herpes 205 was shown to provide good immunity to newborn pups after 2 injections had been administered to their dams. Vaccine should be given to dams during heat or early pregnancy and, again, 1 to 2 weeks before the expected date of whelping.
An experimental attenuated live viral vaccine consisting of a "cold adapted" CHV mutant (small-plaque, SP) also has been developed, but it is not available commercially. There is little information on other suggested prophylactic measures such as the use of avian poxvirus.

The role of canine herpesvirus in neonatal mortality and reproductive failures has not been determined and many closed kennels with 100% seroprevalence have not had problems with CHV disease. However, when pregnant, susceptible (non-immune) bitches, within a month of whelping, are introduced into a kennel together with infected dogs, serious outbreaks may occur in their pups. Notwithstanding, at the present time the general value of CHV vaccines in reducing neonatal mortality cannot be estimated.

Treatment
Antiviral drugs have been generally unsuccessful, although some success has been reported with pups in exposed litters given vidarabine before the onset of symptoms. Antiviral treatment may spare life, but residual damage to the CNS and heart may occur. There has been success in preventing infection in neonatal puppies prior to exposure to CHV during kennel outbreaks by injecting 1 - 2 ml of immune sera from affected dams. Such treatment is effective only if virus has not generalized. Once illness develops in pups, serum treatment is ineffective. Immune serum is not available commercially.

Minute Virus of Canines (MVC, Canine Parvovirus Type-1, CPV01)
Etiology
A small virus called the "minute virus of canines" was isolated from normal fecal samples obtained from military dogs in Germany by Binn et al. in 1967. MVC (CPV-1) was believed to be a "non-pathogenic orphan virus" for about 20 years, until experimental studies revealed its pathogenicity for newborn pups and the fetus. CPV-1 was shown to be a novel canine parvovirus and recent DNA sequence analysis indicates a closer genetic relationship of CPV-1 to bovine parvovirus than to other mammalian parvoviruses studied. Only one cell line (WR 3873-D cells) has been found to support growth of CPV-1. Additional isolates from the lungs or intestinal contents of pups that died between 2 and 5 weeks of age have been made during the past 3 years in Sweden and Italy. MVC also been diagnosed as a cause of abortion in Germany. At the present time, about 30 field cases of neonatal pup death have been documented. Myocarditis has been observed in a few fatal cases of pups that died <1 wk of age.

Epidemiology
Only dogs are known to be susceptible to infection with CPV-1. It is believed that susceptible pups become naturally infected via the oral-nasal route; however direct proof is lacking. Transplacental infections appear to occur most commonly when dams are infected between 20 and 35 days of gestation. As noted above, serologic evidence indicates that CPV-1 is widespread in the dog population, with 50 - 70% seroprevalence rates in areas studied (USA, Japan, Switzerland). Neutralizing antibodies were found in commercial hyperimmune canine serum prepared in 1956.
Clinical Signs and Pathologic Changes
Most cases have been pups at necropsy who died suddenly between 1 and 3 weeks of age with respiratory distress and/or variably severe diarrhea. In litters where dead pups were observed, littermates that survived had vague signs, e.g., anorexia, failure to nurse or eat and mild respiratory illness or diarrhea. Such pups recovered within a few days. Transplacental infections with fetal deaths and abortion have been demonstrated experimentally; therefore, CPV-1 may be a cause of abortions or "failures to conceive". The natural route of infection is believed to be by oral exposure, as with the more pathogenic canine parvovirus type 2.

Principal pathologic changes the small intestine are hyperplasia of villus epithelial cells (duodenum, jejunum), mild necrosis of crypt cells, and numerous inclusion bodies segmentally distributed in duodenal/jejunal villous epithelial cells. In contrast to infection with CPV-2, the intestinal architecture generally remained normal. Viral pneumonia is common, with abundant inclusion bodies in bronchial epithelial cells. Additional changes in nursing pups include thymic edema and atrophy, enlarged and soft lymph nodes and soft, pasty stools. Dyspnea has been reported in ~50% of the cases reported. The principal clinical signs reported are those of "fading pups" - lethargy, loose stools or diarrhea, respiratory distress (dyspnoea), and sudden death in newborn pups attributed to viral myocarditis.

Experimental infections of pregnant bitches resulted in transplacental infections with fetal resorptions or abortions when the dams had been infected by the oral-nasal or parenteral (IV) routes between 25 - 30 days of gestation. MVC infection of dams exposed to MVC during mid-pregnancy (30 - 35 days of gestation) also resulted in myocarditis and anasarca in some of the newborn pups. Recently, we have observed two natural cases of MVC myocarditis in neonatal pups.

The pathogenesis of MVC and its clinical significance are not yet known, but preliminary findings, noted above, suggest that it may be responsible for a portion of deaths in pups less than 4 weeks of age and it may cause reproductive failures. Virus isolation has proved difficult, possibly because of the high level of antibodies in the infected dams at the time they resorbed their fetuses. Much more must be learned to ascertain the role of CPV-1 in canine disease. The reports of puppy deaths and abortions in Sweden, Germany and Italy, where CPV-1 was determined to be the cause, suggest that cases are more common than currently recognized.

Diagnosis
Diagnosis is difficult because of the lack of commercially available reagents. In laboratories where specific antibodies and WR 3873D cells are available, virus can be readily isolated and identified by immunofluorescence or immunocytochemistry. Histopathogic examination of tissues from dead pups may reveal viral inclusion bodies in the small intestinal villus epithelial cells or bronchial epithelial cells; however inclusions may be rare. Tests for neutralizing antibodies may be done where WR 3873D cells are available, but availability this cell line has been restricted to certain research laboratories.

References

Canine Herpesvirus


Minute Virus of Canines (MVC, canine parvovirus-type 1 CPV01)


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