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Canine distemper in Finland

– vaccination and epidemiology

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ABBREVIATIONS AND DEFINITIONS

Abbreviations

CD	canine distemper
CDV	canine distemper virus
CNS	central nervous system
CSF	cerebrospinal fluid
DNA	deoxyribonucleic acid
EELA	National Veterinary and Food Research Institute
EID	egg infectious dose
ELISA	enzyme-linked immunosorbent assay
Evira	Finnish Food Safety Authority
F	fusion (protein)
H	hemagglutinin (protein)
HI	herd immunity
ID_{50}	infectious dose for half (50%) of target population
IF	immunofluorescence
IFA	immunofluorescence assay
IgG	immunoglobulin of type G
IgM	immunoglobulin of type M
L	large (protein)
LD_{50}	lethal dose for half (50%) of target population
M	matrix (protein)
ML	modified live
MLV	modified live vaccine
MS	market share
P	phospho (protein)
PI	post infection
R	net or effective reproduction number
R_0	basic reproduction number
RNA	ribonucleic acid
RT-PCR	reverse transcriptase-polymerase chain reaction
SD	standard deviation
TCID	tissue culture infectious dose
UTR	untranslated region
UV	ultra violet (light)
VELL	State Veterinary Institute
VERO	African green monkey kidney epithelial (cells)
VI	virus isolation
VN	virus neutralisation (assay) or virus neutralising (antibodies or titre)

Definitions

Basic reproduction number, R_0, of an infection is the number of secondary cases a typical single infected case will cause in a population with no immunity to the disease in the absence of interventions to control the infection. The net or effective reproduction number, R, is $R_0 * P_S$, the proportion of susceptible individuals in the population (Anderson and May 1991).

Efficacy or performance of a vaccine is the ability of the vaccine to prevent the adverse effects of the infection to the vaccinated animal itself, or the ability to induce antibodies in the vaccinated animal that are transferred to the offspring, and so provide protection to the newborn animals. In veterinary medicine/vaccinology efficacy is demonstrated under controlled conditions by vaccination-challenge tests using the target animal species (Soulebot et al. 1997).

Herd immunity is here defined as the proportion of subjects with immunity in a given population (herd). The vaccine induced herd immunity depends upon vaccination coverage and efficacy of the vaccine (John and Samuel 2000).

Herd effect is the reduction of infection or disease in the unimmunised segment as a result of immunising a proportion of the population (John and Samuel 2000).

Immunogenicity is the ability of a vaccine to stimulate the immune system, as measured for example by the proportion of individuals that produce specific antibody or T cells, or the amount of antibody produced.

Infectivity is a measure of the ability of the infectious agent to establish itself in the host. This term can be used qualitatively (e.g. low, medium or high), or quantitatively. Attempts to quantify infectivity normally involve the use of a statistic known as infectious dose 50 (ID_{50}). This refers to the individual dose or numbers of the agent required to infect 50% of a specified population of susceptible animals under controlled environmental conditions. It often is expensive or not feasible to determine *in vivo* ID_{50} and the infectivity is expressed using the tissue culture ID_{50} ($TCID_{50}$) as the dimension (Putt et al. 1988).

Pathogenicity is an epidemiological term used to describe the ability of an agent of known virulence to produce disease in a range of hosts under a range of environmental conditions (Putt et al. 1988).

Performance of a vaccine. See efficacy.

Potency testing of a vaccine is done to guarantee that each vaccine batch has the intended effect. In live vaccines, potency can be estimated for example by the number of organisms present. Potency tests must be validated by demonstrating a correlation between the results of the test and the efficacy of the vaccine in the target animal (Soulebot et al. 1997).

Vaccine coverage (%) is the proportion of the population that has been vaccinated.

Vaccine take (%) is the proportion of the vaccinated population in which vaccination has elicited a specific immune response, for example production of specific antibodies.

Virulence is a measure of the severity of the disease caused by the agent. In a strict sense it is a laboratory term, used to measure the varying ability of the agent to produce disease under controlled conditions. It is often quantified by a statistic known as lethal dose 50 (LD_{50}). This refers to the individual dose or numbers of the agent required to kill 50% of a specified population of susceptible animals under controlled environmental conditions (Putt et al. 1988).

ABSTRACT

Canine distemper (CD) is one of the longest-known infectious diseases of dogs and is still prevalent in many parts of the world. Vaccination combined with biosecurity measures is the most productive way to prevent and control infectious diseases. The beneficial effects of vaccination are realized not only on the individual but also on the population level, the latter in the form of herd immunity (HI). Control of CD among dogs relies heavily on vaccination, while in fur farms and zoos with several species or large numbers of CD-susceptible animals in close contact, biosecurity measures in some cases offer the only available means for CD control. Modified live CD virus vaccines have been successfully used to control CD among farmed mink, and since no licensed vaccines for other species kept for fur exist, mink CD vaccines have also been used for foxes and raccoon dogs in CD emergency situations.

CD vaccines for dogs (*Canis familiaris*) and mink (*Mustela vison*) were studied in experimental settings for their ability to induce virus-neutralising (VN) antibodies in target species. Mink vaccines were also assessed in silver foxes (*Vulpes vulpes*), blue foxes (*Alopex lagopus*) and raccoon dogs (*Nyctereutes procyonoides*). Purpose-bred beagle dogs were vaccinated twice with one of three CD vaccines: Candur® SHP, Canlan®-3 or Dohyvac® DA_2P, and the levels of VN antibodies were determined at the time of vaccination and one month after the second vaccination. Fur animals were vaccinated once with Distemink®, Distem®-R-TC or vaccine 3 (which was not licensed in Finland) and the levels of VN antibodies were determined at vaccination and 2-4 times 1-4 months afterwards. Significant differences among vaccine groups were found both in the proportion of animals with measurable levels of VN antibodies and in the mean titres of antibodies.

The levels of VN antibodies were also determined from a large field sample (n = 4 627) of vaccinated dogs. In addition to the three CD vaccines in the seroconversion study above, additional two vaccines, Duramune®-4 and Nobivac® DHP, had been used in the field. Each dog with a known vaccination history, date of birth, sex and breed was sampled once. Based on the overall geometric mean titre of the dogs vaccinated with a single vaccine brand, vaccines were divided into high-take (Candur®, Nobivac® and Duramune®) and low-take (Dohyvac® and Canlan®) groups. The vaccine groups differed significantly among dogs less than two years of age both in the proportion of dogs with detectable VN antibodies and in the mean titres. Both the number of vaccinations and age were associated with the titre and vaccine usage. To control for possible confounding factors, the comparison

of titres among vaccine usage groups was adjusted by classifying them according to the number of vaccinations (one to four) and the age group (less than one, one to two, or over two years old). The same division into low- and high-take vaccines was observed, irrespective of the number of vaccinations the dogs had received. The observations of this seroprevalence study regarding Candur® , Canlan® and Dohyvac® were consistent with the results of the seroconversion study.

CD was reintroduced into Finland in 1990 after 16 years of absence. The disease remained at a low endemic level in 1990-1994, reached epidemic proportions in 1994-1995 and disappeared during 1995. The epidemic also involved vaccinated dogs. Among the virologically-confirmed cases the proportion of Dohyvac®-vaccinated dogs was higher than expected from the market shares on the assumption that all the vaccines had an equal take. As a result of this observation, Dohyvac® was withdrawn from and Nobivac® and Duramune® introduced to the market during 1995. A drastic redistribution of the market shares between the low-take and high-take vaccines took place, and this coincided with the decline and dying out of the outbreak. The observed occurrence pattern of CD from 1990-1996 was largely attributed to the changes in the level of HI, although the possible contribution of other factors, such as developments in the dog demographics, was also recognized. It was concluded that an HI above 75% is needed to keep CD in check, i.e., only sporadic cases of CD, at most, can occur. With the currently used vaccines an HI of 80% corresponds to a vaccine coverage of some 94%.

It was concluded that the development of vaccine-induced immunity is a multifactorial process depending on the properties of the vaccine, on the individual variation, age, species and other factors influencing the immunocompetence of the host. On the individual level the prevention of clinical signs is sufficient, but on the population level, halting the circulation of the virus is crucial for the definitive control of CD. The ultimate test and criterion for a vaccine is its contribution to herd immunity. Heterogeneity in the dog population contributes to the occurrence of CD.

LIST OF ORIGINAL PUBLICATIONS

The present thesis is based on the following original studies, referred to in the text by the Roman numerals I to V

I Rikula U, Sihvonen L, Voipio H-M, Nevalainen T.1996 Serum antibody response to canine distemper virus vaccines in beagle dogs. Scand J Lab Anim Sci, 23: 31-3.

II Rikula U, Pänkälä L, Jalkanen L, Sihvonen L. 2001 Distemper vaccination of farmed fur animals in Finland. Prev Vet Med 49: 125-33.

III Rikula U, Nuotio L, Sihvonen L. 2000 Canine distemper virus neutralising antibodies in vaccinated dogs. Vet Rec 147: 598-603.

IV Ek-Kommonen C, Sihvonen L, Pekkanen K, Rikula U, Nuotio L. 1997 Outbreak of canine distemper in vaccinated dogs in Finland. Vet Rec 141: 380-3.

V Rikula U, Nuotio L, Sihvonen L. 2007 Vaccine coverage, herd immunity and occurrence of canine distemper from 1990-1996 in Finland. Vaccine 25: 7994-8.

1. INTRODUCTION

For centuries, infections by morbilliviruses have imposed a significant burden on both human and animal populations. Measles, which was introduced by the Europeans to America, devastated the populations of native Americans. Rinderpest, a morbillivirus infection of cattle, was introduced to Europe by traders coming from Asia and later to Africa by colonial wars, and it severely affected both domestic and wildlife species. Measles still prevails as an important childhood disease, especially in the developing countries (WHO 2007), but outbreaks also continue to occur in developed countries, when or wherever vaccine coverage wanes (Mossong and Muller 2000, van den Hof et al. 2001). Global eradication of rinderpest is underway, but peste des petits ruminants, a morbillivirus infection of sheep and goat, remains endemic in Africa and has spread to the Middle East and southern Asia (Shaila et al. 1996).

Canine distemper, a morbillivirus infection of dogs and other carnivores, has been recognized for at least 250 years. As reviewed by Blancou (2004), the first report of canine distemper (CD) is from South America by Ulloa in 1746. Heusinger was convinced that CD was introduced in 1760 from Peru to Spain, from where it spread to other parts of Europe and Russia within a few years. Although CD may have occurred in Europe earlier and was possibly confused with rabies, the epidemic spread of CD through Europe started around the 1760s. In 1815 Jenner observed that CD among dogs is as contagious as smallpox, measles and scarlet fever among humans. He attempted vaccination against CD in the way found successful in vaccinating against smallpox. Karle succeeded in experimentally transmitting CD in 1844, by brushing the lips of young dogs with discharge from diseased dogs. The etiology of CD remained controversial until 1905, when Henri Carré demonstrated that CD is caused by a filterable virus. In some connections, CD is still called Carré's disease.

The first vaccine against CD was made in 1923 by Puntoni from the formalin-inactivated brain tissue of a dog suffering from CD encephalitis (reviewed by Appel 1999). The protection obtained with inactivated vaccines was limited, and they are no longer used. The modified live (ML) vaccines by which CD can be successfully controlled were developed in the late 1950s. The CD viruses adapted to chicken embryonated eggs were named Lederle (Cox and Cabasso 1952) and Onderstepoort (Haig 1956) strains. A canine kidney cell culture adapted strain was named the Rockborn strain (Rockborn 1959).

