Review Article — Oorsigartikel

Canine distemper infections, with special reference to South Africa, with a review of the literature

A L Leisewitz[a], A Carter[b], M van Vuuren[c] and L van Blerk[d]

ABSTRACT

Canine distemper virus is a member of the genus *Morbillivirus* of the family *Paramyxoviridae* that causes severe disease in dogs and a range of wild mammals. The clinical signs relate essentially to the respiratory, gastrointestinal and central nervous systems. In South Africa, infection with *Ehrlichia canis* and canine parvovirus may present similarly. Many dogs will initially present with a wide range of central nervous system signs without any history of systemic disease. A recent South African study evaluating *ante mortem* diagnosis highlighted the importance of recognising clinical signs, cerebrospinal fluid IgG titres, serum IgM titres and immunocytochemistry of epithelial tissue. A 2-year retrospective evaluation of cerebrospinal fluid samples collected from dogs presented to the Onderstepoort Veterinary Academic Hospital indicates that distemper infection is common, and this disease should routinely be suspected in cases of diverse neurological disease in dogs. The South African dog population is specifically at high risk for the disease because of the large pool of unvaccinated, reproductively-active dogs that expose the wildlife resources of the country to risk of fatal disease. Outbreaks of disease in dogs continue to occur in developed and developing communities in both vaccinated and unvaccinated dogs worldwide, and have also been described in a wide range of free-ranging wildlife, including seals, dolphins and lions, and in endangered zoo animals. Modified live virus vaccines have contributed markedly to disease control in the dog population but have caused mortality in some wild carnivores. New recombinant vaccines are being developed that will be safe in wild animals. The pathogenesis of CNS demyelination has been compared to various important demyelinating diseases in humans and, amongst other things, relates to down-regulation of the oligodendrocyte gene coding for myelin synthesis and non-immunocyte CNS cell expression of type II major histocompatibility receptors. Early CNS lesions are characterised by demyelination and later lesions by perivascular round cell cuffing. Treatment is supportive.

Key words: canine distemper virus, dog, South Africa, wildlife.

Leisewitz A L, Carter A, Van Vuuren M, van Blerk L Canine distemper infections, with special reference to South Africa, with a review of the literature**.** *Journal of the South African Veterinary Association* (2001) 72(3): 127–136 (En.). Department of Companion Animal Medicine, Faculty of Veterinary Science, University of Pretoria, Private Bag X04, Onderstepoort, 0110 South Africa.

INTRODUCTION

Canine distemper (CD) is a severe, life-threatening disease with a worldwide distribution. It was first reported in Europe in 1761[8]. During the first half of the 19th century it was the most lethal disease of dogs. It affects mainly domestic dogs but has become a serious problem in a wide range of hosts, threatening captive and free-ranging wildlife populations including several marine mammals such as seals, dolphins and whales[18,161]. Outbreaks of disease with significant mortality continue to occur amongst domestic dogs even in developed communities with high levels of vaccination[26,51,82]. It is caused by a *Morbillivirus* (of the family Paramyxoviridae) similar to the human measles virus, which has decimated human populations for centuries and remains a significant cause of childhood mortality in developing countries. Rinderpest, caused by a related *Morbillivirus,* was responsible for great cattle plagues in Europe and Africa where it caused massive die-offs around the turn of the 20th century. It remains a threat to animal health in some developing countries to this day[5]. CD is well established in South Africa and continues to threaten domestic dogs and wild animals[2,5,70,102,130,132,151]. It is incumbent upon the veterinary profession as guardians of animal health to be unrelenting in tackling this problem.

[a]Department of Companion Animal Medicine, Faculty of Veterinary Science, University of Pretoria, Private Bag X04, Onderstepoort, 0110 South Africa.

[b]Fourways Veterinary Hospital, PO Box 68159, Bryanston, 2021 South Africa.

[c]Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria, Private Bag X04, Onderstepoort, 0110 South Africa.

[d]Kenilworth Veterinary Hospital, 47 Kenilworth Road, Cape Town, 7708 South Africa.

Received: October 2000. Accepted: August 2001.

AETIOLOGY

Canine distemper virus is an enveloped virus with a single-stranded, linear, negative polarity RNA genome. The latter is enclosed in a rod-shaped, helical nucleocapsid. The morphology is pleomorphic and the size varies between 100–250 nm, although many virions can be much larger and filamentous forms have been observed. It is a labile virus that is sensitive to heat, UV irradiation, lipid solvents, detergents and oxidising agents[86]. It can survive at room temperature in tissues and exudates for between 20 minutes and 3 hours. In environmental temperatures below zero it will survive several days if protected by organic material[62].

EPIDEMIOLOGY

Canine distemper has a worldwide distribution and affects a wide range of mammalian hosts (domestic and wild, land and sea-living)[13,23]. The virus is readily transmitted between susceptible hosts and the dog remains the most important reservoir for the infection. Because morbilliviruses do not persist in an infectious form following an acute infection, and infection results in life-long immunity in recovered hosts, the virus needs a constant supply of susceptible hosts for its maintenance[17]. Virus is shed in the stool, saliva, urine and conjunctival and nasal exudates during the acute phase of the infection.

In a developing peri-urban community of southern Africa, disease incidence was highest in the spring and early summer months of August to November[49]. The age at which the highest incidence of infection occurs depends on whether the disease is enzootic or epizootic. In a susceptible population, all ages are susceptible[26]. Enzootic disease, on the other hand, tends to affect mainly young dogs[96]. Aerosol and droplets are the most important means of transmission[59]. Virus may be shed for between 60 and 90 days following infection, although shorter periods are more typical[62]. This has bearing on the decision

clinicians need to make about the admission of suspect cases to their hospitals that could expose the hospital population to the CDV. As a rule, cases that present with acute catarrhal disease should not be admitted to an in-patient facility unless very strict isolation and barrier nursing can be applied. Specimens should be collected from these cases for diagnosis, and the dogs should then be managed as outpatients. A scenario that becomes very difficult to control is an isolation ward where puppies with *Parvovirus* infection are kept. The 2 diseases have clinical features in common and CD can be devastating in a ward of *Parvovirus*-infected puppies. Chronic CD that manifests with neurological signs or nasodigital hyperkeratosis is far less likely to be a source of infection. Because CDV remains a differential diagnosis in many cases showing CNS disease, it becomes impractical to separate these cases from the rest of the hospital population. The risk in such situations is so low that for practical purposes it can be regarded as insignificant. Dogs recovering from the infection are usually immune, are not persistently infected and do not shed the virus. Transplacental infection has been reported[93], although the general consensus is that morbilliviruses are not transmitted vertically[86].

The infection rate is significantly higher than the disease rate, with between 25 and 75 % of susceptible dogs clearing the infection without showing any clinical disease[62]. A constant supply of puppies or susceptible dogs is required to maintain the infection in a population. Several outbreaks of CDV infection have been described in both vaccinated and unvaccinated dog populations in developed and developing environments[26,51,82,130]. In a cross-sectional study of the canine population in a rural town in southern Africa, 5.5 % of the dogs examined were diagnosed with active CDV infection[130]. In a second study that compared the disease status of canine hospital patients from developed communities with those from developing communities over a 3-year period, a remarkable difference was found between the 2 groups. While 43.6 % of the dogs from the developing community had infectious disease, only 8.2 % of dogs from the developed community were similarly diagnosed. CD was diagnosed in 4.1 % of the infectious disease cases in the developing community dogs, but infection with CDV was rarely diagnosed in the dogs from the developed community[49]. This highlights the epidemiological requirement for CDV to persist in dogs, and the risk to certain southern African dog populations. The disease is constantly active in developing communities, where outbreaks can be expected. Owners in these communities must be encouraged to be especially vigilant in keeping to annual vaccination practices. The estimated dog population in South Africa varies widely but conservative estimates place it at around 4 million (1999/2000). Approximately 1 million dogs will visit a veterinarian at least once a year (Pfizer and Intervet companies, South Africa, pers. comm., 1999). This implies that the greater proportion of the South African dog population is unvaccinated and probably also reproductively active. The conditions for persistent large-scale CDV disease are therefore ideal, as it has been estimated that at least 300 000 individuals are required to maintain a *Morbillivirus* in circulation[23]. This situation poses a threat to the domestic dog population, but the threat it poses to the wildlife resource of the country is equally serious.

PATHOPHYSIOLOGY

Dogs are exposed to the virus by aerosol droplets contacting the upper respiratory tract epithelium[11,62]. The virus multiplies there in tissue macrophages and spreads *via* the lymphatics to the tonsils and respiratory tract lymph nodes within 24 hours[16,92]. By d 2-4 other lymphoid tissues are infected, and by day 6 the spleen, gastrointestinal mucosa and hepatic Kupffer cells are infected. By this time there is a systemic reaction to the infection characterised by pyrexia and lymphopaenia. Further haematogenous spread of virus occurs to involve other epithelial tissues and the central nervous system (CNS). Virus has been demonstrated to travel cell-free in plasma as well as associated with leukocytes and thrombocytes[91,95]. By 2 weeks after infection, dogs with an adequate cellular and humoral response clear the infection, and no clinical signs of illness persist[75]. Intermediate immune responsiveness allows further spread of virus to epithelial tissues. Virus persists in some tissues (such as nervous tissue and epithelium, especially foot pads), resulting in delayed clinical signs in these tissues. A poor immune response at this point allows pan-systemic viral dissemination that causes the gastrointestinal and respiratory signs seen with acute infection[100,140]. Serum antibody response varies inversely with the severity of the disease[75]. The mortality in gnotobiotic dogs is similar to that caused by natural infections, which discounts the importance of secondary bacterial infections in disease severity despite their obvious involvement[96–98].

