more recent report suggests that infectivity is reduced considerably after 2 months' storage at room temperature. No apparent reduction in infectivity occurs over 12 months' storage at a temperature of −20 °C (Gordon and Angrick 1986).

In infected cells CPV characteristically multiplies in nuclei and the cytopathological changes (margination of the heterochromatin, vacuolation of the nucleolar fibrous components, and association of virus capsids with chromatin fibres) are considered to be diagnostic of parvovirus infection (Paradiso et al. 1982).

2 *Canine Parvovirus Disease Syndromes*

There are two distinct disease syndromes caused by CPV infection. These are the enteric form, a haemorrhagic enteritis, and the cardiac form, generally described as a non-suppurative myocarditis. A third manifestation, generalised necrotizing vasculitis, is occasionally diagnosed in puppies (Johnson and Castro 1984). With the pig parvovirus (PPV) and some other parvoviruses, infection of breeding stocks can lead to a further syndrome resulting in reduced fertility. However, despite worries voiced by owners and breeders (e.g. Thompson et al. 1985), there is no evidence that either CPV or its vaccine strains seem to be linked with these problems in dogs. The pathology of the disease is related to the age at which the animal succumbs to infection. Sites of virus multiplication are determined to a large extent by the fact that virus replication can only be supported by host cells which are actively dividing and so may be modified by any factor which influences the mitotic index of host tissue.

2.1 Canine Parvovirus-Induced Myocarditis

In utero or neonatal infection results in the cardiac form of the disease. This has been correlated with the rapid expansion of the myocardium during the later stages of foetal development, and with the fact that during the first 9 days of life, 2 %–4 % of myocytes are undergoing mitosis (Bishop and Hine 1975). There are three clinical presentations:

1. Acute non-suppurative myocarditis is the most common clinical form, seen in very young pups of up to 8 weeks of age. Death occurs suddenly, frequently without premonitory symptoms, and is thought to be due to terminal cardiac arrhythmias (Kelly and Atwell 1979; Atwell and Kelly 1980).

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2. Subacute heart failure with respiratory distress is seen in pups over the age of 8 weeks. In general, there is a sudden onset of dyspnoea followed by collapse. Symptoms which include tachycardia and cardiomegaly may be demonstrated on X-rays. Death usually follows within 24–48 h due to severe multifocal necrosis (Kelly and Atwell 1979; Robinson et al. 1980).
3. Cardiac myopathies and congestive heart failure are often seen in adolescent pups and adult dogs of between 5 months and 3 years. Clinical symptoms follow a history of exercise intolerance and periodic anorexia and include irregular pulse, regurgitant heart murmur, and moist crepitant rale of the lungs. There is abdominal distension with hepatomegaly and accumulation of ascitic fluid. Gross cardiomegaly is demonstrable on X-rays (Atwell and Kelly 1980; Robinson et al. 1980) and fibrosis, especially of the left ventricle, is seen at autopsy.

The primary lesion of the myocardium is caused by lytic viral replication which results in necrosis of the myofibres. This is accompanied by an inflammatory response causing infiltration of mononuclear cells into the interstitium (Robinson et al. 1980). Basophilic inclusion bodies can be demonstrated in myocardial nuclei in the acute and subacute stages. This is not possible during chronic disease when it appears the dogs have survived the phase of primary necrosis, only to develop extensive myocardial fibrosis and later die of congestive heart failure (Atwell and Kelly 1980).

Electron microscopic and immunofluorescent studies with myocardial nuclei from dogs that had died from CPV-induced myocarditis (Robinson et al. 1980; Carpenter et al. 1980) have revealed ultrastructural changes that are strikingly similar to the cytopathic effects observed in cultures of CPV-infected DKSV (dog kidney SV40 transformed) cells (Paradiso et al. 1982).

Much of the pathogenesis of CPV myocarditis is not fully understood, although the dependency of the virus on active host DNA synthesis has been well established (Kollek et al. 1982). Between 0 and 9 days of age, only 2 %–4 % of myocardial cells are dividing, and this may correspond with the most active phase of myocardial necrosis. However, in the developing foetus many other tissues are in active growth, yet a generalised infection is not seen. Experimentally induced myocarditis has been achieved in puppies that were infected *in utero* 8 days pre-partum (Lenghaus et al. 1980). If infected post-partum, then usually the enteric form of the disease develops (Robinson et al. 1980). However, Meunier et al. (1984) induced

myocarditis in seronegative 5-day-old pups by oral or intraperitoneal inoculation of CPV (at 10^5 $TCID_{50}$). The resulting infection was subclinical, although histological lesions were found and virus-infected myocytes were demonstrated.