Although the use of ML vaccines has significantly reduced the incidence of CD, the circulation of CD virus in populations of dogs and other susceptible carnivores continues. This circulation manifests itself as the sporadic, endemic or epidemic occurrence of CD, and outbreaks of CD also involve vaccinated dogs (Glardon and Stöckli 1985, Adelus-Neveu et al. 1991, Blixenkrone-Møller et al. 1993). The neglecting of vaccinations, leading to poor vaccine coverage and herd immunity, is an obvious reason for outbreaks on the population level. Antigenic shift in the wild CD viruses, making the current vaccines unprotective, has been suggested as a cause, but never proven. On the individual level, interference by maternal antibodies, immunosuppression caused by concurrent infections such as canine parvovirus or a heavy load of internal parasites, and improper storage and handling of the vaccine have been blamed for vaccine failures (Povey 1986, Tizard 2000, Greene and Appel 2006). However, the ultimate reason for the vaccine failures may lie in the inherent properties of the vaccines themselves.

In the development of an ML vaccine, a balance must be reached between two opposing aims: safety and efficacy. On the individual level, vaccination can be considered to have succeeded when the vaccine itself has not induced disease and no signs of a disease are observed after challenge. However, on the population level, the circulation of a pathogen should be stopped. This is possible if vaccines that induce a sufficiently vigorous immune response to prevent the replication of a wild CDV, and not only the clinical signs, are used and a high enough vaccine coverage is maintained. Unfortunately, the current requirements for a vaccine to be accepted in the market are concentrated on the prevention of clinical disease after challenge (European Pharmacopoeia). The importance of field trials before accepting the vaccine in the market has only recently been recognized.

Two seroconversion studies (I, II) were conducted in order to determine whether commercial CD and mink distemper vaccines differ in their take or in the level of antibodies induced by vaccination. The performance of the vaccines under field conditions according to the above-mentioned criteria was explored in a seroprevalence study (III). In a preliminary report (IV) the causes of the outbreak among vaccinated dogs were sought, and finally, an attempt was made to explain the observed pattern of CD occurrence in Finland from 1990-1996 by using vaccine coverage and herd immunity as explanatory factors (V). The level of herd immunity (%) that is critical for the control of CD in Finland was pinpointed and the vaccine coverage needed to sustain this level with currently available vaccines was suggested.

2. REVIEW OF LITERATURE

2.1 Canine distemper

2.1.1 Etiology

Canine distemper virus (CDV) belongs to the genus *Morbillivirus* in the *Paramyxoviridae* family. The type virus of the genus is measles virus. The morbilliviruses, the diseases that they cause and their natural hosts are presented in Table 1. Morbilliviruses consist of a non-segmented linear single-stranded RNA genome of negative polarity comprising about 15 900 bp. The RNA is enclosed in a helical nucleocapsid formed by the N protein. In addition, mature ribonucleoprotein complexes also contain copies of the phospho- (P) and large (L) proteins. The host-cell-derived lipid envelope is spiked with transmembrane haemagglutinin (H) and fusion (F) glycoproteins. Internally, the envelope is stabilized by a layer of the matrix (M) protein (Figure 1). Genes in the genome are in the following order: 3'-UTR-N-P(C,V)-M-UTR-F-H-L-UTR-5'. P gene encodes two non-structural proteins C and V in addition to P protein (Griffin, 2001).

Table 1 Viruses belonging to the genus *Morbillivirus* and diseases that they cause in their natural hosts (modified from Osterhaus et al. 1995 and Di Guardo et al. 2005).

Virus	Disease	Natural host
Measles virus (MV)	Measles	Human
Rinderpest virus (RPV)	Rinderpest	Cattle, goat, sheep, pig
Peste des petits ruminants virus (PPRV)	Peste des petits ruminants	Goat, sheep
Dolphin morbillivirus (DMV)		Dolphin
Porpoise morbillivirus (PMV)		Porpoise
Canine distemper virus (CDV)	Canine distemper	Dog
Phocine distemper virus (PDV)		Seal

DMV and PMV are currently gathered under the common denomination of 'cetacean morbilliviruses' (CMV).

CDV is susceptible to visible and UV light and extremely susceptible to heat and drying. It is destroyed by temperatures above 50 °C in 30 minutes, but it can survive for 48 hours at +25 °C and for 14 days at + 5 °C (Shen and Gorham 1980). At near freezing temperatures (0–4°C) it survives in the environment for weeks. Viral infectivity is lost above pH 10.4 or below pH 4.4 (Zee, 1999). Routine disinfection procedures are effective in destroying CDV in kennel, clinic or hospital environments (Greene and Appel 1998).

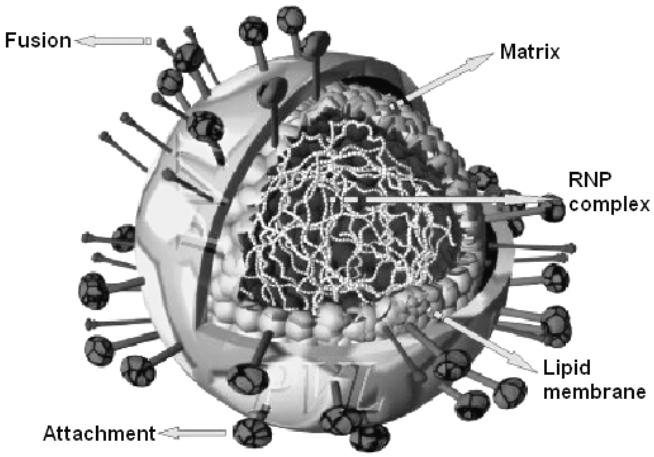

Figure 1 Schematic picture of a structure of morbillivirus (ICTVdb, 2006).

CDV is considered to have one antigenic type (Zee 1999). On the other hand, based on phylogenetic analysis of subgenomic F, P, and complete H gene sequences, CDV strains can be divided into distinct CDV lineages, which are mainly associated with the geographical area from which the strain is isolated (Lednicky et al. 2004, Lan et al. 2005, Martella et al. 2006).

2.1.2 Pathogenesis and clinical picture

Both the pathogenesis and the clinical picture of CD depend on the intrinsic determinants of both the agent and the host animal (see 2.1.4 below).

Pathogenesis of CD is best studied in the dog (Fig. 2, Appel 1969, Greene and Appel 2006). Briefly, invasion of the body is followed within 24 hours by multiplication of CDV in local tissue macrophages, spread within these cells to the tonsils and bronchial lymph nodes, further replication from 2-4 days postinfection (PI), and spread to other lymphoid organs. The virus multiplies from 4-6 days PI in the lymphoid follicles of the spleen, in the lamina propria of the stomach and small intestine, and in the Kupffer's cells in the liver, which is accompanied by an initial fever 3-6 days PI. Further spread of CDV

to epithelial and central nervous system (CNS) tissues 8-9 days PI depends on the immune status of the dog, and most likely takes place both as a cell-associated and plasma-phase viremia.

The clinical picture in all susceptible species manifests most frequently in respiratory, gastro-intestinal, integumentary, and CNS systemic signs. Biphasic fever and general malaise are often associated with viremia (Deem et al. 2000).

The first systemic sign is an initial febrile response at 3-6 days PI, which usually goes unnoticed. Mild forms of clinical illness are common, and the signs include apathy, loss of appetite, fever, and upper respiratory tract infection. Bilateral serous oculonasal discharge may become mucous with coughing and dyspnea. In more severe cases the dry cough rapidly becomes

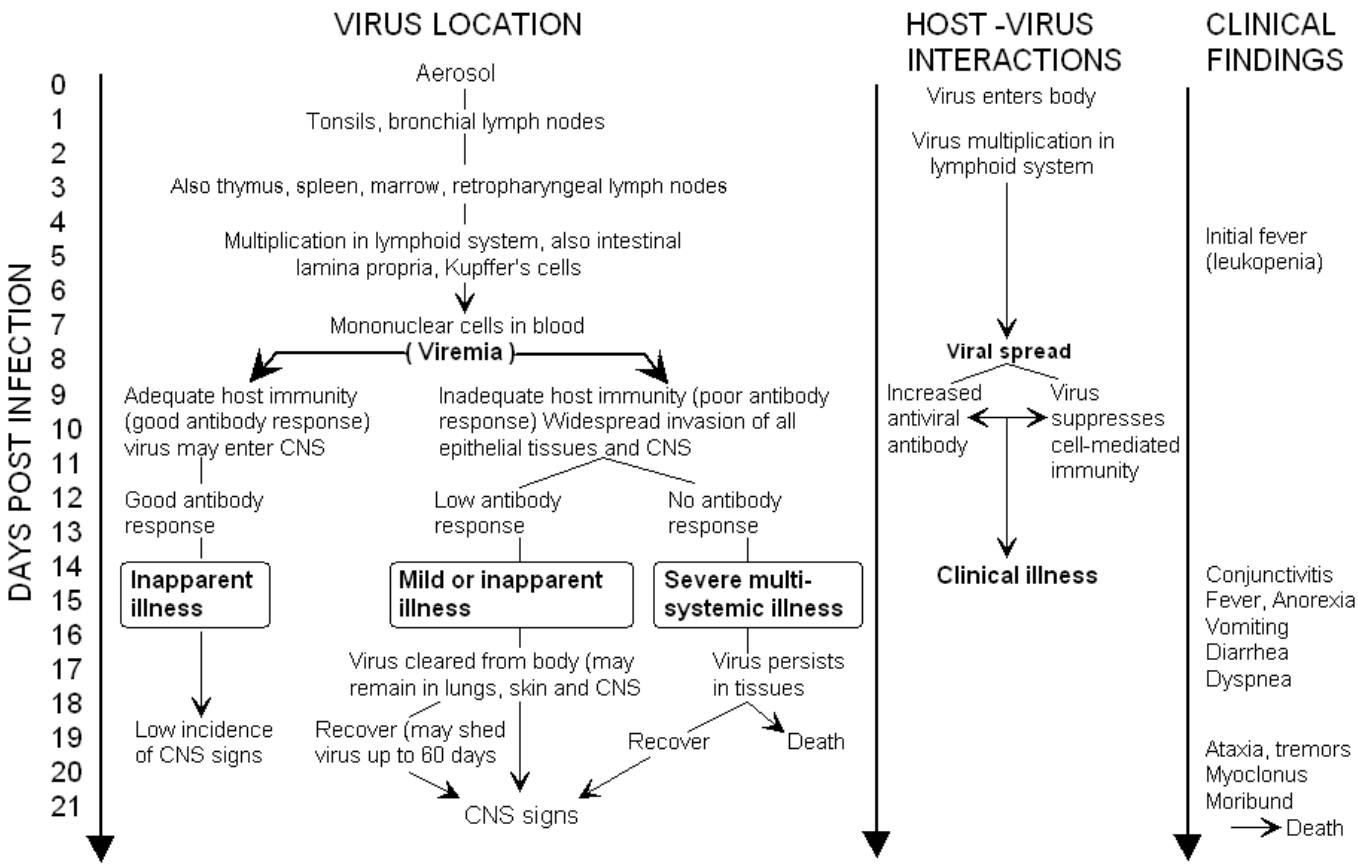

Figure 2 Pathogenesis of CD and associated clinical signs (modified from Green and Appel 2006)

moist and productive. Lower respiratory sounds will be increased. Vomiting will follow depression and anorexia, and diarrhea, which may vary in consistency from watery to frank blood and mucus, develops. The neurological signs, which vary according the CNS areas involved, can coincide with the systemic signs, but usually begin 1-3 weeks after recovery from systemic illness, and are typically progressive. The neurological signs may emerge several months later, and without any preceding systemic signs. The presence of neurological signs strongly determines the prognosis for CD.

Other signs associated with CD infections of dogs include vesicular or pustular dermatitis in puppies, and nasal and digital hyperkeratosis ('hard pads'). CDV infection before the eruption of permanent dentition may cause enamel hypoplasia characterized by irregularities in the dental surface of permanent teeth.