Canine distemper virus is a significant cause of immune suppression in host animals[100,124]. Although the molecular basis for immune suppression is known for only a few viral diseases, canine distemper is characterised by viral replication or the presence of virus during the viraemic phase in T lymphocytes, B lymphocytes and macrophages. Necrosis and lymphoid depletion are the essential lesions in the lymphoid tissues[121]. Infection of cells involved in phagocytosis, antigen presentation and the non-specific effector aspects of cell-mediated immunity in dogs with distemper leads to an inability to generate an effective *in vivo* immune response[134]. Acutely-infected dogs fail to respond to intradermal skin tests with phytomitogens, and do not develop significant antibody responses. The latter suggests a direct viral effect on B cells or their precursor cells in the bone marrow[124].

Spread to the CNS depends on the efficacy of the initial immune response and many dogs probably develop transient CNS infections without clinical signs. Virus probably gains access to the CNS *via* infected white cells in the CSF and thrombocytes[16,94]. Antigen is first detected in CNS capillary and venular endothelium and perivascular astrocytic foot processes[21,157], but this occurs long before signs of CNS disease occur. It seems plausible that viral access is *via* CSF, as the first lesions seen are in the periventricular sub-pial tissue and in the choroid plexus. Areas of predilection for infection include the cerebral cortex, optic tracts, cerebral peduncles, cerebellum and spinal cord[21,131,135,153,157]. Many factors affect the type of CNS lesions observed, including the efficacy of the immune response and the neurotropic and immune-suppressive characteristics of the particular virus strain[144]. Chronic lesions are classically inflammatory and develop in dogs unsuccessful at clearing the virus from the CNS[27,120,157,165,166].

The neurobiology of demyelination in CDV infection is of particular interest and has been the topic of several studies, as it is regarded as a model of human demyelinating disease[36,37,42,142]. The acute demyelinating lesions are non-inflammatory, develop during a period of severe immune suppression, and coincide with replication of virus in glial cells of the white matter[118,122,153,157]. An obvious explanation for this would be infection of the oligodendrocytes, which are the myelin-producing cells. Viral protein is, however, only very rarely demonstrated in these cells, and in fact it was initially believed that oligodendrocytes could not become infected with virus[158]. Astrocytes are the most commonly-infected cells. Although viral protein is not produced in oligoden-

drocytes, viral mRNA corresponding to all viral genes has been demonstrated by *in situ* hybridisation in oligodendrocytes, and it has been shown that these cells will support transcription of all CDV genes[167,168,168]. Thus a restricted form of oligodendrocyte infection occurs and may well be the mechanism responsible for demyelination. Down-regulation of myelin gene transcription (the effective shutting-down of the oligodendroglial cell metabolic machinery) in the areas of demyelination in the CNS has been demonstrated[61].

The mechanisms of chronic demyelination are varied, but appear to be related to the inflammation that characterises this phase of the disease[154,157]. As the immune competence of the host recovers, lymphocytes, plasma cells and macrophages accumulate in the perivascular regions, leading to tissue damage and even necrosis. This inflammation is characterised by intrathecal immunoglobulin production. Dogs that recover have decreasing levels of immunoglobulin production in the CSF whereas increasing levels are associated with progressive disease. Intrathecal production of antibodies to CDV is clearly an important mechanism of immunity, as clearance of the virus from the CNS is associated with recovery[41,80,139,147,159,162]. CSF anti-CDV titres may be higher than in the blood[155]. Intrathecally-produced antibodies to myelin are not a consistent feature of CNS CDV infection, and titres are also not related to disease severity[162]. A cell-mediated response to myelin basic protein has been demonstrated in experimental disease but is poorly correlated to the occurrence of demyelination[145,155]. Thus, although autoimmunity has been suggested as a mechanism of progressive neural injury in CDV infection, it remains a topic of debate and is regarded by some to be an epiphenomenon not primarily involved in the demyelinating process[143,157]. On the other hand, major histocompatibility complex class II (MHC II) expression has been demonstrated in the CNS of CDV-infected dogs, especially in the microglia and astrocytes of the white matter[3,160]. The brain is usually regarded as an immunologically-privileged site that cannot express MHC II and thus cannot mount its own immune response. Aberrant antigen-presenting capacity by cells of the CNS has been reported in autoimmune-like diseases of the CNS such as multiple sclerosis, experimental allergic encephalitis and Theiler's murine encephalomyelitis virus infection[103]. Expression of MHC II by microglia and astrocytes in CDV infections is probably a direct viral effect. The significance of this lies in the fact that microglia and astrocytes expressing MHC II would allow presentation of 'self antigens' that could lead to virus-independent demyelination. In chronic lesions, MHC II was found to be highly expressed despite the absence of viral antigen, suggesting that demyelination may be triggered and perpetuated by non-viral (self) antigens.

Antiviral immune responses have been blamed for 'bystander' demyelination[28,65,66]. Macrophages constitute an important proportion of the cells that respond to infection. These cells have been shown to play an important role in producing reactive oxygen species that lead to selective destruction of oligodendrocytes as 'innocent bystander' cells[65,67]. It has also been shown that CDV infection leads to enhanced macrophage activity and hence enhanced destructive activity[30].

It is important to realise that viral clearance results in CNS recovery and only persistent infection of the CNS can lead to a progressive smouldering inflammatory reaction. It is possible for virus to persist in white matter areas outside the inflammatory demyelinating lesions or even in the periphery of such lesions. Mechanisms allowing such persistence are poorly understood but are obviously important to the evolution of disease and are the topic of ongoing investigation[27].

Cytokines play a significant role in any disease in which inflammation contributes to the disease process. To date there have been few studies evaluating the effect of cytokines in CDV infections[55,68]. One of the hindrances to this type of work in dogs is the lack of species-specific reagents. One study evaluated mRNA expression of interleukin 1(IL-1) , IL-6, IL-8, IL-12, tumour necrosis factor α (TNF), interferon γ (IFN)and TGFβin whole blood using reverse transcriptase polymerase chain reaction (rt-PCR)[68]. No significant correlation between disease and cytokine mRNA expression was demonstrated. No cytokine transcripts could be found in the blood of any of the acutely viraemic dogs with a high viral load. This may have been due to immune suppression. Pro- and anti-inflammatory cytokines revealed no clear pattern of expression. It is highly probable that the form of disease, immune status of the individual and time of sampling all impact strongly on cytokine expression. Circulating levels of cytokine mRNA may also be poorly correlated with cytokine protein production at tissue level. Another study examined mRNA expression of various cytokines in the CSF of naturally-infected dogs[55]. IL-10 was found to be the dominantly-expressed cytokine, although both pro- and anti-inflammatory cytokines were co-expressed in most cases, making it very difficult to link expression with pathogenic effect. A further problem is the heterogeneous nature of CNS lesions at any time. Both non-inflammatory and chronic inflammatory lesions co-exist in the same brain at any particular time. Furthermore, determining cytokine mRNA expression in CSF may not relate to what is actually taking place in the brain tissue. One case with advanced chronic lesions expressed only TNF and IL-12. Ideally, it would be possible to study the cytokine kinetics in brain tissue over time at the same time as being able to evaluate inflammatory cell phenotype.

T-cell subsets in the brains of natural CDV infections have been evaluated[163,164]. Acute demyelination (histologically non-inflammatory) is accompanied by a diffuse influx of CD8+ cells. It is suggested that these cells may be responsible for early antibody-independent anti-viral cytotoxicity. The chronic inflammatory perivascular cuffs consist mainly of CD4+ lymphocytes, plasma cells and macrophages. This may represent a phase of antibody-dependent demyelination. These findings highlight the heterogeneous and possibly, in part, immune-mediated plaque pathogenesis of CDV demyelination.

CLINICAL SIGNS

Signs vary depending on a number of factors such as the age of the host, host immune competence, virus strain and organ system affected[45,74,96]. It is estimated that between 50 and 70 % of CDV infections are sub-clinical[62]. Mild forms of the disease present with non-specific signs such as listlessness, loss of appetite and fever. More specific presentations include oculonasal discharge, coughing, dyspnoea, diarrhoea and vomiting. These signs are not specific for CD and in the experience of the authors *Ehrlichia canis* infection may be an important differential diagnosis for practitioners in areas where both diseases are present. Both diseases can present with similar secondary skin and chest infections and the typical haematological findings of leukopaenia, thrombocytopaenia and possibly a non-regenerative anaemia. Kennel cough may be another differential diagnosis. It has been the experience of the first author at the Onderstepoort Veterinary Academic Hospital (OVAH) that some puppies admitted to the isolation ward for suspected parvovirus-induced diarrhoea were subsequently found to suffer from CDV infection. This presents a particularly severe problem, as the introduction of CDV to a group of severely immune-suppressed *Parvovirus*-infected puppies

can be catastrophic. Problems with CDV infection following discharge from hospital of puppies that had parvovirus infection is likely in these circumstances.