Generalised infection is unusual but has been reported in one litter of 3- to 10-day-old pups that were dying with CPV-induced pneumonitis, hepatitis, gastritis, nephritis and enteritis (Lenghaus and Studdert 1982). This has not been reported in older puppies and may suggest that congenital infection results in myocarditis because the myocardium, unlike other foetal organs, is unable to undergo substantial repair. This situation is comparable with the ataxic syndrome seen in prenatal infection of kittens with FPV (Csizar et al. 1971b) (see Fig. 1).

Lenghaus et al. (1980) have suggested that the apparent predilection for myocytes in neonatally infected pups may be explained by "permissive differentiation." In beagle pups myocytes are essentially mononuclear at birth, but from 2–8 weeks post-partum, there is a gradual transition to the binuclear state (Bishop and Hiner 1975). The pups in Lenghaus's study were experimentally infected *in utero* and, although they appeared clinically normal at birth, they had very high antibody titres on day 1, indicating that they had mounted an immune response prior to birth. This antibody level was too high to have been due solely to maternal antibodies. The pups started to develop symptoms after 2 weeks, and these could be attributed to myocarditis. This led Lenghaus to conclude that CPV or its viral DNA had remained "dormant" and protected from antibody attack in myocyte nuclei until after birth when transition to the binucleate state permitted virus multiplication.

Tattersall (1978) investigated susceptibility as a function of host cell differentiation in studies with a particular isolate of MVM. He compared infection of non-differentiating cells with cells of the same line that were induced to differentiate. Even though both types had receptors for the virus, multiplication was blocked in the non-differentiating cells. More recently, it has been confirmed with different isolates of MVM that replication requires certain tissue-specific factors expressed by cells of a particular differentiated phenotype (Guetta et al. 1986). MVM may not be unusual in this respect, for factors other than dependence on nuclear division have also been described for other parvoviruses, including H-1 and Kilham rat parvovirus (Lipton and Johnson 1983). If differentiating cells similarly provide a particularly favourable environment for CPV replication, then this could explain both the absence of active necrosis

of the myocardium after 8 weeks of age, and the age limitations for susceptibility to myocardial infection. However, when seronegative pups of 3–4 weeks of age are experimentally infected, they develop the enteric form of the disease (Lenghaus et al. 1980; Robinson et al. 1980). At this age pups are still feeding from the dam, and so the mitotic index of gut epithelium is very low (Koldovsky et al. 1966). Virus should therefore preferentially localise in heart tissue at this stage.

It is generally accepted that parvoviruses only multiply in dividing cells in which replication is thought to depend upon a requirement for cellular DNA polymerases, particularly polymerases α and γ (Kollek et al. 1982). It is of interest that Lenghaus et al. (1985) have reported that FPV can replicate in cells in which cellular DNA synthesis is blocked. These observations, considered together with the close study of the pathogenesis of CPV, suggest that it is obviously an oversimplification to associate sites of virus multiplication with mitotic index alone.

2.2 Canine Parvovirus Enteric Disease

In weaned pups and non-immune older dogs, oral or nasal infection results in enteritis. The morbidity and mortality rates vary according to the age of the animal, pups of 6–20 weeks being the most susceptible. The disease is described as haemorrhagic enteritis which is accompanied by a leucopenia. Enteric lesions and changes in the number and type of circulating white blood cells resemble those symptoms produced in cats by FPV infection (Carlson and Scott 1977) and for this reason CPV has also been called canine panleucopenia by some workers (see. Fig. 1).

According to its severity, the disease is described as 'mild', 'acute' or 'peracute', when it is fatal. Hoffman and von Pock (1981) examined 111 cases, of which 63 % were between 7 and 23 weeks old. Of the total, 30 % suffered the mild form, 63 % the acute form and 7 % the peracute form. Stann et al. (1984) compared CPV enteritis in 40 'pound-source' dogs (procured from a commercial vendor) whose previous history was unknown. Clinical signs were uniformly severe, with a rapid onset of disease which resulted in death or else required euthanasia.

Kramer et al. (1980) have reported on the frequency with which different symptoms are observed, with depression and anorexia in 48 % of cases, vomiting in 85 %, diarrhoea in 100 % (but only

haemorrhagic in 55%), pyrexia in 45%, leucopenia in 43% and dehydration in 43%. In addition, these authors noted encephalitis, pancreatitis and cardiopulmonary signs with a frequency of 5%. In peracute infection, rapid dehydration and death follow within hours; in milder disease, recovery may occur within a week of onset (Kramer et al. 1980; Hoffman and von Pock 1981; McCandlish et al. 1981).