2.1.3 Laboratory confirmation of the clinical suspicion of CD

Clinical suspicion of distemper can be confirmed by detecting either CDV or a specific immune response in samples from the affected animal. Detection of CDV from smears of the conjunctiva, tonsillar or genital epithelium using immunofluorescent (IF) techniques is possible only within the first 3 weeks PI, while systemic clinical signs are apparent. As antibody titres rise in association with clinical recovery, the virus will either be masked by antibodies or will disappear from the epithelium. The sensitivity of the IF technique is no more than 40% (Blixenkrone-Møller et al. 1993, Leisewitz et al. 2001). Immunohistochemistry can be used to demonstrate CDV antigens in foot pad or skin biopsies, or in samples of spleen, tonsils, lymph nodes, stomach, duodenum, bladder and brain taken post-mortem (Greene and Appel 2006). The reverse-transcriptase-polymerase chain reaction (RT-PCR) can demonstrate CDV from buffy coat cells of acutely-infected dogs and from serum, whole blood, cerebrospinal fluid (CSF) or urine of dogs with systemic or neurological CD (Shin et al. 1995, Frisk et al. 1999, Saito et al. 2006). RT-PCR can be applied for the detection of CDV from smears of epithelial cells and from other tissue samples. A positive RT-PCR result is indicative of CD infection, whereas a negative one can result from various reasons. In the case of recently spray-vaccinated fur animals, a positive IF or RT-PCR result from epithelial smears of the respiratory tract may also be due to a vaccine strain.

Although virus isolation (VI) is the gold standard for the detection of the agent, it is not straightforward with CDV. Virulent CDV requires adaptation before it grows in routinely-used epithelial or fibroblast cell lines. The best

results with virus isolation are achieved by direct cultivation of buffy coat cells or other target tissues from the infected host together with mitogen-stimulated dog lymphocytes (Greene and Appel 2006). Ferret inoculation has been used in the past, when other laboratory procedures have not been available (Pearson and Gorham 1987).

CDV infection can be confirmed by demonstrating specific antibodies to the agent. A four-fold rise in the antibody level of paired sera taken 10 to 21 days apart is indicative of the infection. However, this method is not suitable for detecting a recent infection, since titres are often already high at the first sampling and a four-fold rise cannot therefore be demonstrated. Instead, the detection of CDV-specific IgM is indicative of a recent infection. IgM is measurable for up to 3 months PI and 3 weeks after the first vaccination. ELISA methods for measuring IgM are available (Blixenkrone-Møller et al. 1991, von Messling et al. 1999, Soma et al. 2003, Latha et al. 2007). Increased CDV antibody in CSF is definitive evidence of a neurological CDV infection provided that the blood-brain barrier is intact (Greene and Appel 2006).

2.1.4 Epidemiology

Because CDV does not persist in an infectious form after the resolution of an infection, and both infection and vaccination result in long-lasting immunity, a constant source of susceptible individuals is required for proliferation of CDV in the population. It has been estimated that at least 300 000 individuals are needed to maintain measles virus in circulation (Black, 1991). Considering the wide host range of CDV, the circulation of the virus does not solely depend on the size of the dog population but on the size of the combined total population of all susceptible species in the area. Furthermore, the contact structures of among those species will be crucial for the continued presence of the virus.

Intrinsic determinants of the hosts and the agent

The infection rate is estimated to be significantly higher than the disease rate, and over 50% of infections in domestic dogs may be subclinical (Rockborn 1958a, Greene and Appel 2006). The prevalence of CD in urban dogs is highest between 3 and 6 months of age. However, in fully susceptible populations CDV is capable of causing mortality in dogs of all ages (Gorham 1966, Böhm et al. 1989). Brachiocephalic breeds of dogs have been reported to have a lower prevalence of disease, mortality and sequelae compared with dolichocephalic breeds (Greene and Appel 1998). Among farmed mink, pastel mink is more susceptible to CD than the ordinary dark form of the species (Pearson and Gorham, 1987). Gender does not play a significant

role in the susceptibility to CD. CD produces a long-lasting immunity in dogs that survive the infection. Maternal antibodies received mainly in colostrum have a half-life of 8.4 days and will usually be absent by the age of 12 to 14 weeks (Greene and Appel 1998). *In utero* or tranplacental infections of CD do occur. The outcome of the infection in these cases depends on the stage of gestation.

There is no published information of *in vivo* ID_{50} of the virus. However, in experimental conditions clinical disease has been induced by inoculating 5 x 10^3 dog lung macrophage ID_{50} of virulent CDV strain intranasally in specific-pathogen-free 4-month-old male beagle dogs (Appel et al. 1982). No quantitative data on the **virulence** of the agent, for example in the form of LD_{50}, have been published. The mortality rate in naïve dog populations may rise to 80% (Böhm et al. 1989), so that qualitatively the virulence can be regarded as at least moderate to high. The case fatality rate in domestic ferrets (*Mustela putorius furo*) approaches 100% (Deem et al. 2000). However, virulence differs between CDV strains (Appel et al. 1984a).

Host-agent relationship in the disease

The length of the latent period (the time from infection to when the individual becomes infectious to others) is typically 1 week, while that of the infectious period is 2-3 weeks (1 week before and 1-2 weeks after the onset of signs), and in rare cases 60-90 days. The incubation period (the time from infection to clinical signs) is frequently 1-2 weeks. Urine and saliva of experimentally-infected dogs have been shown to be infective from day 6 to day 22 PI, and from day 7 to day 41 PI, respectively (Shen et al. 1981).

CDV does not establish true carrier states but the virus may be demonstrated after the clinical illness for longer periods in epithelial cells and macrophages of the lower respiratory tract. It can also persist for at least 60 days in the skin, footpad and CNS (Greene and Appel 2006). The epidemiological significance of these findings remains inconclusive.

Antigenic drift in the wild-type CDV strains could cause increasing numbers of outbreaks in dog and wild animal populations. Several genotypes of CDV have been shown to simultaneously circulate in a population (Gemma et al. 1996, Haas et al. 1999, Lednicky et al. 2004, Martella et al. 2006). However, CDV is considered to have only one antigenic type (Zee 1999). Haas et al. (1999) found no major diversity in H genes and neutralisation assays between recent wild-type isolates and the vaccine strain. On the other hand, serum from a dog infected with the Onderstepoort strain reacted at a low level against two Japanese field CDV isolates in an immunoperoxidase

assay (Gemma et al. 1996). The biological significance of these findings needs verification.

Transmission of CDV

CDV is most abundant in the respiratory exudates of infected animals and is mainly transmitted by aerosol or droplet exposure. Direct or indirect contacts between recently infected (subclinical or clinical) and susceptible animals sustain the virus in the population. In temperate climates the highest incidence has been reported during the colder months (Rockborn 1958b, Gorham 1966, Glardon and Stöckli 1985). This may be attributed to the ability of the virus to survive longer in a cool, shady environment, which may increase the chances of indirect transmission.

The basic reproduction number R_0, defined as the number of secondary cases caused by one primary case in a population consisting entirely of susceptible individuals (Anderson and May 1982), is a useful measure of the transmission of a pathogen in a population. As a formula, R_0 can be thought to be composed of the probability of transmission during a contact (β) multiplied by the frequency of contacts per time unit (c), multiplied by the duration of the infectious period (D) (Woolhouse and Bundy 1997). R_0, or rather the net or effective R in real-life situations, is frequently considered as a threshold parameter: when $R < 1$ the infection will tend to die out without a major outbreak. When $R > 1$, the chance of a major outbreak exists (De Jong and Bouma 2001). However, R is extremely sensitive to heterogeneities created by the spatial structure of populations (Dobson and Foufopoulos 2001), by the age and contact structures in populations, and by a variety of ill-defined management and behavioural factors. There are no published assessments of R for CD in any settings, but the R_0 for phocine distemper is 2.8 and for measles in the range of 11-18 (Swinton et al. 1998, Woolhouse and Bundy 1997).

Occurrence and host range

CD has a worldwide distribution. CDV is able to infect practically all the families of terrestrial carnivores of the order *Carnivora*. It has also been associated with mass-mortalities of Baikal seals (*Pusa* former *Phoca sibirica*) and Caspian seals (*Pusa* former *Phoca caspica*), which belong to the family *Phocidae* of the *Carnivora* (Osterhaus et al. 1989, Kennedy et al. 2000, Figure 3, Table 2). Furthermore, CDV-induced fatal encephalitis has been reported in a Japanese macaque (*Macaca fuscata*) and collared peccaries (*Tayassu tajacu*), which belong to the family *Cercopithecidae* in the order *Primates* and to the family *Tayassuidae* in the order *Artiodactyla*, respectively (Deem et al. 2000). Experimental CDV infection in domestic cats

(*Felis silvestris catus*) and pigs (*Sus scrofa)* resembles the infection of dogs with attenuated CDV, but neither natural infection nor clinical disease in the cat has been reported (Appel et al. 1974, Harder and Osterhaus 1997). Despite the wide host range, dogs are the principal reservoir host for CDV (Greene and Appel 2006).

In Finland, CD is known to have occurred in both dogs and fur animals (mink) as early as in the 1950s and the 1970s (Estola 1964, Loikala and Kangas 1988). In 1985-1987, fur farms suffered from a widespread epidemic that originated from imported foxes, but the disease did not spill over to dogs and was finally controlled by mass vaccinations of all fur animals in the most important fur-farming areas. As a consequence, distemper in fur animals became a notifiable disease in Finland (Loikala and Kangas 1988).

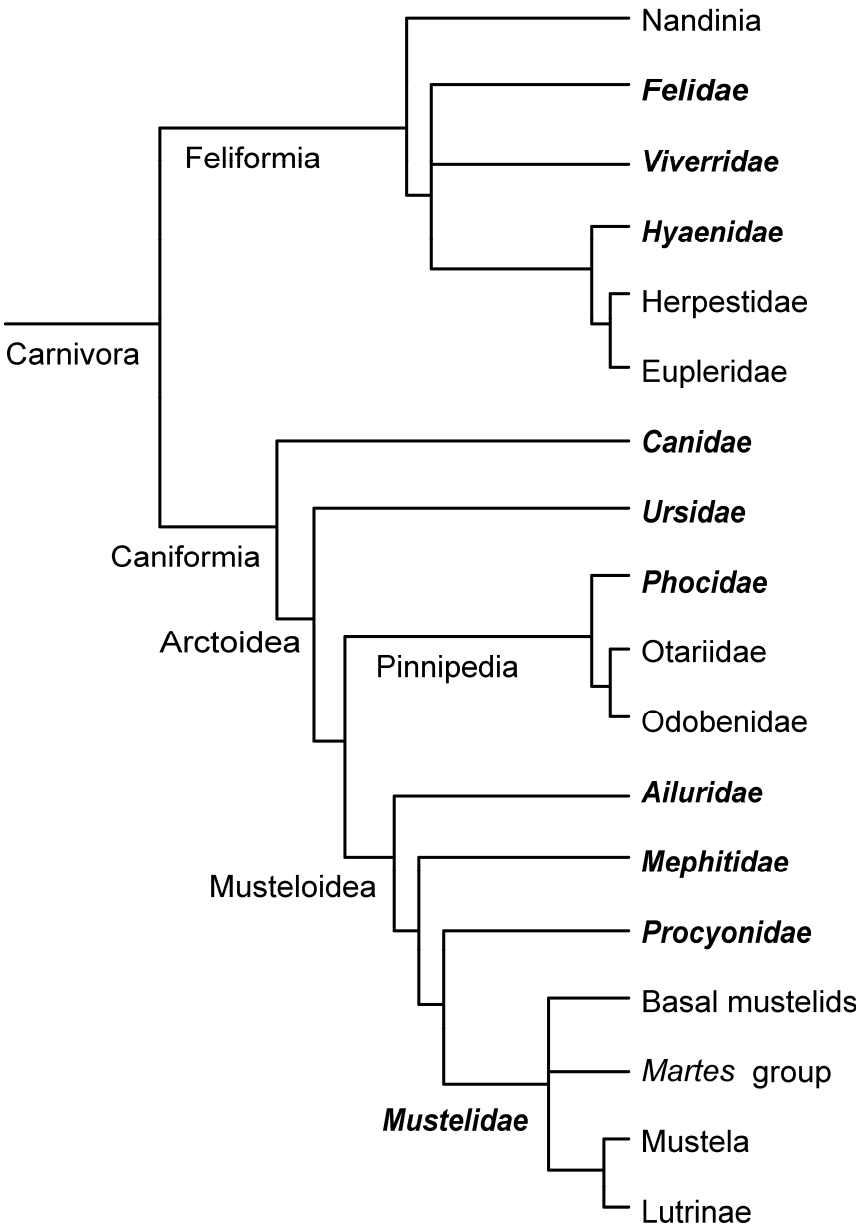

Figure 3 Phylogenetic tree of the families in the order *Carnivora*. The families with species reported to be susceptible to CDV are in ***bold italic*** (adopted from Flynn et al. 2005, modified according to Appel and Summers 1995, Deem et al. 2000).