The form of the disease that presents most commonly depends especially on the level of immune competence of the population and age of the affected dogs. Clinical signs therefore vary widely[154]. In unvaccinated dogs and unvaccinated puppies (of between 12 and 16 weeks of age when maternal antibody titres have waned) the severe generalised catarrhal form is most common. These dogs are febrile, with mucopurulent conjunctivitis, upper respiratory tract signs, depression, anorexia, vomiting and diarrhoea (which may be bloody). Severe dehydration and weight loss may be present. Adequate treatment in this group may reduce the mortality rate.

The most common form of the disease seen at the OVAH is the neurological form in older dogs. The disease occurs frequently enough to justify investigation for CDV infection in all dogs showing non-surgical spinal cord disease and in all dogs with brain signs.

In an outbreak described in an urban dog population of the Copenhagen area in Denmark, more than half of the cases presented with the catarrhal form. Many of these dogs also had CNS involvement and digital hyperkeratosis (so called 'hard-pad')[25]. In an outbreak described amongst unvaccinated sled dogs in northern Greenland (with a mortality of 1000 of a population of around 3000), most died within a week with classic catarrhal and nervous signs. Digital hyperkeratosis was not observed. Blindness was observed in a few survivors[26]. In a Finnish study of an outbreak in approximately 5000 vaccinated dogs with a mortality of 30 %, the majority showed classic catarrhal disease. A few cases of digital hyperkeratosis were noted. Nervous signs were noted but the proportion was not reported[51].

Typically, neurological signs follow the catarrhal signs within about 2–3 weeks[10,11,62]. As demonstrated by previous studies and observations cited above, many dogs will present for the first time with neurological signs preceded by no or only very mild signs. In one report, only one third of cases with neurological disease had a previous history of systemic signs, and another third had no previous history of disease at all[148]. Once CNS disease manifests, it is usually progressive, without a relapsing course, although a chronic relapsing course has been described[73]. Neurological disease often appears localised according to clinical examination, despite the diffuse viral and lesion localisation[148]. Signs are very varied but commonly include seizures[105], blindness, central vestibular disease[52], cerebellar[87,118,142,154], brain stem and spinal cord disease[135]. Myoclonus occurs in about one third of cases and is most commonly associated with CDV, but should not be regarded as pathognomonic, as it is seen in other causes of menigoencephalomyelopathies[77,78,146]. It is seen most commonly in the muscles of mastication and the limbs, but may be seen in any muscle group[77,78]. Naso-digital keratosis is reported to occur in about 8 % of cases with neurological disease[148]. Bi- or unilateral optic neuritis is often the cause of blindness. Swelling of the optic disc may be seen in the acute disease. Ill-defined grey to pink densities may be seen in the tapetal and non-tapetal regions. Chronic disease may cause well-delineated hyperrefletic retinal areas[24,57,88,107].The immune compromising nature of the disease[92,100,140] results in the frequent occurrence of secondary bacterial infection of the skin and respiratory tract which are features of the acute phase of the disease. The activation of other unusual and CNS infections owing to the immune suppression caused by CDV has been reported, and these infections include combinations of CDV and *Toxoplasma gondii*[50], *Neospora caninum*[148], *Filaroides hirthi*[32], cryptosporidiosis[149], *Pneumocystis carinii*[141] and canine infectious hepatitis virus[89].

CDV infection has been associated with inflammatory joint diseases and hypertrophic osteodystrophy (HOD) in dogs[20,116]. In a study of experimental infection of gnotobiotic dogs CDV infection of the proximal metaphyses of the humerus was prominent. Antigen was abundant in the marrow, osteoclasts and osteoblasts, where it had a direct cytolytic effect, even causing cell necrosis. There was a strong association between CDV antigen presence and osseous changes. CDV antigen has been reported in the bone of humans with Paget's disease and an association between dog ownership and this disease in man has been postulated[58]. There is, however, very little evidence for this, and other studies have failed to support this hypothesis[54,85,114,115,123,129]. A similar link between CDV infection and multiple sclerosis in man has been suggested, but there is no proof of this association, and CDV should be regarded as an animal disease with no public health risk[36,37,39,60,76,101,133]. In an earlier study linking CDV infection and HOD, dogs injected with serum from HOD patients developed CDV signs and the infection was confirmed at necropsy[69]. CDV has also been demonstrated by rt-PCR, *in situ* hybridisation and southern blotting in the bone tissue of dogs with HOD[112,113]. It is possible that all dogs with CDV infection develop sub-clinical bone disease, especially considering that dogs with HOD develop constitutional signs of disease such as fever and malaise. Infection during puppyhood may cause enamel hypoplasia in the permanent teeth seen in adulthood[22,45].

A rare progressive condition that has come to be called 'old dog encephalitis' is purported to occur in dogs that have survived CDV infection. It is characterised by widespread perivascular lymphoplasmacytic infiltration in areas of demyelination and neuronal degeneration. In some cases it has also been described as a sclerosing panencephalitis with infiltration and replacement of nervous tissue by a dense astrocytic network. Clinical signs are typically restricted to lesions typical for the cerebrum and exclude involvement of the brain stem, spinal cord and cerebellum[104].

A link between juvenile pyoderma and CDV has been suggested. A pustular skin disease is not uncommon in CDV infection and has been described as an 'allergic response to the virus'[6]. In a recent report, 3 of 4 dogs recently vaccinated with a modified live CDV vaccine developed juvenile cellulitis and radiographic evidence of HOD. The skin disease was successfully treated in all 4 dogs with prednisolone. Distemper-like skin lesions have also been described following inoculation with a modified live virus vaccine[34]. The concurrence of HOD and juvenile cellulitis has been reported previously[35,106,117].

EXPERIENCE WITH THE DISEASE IN SOUTH AFRICA

A retrospective study of cerebrospinal fluid samples submitted to the Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, from dogs with clinical signs compatible with CDV infection was conducted during 1998 and 1999.

One hundred and thirty-three CSF samples were submitted during this 2-year period. Thirty-four (25 %) were found to be positive for CDV-specific IgG. Of these 34 cases, 23 showed nervous signs only (including tremors, vestibular syndrome, hind-limb ataxia, progressive ataxia, seizures, myoclonus, depression, periodic neck pain, generalised body pain and dementia). A combination of neurological signs with gastrointestinal signs was seen in 4 cases. No dogs were observed to have a combination of neurological and respiratory signs. The typical non-neurological signs seen were purulent oculonasal discharge (4 dogs) and respiratory signs (2 dogs) that included

pneumonia-induced coughing and increased respiratory rate. The gastrointestinal signs observed were anorexia, diarrhoea and vomiting (4 dogs). No cases of digital hyperkeratosis (so called 'hard-pad disease') were noted. Nineteen of the 34 CSF CDV-specific IgG-positive dogs were less than a year old, and 12 of these presented with only neurological signs. The remaining 15 dogs were older than a year, 2 being older than 7 years. Fourteen of these 15 dogs older than a year showed only neurological signs. No seasonal incidence was noted over the 2 years. The results of this study highlighted several important issues about the disease as it is seen at the OVAH. These include:

- CDV infection is sufficiently common in the OVAH environment to justify ruling out this disease early in the course of the investigation of diverse neurological signs. (See 'Diagnosis' below).
- Neurological signs are the most common presentation. Catarrhal signs combined with neurological signs are the next most common presentation. Catarrhal signs alone are an infrequent presentation. The neurological presentation is the most common in all age groups and especially common in dogs older than one year.
- Digital hyperkeratosis is an uncommon sign.

At a satellite clinic of the Medical University of Southern Africa that served a more resource-deprived community than the OVAH, catarrhal signs alone and in combination with neurological signs were more common than neurological signs alone [33,49,130].

In a retrospective evaluation of 154 dog brains presented to the OVI for rabies diagnosis between July 1992 and July 1993, a final diagnosis of CDV was made in 26 cases. Diagnosis was based on immunoperoxidase staining for inclusion bodies in at least 1 of 6 standard sections. Other common diagnoses included cerebral babesiosis, *E. canis* infection and granulomatous meningoencephalitis (J J van der Lugt, pers. comm., 1999).

DIAGNOSIS

Diagnosing canine distemper in the live animal with confidence remains difficult, yet very important because of the implications of the disease. Identifying typical clinical signs still remains the most important initial diagnostic step. Because the disease often has a poor prognosis and has a high infectious potential, *ante mortem* confirmation of the diagnosis is preferred. The many varied clinical presentations of the disease (especially in its neurological form) may also dictate that clinical signs are by no means typical, further emphasising the need for a definitive diagnostic test. Numerous different methods that have been used for diagnosis testify to the difficulties experienced in this regard.

The most common haematological finding is lymphopaenia. In a local study 86 % of 30 dogs with CDV infection were lymphopaenic[33]. Thrombocytopaenia may also be present[15]. A left shift neutrophilia may be helpful in confirming the presence of significant secondary bacterial infection. In the South African setting, because of the frequency of other concurrent infectious diseases, haematological findings may be varied if mixed infections are present. Other immune-suppressive infections (such as ehrlichiosis or parvovirus infections) can induce very similar haematological abnormalities. This is especially relevant because many of the dogs suspected of having distemper infection live in developing communities where infectious diseases are very common and vaccination is seldom practised. Viral inclusions in the white or red cells are rarely observed. Serum biochemistry is usually unremarkable. Radiology may demonstrate an interstitial (viral) pneumonia in early cases and an alveolar pattern with bacterial pneumonia later in the course of the disease.

CSF and serological abnormalities and the demonstration of viral inclusions in epithelial cell cytology (by fluorescent antibody techniques) are usually the most practical and useful means of confirming a diagnosis.