Studies such as those of Macartney et al. (1984) and Carman and Povey (1985) have characterised the pathological features of CPV enteric disease by following the course of infection in dogs orally infected with virus. These workers have shown how the sequential development of lesions in myeloid, lymphoid and intestinal tissues are related to the clinical picture. Although the disease presents clinically as an enteritis, it would appear that the lymphocyte is the primary target cell. Following oronasal infection, there is extensive replication in the lymphoid tissue of the oropharynx and mesenteric lymph nodes, from which virus is shed and carried in the blood. Virus multiplication then continues in other lymphoid tissues such as thymus and spleenic white pulp. Lytic damage is reflected in the characteristic leucopenia seen during early infection. A marked viraemic stage follows at 4–5 days post-infection, when virus can be isolated at lower titres from most other tissues and in serum, although it is not cell associated. Gross and microscopic lesions involving the upper small intestine first appear at this stage, and the degree of damage to the crypt/villus architecture subsequently dictates the clinical severity of enteric disease. In fatal cases there is complete breakdown of normal architecture and considerable necrosis of epithelium. The neutropenia seen in severe cases may be attributable to increased loss of neutrophils through damaged intestinal walls.

The role of the lymphocyte as a primary target for virus infection has been inferred from experimental studies which show that CPV inoculated by routes other than by mouth produces milder symptoms than when given orally. This presumably indicates the importance of initial virus multiplication in oropharyngeal and mesenteric lymph nodes (Pollock 1982; Potgieter et al. 1981; O'Sullivan et al. 1984).

Pathological changes are only seen in tissues which have relatively high mitotic indices, and this may be used to correlate susceptibility to host cell DNA synthesis. This is a critical factor in determining the severity of infection in gut epithelium. In cats (and rats) it is known that cellular proliferation rates are greater in the upper small intestine than in the lower regions (Carlson and Scott 1977). This is presumed to be the case for other mammals and would be in agreement with the finding that duodenal and jejunal lesions are more advanced and

involve larger areas of tissue than those of the ileum. Other factors such as age, diet and intestinal flora also influence the course of enteric disease.

The mitotic index of gut epithelium is very low in suckling animals but increases sharply on weaning (Koldovsky et al. 1966). Weaning pups are therefore more susceptible to enteric infection, whereas perinatal infection results in myocarditis. As the animal ages, there is a decrease in cell turnover of gut epithelium (Thrasher and Greulich 1964a, b) and this could explain why infection in adult dogs is frequently less severe and often asymptomatic. It is of interest that difficulties encountered earlier when experimentally infecting adult dogs with CPV were overcome by oral inoculation following a period of starvation (Carman and Povey 1982). A possible explanation is that starvation causes a reduction in cell turnover, whilst re-feeding is accompanied by a surge of mitotic activity (Aldewachi et al. 1975). Inoculation of gnotobiotic and specific pathogen-free dogs rarely causes development of enteric symptoms and may only lead to seroconversion and some degree of leucopenia. Similar signs of infection are also seen in specific pathogen-free cats challenged with FPV (Carlson et al. 1977).

The presence of other gut pathogens may also influence the outcome of CPV infection. For example, a heavy round worm burden, Giardia salmonellae (Prange et al. 1982) and E. Coli infections (Isogai et al. 1989), and recent canine corona virus infection (Appel 1988) can increase the severity of CPV disease. Such pathogens seem to enhance susceptibility to CPV by stimulating cellular proliferation in gut epithelia providing sites for virus replication.

2.3 Confirmation of Canine Parvovirus Infection

The serological techniques that have been most commonly used for routine CPV diagnosis are haemagglutination (HA) activity for virus in faecal samples and haemagglutination-inhibition (HI) for sera (Walker et al. 1979; Carmichael et al. 1980; McCandlish et al. 1981; Senda et al. 1986). In the enteric form of the disease, virions can only be detected in faeces during a period of peak virus shed, and this may not coincide with maximum circulating antibody (Fig. 3).

For the sensitive identification of CPV-specific antibodies in stored sera, HI methods may not yield reproducible results, especially if comparisons are made between different laboratories. One of the