Table 2 Species that have been reported to be susceptible to CDV in the order *Carnivora* (Appel and Summers 1995, Deem et al. 2000, Loikala and Kangas 1988, Mos et al. 2003).

Family	Genus	Species
Felidae	Panthera	African lion (*Panthera leo*)
		Tiger (*Panthera tigris*)
		Leopard (*Panthera pardus*)
		Jaguar (*Panthera onca*)
	Puma	Cougar, mountain lion, puma (*Puma concolor*)
Viverridae	Arctictis	Binturong (*Arctictis binturong*)
	Paguma	Masked palm civet (*Paguma larvata*)
Hyaenidae	Crocuta	Spotted hyena (*Crocuta crocuta*)
Canidae	Alopex	Arctic/blue fox (*Alopex lagopus*)
	Vulpes	Red fox (*Vulpes vulpes*)
		Kit fox (*Vulpes macrotis macrotis*)
		Fennec fox (*Vulpes zerda*)
	Nyctereutes	Raccoon dog (*Nyctereutes procyonoides*)
	Otocyon	Bat-eared fox (*Otocyon megalotis*)
	Urocyon	Grey fox (*Urocyon cinereoargentus*)
	Chrysocyon	Maned wolf (*Chrysocyon brachyurus*)
	Speothos	South American bush dog (*Speothos venaticus*)
	Canis	Wolf (*Canis lupus*)
		Domestic dog (*Canis lupus familiaris*)
		Australian dingo (*Canis lupus dingo*)
		Coyote (*Canis latrans*)
	Lycaon	African wild dog (*Lycaon pictus*)
Ursidae	Ailuropoda	Giant panda (*Ailuropoda melanoleuca*)
	Ursus	Black bear (*Ursus americanus floridanus*)
		Grizzly bear *(Ursus arctos horribilis*)
		Marsican bear (*Ursus arctos marsicanus*)
		Polar bear (*Ursus maritimus*)
	Tremarctos	Spectacled bear *(Tremarctos omatus*)
Phocidae	Pusa	Baikal seal (*Pusa sibirica*)
		Caspian seal (*Pusa caspica*)
Ailuridae	Ailurus	Red panda (*Ailurus fulgens*)
Mephitidae	Mephitis	Striped skunk (*Mephitis mephitis*)
Procyonidae	Procyon	Raccoon (*Procyon lotor*)
	Potos	Kinkajou (*Potos flavus*)
Mustelidae	Lutra	European otter (*Lutra lutra*)
		River otter (*Lutra canadiensis*)
	Meles	European badger (*Meles meles*)
	Taxidea	American badger (*Taxidea taxus*)
	Mustela	American mink (*Mustela vison*)
		European mink (*Mustela lutreola*)
		Ferret (*Mustela putorius*)
		Black-footed ferret (*Mustela nigripes*)

Control of CD

Vaccination remains the principal means of controlling the disease. The widespread use of modified live vaccines (MLV) has greatly reduced the incidence in dogs (Chappuis, 1995). Vaccination is also used to control CD

among several species of farmed fur animals, ferrets kept as pets and among a wide range of other susceptible animal species kept in zoos (Appel and Summers 1995, Gorham and Wilson 1997). On the other hand, post-vaccinal encephalitis in dogs (Cornwell et al. 1988) and vaccination-induced cases of CD have been reported in several species (Bush et al. 1976, Carpenter et al. 1976, Appel and Summers 1995, Halbrooks et al. 1981, Saari et al. 1999, Ek-Kommonen et al. 2003a). Maintaining a high vaccine coverage in populations of dogs and other susceptible species, as well as the use of the most potent vaccines available, is vital for the control of CD (Chappuis 1995, Harder and Osterhaus 1997). Dogs infected with CDV should be isolated from healthy ones (Greene and Appel 2006). Furthermore, as no vaccination strategy can eliminate the gap in protection between the passive maternal immunity and an active immunity, prophylactic measures should include the isolation of young dogs from the general dog population until vaccine-induced protection has been reached (Blixenkrone-Møller et al. 1993).

For CD control, fur animal farms form a very specific setting: tens to thousands of various CD susceptible species are kept at a high density. Furthermore, the population structure varies greatly according to the season: the size of a population more than triples in March-May, and after pelting in December only breeding animals are left. In addition to vaccination, strict biosecurity measures such as isolation and quarantine are necessary at fur farms (Pearson and Gorham 1987). The risk period for CD (immunity gap) varies from a few weeks in mink farms to some months in fox farms, because the breeding period is longer in fox farms compared to that in mink farms (Loikala and Kangas 1988).

In Finland, dogs are recommended to be vaccinated as follows: the first two vaccinations are given with a 4-week interval starting at the age of 12 weeks, and the third vaccination is given at the age of 12 months. Thereafter, booster vaccinations are given 1- to 3-year intervals. If the infectious pressure is high, puppies can be vaccinated starting at the age of 6-8 weeks, followed by vaccination at 3- to 4-week intervals until the puppy is 12-16 weeks of age. Mink, fox and raccoon dogs from unvaccinated dams can be vaccinated at 8-9 weeks of age, and those from vaccinated dams at 10-14 weeks of age. Dams should be vaccinated at latest 3 weeks before the beginning of the breeding season. Annual boosters are recommended.

The impact of vaccination can also be examined in terms of the reproduction number R. Vaccination evidently reduces the number of susceptible individuals in the population and thus decreases the probability of transmission (β) and furthermore shortens the duration of the infectious

period (D). By vaccinating a large enough portion of the population it is possible to reduce R below 1 (De Jong and Bouma 2001).

2.2 Distemper vaccines

Active immunization against CD has been practised since Puntoni in 1923 described the use of formalin-inactivated CDV-infected dog brain tissue (reviewed by Appel 1999). However, active immunization was not successful before MLVs became available in the 1950s. All commercial CD vaccines available for dogs are multivalent vaccines that, besides the CDV component, also contain some of the following components: inactivated canine adenovirus type 1 (CAV-1) or attenuated CAV-2, attenuated or inactivated canine parvovirus, attenuated canine parainfluenza viruses and *Leptospira canicola-icterohaemorrhagiae* bacterin. Nowadays, most of the virus antigens included in the vaccines tend to be of the modified live type. CD vaccines are administered either subcutaneously or intramuscularly. Canine distemper vaccines registered to be used in mink usually contain only CDV antigen and are administered subcutaneously, intramuscularly or by aerosol-spray. Since no registered CD vaccines exist for other farmed fur animal species, mink CD vaccines are used.

2.2.1 Attenuated live viral vaccines

In MLV the micro-organism is rendered avirulent by attenuation, but it is still able to replicate in the host. Conventional attenuation is achieved by serial passage of the virus in a cell culture. Vaccination with an MLV closely mimics natural infection; it stimulates both humoral and cellular immune responses, and induces immunological memory (van Oirschot 1997). The majority of CD vaccines currently contain either the egg-adapted, avian cell culture-adapted or Vero cell-adapted Onderstepoort strain (Haig 1956), or the Rockborn strain, which is produced in canine cell cultures (Rockborn 1959). As there are problems with the safety of some MLVs in dogs and especially in other species, a new generation of CD vaccines has been and is being developed.

2.2.2 Recombinant and DNA vaccines

Since antibodies raised against H and F glycoproteins of CDV play an important role in the protection against CD (Norrby et al. 1986), it is clear that these antigens should be included in the new generation of CD vaccines. Improved adjuvants or other immune-stimulating complexes are needed in formulation of these recombinant protein vaccines in order to reach sufficient efficacy (de Vries et al. 1988, Visser et al. 1992, Fischer et

al. 2003). Vaccines produced by recombinant techniques have been shown to be efficient and safe (Taylor et al. 1991, Pardo et al. 1997, Welter et al. 2000). The canarypox vector, which is non-replicating in mammals, has been used to express genes of CDV H and F glycoproteins in a recombinant CD vaccine currently available for dogs in the USA (Pardo et al. 1997). This vaccine has been successfully tested in ferrets (Stephensen et al. 1997). The VN antibody levels induced by recombinant vaccines are not as high as with well-performing MLVs (Pardo et al. 1997), and the duration of immunity is probably shorter than that of MLV (Schultz 2006). DNA vaccines are in the experimental stage (Sixt et al. 1998, Cherpillod et al. 2000, Fischer et al. 2003, Dahl et al. 2004).

2.3 Response to vaccination on individual and population levels

The outcome of an infection depends on the properties of the virus and the host's immune responses. Naïve individuals can recover from the infection caused by virulent CDV provided that their humoral and especially cellular immune reactions are vigorous enough (Appel et al. 1982). The MLVs induce immune responses that are in principle very similar to those occurring after natural infection. The outcome of the vaccination depends on the properties of the vaccine strain, on the formulation of the vaccine, and on several other factors. Both cellular and humoral immunity are important in the protection against CDV (Appel 1969, Krakowka et al. 1975, Appel et al. 1984b).

2.3.1 Individual level

Humoral immunity can be demonstrated by measuring the titre of virus neutralising (VN) antibodies against CDV in the serum. These antibodies are raised against the viral glycoproteins H and F. The presence and titre of the neutralising antibodies correlate with the level of protection against CD (Norrby et al. 1986). Vaccination with MLVs also elicits antibodies against other viral antigens, but the role of these in protective immunity is inconclusive. The neutralising antibodies can be detected 6-10 days post vaccination in the serum, and their titres peak between 14-21 days post vaccination (Appel 1987), and persist for several years (Olson et al. 1997b, Coyne et al. 2001). Puppies with maternal antibody titres higher than 1:100 were protected against CDV infection (Gillespie 1996). Susceptible dogs that developed titres of at least 1:100 by day 14 after challenge with virulent CDV survived (Appel 1969). In another study, susceptible dogs that on average developed a titre of 1:8 between 10-21 days PI survived (Appel et al. 1982). According to Greene and Appel (2006), a VN antibody titre of 1:20 is considered protective after vaccination. Among the variety of serological

methods applicable for the detection of the humoral response or measurement of antibody titres, immunofluorescence assay and ELISA–based methods are nowadays perhaps the most common. The efficacy of a vaccine can be indirectly assessed by determining the average level of VN antibodies in the vaccinated population.

Several methods have been used to measure **cellular immunity** against CDV (Appel et al. 1984b, Krakowka and Wallace 1979, Shek et al. 1980). Virus-specific cell-mediated immunity can be demonstrated from 6-18 days post vaccination. It reaches maximal levels between 7-10 days post vaccination (Krakowka and Wallace 1979, Shek et al. 1980). However, the use of these methods is hampered by the overall short duration of the responses (Appel et al. 1982); furthermore, the methods require considerable expertise and are not easily amenable to high-throughput procedures.