The diagnosis of inflammatory diseases of the canine CNS is usually difficult, with at least one third of cases remaining undiagnosed in the live animal despite thorough investigation[146]. However, results of analysis of the CSF of CDV-infected dogs are frequently useful in diagnosis. Raised protein content and lymphocytic pleocytosis are common[1,41,64,81,139,146,156]. In the study by Carter, 89 % of the 30 CDV positive dogs examined had CSF protein concentrations in excess of 0.5 g/ℓ (normal is <0.25 g/ℓ), and lymphocytes were also the most frequently-encountered cells[33]. Finding anti-CDV IgG antibodies in the CSF is definitive in CDV encephalitis, as the antibody is locally produced[41,64,80,146,147,156]. This is not the case in vaccinated dogs or dogs without CNS CDV infection. Vaccinated or exposed and cured dogs should all have a blood IgG titre that, if present in CSF, would indicate blood contamination during CSF collection. This problem can be overcome by determining concomitant *Parvovirus* titres in both serum and CSF. If there has been blood contamination of the CSF, a *Parvovirus* titre will be present in the CSF if it is present in the blood. The only way that this could have occurred is by contamination, as anti-*Parvovirus* antibodies are not synthesised intrathecally. An IgG index may also be calculated comparing CSF CDV IgG titres to CSF albumin concentrations[147].

Immunocytological examination of smears from the conjunctiva, tonsillar, genital or respiratory epithelium may demonstrate virus if smears are made early in the course of disease (within 2 weeks post-infection)[1,19]. Finding antigen by this method in buffy coat smears would also confirm a diagnosis, but only during the early brief viraemic phase of the disease (usually within 10 days post-infection). As antibody titres rise, antigen is either bound and masked from immuno-staining, or disappears from the epithelial surfaces altogether. A recent report has demonstrated the usefulness of CDV-specific immunoperoxidase histochemical test in skin biopsies (foot pad, nasal mucosa and haired skin form the neck) in diagnosis. Biopsies from the haired skin from the neck were as likely to be positive as epithelium from the other sites in this study. This method proved useful in acute and subacute disease[71]. Although the experience base with this method is small, its efficiency, ease and cost-effectiveness should encourage wider use. Immunochemical staining for antigen has also been successful in cells from CSF[4] and urine[29,136].

Detection of serum IgG and IgM titres has been widely used. Because of the practice of vaccination and the frequent exposure of dogs to self-limiting infection, detecting a positive IgG titre in serum is of very little value in confirming a diagnosis. A positive IgM titre, on the other hand, should be interpreted as recent exposure (during the last 3 weeks) to vaccine virus or natural infection[62].

With the advent of molecular biological technology, rt-PCR has been used to detect viral nucleic acid in blood, serum and CSF[56,68,137]. This highly sensitive and specific mode of diagnosis can be expected to be widely used in future.

Virus isolation is useful, but special arrangements need to be made with the diagnostic laboratory concerned. This technique is usually reserved for research environments and has proven useful to differentiate natural infections from vaccine-induced disease in exotic species. Vaccine strains can replicate in macrophage, lymphocyte and epithelial cell cultures, whereas field strains require several blind passages to replicate in epithelial cell lines[72]

In a South African study Carter[33] examined 30 dogs with clinical signs suggestive

of CDV from a developing community. All dogs were euthanased and necropsies performed and CDV infection confirmed on histology of the brain. Analysis of the CSF for distemper yielded the following information:

- The direct fluorescent antibody test for viral antigens in cells from the CSF had a sensitivity of 27 % and a specificity of 100 %.
- The indirect fluorescent antibody test for IgG in the CSF had a sensitivity of 64 % and a specificity of 100 %. No IgM was detected in any of the cases by using this method.
- 89 % of dogs had a protein concentration of 0.5 g/ℓ or more.
- Direct fluorescent antibody testing on conjunctival scrapings had a sensitivity of 37 % and a specificity of 100 %. Impressions made of the tissue of the footpad had a sensitivity of 23 % and specificity of 100 %.

Blood analysis showed:

- An indirect fluorescent antibody (IFA) test for IgG in serum had a sensitivity of 96 % but a specificity of 0 %.
- IFA for IgM in the serum had a sensitivity of 75 % and a specificity of 80 %.
- 86 % of cases had haematocrits below 0.4 ℓ/ℓ and 49 % were thrombocytopaenic.
- 86 % were lymphopaenic.

This study concluded that the following tests, in order of preference, were helpful in confirming the *ante mortem* diagnosis of CDV:

1. IFA for IgG in the CSF in cases of CDV encephalopathy.
2. IFA for IgM in the serum.
3. Direct fluorescent antibody testing for CDV antigen in conjunctival scrapings.
4. Direct fluorescent antibody testing for CDV antigens in cells in the CSF of cases with CDV encephalopathy.

These findings have proven useful and practical in the resource-deprived communities of South Africa. A similar survey of diagnostic tests in dogs from developed areas has not been performed, and would probably yield slightly different results because the acute catarrhal form of the disease is rare in this population of dogs. Most CDV disease in developed communities is of the chronic neurological type. In this setting, CSF IgG titres are most useful, but fluorescent antibody testing of cellular material for analysis is less useful. Reverse transcriptase PCR of CSF fluid may prove to be useful in the future.

CANINE DISTEMPER VIRUS INFECTION IN WILDLIFE

Although CD is an important disease in dogs, there have been some alarming distemper outbreaks in non-domestic species, which further highlights the importance of control of the dog reservoir. Eight of the 11 families of the order Carnivora are susceptible to CDV, with a mortality rate that varies widely between species[5,13,72]. Of the species in which CDV infection has been reported, the following occur in South Africa: lion, leopard, wild dog, hyaena, foxes and otters[70,102,132]. Of these species, exposure to CDV has been demonstrated serologically in lions, leopards and wild dogs. However, no catastrophic mortality due to CDV infection has been reported in southern Africa[102,152]. Distemper outbreaks have been reported in captive lions, tigers, leopards and jaguars in North America[14]. Most of these animals die of CNS disease following respiratory and gut involvement.

Outbreaks have been reported in marine mammals, including seals (so called phocine distemper)[125] and striped dolphins. Thousands died in the Mediterranean between 1990 and 1992[43,46,84,125,150,161]. It is most likely that these outbreaks originated in terrestrial carnivores[53].

A massive CD outbreak in the lion population of Serengeti (Tanzania) killed about 30 % of the lion population of that area in 1994. The same area experienced CDV-related deaths in bat-eared foxes, African wild dogs, spotted hyaena, common jackal and silver-backed jackal. The source of these infections is purported to be the domestic dog population of about 30 000 that surrounds the park[132]. Several years later 55 % of the lions of Maasai-Mara were found to be serologically positive for CDV, indicating exposure[90]. Preventing the spread of this disease to wildlife would require vaccinating all dogs around the conserved areas or vaccinating the wildlife, which is not practical in free-ranging wild animals. Developing a vaccine that is safe in captive wildlife remains a challenge, as there have been several reports of disease caused by the use of modified live CDV vaccines[40,47,79,110,119]. It would be ill-advised to use a live vaccine in captive wild animals. In this regard, inactivated vaccines (which are no longer commercially available) are best used. Recombinant or subunit vaccines would be desirable for captive wildlife[83,126,127,138].

PREVENTION

Vaccination remains the mainstay of preventing CDV infection in dogs, and therefore a large amount of research and a large industry has developed around this practice. Maternally-derived antibody provides temporary protection to neonates for a period that varies from days to months. This phenomenon has been responsible for the use of a modified live measles virus vaccine. The 2 viruses are antigenically closely related, and the measles virus is not neutralised by the maternal antibody in 6-week-old puppies[12]. The second and all subsequent vaccines are modified live virus vaccines, as by this time maternal antibody titre has waned to the point of being inconsequential. This long-accepted practice of 'heterotypic' vaccine use has been questioned, as ongoing usage of measles vaccine means that progeny of bitches vaccinated with measles vaccine may possess maternal antibodies against measles virus that would interfere with early measles vaccine in puppies born to them. It has been shown that a high-titre CDV vaccine is even more effective in protecting 6-week-old puppies against experimental distemper challenge than the traditional human measles vaccine[34].It is generally accepted that vaccination should take place every 2–4 weeks from the age of 6 weeks until 12 weeks, and then yearly thereafter with a modified live virus vaccine[7,31,44]. Frequent vaccination has been practised in an attempt to reduce losses early in life. It has been shown that 'maximal' vaccination (every week from the age of 2 weeks until 10 weeks and then at 12, 16, 18 and 24 weeks) gave no better protection than a 'minimal' regime (5, 9, 13, 17 and 22 weeks) in Greyhound puppies. There was also no adverse effect on cellular immunity of the maximal protocol[111]. Inactivated vaccines do not provide protection against experimental challenge infection[9]. A canary pox recombinant vaccine has recently been developed that provided good protection[127] and is now commercially available.

The need for yearly booster vaccination is often questioned, and in fact in developed countries the recommendation is now that booster vaccinations are only required every 3 years following the initial puppy series and a booster at one year[44]. Boosters for geriatric animals are discouraged[44]. Sufficient levels of neutralising antibodies remain in serum for longer than 6 years after vaccination[31]. It has, however, been claimed that, on the basis of serum antibody titres, annual vaccination should be maintained[109]. On the other hand, it has been suggested that humoral immune mechanisms alone do not account for immunity and that protection lasts much longer than indicated by antibody titres[63]. In a country such as South Africa, where most dogs are unvaccinated and reproductively active, and where a large wildlife resource is potentially at risk, it would be unwise to

recommend anything other than yearly booster vaccination.