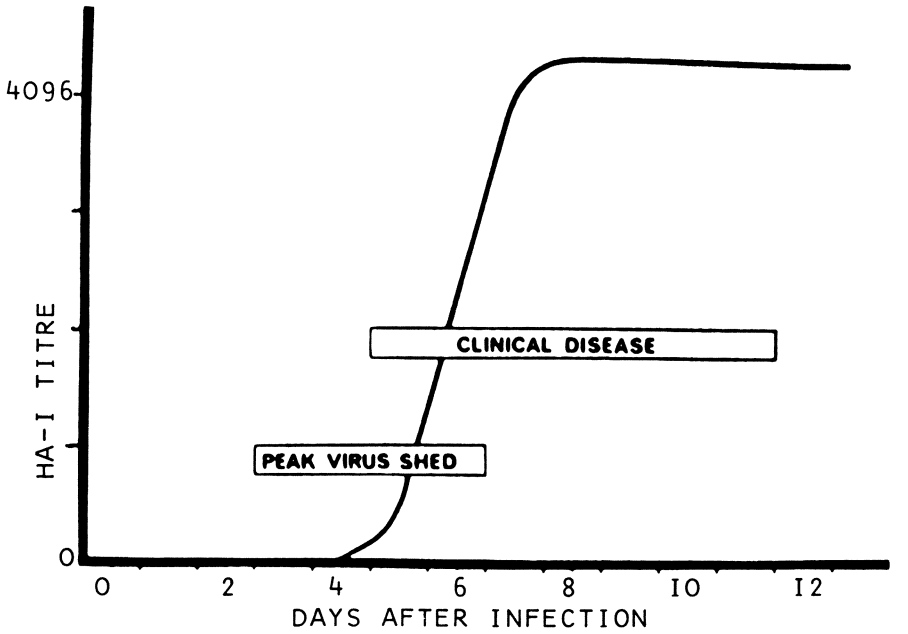

Fig. 3. Canine parvovirus enteritis – virus shedding and antibody response in relation to clinical signs (McCandlish et al. 1981)

reasons for such discrepancies seems to be that conditions for the HI test are not generally standardised, although the use of a standard CPV antiserum improves reproducibility between laboratories (Luff et al. 1987). For the sensitive detection of CPV-specific antibodies, serum-neutralisation tests are at least an order of magnitude more sensitive than HI tests, but obviously such assays require more specialised laboratory facilities. For rapid diagnosis of CPV infection, the assay of serum IgM is now probably the most useful measure. Stann et al. (1984) have made a study of 80 dogs with nonparvoviral enteritis and detected serum CPV-specific IgG antibodies (at titres of >1 : 25) in 85 % of them, whereas none had IgM antibodies against CPV, thus indicating the value of the IgM assay for diagnosis. Enzyme-linked immunosorbent assays (ELISA) have been developed for the class-specific measurement of IgM (Florent 1986). The presence of IgM is diagnostic of primary infection and allows differentiation between maternally derived antibody, vaccine-stimulated antibody, past infections and reinfection. Other ELISA test kits have also become available for the detection of viral antigen in faecal samples and similarly provide a rapid means of detecting CPV in

practice (Herbst et al. 1986). Bartkoski et al. (1988) describe the use of a commercial ELISA kit which utilises CPV-specific monoclonal antibodies with which both pre- and post-1980 isolates can be detected and Rimmelzwann et al. (1990) and Burtonboy et al. (1991) report that ELISAs are more sensitive than either HA or HI.

3 Transmission

There are two major routes of transmission: the faecal-oral route and the transplacental route. During acute disease as many as 10^9 $TCID_{50}$ virus particles are shed per gram of faeces (Pollock 1982), and these may be passed 2–3 days post-infection, before symptoms become apparent (see Fig. 3). The minimum infectious dose for wild strains of CPV is unclear, although experience with an attenuated strain suggests that it may be a very small dose, since vaccination with this strain is accomplished by intramuscular inoculation with 16 $TCID_{50}$ (Carmichael et al. 1983). The scale of virus shed during infection and the intrinsic stability of CPV particles must have contributed to the enzootic status of the virus. Mechanical (passive) transmission of CPV is also important: infective virus may be carried out on dogs' hair and feet, on clothing and footware of dog owners, on fomites (such as feeding dishes) and by insects, notably flies. In the past this means of transmission may have been particularly significant at dog shows and in kennels where large numbers of dogs were gathered together. It must have contributed significantly to the high mortality rates recorded for young pups in the original pandemic.

In the non-immune pregnant bitch virus is transmitted vertically. Predilection for the foetus *in utero* is more widespread in parvoviruses than in any other virus group, and this is well known with MVM (Kilham and Margolis 1971), FPV (Csizar et al. 1971c), PPV (Mengeling et al. 1980) and H-1, a rodent parvovirus (Ferm and Kilham 1964). This may appear to be an efficient means of transmission for, with each infected pregnant bitch, the virus has the potential of infecting several offspring *in utero*. However, the majority of infected pups so born die of severe heart disease, and because the virus localises in myocardial nuclei, it will not be shed.

Virions may also be shed to the environment by asymptomatic carriers, and Appel et al. (1980b) have reported transmission of CPV to contact-controlled dogs by a persistent carrier.