2.3.2 Population level

On the population level, the response to vaccination can be described as the proportion of the vaccinated population that has developed sufficient immunity against infection, i.e. the vaccine take (Woolhouse and Bundy 1997). In addition to the average levels of VN antibodies elicited by a vaccine, its field efficacy is reflected in the take. Herd immunity (HI), defined as the proportion of subjects with immunity in a given population (John and Samuel 2000), is a result of the immunity induced both by vaccination and natural infection in the population. The part of HI induced by vaccination heavily depends on both the overall vaccine coverage and on the takes of the employed vaccines. Other factors that contribute to vaccine-based HI are the duration of the induced immunity and the average life expectancy of the vaccinees. The higher the HI, the less probable it will be that a susceptible individual encounters and perpetuates the infection (De Jong and Bouma 2001).

2.4 Reported assessments of vaccines, vaccine coverage and outbreaks

2.4.1 CD vaccines

There have been only a few reports of the average levels antibodies reached with vaccination. Floss and Schrag (1995) reported significantly higher titres in 13 puppies vaccinated with a Rockborn-strain vaccine compared with the Onderstepoort-strain vaccinated ones. Gore et al. (2005) reported a geometric mean VN titre of 1:193 thirty six months after vaccination among

23 beagles vaccinated at 7 and 11 weeks of age. Olson et al. (1988) reported geometric mean titres of 1:51 in vaccinated dogs less than 12 months of age, 1:27 in vaccinated dogs more than 12 months of age, and 1:5 in unvaccinated dog less than 12 months of age.

On the population level, Kölb et al. (1995) reported IFA titres $\geq$ 1:20 against CDV in 63% of Dohyvac® , 91.7% of Canimed® and 100% of Canlan®, Enduracell® and Vetamun® vaccinated groups of puppies (n = 12-13 per group). Olson et al. (1988) reported that 78.3% and 80.7% of dogs (n = 259) less than 12 months of age vaccinated once and twice, respectively, and 60-88.2% of vaccinated dogs (n = 244) more than 12 months of age had a titre of $\geq$ 1:16. Among randomly-selected vaccinated dogs (n = 176) representing six breeds, 86.1% had a titre of $\geq$ 1:16 (Olson et al. 1996a). In a large population study of vaccinated dogs (n = 1848), the proportion of dogs with a titre higher than 1:16 were 66.4% and between 86.7–92% in those vaccinated with Dohyvac® and Nobivac® or Candur®, or with several vaccines, respectively (Olson et al. 1997a). McCaw et al. (1998) reported that 79% of dogs (n = 117) coming to revaccination had titres of at least 1:96. According to Böhm et al. (2004), 89.6% of dogs (n = 144) that had been vaccinated more than three years previously had a VN titre higher than 1:16 against CDV. In a population based study of 207 dogs vaccinated 1 or more years previously, Ottiger et al. (2006) observed that 83% had a titre higher than 1:16.

2.4.2 Vaccine coverage and outbreaks

In a questionnaire-based study (n = 538) a vaccine coverage of 95.8% was observed in Sweden (Olson et al. 1996a). A slightly lower level of 85.4% of mixed breed dogs were vaccinated against CD compared to the 97.6% of pure-bred dogs. Glardon and Stöckli (1985) reported 179 out of 280 cases (63.9%) among vaccinated dogs. Of these, 83% were regarded as properly vaccinated. The vaccine coverage before the Danish CD outbreak was estimated to be 50%, and 65% of confirmed CD cases were among dogs less than 2 years of age. Among these 50 cases, 17 were unvaccinated, 18 vaccinated and 15 had obscure vaccination records (Blixenkrone-Møller et al. 1993). Jozwik and Frymus (2002) reported that 72% and 22% of all CD cases occurred among dogs less than 12 months of age and among vaccinated dogs, respectively.

2.5 Vaccine failures

Despite vaccination, outbreaks of CD continue to occur among vaccinated individuals and populations (Glardon and Stöckli 1985, Harder et al. 1991, Blixenkrone-Møller et al. 1993, Mori et al. 1994). General reasons for vaccine failures are schematically presented in Figure 4. The viability of the modified live CDV vaccine strain is essential to successful vaccination. Lyophilized tissue culture vaccine strains are stable for 16 months under refrigeration (0–4 ^{o}C), 7 weeks at 20 ^{o}C and 7 days when exposed to sunlight at 47 ^{o}C. After reconstitution, a vaccine virus remains stable for 3 days at 4 ^{o}C and 24 hours at 20 ^{o}C, However, a reconstituted vaccine should be used within one hour (Greene and Appel 2006).

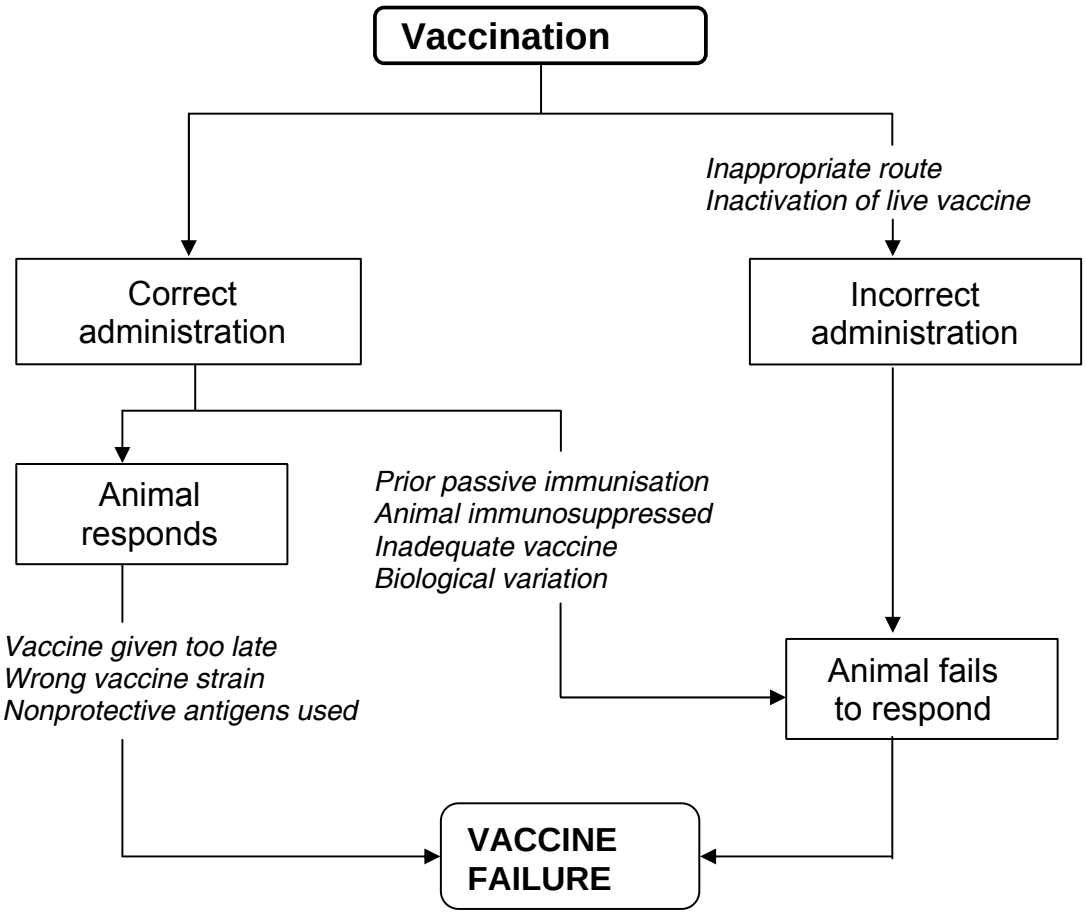

Figure 4 General reasons for vaccine failures (Povey 1986, Roth 1999, Tizard 2000)

Results from field studies of CDV vaccinations suggest that even minimal levels of maternal antibodies that persist at the time of vaccination may impair the ability of dogs to respond to both primary and subsequent vaccinations (Blixenkrone-Møller, unpublished data, 1993). On the other hand, some CDV vaccines have been shown to break through maternal immunity in experimental conditions when basic vaccination was started at

the age of six weeks and completed at the age of 10 weeks (Bergman et al. 2006). Nevertheless, interference by the maternal antibodies is considered to be one of the most important reasons for vaccine failures in dogs.

Due to intensive inbreeding, inherited immunodeficiency syndromes are probably more common in dogs than has been recognized. They are likely to account for an unknown, but important number of vaccination failures (Povey 1986).

Concurrent infections at the time of vaccination may stimulate the production of interferon, block the replication of vaccine virus, or be immunosuppressive. Environmental conditions that can be regarded as stressful, such as overcrowded conditions or transportation, may inhibit the immune response to vaccination. A high environmental temperature and humidity that raised the body temperature above the normal average had an adverse effect on the immune response of puppies after vaccination (Webster 1975).

Antigens applied simultaneously can interact with each other and with the vaccinated host. These interactions may enhance or reduce the immunogenicity of a particular antigen (Strube 1997). Phillips et al. (1988) demonstrated that ML CDV and CAV-1 or -2 in a multivalent vaccine suppressed lymphocyte responsiveness. ML parvovirus antigens in multivalent vaccines have also been suspected but not proven to be immunosuppressive (Greene 1998).

3. AIMS OF THE STUDY

The aim of the present thesis was to investigate the immunogenicity of canine distemper (CD) vaccines both in experimental and in field conditions, as well as the occurrence and epidemiological features of CD. More specifically, the aim was to

- study the immunogenicity of CD vaccines in dogs and farmed fur animals in experimental settings (I, II);

- explore the immunogenicity of CD vaccines in dogs in a sample obtained from the field, with special reference to the age, number of vaccinations, time since the last vaccination and the vaccine used (III);

- describe the background and causes that led to a severe CD outbreak in a vaccinated population (IV, V);

- estimate the level of herd immunity induced by vaccination against CD in the contemporary young dog population from 1988-1996 (V);

- pinpoint the critical level of herd immunity for the control of CD (V).

4. MATERIALS AND METHODS

4.1 Materials

4.1.1 Seroconversion studies (**I, II**)

Animals and vaccinations

Study I. Three groups of 25 purpose-bred beagle dogs (altogether 15 litters) were each vaccinated with one of the three commercially-available triple vaccines at the age of 3 and 4 months at the National Laboratory Animal Centre, University of Kuopio.

Study II. Healthy American mink (*Mustela vison)*, raccoon dog (*Nyctereutes procyonoides*), silver fox (*Vulpes vulpes*) and blue fox (*Alopex lagopus*) young born within a time span of 2-3 weeks were chosen from the population of University of Kuopio Research Fur Farm. The young of each species were weaned at the age of 5-7 weeks, and placed in cages of the same shedhouse at the age of 10-12 weeks. Every other mink female and male of the same litter were placed together on opposite sides of the shedhouse. With the other species, every other litter according to age was placed on opposite sides of the shedhouse. If there were more males than females, the extra males were kept alone. Animals on one side of the shedhouse were vaccinated with one vaccine and those on the opposite side with another. Each fur animal was vaccinated once subcutaneously in the neck, using the dose recommended for mink by the manufacturer (1 ml). Two trials using two CD vaccines in four (first trial) and two fur animal species (second trial) were run. Ages varied within trials between species because all animals and all species were vaccinated simultaneously, and breeding seasons differ slightly (II). In the first trial, mink (n = 20 + 20) were vaccinated at 12-14 weeks, raccoon dogs (19 + 20) at 15-17 weeks, blue fox (20 + 22) at 12-15 weeks and silver fox (20 + 20) at 14-16 weeks of age. In the second trial, mink (20 + 20) were vaccinated at 12-13 weeks of age and silver fox (20 + 20) at 12-15 weeks of age.