Not all vaccines are equally effective, as has been demonstrated in numerous comparative studies on the efficacy of various products. Outbreaks of disease have been described in well-vaccinated populations, as indicated above. Despite these reports, the chances of outbreaks are greatly reduced in well-vaccinated populations[128]. It should, however, always be remembered that no vaccine is 100 % effective in 100 % of animals. When individuals present with signs of CDV infection, the disease cannot be ruled out on the basis of an adequate vaccination history.

There are numerous reports of adverse effects of CDV vaccination. Introduction of a foreign protein will always be associated with a certain amount of risk ranging from mild urticarial skin reactions to life-threatening anaphylaxis. It has been recommended that caution should be exercised when using a modified live vaccine in dogs infected with *Parvovirus,* as this may predispose dogs to neurological disease[99]. Cases of post-vaccinal encephalitis were recorded in dogs in various parts of Britain after the administration of a particular batch of vaccine[38,108]. It has also been shown that there is a temporal relationship between the use of canine vaccines (not specifically CDV) and immune-mediated haemolytic anaemia[48]. Full-blown CDV infection with mortality has been reported following the use of modified live virus vaccines in various wildlife species, as described above.

MANAGEMENT

There is no effective specific antiviral treatment. All treatment focuses on patient support and nursing care. Before treating an infected dog, the infectious nature of the disease and prognosis should be discussed with the owner. The possibility of neurological signs developing should be discussed with owners. This remains an important reason for euthanasia but before this is done, it is worth considering treatment for a period of time in a select group of patients, as some dogs may recover. Patients for which euthanasia should be discussed include: 1) those dogs with neurological disease that compromises quality of life (such as intractable seizure activity, severe myoclonus or incapacitating spinal cord disease), 2) severe and worsening catarrhal forms of the disease, or 3) dogs that come from multiple-dog households where other dogs may be at risk of infection.

Supportive treatment for dogs likely to be shedding virus should be administered on an outpatient basis as far as possible unless the dogs can be barrier nursed in isolation. This is frequently a problem in the private practice situation in South Africa where most isolation facilities are still used for *Parvovirus*-infected puppies. CDV suspect dogs should not be treated in proximity to these patients. Antibiotic cover (broad spectrum and effective in the respiratory tract), fluid and electrolyte balance (including anti-emetics if required), nutritional support and seizure control form the basis of a management plan. Good nursing care such as keeping the animal warm and dry and cleaning oculonasal discharges should be applied. Various homeopathic treatment claims have been made. These are scientifically unsubstantiated.

REFERENCES

1. Abate O, Bollo E, Lotti D, Bo S 1998 Cytological, immunocytochemical and biochemical cerebrospinal fluid investigations in selected central nervous system disorders of dogs. *Zentralblatt für Veterinärmedizin Reich B* 45: 2 73–85
2. Alexander K A, Kat P W, Wayne R K, Fuller T K 1994 Serologic survey of selected canine pathogens among free-ranging jackals in Kenya. *Journal of Wildlife Diseases* 30: 4 486–491
3. Alldinger S, Wunschmann A, Baumgartner W, Voss C, Kremmer E 1996 Up-regulation of major histocompatibility complex class II antigen expression in the central nervous system of dogs with spontaneous canine distemper virus encephalitis. *Acta Neuropathologica* (*Berlin)* 92: 3 273–280
4. Alleman A R, Christopher M M, Steiner D A, Homer B L 1992 Identification of intracytoplasmic inclusion bodies in mononuclear cells from the cerebrospinal fluid of a dog with canine distemper. *Veterinary Pathology* 29: 1 84–85
5. Anderson E C 1995 *Morbillivirus* infections in wildlife (in relation to their population biology and disease control in domestic animals). *Veterinary Microbiology* 44: 2–4 319–332
6. Appel M Canine distemper 1977 In Kirk R W (ed), *Current veterinary therapy* Vol. 6. W B Saunders, Philadelphia: 1308
7. Appel M, Schultz R D 1999 Forty years of canine vaccination. *Advances in Veterinary Medicine* 41: 309–324
8. Appel M, Gillespie J H 1972. In Gard S, Hallauer C, Meyer K F (eds) *Virology monographs, 11.* Springer-Verlag, New York: 1–96
9. Appel M, Shek W R, Shesberadaran H, Norrby E 1984 Measles virus and inactivated canine distemper virus induce incomplete immunity to canine distemper. *Archives of Virology* 82: 73–82
10. Appel M J 1969 Pathogenesis of canine distemper. *American Journal of Veterinary Research* 30: 7 1167–1182
11. Appel M J 1970 Distemper pathogenesis in dogs. *Journal of American Veterinary Medical Association* 156: 12 1681–1684
12. Appel M J, Shek W R, Shesberadaran H Norrby E 1984 Measles virus and inactivated canine distemper virus induce incomplete immunity to canine distemper. *Archives of Virology* 82: 73–82
13. Appel M J, Summers B A 1995 Pathogenicity of morbilliviruses for terrestrial carnivores. *Veterinary Microbiology* 44: 2–4 187–191
14. Appel M J, Yates R A, Foley, G L, Bernstein J J, Santinelli S, Spelman L H, Miller L D, Arp L H, Anderson M, Barr M 1994 Canine distemper epizootic in lions, tigers, and leopards in North America. *Journal of Veterinary Diagnostic Investigation* 6: 3 277–288
15. Axthelm M K, Krakowka S 1987 Canine distemper virus-induced thrombocytopenia. *American Journal of Veterinary Research* 48: 8 1269–1275
16. Axthelm M K, Krakowka S 1987 Canine distemper virus: the early blood–brain barrier lesion. *Acta Neuropathologica (Berlin)* 75: 27–33
17. Barrett T 1999 Morbillivirus infections, with special emphasis on morbilliviruses of carnivores. *Veterinary Microbiology* 69: 1–2 3–13
18. Barrett T, Blixenkrone-Moller M, Di Guardo G, Domingo M, Duignan P, Hall A, Mamaev L, Osterhaus A D 1995 Morbilliviruses in aquatic mammals: report on round table discussion. *Veterinary Microbiology* 44: 261–265
19. Baumann G 1967 Demonstration of distemper antigen in impression and section preparations using the fluorescent antibody technique. *Gegenbaurs Morphologisches Jahrbuch* 111: 187–194
20. Baumgartner W, Boyce R W, Weisbrode S E, Aldinger S, Axthelm M K, Krakowka S 1995 Histologic and immunocytochemical characterization of canine distemper-associated metaphyseal bone lesions in young dogs following experimental infection. *Veterinary Pathology* 32: 702–709
21. Baumgartner W, Orvell C, Reinacher M 1989 Naturally occurring canine distemper virus encephalitis: distribution and expression of viral polypeptides in nervous tissues. *Acta Neuropathologica (Berlin)* 78: 504–512
22. Bittegeko S B, Arnbjerg J, Nkya R, Tevik A 1995 Multiple dental developmental abnormalities following canine distemper infection. *Journal of the American Animal Hospital Association* 31: 42–45
23. Black F L 1991 Epidemiology of paramyxoviridae. In Kingbury D (ed.) *The paramyxoviruses.* Plenum Press, New York: 509–536
24. Blanchard G L, Howard D R, Krehbiel J D, Keller W F 1973 Amaurosis and associated electroretinographic alterations in canine distemper. *Journal of the American Veterinary Medical Association* 163: 976–978
25. Blixenkrone-Moller M, Svansson V, Have P, Orvell C, Appel M, Pedersen I R, Dietz H H, Henriksen P 1993 Studies on manifestations of canine distemper virus infection in an urban dog population. *Veterinary Microbiology* 37: 163–173
26. Bohm J, Blixenkrone-Moller M, Lund E 1989 A serious outbreak of canine distemper among sled-dogs in northern Greenland. *Arctic Medical Research* 48: 195–203
27. Bollo E, Zurbriggen A, Vandevelde M, Fankhauser R 1986 Canine distemper virus clearance in chronic inflammatory demyelination. *Acta Neuropathologica (Berlin)* 72: 69–73
28. Botteron C, Zurbriggen A, Griot C, Vandevelde M 1992 Canine distemper virus-immune complexes induce bystander degeneration of oligodendrocytes.