Blood samples

Study I. Blood samples were drawn from all 75 dogs at the age of 3, 4 and 5 months and from 8, 6 and 3 dogs in vaccine groups 1, 2 and 3, respectively, at the age of 1 year.

Study II. Blood samples were collected from the cephalic vein of Canidae species with a vacuum-sampling device; for mink, a claw was cut and capillary blood collected openly. Adequate blood samples could not be drawn

from all of the animals at every sampling. Animals were monitored daily for changes in appetite, growth or any other signs of unthriftiness or clinical disturbances by the animal attendants. The staff had no knowledge of which animals received the vaccines used in these trials. In the first trial, blood samples were collected three times: before vaccination and 1.5 and 2.5-4 months after vaccination. Some animals were sampled two further times: 8-10 and 11-12 months after vaccination. In the second trial, blood samples were collected five times: before vaccination and 4 times at monthly intervals starting one month after vaccination. Some animals were sampled two further times: 5 and 6 months after vaccination.

4.1.2 Prevalence studies (**III, IV, V**)

***Laboratory-confirmed CD cases* (IV, V)**
Information on the occurrence of CD in dogs in Finland is based on clinical samples sent to the National Veterinary and Food Research Institute (EELA).

***Vaccinated dogs sampled for the determination of VN titres* (III, IV)**
Serum samples were collected from CD-vaccinated dogs between November 1994 and December 1995 by 230 small animal clinics or clinicians in urban and suburban areas of Finland. The name of the owner and the dog, its date of birth, gender and breed, the dates of vaccinations, the vaccines used and the date of sampling were recorded. The clinicians were also asked to comment on the health of the dogs. A total of 5 734 samples were received, but as a result of incomplete information and multiple sampling of some of the dogs, the number of dogs used in the analysis was reduced to 4 627. The dogs sampled several times were included only when they were first sampled. The most recent vaccination was ignored if it had been given less than three weeks before sampling.

Vaccines and their market shares
Studies I, III - V. The CD vaccines registered for dogs and available from 1988-1996 are shown in Table 3. Three CD vaccine brands were in use from 1987 until 1994: Candur® (Behringwerke), Canlan® (Langford Laboratories) and Dohyvac® (Solvay Animal Health). In February 1995, Dohyvac® was withdrawn from the market. Nobivac® (Intervet) and Duramune® (Fort Dodge Laboratories) were introduced to the market in February and July 1995, respectively. The annual statistics on CD vaccines sold in 1984-1996 and monthly statistics on CD vaccines sold in 1994-1996 were obtained from the Statistics of the National Veterinary Institute, later the National Veterinary and Food Research Institute (EELA). The market shares of the high-take CD vaccine brands are presented in Table 9.

Table 3 Canine distemper vaccines registered for dogs in 1988-1996 in Finland. CDV strains abbreviated as Rockborn (RO) and Onderstepoort (OP). Minimum titre of CDV per dose expressed as $TCID_{50}$ (tissue culture infectious dose) except EID_{50} (egg infectious dose) for Dohyvac®.

Vaccine	CDV strain Cell line	Minimum titre of CDV per dose	Other antigens in the vaccine	Adjuvant	Manufacturer
Candur® SH	RO Canine kidney	10^3	inact.[1] freeze-dried CAV-1[2]	$Al(OH)_3$	Behringwerke
Candur® SHP			as above + inact.CPV[3]	$Al(OH)_3$ and $Al_2(PO_4)_3$	
Canlan®-3	OP type Vero	$10^{3.7}$	inact.CAV-1 and CPV	$Al(OH)_3$ and L-80	Langford Laboratories
Canlan®-4			as above + ML[4] CPI[5]		
Dohyvac® DA2	OP type Chicken embryofibroblasts	$10^{2.5}$	ML CAV-2	No adjuvant	Solvay Animal Health
Dohyvac® DA2+P			as above + inact.CPV	$Al(OH)_3$	
Dohyvac® DA2Pi			ML CAV-2 and CPI	No adjuvant	
Dohyvac® DA2Pi+P			as above + inact.CPV	$Al(OH)_3$	
Duramune®-4	RO type Canine kidney	$10^{3.3}$	ML CAV-2, CPV and CPI	No adjuvant	Fort Dodge Laboratories
Nobivac® DHP	OP type Vero	$10^{3.3}$	ML CAV-2 and CPV	No adjuvant	Intervet

[1] inactivated

[2] canine adenovirus 1 or 2

[3] canine parvovirus

[4] modified live

[5] canine parainfluenza virus

Study II. The three commercial mink distemper vaccines containing freeze-dried modified live egg-adapted canine distemper virus are shown in Table 4.

Table 4 Commercial mink distemper vaccines containing a freeze-dried modified live egg-adapted CDV strain used in seroconversion study II. Vaccine 3 was intended for use in both mink and ferret. Minimum titre of CDV per dose is expressed as EID_{50} (egg infectious dose).

Vaccine	Minimum titre of CDV per dose	Manufacturer
Distemink®	$10^{3.0}$	United Vaccines
Distem®-R-TC	$10^{3.5}$	Schering Corporation
Vaccine 3	$10^{3.7}$	Gift from a manufacturer

Dog demographics 1985-2006 **(V)**

The annual statistics on the numbers of dogs registered in 1975 to 2006 were provided by the Finnish Kennel Club. The proportion of non-registered dogs in the population was estimated to e a constant 20% and each dog was assumed to have a life-expectancy of 10 years. These estimates were obtained from an expert in the Finnish Kennel Club. The age structure of the population was determined using the annual numbers of registered dogs, starting from 1975. In this way the total size and age structure of the registered population could be calculated from 1985 onwards. The calculations were performed using the spreadsheet program MS Excel 2000 (Microsoft Corporation, USA).

4.2 Methods

Laboratory confirmation of CD **(IV, V)**

Clinical suspicion of CD was confirmed by demonstrating the presence of CDV in the epithelial cells from the mucous membranes of conjunctiva, genital tract, trachea or urinary bladder. The epithelial cells were transferred onto microscope slides, air-dried and fixed in acetone. An indirect immunofluorescence assay (IFA) was performed, using a mixture of monoclonal antibody clones 4.100 and 3.851 (Claes Örvell, Huddinge University Hospital, Sweden) directed against the nucleoprotein of the CD virus and rabbit anti-mouse IgG fraction conjugated with fluorescein isothiocyanate (Dako, Denmark).

Determination of virus-neutralising antibodies **(I, II, III, IV)**

The level of virus-neutralising antibodies against CDV was determined with a modified version of the microneutralisation test described by Appel and Robson (1973). Briefly, the heat inactivated sera were diluted fourfold (1:8, 1:32, 1:128 and 1:512) and mixed with an equal volume of medium containing 100 $TCID_{50}$/ml of the Onderstepoort strain of CDV and incubated at 37 ^{o}C for one hour. The mixture was then inoculated into Vero cells and incubated again at 37 ^{o}C for one hour; after this incubation, maintenance medium was added to the wells. A standard virus titration and a positive control serum were included in each test series. The test was read microscopically after six days. The highest serum dilution without a cytopathogenic effect was recorded as its reciprocal (titre < 1:8 = 1, 1:8 = 8, 1:32 = 32, 1:128 = 128 and $\geq$ 1:512 = 512). The value 1 was used for titres < 1:8 to simplify the statistical analysis. Titres below 8 were classified as undetectable for virus-neutralising antibodies, and those of 8 or above as detectable.

Vaccine usage and immunogenicity groups, and redistribution of the vaccines into low- and high take groups **(III)**

The dogs were grouped according to the canine distemper vaccine used into vaccine usage groups. Dogs vaccinated with a single vaccine brand were grouped together and designated according to the brand. The geometric mean titre of neutralising antibodies for all single vaccines was 29. This reciprocal value was used as a cut-off point to divide the vaccines according to their geometric mean titres. Canlan® and Dohyvac® induced low titres and are here referred to as low-take, whereas Candur® , Duramune® and Nobivac® induced high titres and are referred to as high-take vaccines. Dogs vaccinated with more than one brand of vaccine were designated as mixed low-take or mixed high-take; the former consisted of dogs vaccinated with Canlan® and Dohyvac® and the latter consisted of dogs vaccinated with any other combinations. These single low and high, and mixed low and high groups are referred to as immunogenicity groups.

Vaccine coverage **(IV, V)**

Estimation of the number of vaccines needed annually was based on contemporary vaccination recommendations. In the 1980s, puppies were vaccinated for the first time at the age of 3-4 months and the second vaccination was given one year later. In the 1990s, two vaccinations with a 4-week interval were given, starting at the age of 3 months, and a third vaccination was administered at the age of one year. After these basic vaccinations each dog was assumed to receive 3 booster vaccinations approximately triennially. This yielded the number of vaccines needed, which was then compared with the number of vaccines sold to obtain an estimate

of the annual vaccine coverage from 1988-1993 and the monthly coverage from 1994-1996. Uniform vaccine coverage was assumed in the population.

***Herd immunity* (V)**

The take of each vaccine was calculated as the proportion of dogs with detectable levels of neutralising antibodies against CDV, as examined in **III**. The vaccine takes were calculated separately for the age groups < 1 year and 1-2 years, and are shown in Table 5.

Table 5 Take (proportion of vaccinated dogs with a detectable level of neutralising antibodies against CDV, titre $\geq$ 1:8) of the CD vaccine brands among dogs < 1 year of age and dogs 1-2 years of age in 1994-1995 (study **III**), which was used in calculating herd immunity (study **V**).

Age	Candur®	Canlan®	Dohyvac®	Nobivac®	Duramune®
< 1 year	0.97	0.56	0.46	0.89	0.87
1-2 years	1	0.8	0.62	NT*	NT

* not tested, take of Nobivac® and Duramune® in dogs < 1 years was used for dogs 1-2 years of age as well

To obtain a uniform time scale for the calculations, the annual numbers of registered dogs were divided by 12 to determine the annual average monthly values and the monthly estimates were interpolated linearly between these annual average values. The numbers of immune dogs in age groups < 1 year and 1-2 years were calculated separately for each monthly time point with:

$$Nr_{immunes} = 1.25 * n * vc * \sum_{j=1}^{5} (m_j * vt_j)$$

where

the constant 1.25 is used to obtain the total size of the age group using the known number of registered dogs (20% of the population estimated to be non-registered)

n = number of registered dogs in the age group

vc = calculated vaccine coverage (upper limit 1)

m_j= market share of the j^{th} vaccine

vt_j = take of the j^{th} vaccine in the age group

The HI was then calculated on the monthly level among dogs less than 2 years of age with:

$$HI\ (\%) = 100 * (Nr_1 + Nr_2) / N$$

where

Nr_1 = number of immune dogs < 1 year of age
Nr_2 = number of immune dogs 1-2 years of age
N = total number of dogs less than 2 years of age

Calculation of the number of immunes and HI was performed with the spreadsheet program MS Excel 2000 (Microsoft Corporation, USA).

***Grouping variables and the variables compared between groups* (III)**

The groups used for the statistical comparisons and the variables compared among the groups are summarized in Table 6.

Table 6 The grouping variables and the variables compared between groups in statistical analysis in the seroprevalence study (**III**). The three **age groups** of dogs were: 1 = less than 1 year, 2 = one to two years, and 3 = over two years. The three groups for the time since the latest vaccination were: 1 = less than year, 2 = one to two years, and 3 = over two years. **Immunogenicity groups** were single high = dogs vaccinated with a single high-take vaccine, single low = dogs vaccinated with a single low-take vaccine, mixed high = dogs vaccinated with at least two vaccine brands and at least once with a high-take vaccine, and mixed low = dogs vaccinated only with low-take vaccines.