Acta Neuropathologica (Berlin) 83: 402–407
29. Brown R A L, Morrow A, Heron I, Chong S N 1987 Immunocytological confirmation of a diagnosis of canine distemper using cells in urine. *Journal of Small Animal Practice* 28: 845–851
30. Burge T, Griot C, Vandevelde M, Peterhans E 1989 Antiviral antibodies stimulate production of reactive oxygen species in cultured canine brain cells infected with canine distemper virus. *Journal of Virology* 63: 2790–2797
31. Carmichael L E 1999 Canine viral vaccines at a turning point – a personal perspective. *Advances in Veterinary Medicine* 41: 289–307
32. Carrasco L, Hervas J, Gomez-Villamandos J C, de Lara F C, Sierra M A 1997 Massive *Filaroides hirthi* infestation associated with canine distemper in a puppy. *Veterinary Record* 140: 72–73
33. Carter A 1997 Antemortem diagnosis of canine distemper virus infection in dogs. M.Med.(Vet.) thesis, Medical University of South Africa, Pretoria
34. Chalmers W S, Baxendale W 1994 A comparison of canine distemper vaccine and measles vaccine for the prevention of canine distemper in young puppies. *Veterinary Record* 135: 349–353
35. Clarke R E 1978 Hypertrophic osteodystrophy in the canine associated with a lowered resistance to infection – an unusual case history. *Australian Veterinary Practitioner* 8: 39
36. Cook S D, Blumberg B, Dowling P C 1986 Potential role of paramyxoviruses in multiple sclerosis. *Neurologic Clinics* 4: 303–319
37. Cook S D, Rohowsky-Kochan C, Bansil S, Dowling P C 1995 Evidence for multiple sclerosis as an infectious disease. *Acta Neurologica Scandinavian Supplement* 161: 34–42
38. Cornwell H J, Thompson H, McCandlish I A, Macartney L, Nash A S 1988 Encephalitis in dogs associated with a batch of canine distemper (Rockborn) vaccine *Veterinary Record* 122: 54–59
39. Cosby S L, McQuaid S, Taylor M J, Bailey M, Rima B K, Martin S J, Allen I V 1989 Examination of eight cases of multiple sclerosis and 56 neurological and non-neurological controls for genomic sequences of measles virus, canine distemper virus, simian virus and rubella virus. *Journal of Genetic Virology* 70: 2027–2036
40. Curlee J F 1999 Cross-species vaccination in wild and exotic animals. *Advances in Veterinary Medicine* 41: 551–556
41. Cutler R W Averill D R 1969 Cerebrospinal fluid gamma globulins in canine distemper encephalitis. An immunoelectrophoretic study. *Neurology* 19: 1111–1114
42. Dal Canto M C, Rabinowitz S G 1982 Experimental models of virus-induced demyelination of the central nervous system. *Annals of Neurology* 11: 109–127
43. de Swart R L, Harder T C, Ross P S, Vos H W, Osterhaus A D 1995 Morbilliviruses and morbillivirus diseases of marine mammals. *Infectious Agents and Disease* 4: 125–130
44. Dodds W J 1999 More bumps on the vaccine road. *Advances in Veterinary Medicine* 41: 715–732
45. Dubielzig R R 1979 The effect of canine distemper virus on the ameloblastic layer of the developing tooth. *Veterinary Pathology* 16: 268–270
46. Duignan P J, House C, Geraci J R, Duffy N, Rima B K, Walsh M T, Early G, St Aubin D J, Sadove S, Koopman H 1995 Morbillivirus infection in cetaceans of the western Atlantic. *Veterinary Microbiology* 44: 241–249
47. Durchfeld B, Baumgartner W, Herbst W, Brahm R 1990 Vaccine-associated canine distemper infection in a litter of African hunting dogs (*Lycaon pictus*). *Zentralblatt für Veterinärmedizin Reich B* 37: 203–212
48. Duval D, Giger U 1996 Vaccine-associated immune-mediated hemolytic anemia in the dog. *Journal of Veterinary Internal Medicine* 10: 290–295
49. Eckersley G N, Hohn E, Reyers F, Turner G V, Wolmarans L 1992 A comparison between the disease status of hospitalised dogs from developed and developing communities. *Journal of the South African Veterinary Association* 63: 2–6
50. Ehrensperger F, Pospischil A 1989 Spontaneous mixed infections with distemper virus and toxoplasma in dogs. *Deutsche Tierarztliche Wochenschrift* 96: 4 184–186
51. Ek-Kommonen C, Sihvonen L, Pekkanen K, Rikula U, Nuotio L 1997 Outbreak off canine distemper in vaccinated dogs in Finland. *Veterinary Record* 141: 380–383
52. Forbes S, Cook J R 1991 Congenital peripheral vestibular disease attributed to lymphocytic labyrinthitis in two related litters of Doberman pinscher pups. *Journal of the American Veterinary Medical Association* 198: 447–449
53. Forsyth M A, Kennedy S, Wilson S, Eybatov T, Barrett T 1998 Canine distemper virus in a Caspian seal. *Veterinary Record* 143: 662–664
54. Fraser W D 1997 Paget's disease of bone. *Current Opinion in Rheumatology* 9: 347–354
55. Frisk A L, Baumgartner W, Grone A 1999 Dominating interleukin-10 mRNA expression induction in cerebrospinal fluid cells of dogs with natural canine distemper virus induced demyelinating and non-demyelinating CNS lesions. *Journal of Neuroimmunology* 97: 102–109
56. Frisk A L, Konig M, Moritz A, Baumgartner W 1999 Detection of canine distemper virus nucleoprotein RNA by reverse transcription-PCR using serum, whole blood, and cerebrospinal fluid from dogs with distemper. *Journal of Clinical Microbiology* 37: 3634–3643
57. Gaskin J M 1980 Microbiology of the canine and feline eye. *Veterinary Clinics of North America (Small Animal Practice)* 10: 303–316
58. Gordon M T, Bell S C, Mee A P, Mercer S, Carter S D, Sharpe P T 1993 Prevalence of canine distemper antibodies in the pagetic population. *Journal of Medical Virology* 40: 313–317
59. Gorham J 1966 The epizootology of distemper. *Journal of the American Veterinary Medical Association* 149: 610–622
60. Gorman N T, Habicht J, Lachmann P J 1980 Intracerebral synthesis of antibodies to measles and distemper viruses in patients with subacute sclerosing panencephalitis and multiple sclerosis. *Clinical and Experimental Immunology* 39: 44–52
61. Graber H U, Muller C F, Vandevelde M, Zurbriggen A 1995 Restricted infection with canine distemper virus leads to down-regulation of myelin gene transcription in cultured oligodendrocytes. *Acta Neuropathologica (Berlin)* 90: 312–318
62. Greene C E, Appel M 1984 Canine distemper. In Greene C E (ed.) *Clinical microbiology and infectious disease of the dog and cat.* W B Saunders, Philadelphia: 386–405
63. Greene C E, Appel M 1990 Canine distemper. In Greene C E (ed.) *Infectious diseases of the dog and cat.* W B Saunders, Philadelphia: 226–241
64. Grevel V, Machus B 1992 Cytology of the cerebrospinal fluid of dogs and cats with symptoms of meningitis/meningoencephalitis. Part 3. *Tierärztliche Praxis* 20: 199–214
65. Griot C, Burge T, Vandevelde M, Peterhans E 1989 Antibody-induced generation of reactive oxygen radicals by brain macrophages in canine distemper encephalitis: a mechanism for bystander demyelination. *Acta Neuropathologica (Berlin)* 78: 396–403
66. Griot C, Burge T, Vandevelde M, Peterhans E 1989 Bystander demyelination through antibody induced macrophage activation in canine distemper virus infection. *Schweizer Archiv für Neurologie und Psychiatrie* 140: 39–41
67. Griot C, Vandevelde M, Richard A, Peterhans E, Stocker R 1990 Selective degeneration of oligodendrocytes mediated by reactive oxygen species. *Free Radical Research Communications* 11: 181–193
68. Grone A, Frisk A L, Baumgartner W 1998 Cytokine mRNA expression in whole blood samples from dogs with natural canine distemper virus infection. *Veterinary Immunology Immunopathology* 65: 11–27
69. Grøndalen J 1976 Metaphyseal osteopathy (hypertrophic osteodystrophy) in growing dogs. A clinical study. *Journal of Small Animal Practice* 17: 721
70. Haas L, Hofer H, East M, Wohlsein P, Liess B, Barrett T 1996 Canine distemper virus infection in Serengeti spotted hyenas. *Veterinary Microbiology* 49: 147–152
71. Haines D M, Martin C L, Chelack B J, Sargent R A, Outerbridge C A, Clark E G 1999 Immunohistochemical detection of canine distemper virus in haired skin, nasal mucosa, and footpad epithelium: a method for antimortem diagnosis of infection. *Journal of Veterinary Diagnostic Investigation* 11: 396–399
72. Harder T C, Osterhaus A D 1997 Canine distemper virus – a morbillivirus in search of new hosts? *Trends in Microbiology* 5: 120–124
73. Higgins R J, Child G, Vandevelde M 1989 Chronic relapsing demyelinating encephalomyelitis associated with persistent spontaneous canine distemper virus infection. *Acta Neuropathologica (Berlin)* 77: 441–444
74. Higgins R J, Krakowka S, Metzler A E, Koestner A 1981 Canine distemper virus-associated cardiac necrosis in the dog. *Veterinary Pathology* 18: 4 472–486
75. Ho C K, Babiuk L A 1979 Immune mechanisms against canine distemper. II. Role of antibody in antigen modulation and prevention of intercellular and extracellular spread of canine distemper virus. *Immunology* 38: 765–772
76. Hodge M J Wolfson C 1997 Canine distemper virus and multiple sclerosis. *Neurology* 49: S62–S69
77. Inada S 1989 Electromyographic analysis of canine distemper myoclonus. *Electromyography and Clinical Neurophysiology* 29: 323–331
78. Inada S, Nomoto H, Kawasaki Y 1993 Canine distemper myoclonus and sleep: observation of a case. *Electromyography and Clinical Neurophysiology* 33: 137–141