Grouping variable	Variable compared between groups
vaccine usage	titre*
age (1-3)	titre*
gender	titre
time since the latest vaccination (1-3)	titre
number of vaccinations (1-4)	titre*
vaccine usage	mean no of vaccinations*
vaccine usage	age in days*
immunogenicity groups stratified by number of vaccinations	titre*
immunogenicity groups stratified by age	titre*

* significant association

Statistical analysis

Study I. The Yates-corrected Chi-square test was used to compare the number of dogs with titre $\geq$ 1:8 and < 1:8 after vaccination with different vaccines.

Study II. The mean and standard deviation (S.D.) of the titres were calculated from $\log_{10}$-transformed reciprocal values. Statistical tests were applied to the transformed values. The antibody levels among the groups of vaccinees stratified according to sampling time, species and trial were compared with the Student's two-sample *t*-test (two-tailed). Statistical significance was inferred with $p < 0.05$.

Study III. The geometric mean titres and the proportion of dogs with a detectable VN antibody titre were calculated and compared among the groups displayed in Table 6. Kruskal- Wallis one-way nonparametric analysis of variance was used with the titre data and one-way analysis of variance with Tukey's comparison of means was used to compare the number of vaccinations and age among the vaccine usage groups (Sokal and Rohlf 1995). The representativeness of the sample was tested by comparing the vaccine usage and breed distribution of the sample with those of the whole country, by using the Kolmogorov-Smirnov equality of distribution test (Sokal and Rohlf 1995).

Study IV. The observed numbers of diseased dogs vaccinated against CD with available vaccines were compared with those which would have been expected from the market shares of the vaccines, on the assumption of uniform efficacy, by the Chi-square test.

Studies I – IV. The software packages used were Statistix for Windows (Analytical Software, USA) and Unistat Statistical Package, Version 4 (Unistat, UK).

5. RESULTS

5.1 Seroconversion studies (I, II)

Results of the seroconversion studies in beagle dogs (**I**) and four fur animal species (**II**) are combined in Figures 5 and 6. From study **II** the sampling times with the highest number of samples were chosen. Altogether, 10 out of 161 and 3 out of 80 samples were missing from the sampling at 2.5-4 and 3 months, respectively.

Candur® induced significantly higher VN titres than Dohyvac® or Canlan® ($p < 0.001$). All the dogs in Candur® group had a VN antibody titre $\geq$ 1:32 1 month after the first vaccination, whereas nine out of twenty-five and four out of twenty-five dogs in Dohyvac®- and Canlan®-vaccinated groups failed to produce VN antibodies even one month after the second vaccination. The proportions of dogs with VN antibodies differed significantly between Dohyvac® and Canlan® vaccinated groups; both vaccines contained the Onderstepoort strain.

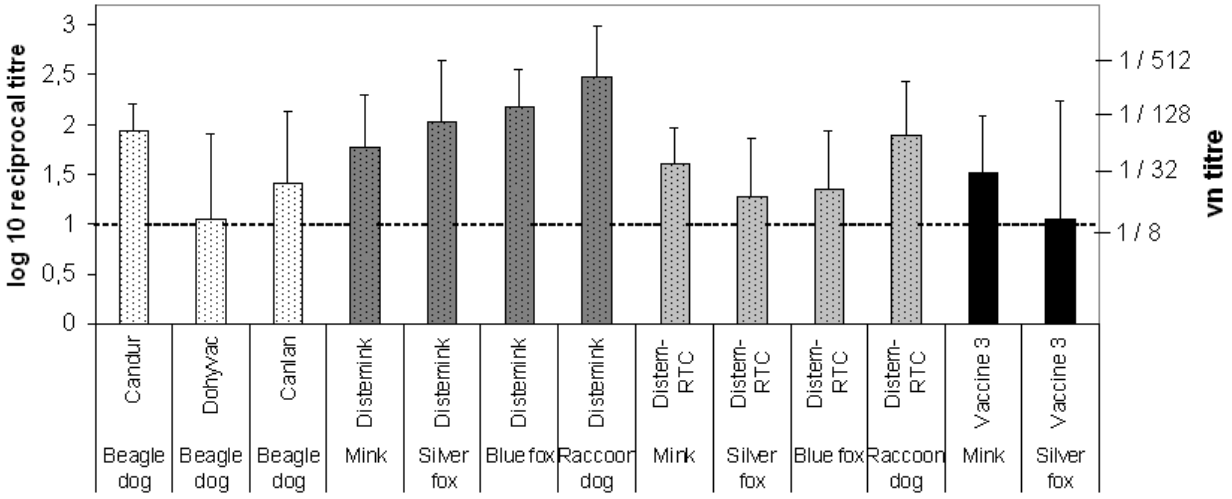

Figure 5 The $\log_{10}$ reciprocal geometric mean titres achieved with the vaccines in the seroconversion studies (**I** and **II**). Bars represent the respective standard deviations. Beagle dogs were sampled one month after the second vaccination, blue fox and raccoon dog 2.5–4 months after vaccination, and vaccine 3 vaccinated mink and silver fox 3 months after vaccination. The results for Distemink®-vaccinated mink and silver fox are combined from two trials in which animals were sampled 2.5–4 and 3 months after vaccination.

Distemink® induced high geometric mean titres in all four species, and the proportion of animals with a detectable level of VN antibodies was > 95%. Although the proportion of Distem®-R-TC vaccinated animals with a

detectable level of VN antibodies was high (> 90%) in all species, the geometric mean antibody titres in the silver fox and blue fox were less promising. Distemink® induced a significantly higher level of VN antibodies than Distem®-R-TC in all four species: mink, silver fox, blue fox and raccoon dog. Although performing satisfactorily in vaccinated minks, vaccine 3 induced antibodies at best in only 60% of silver foxes four months after vaccination. Samples were obtained from all silver foxes vaccinated with Distem®-R-TC or vaccine 3 at every sampling. However, difficulties in sampling the other fur animals meant that samples were not obtained from all the individuals at each sampling.

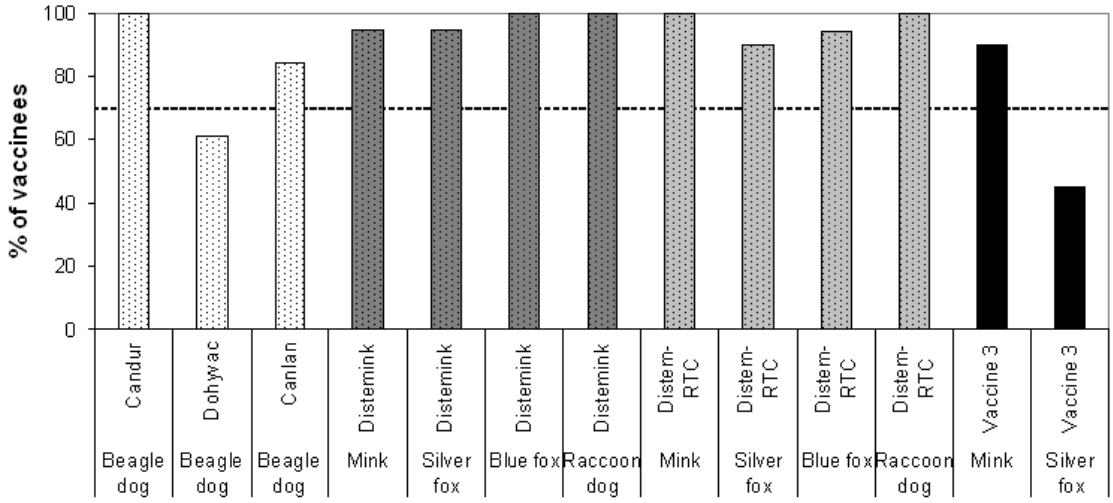

Figure 6 Proportions of vaccinated animals with detectable antibody titres in the seroconversion studies (**I** and **II**). Sampling times are the same as in Fig. 5.

5.2 Seroprevalence study (III)

5.2.1 Representativeness of the field sample

According the registration statistics of the Finnish Kennel Club, dogs from 262 breeds were registered from 1990-1994. Ten or more individuals were registered for 231 breeds during these five years. Samples were obtained from 208 breeds of dogs. No significant differences were found between proportions of the vaccines used in the sample and in the country as a whole. The 30 breeds used for the comparison constituted 63% of the sample and 61% of the registrations in the whole country from 1990 to 1994. No significant differences between the breed distribution of the sample and that of the whole country were found, even though the breeds used for hunting, such as the Finnish hound, Norwegian grey elkhound, Finnish spitz,

Karelian bear dog and beagle, were under-represented in the sample. These breeds represented only 2% of the sample, but 21% of all the registrations.

5.2.2 Virus neutralising antibodies in the field sample

Grouping variables, the variables compared between groups and significant associations found in the comparisons are presented in Table 6. Significant differences (overall $p < 0.001$) in titre were detected between the unstratified vaccine usage groups. The dogs less than a year old had significantly lower titres than those aged one to two years ($p = 0.01$) and over two years ($p < 0.001$), but the titres of dogs aged one to two years and over two years did not differ significantly from each other. The titres of the dogs that had been vaccinated once or twice were significantly lower than those of the dogs that had been vaccinated three or four times ($p < 0.001$). The mean number of vaccinations among the dogs less than two years of age differed significantly between the vaccine usage groups ($p < 0.001$). Mixed high and low groups had the most vaccinations (mean 2.8), followed by groups vaccinated with Dohyvac® (2.6), Canlan® (2.1), Candur® (2.0), Nobivac® (1.2) and Duramune® (1.0). Overall, there were significant differences in mean age among three age groupings ($p < 0.001$), with the dogs vaccinated with mixed low and high vaccines being the oldest, and the dogs vaccinated with Candur® , Canlan®, Dohyvac®, Nobivac® and Duramune® being successively younger.

Table 7 Proportion of dogs under 2 years of age with a detectable antibody titre according to their immunogenicity group and the number of vaccinations they received (**III**). Immunogenicity groups as in Table 6.

Immunogenicity group	Number of vaccinations			
	1	2	3	4
	% (n)	% (n)	% (n)	% (n)
Single high	90 (257)	99 (283)	97 (78)	100 (3)
Single low	39 (183)	47 (658)	61 (775)	78 (94)
Mixed high	-	93 (162)	97 (181)	98 (61)
Mixed low	-	62 (113)	74 (163)	63 (40)

(n) is the number of dogs in each group

Both the number of vaccinations and age were associated with the titre and vaccine usage group. To control for possible confounding factors, the comparison of titres among vaccine usage groups was adjusted by classifying according to the number of vaccinations (one to four) and the age group (1, 2 and 3). The proportions of dogs with detectable titres in the different immunogenicity groups, grouped by the number of vaccinations, are presented in Table 7. The same division of single vaccines into low- and high-take vaccines was observed, irrespective of the number of vaccinations the dogs had received ($p < 0.001$ in each case). Similarly, consistent differences in the take were observed between the mixed low- and mixed high-take groups. The $\log_{10}$ reciprocal geometric mean titres with standard deviations for the age-specific immunogenicity groups are illustrated in Figure 7, and the proportions of dogs with detectable antibody titres in these groups are presented in Figure 8.