79. Itakura C, Nakamura K, Nakatsuka J, Goto M 1979 Distemper infection in lesser pandas due to administration of a canine distemper live vaccine. *Journal of the Nippon Medical School* 41: 561–566
80. Johnson G C, Fenner W. R, Krakowka S 1988 Production of immunoglobulin G and increased antiviral antibody in cerebrospinal fluid of dogs with delayed-onset canine distemper viral encephalitis. *Journal of Neuroimmunology* 17: 237–251
81. Johnson G C, Krakowka S, Axthelm M K 1987 Albumin leakage into cerebrospinal fluid of dogs lethally infected with R252 canine distemper virus. *Journal of Neuroimmunology* 14: 61–74
82. Johnson R, Glickman L T, Emerick T J, Patronek G J 1995 Canine distemper infection in pet dogs: I. Surveillance in Indiana during a suspected outbreak. *Journal of the American Animal Hospital Association* 31: 223–229
83. Jones L, Tenorio E, Gorham J, Yilma T 1997 Protective vaccination of ferrets against canine distemper with recombinant pox virus vaccines expressing the *H* or *F* genes of rinderpest virus. *American Journal of Veterinary Research* 58: 590–593
84. Kennedy S 1998 Morbillivirus infections in aquatic mammals. *Journal of Comparative Pathology* 119: 201–225
85. Khan S A, Brennan P, Newman J, Gray R E, McCloskey E V, Kanis J A 1996 Paget's disease of bone and unvaccinated dogs. *Bone* 19: 47–50
86. Kingbury D, Bratt M A, Choppin P W, Hanson R P, Hosaka Y, Meulen V, Norrby E, Plowright W, Rott R, Wunner W H 1978 Paramyxoviridae. *Intervirology* 10: 137–152
87. Kipar A, Baumgartner W, Vogl C, Gaedke K, Wellman M 1998 Immunohistochemical characterization of inflammatory cells in brains of dogs with granulomatous meningoencephalitis. *Veterinary Pathology* 35: 43–52
88. Klintworth G K 1983 Ophthalmological features of inflammatory demyelinating disorders. *Bullétin de la Societé Belge d'Ophtalmologie* 208 Pt 1: 437–452
89. Kobayashi Y, Ochiai K, Itakura C 1993 Dual infection with canine distemper virus and infectious canine hepatitis virus (canine adenovirus type 1) in a dog. *Journal of Veterinary Medical Science* 55: 699–701
90. Kock R, Chalmers W S, Mwanzia J, Chillingworth C, Wambua J, Coleman P G, Baxendale W 1998 Canine distemper antibodies in lions of the Masai Mara. *Veterinary Record* 142: 662–665
91. Krakowka S, Axthelm M K, Gorham J R 1987 Effects of induced thrombocytopenia on viral invasion of the central nervous system in canine distemper virus infection. *Journal of Comparative Pathology* 97: 441–450
92. Krakowka S, Cockerell G, Koestner A 1975 Effects of canine distemper virus infection on lymphoid function *in vitro* and *in vivo*. *Infection and Immunity* 11: 1069–1078
93. Krakowka, S, Confer A, Koestner A 1974 Evidence for transplacental transmission of canine distemper virus: two case reports. *American Journal of Veterinary Research* 35: 1251–1253
94. Krakowka S, Cork L C, Winkelstein J A, Axthelm M K 1987 Establishment of central nervous system infection by canine distemper virus: breach of the blood–brain barrier and facilitation by antiviral antibody. *Veterinary Immunology and Immunopathology* 17: 471–482
95. Krakowka, S, Higgins, R J, ad Metzler, A E 1980 Plasma phase viremia in canine distemper virus infection. *American Journal of Veterinary Research* 41: 144–146
96. Krakowka S, Koestner A 1976 Age-related susceptibility to infection with canine distemper virus in gnotobiotic dogs. *Journal of Infectious Disease* 134: 629–632
97. Krakowka S, Koestner A 1977 Comparison of canine distemper virus strains in gnotobiotic dogs: effects on lymphoid tissues. *American Journal of Veterinary Research* 38: 1919–1922
98. Krakowka S, Olsen R, Confer A, Koestner A, McCullough B 1975 Serologic response to canine distemper viral antigens in gnotobiotic dogs infected with canine distemper virus. *Journal of Infectious Disease* 132: 384–392
99. Krakowka S, Olsen R G, Axthelm M K, Rice J, Winters K 1982 Canine parvovirus infection potentiates canine distemper encephalitis attributable to modified live-virus vaccine. *Journal of the American Veterinary Medical Association* 180: 137–139
100. Krakowka S, Ringler S S, Lewis M, Olsen R G, Axthelm M K 1987 Immunosuppression by canine distemper virus: modulation of in vitro immunoglobulin synthesis, interleukin release and prostaglandin E2 production. *Veterinary Immunology and Immunopathology* 15: 181–201
101. Kurtzke J F, Hyllested K, Arbuckle J D, Baerentsen D J, Jersild C, Madden D L, Olsen A, Sever J L 1988 Multiple sclerosis in the Faroe Islands. IV. The lack of a relationship between canine distemper and the epidemics of MS. *Acta Neurologica Scandinavia* 78: 484–500
102. Laurenson K, Van Heerden J, Stander P, Van Vuuren M J 1997 Seroepidemiological survey of sympatric domestic and wild dogs (*Lycaon pictus*) in Tsumkwe District, north-eastern Namibia. *Onderstepoort Journal of Veterinary Research* 64: 313–316
103. Lechi A 1992 Viruses and demyelination in the central nervous system. *Italian Journal of Neurological Sciences* 13: 23–26
104. Lincoln S D, Gorham J R, Ott R L, Hegreberg G A 1971 Etiologic studies of old dog encephalitis. I. Demonstration of canine distemper viral antigen in the brain in two cases. *Veterinary Pathology* 8: 1–8
105. Lisiak J A, Vandevelde M 1979 Polioencephalomalacia associated with canine distemper virus infection. *Veterinary Pathology* 16: 650–660
106. Maeda H, Ozaki K, Takagi Y, Sawashima K, Narama I 1994 Distemper skin lesions in a dog. *Zentralblatt für Veterinärmedizin Reich A* 41: 247–250
107. Martin C L 1982 Ocular signs of systemic diseases. *Modern Veterinary Practice* 63: 689–694
108. McCandlish I A, Cornwell H J, Thompson H, Nash A S, Lowe C M 1992 Distemper encephalitis in pups after vaccination of the dam. *Veterinary Record* 130: 27–30
109. McCaw D L, Thompson M, Tate D, Bonderer A, Chen Y J 1998 Serum distemper virus and parvovirus antibody titers among dogs brought to a veterinary hospital for revaccination. *Journal of the American Veterinary Medical Association* 213: 72–75
110. McInnes E F, Burroughs R E, Duncan N M 1992 Possible vaccine-induced canine distemper in a South American bush dog (*Speothos venaticus*). *Journal of Wildlife Diseases* 28: 614–617
111. McMilen G L, Briggs D J, McVey D S, Phillips R M, Jordan F R 1995 Vaccination of racing greyhounds: effects on humoral and cellular immunity. *Veterinary Immunology and Immunopathology* 49: 101–113
112. Mee A, Gordon M T, May C, Bennett D, Anderson D C, Sharpe P T 1993 Canine distemper virus transcripts detected in bone cells of dogs with metaphyseal osteopathy. *Bone* 14: 59
113. Mee A, Webber D M, May C, Bennett D, Sharpe P T, Anderson, D C 1992 Detection of canine distemper virus in bone cells in the metaphyses of distemper infected dogs. *Journal of Bone and Mineral Research* 7: 829
114. Mee A P, Dixon J A, Hoyland J A, Davies M, Selby P L, Mawer E B 1998 Detection of canine distemper virus in 100 % of Paget's disease samples by in situ-reverse transcriptase-polymerase chain reaction. *Bone* 23: 171–175
115. Mee A P, Sharpe P T 1993 Dogs, distemper and Paget's disease. *Bioessays* 15: 783–789
116. Mee A P, Webber D M, May C, Bennett D, Sharpe P T, Anderson D C 1992 Detection of canine distemper virus in bone cells in the metaphyses of distemper-infected dogs. *Journal of Bone and Mineral Research* 7: 829–834
117. Meier H, Clark S T, Schnelle G B, Will D H 1957 Hypertrophic osteodystrophy associated with disturbance if vitamin C synthesis in dogs. *Journal of the American Veterinary Medical Association* 130: 483
118. Mitchell W J, Summers B A, Appel M J 1991 Viral expression in experimental canine distemper demyelinating encephalitis. *Journal of Comparative Pathology* 104: 77–87
119. Montali R J, Bartz C R, Teare J A, Allen J T, Appel M J, Bush M 1983 Clinical trials with canine distemper vaccines in exotic carnivores. *Journal of the American Veterinary Medical Association* 183: 1163–1167
120. Muller C F, Fatzer R S, Beck K, Vandevelde M, Zurbriggen A 1995 Studies on canine distemper virus persistence in the central nervous system. *Acta Neuropathologica (Berlin)* 89: 438–445
121. Muneer M A, Farah I O, Newman J A, Goyal S M 1988 Immunosuppression in animals. *British Veterinary Journal* 144: 288–301
122. Mutinelli F, Vandevelde M, Griot C, Richard A 1989 Astrocytic infection in canine distemper virus-induced demyelination. *Acta Neuropathologica (Berlin)* 77: 333–335
123. O'Driscoll J B, Anderson D C 1985 Past pets and Paget's disease. *Lancet* 2: 919–921
124. Olsen R G, Krakowka S 1984 Immune dysfunctions associated with viral infections. *Compendium of Continuing Education for the Practicing Veterinarian* 6: 422–430
125. Osterhaus A 1989 A morbillivirus causing mass mortality in seals. *Vaccine* 7: 483–484
126. Paoletti E 1996 Applications of pox virus vectors to vaccination: an update. *Proceedings of the National Academy of Sciences USA* 93: 11349–11353
127. Pardo M C, Bauman J E, Mackowiak M 1997 Protection of dogs against canine distemper by vaccination with a canarypox virus recombinant expressing canine distemper virus fusion and hemagglutinin glycoproteins. *American Journal of Veterinary Research* 58: 833–836
128. Patronek G J, Glickman L T, Johnson R,

Emerick T J 1995 Canine distemper infection in pet dogs: II. A case-control study of risk factors during a suspected outbreak in Indiana. *Journal of the American Animal Hospital Association* 31: 230–235
129. Ralston S H, Digiovine F S, Gallacher S J, Boyle I T, Duff G W 1991 Failure to detect paramyxovirus sequences in Paget's disease of bone using the polymerase chain reaction. *Journal of Bone and Mineral Research* 6: 1243–1248
130. Rautenbach G H, Boomker J, de Villiers I 1991 A descriptive study of the canine population in a rural town in southern Africa. *Journal of the South African Veterinary Association* 62: 158–162
131. Raw M E, Pearson G R, Brown P J, Baumgartner W 1992 Canine distemper infection associated with acute nervous signs in dogs. *Veterinary Record* 130: 291–293
132. Roelke-Parker, M E, Munson L, Packer C, Kock R, Cleaveland S, Carpenter M, O'Brien S J, Pospischil A, Hofmann-Lehmann R, Lutz H 1996 A canine distemper virus epidemic in Serengeti lions (*Panthera leo*). *Nature* 379: 441–445
133. Rohowsky-Kochan C, Dowling P C, Cook S 1995 Canine distemper virus-specific antibodies in multiple sclerosis. *Neurology* 45: 1554–1560
134. Rouse B T Horohov D W 1986 Immunosuppression in viral infections. *Reviews of Infectious Diseases* 8: 850–873
135. Schoning P, Layton C E 1992 Canine distemper with spinal cord lesions. *Zentralblatt für Veterinärmedizin Reich B* 39: 571–574
136. Shen D T, Gorham J R, Pedersen V 1981 Viruria in dogs infected with canine distemper. *Veterinary Medicine (Small Animal Clinics)* 76: 1175–1177
137. Shin Y, Mori T, Okita M, Gemma T, Kai C, Mikami T 1995 Detection of canine distemper virus nucleocapsid protein gene in canine peripheral blood mononuclear cells by RT-PCR. *Journal of Veterinary Medical Science* 57: 439–445
138. Sixt N, Cardoso A, Vallier A, Fayolle J, Buckland R, Wild T F 1998 Canine distemper virus DNA vaccination induces humoral and cellular immunity and protects against a lethal intracerebral challenge. *Journal of Virology* 72: 8472–8476
139. Sorjonen D C, Cox N R, Swango L J 1989 Electrophoretic determination of albumin and gamma globulin concentrations in the cerebrospinal fluid of dogs with encephalomyelitis attributable to canine distemper virus infection: 13 cases (1980–1987). *Journal of the American Veterinary Medical Association* 195: 977–980
140. Stevens D R, Osburn B I 1976 Immune deficiency in a dog with distemper. *Journal of the American Veterinary Medical Association* 168: 493–498
141. Sukura A, Laakkonen J, Rudback E 1997 Occurrence of *Pneumocystis carinii* in canine distemper. *Acta Veterinaria Scandinavica* 38: 201–205
142. Summers B A, Appel M J 1994 Aspects of canine distemper virus and measles virus encephalomyelitis. *Neuropathology and Applied Neurobiology* 20: 525–534
143. Summers B A, Greisen H A, Appel M J 1984 Canine distemper and experimental allergic encephalomyelitis in the dog: comparative patterns of demyelination. *Journal of Comparative Pathology* 94: 575–589
144. Summers B A, Greisen H A, Appel M J 1984 Canine distemper encephalomyelitis: variation with virus strain. *Journal of Comparative Pathology* 94: 65–75
145. Summers B A, Whitaker J N, Appel M J 1987 Demyelinating canine distemper encephalomyelitis: measurement of myelin basic protein in cerebrospinal fluid. *Journal of Neuroimmunology* 14: 227–233
146. Tipold A 1995 Diagnosis of inflammatory and infectious diseases of the central nervous system in dogs: a retrospective study. *Journal of Veterinary Internal Medicine* 9: 304–314
147. Tipold A, Pfister H, Vandevelde M 1993 Determination of the IgG index for the detection of intrathecal immunoglobulin synthesis in dogs using an ELISA. *Research in Veterinary Science* 54: 40–44
148. Tipold A, Vandevelde M, Jaggy A 1992 Neurological manifestations of canine distemper virus infection. *Journal of Small Animal Practice* 33: 466–470
149. Turnwald G H, Barta O, Taylor H W, Kreeger J, Coleman S U, Pourciau S S 1988 Cryptosporidiosis associated with immunosuppression attributable to distemper in a pup. *Journal of the American Veterinary Medical Association* 192: 79–81
150. Uchida K, Muranaka M, Horii Y, Murakami N, Yamaguchi R, Tateyama S 1999 Nonpurulent meningoencephalomyelitis of a Pacific striped dolphin (*Lagenorhynchus obliquidens*). The first evidence of morbillivirus infection in a dolphin at the Pacific Ocean around Japan. *Journal of Veterinary Medical Science* 61: 159–162
151. Van Heerden J, Bainbridge N, Burroughs R E, Kriek,N P 1989 Distemper-like disease and encephalitozoonosis in wild dogs (*Lycaon pictus*). *Journal of Wildlife Diseases* 25: 70–75
152. Van Vuuren M J, Stylianides E, du Rand A 1997 The prevalence of viral infections in lions and leopards in southern Africa. *Proceedings of a Symposium on Lions and Leopards as Game Ranch Animals*,Onderstepoort, 24–25 October 1997: 168–173
153. Vandevelde M, Kristensen B 1977 Observations on the distribution of canine distemper virus in the central nervous system of dogs with demyelinating encephalitis. *Acta Neuropathologica (Berlin)* 40: 233–236
154. Vandevelde M, Kristensen B, Braund K G, Greene C E, Swango L J, Hoerlein B F 1980 Chronic canine distemper virus encephalitis in mature dogs. *Veterinary Pathology* 17: 17–28
155. Vandevelde M, Kristensen F, Kristensen B, Steck A J, Kihm U 1982 Immunological and pathological findings in demyelinating encephalitis associated with canine distemper virus infection. *Acta Neuropathologica (Berlin)* 56: 1–8
156. Vandevelde M Spano J S 1977 Cerebrospinal fluid cytology in canine neurologic disease. *American Journal of Veterinary Research* 38: 1827–1832
157. Vandevelde M, Zurbriggen A 1995 The neurobiology of canine distemper virus infection. *Veterinary Microbiology* 44: 271–280
158. Vandevelde M, Zurbriggen A, Dumas M, Palmer D 1985 Canine distemper virus does not infect oligodendrocytes *in vitro*. *Journal of Neurological Science* 69: 133–137
159. Vandevelde M, Zurbriggen A, Steck A, Bichsel P 1986 Studies on the intrathecal humoral immune response in canine distemper encephalitis. *Journal of Neuroimmunology* 11: 41–51
160. Vilafranca M, Tello M, Pumarola M, Domingo M 1996 Neural cells from dogs with spontaneous distemper encephalitis express class II major histocompatibility complex molecules. *Journal of Comparative Pathology* 114: 43–50
161. Visser I K, van Bressem M F, Barrett T, Osterhaus A D 1993 Morbillivirus infections in aquatic mammals. *Veterinary Research* 24: 169–178
162. Williams R M, Krakowka S, Koestner A 1980 *In vivo* demyelination by antimyelin antibodies. *Acta Neuropathologica (Berlin)* 50: 1–8
163. Wunschmann A, Alldinger S, Kremmer E, Baumgartner W 1999 Identification of CD4+ and CD8+ T cell subsets and B cells in the brain of dogs with spontaneous acute, subacute-, and chronic-demyelinating distemper encephalitis. *Veterinary Immunology and Immunopathology* 67: 101–116
164. Wunschmann A, Kremmer E, Baumgartner W 2000 Phenotypical characterization of T and B cell areas in lymphoid tissues of dogs with spontaneous distemper. *Veterinary Immunology and Immunopathology* 73: 83–98
165. Zurbriggen A, Graber H U, Vandevelde M 1995 Selective spread and reduced virus release leads to canine distemper virus persistence in the nervous system. *Veterinary Microbiology* 44: 281–288
166. Zurbriggen A, Graber H U, Wagner A, Vandevelde M 1995 Canine distemper virus persistence in the nervous system is associated with noncytolytic selective virus spread. *Journal of Virology* 69: 1678–1686
167. Zurbriggen A, Muller C, Graber H U, Vandevelde M 1994 Canine distemper virus and myelin mRNAs in oligodendrocytes. *Schweizer Archiv für Neurologie und Psychiatrie* 145: 17
168. Zurbriggen A, Yamawaki M, Vandevelde M 1993 Restricted canine distemper virus infection of oligodendrocytes. *Laboratory Investigation* 68: 277–284