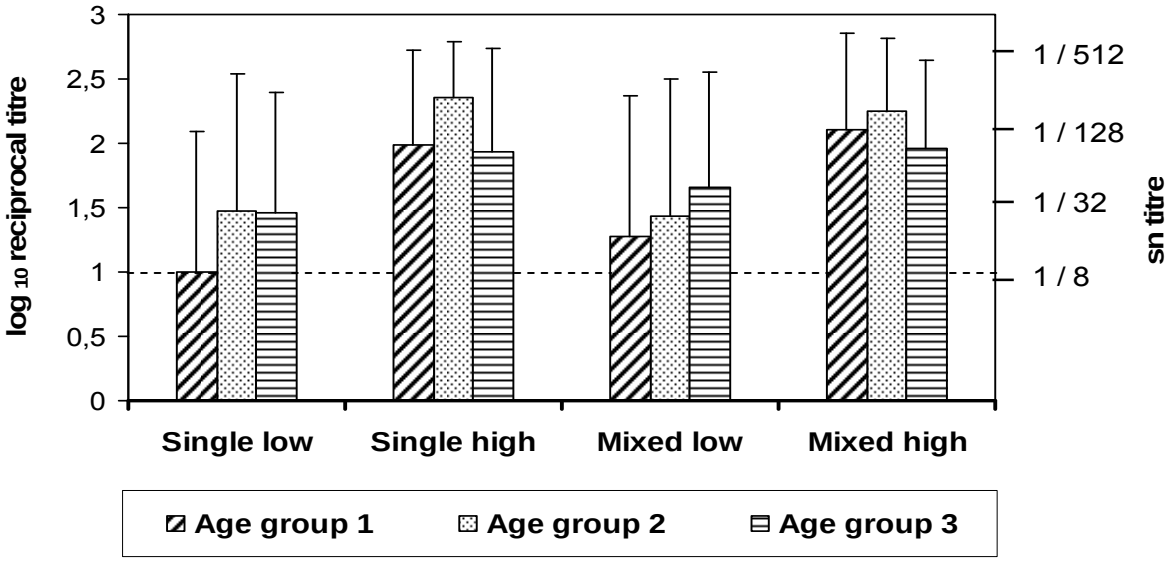

Figure 7 The $\log_{10}$ reciprocal geometric mean titres in age-specific immunogenicity groups in the seroprevalence study (**III**). Bars represent the respective standard deviations. Grouping as in Table 6.

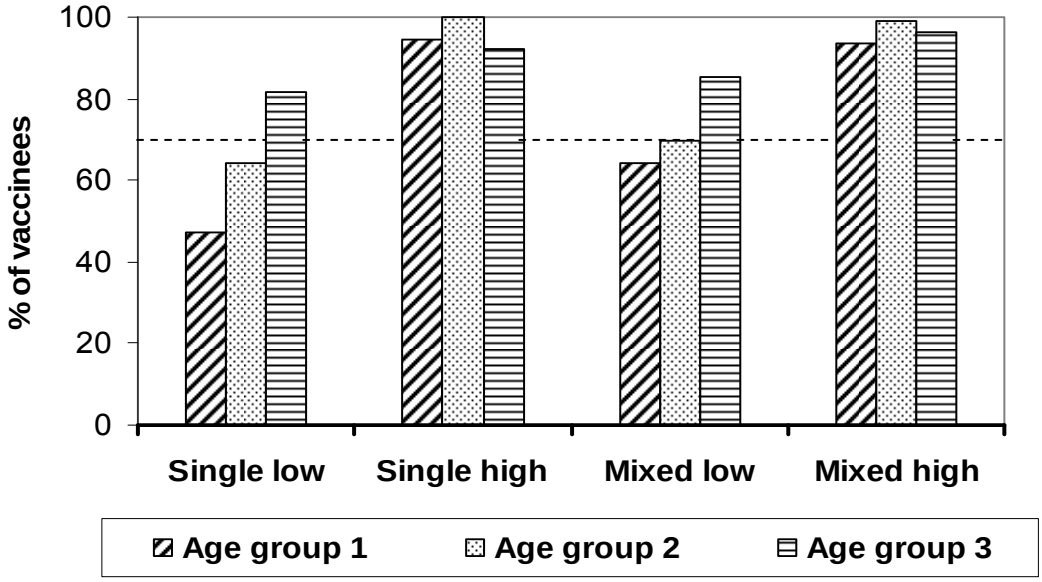

Figure 8 Proportions of dogs with a detectable antibody titre in age-specific immunogenicity groups in the seroprevalence study (**III**). Grouping as in Table 6.

5.3 Epidemiological observations (IV and V)

5.3.1. Clinical signs (**IV**)

The clinical signs reported during the 1994-1995 outbreak were anorexia, conjunctivitis and fever that frequently fluctuated. Respiratory illness was characterised by rhinitis, tracheobronchitis and, in the most severe cases, by pneumonia. Gastrointestinal signs were manifested in the early stages of the disease by vomiting and diarrhoea, usually lasting for one to two days. In severe cases the animals remained depressed and anorexic for several weeks and suffered from serous-mucopurulent nasal and ocular discharges. Some dogs exhibited a reddening of the skin followed by a pustular rash. Hyperkeratosis of the pads and nose, and central nervous system signs were also observed. On rare occasions vesicles developed on the earflaps and a vesicular stomatitis was observed. Mortality rate was estimated at 30%.

5.3.2 Confirmed CD cases (**IV**)

Of the 3 649 samples examined between 1 January 1994 and 31 August 1995, a total of 865 (23.7%) tested positive in the IFA. A total of 71.7% of the CD cases were among vaccinated dogs, 4.3% among unvaccinated dogs and 24% among dogs with no information on the vaccinations. Information on

the age of 42 dogs was lacking. Of the remaining 823 CD cases, 2% were among dogs less than 3 months of age, 76.7% among dogs 3-24 months of age and 21.3% among dogs more than 24 months of age. A complete vaccination history was available for only 351 of the cases aged 3-24 months. Among these the proportion of dogs vaccinated with Dohyvac® was significantly higher than would have been expected from the market shares, around 70% through 1992-1994, on the assumption that all the vaccines had an equal take ($p < 0.001$). The number of confirmed CD cases among dogs from 1988-1996 and among dogs and other species from 1997-2007 are presented in Figure 10 and Table 8, respectively.

Table 8 Laboratory-confirmed CD cases among dogs and other species versus samples tested from 1996-2007 in Finland (EELA and Evira).

	Number of CDV positive (number tested)				
Year	Dog	Fox	Mink	Others	Other species tested
1996	0 (184)	0 (27)	**1** (14)	0 (3)	a
1997	**2** (84)	0 (19)	0 (5)	0 (4)	a, b
1998	0 (45)	0 (16)	0 (10)	**1** (8)	**c**, d, e
1999	**2** (48)	0 (17)	0 (19)	0 (9)	a, f, g
2000	0 (40)	0 (5)	0 (2)	0 (4)	a, g, h
2001	0 (17)	0 (9)	0 (7)	0 (3)	g, h, i
2002	0 (18)	0 (20)	0 (14)		
2003	**2** (26)	0 (16)	0 (4)		
2004	**4** (43)	0 (10)	0 (6)	0 (1)	a
2005	0 (43)	0 (34)	0 (6)	0 (2)	a
2006	**1** (36)	0 (11)			
2007	**8** (82)	0 (16)		0 (3)	a, j

a raccoon dog (*Nyctereutes procyonoides*)
b pine marten (*Martes martes*)
c European mink (*Mustela lutreola*)
d ferret (*Mustela putorius*)
e sable (*Martes zibellina*)
f European badger (*Meles meles*)
g arctic fox (*Alopex lagopus*)
h wolf (*Canis lupus*)
i wolverine (*Gulo gulo*)
j Saimaa seal (*Phoca hispida saimensis*)

Table 9 Number of dogs registered (with the Finnish Kennel Club), total number of CD vaccines sold (statistics of VELL and EELA), market share of the high-take vaccines and calculated vaccine coverage in Finland in 1988-1996.

Year	Number of dogs registered	Number of CD vaccines sold	Market share of the high-take vaccines %	Vaccine coverage %
1988	33 885	74 626	31.5	49
1989	37 547	83 684	27.3	53
1990	41 669	249 263	23.7	100
1991	46 003	186 114	20.3	82
1992	46 623	229 860	13.6	96
1993	47 093	214 622	13.5	87
1994	44 950	292 437	9.9	100
1995	40 062	274 533	59.6	100
1996	35 932	201 733	75.7	83

5.3.3 Dog demographics, vaccine coverage and herd immunity (V)

The number of dogs registered, the total number of CD vaccines sold, the market share of the high-take vaccines and the calculated vaccine coverage in Finland during 1988-1996 are summarized in Table 9. The number of annually registered dogs increased rapidly from 1988 to 1991 and remained high until 1994. The market share of the high-take vaccine simultaneously decreased. The size of the registered dog population and the proportion of dogs less than two years of age in 1988-2006 are illustrated in Figure 9. The increase in the size of registered dog population and changes in the age structure (high proportion of young dogs) preceded the major outbreak. Figure 10 depicts the number of virologically-confirmed CD cases in 1990-1996 and the HI (%) as the proportion of dogs under two years of age in 1988-1996 with detectable levels of antibodies. Two periods with a steep increase in the calculated HI are apparent. Despite the fairly high vaccine coverage (Table 9), the HI shows a slight decreasing trend during 1990-1994. Table 10 summarizes the market shares of the high-take vaccines in Finland, Oulu and Turku area and the numbers of virologically-confirmed CD cases in Finland, Helsinki metropolitan area, Tampere, Oulu and Turku area from 1990-1996. Unlike the Helsinki metropolitan and Tampere areas, a

high-take vaccine was the market leader in Oulu and Turku, except in 1994. The number of confirmed CD cases among dogs per county in Finland from 1990-1996 is shown in Table 11.

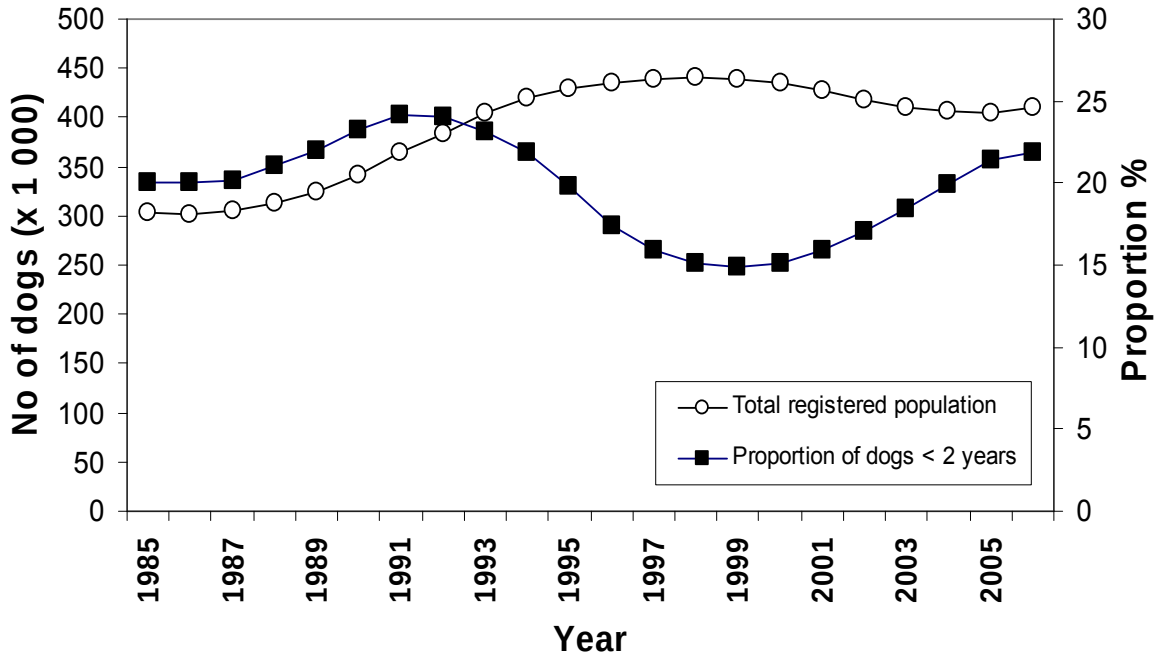

Figure 9 The size of the registered dog population and the proportion of dogs under 2 years of age from 1985-2006 in Finland.

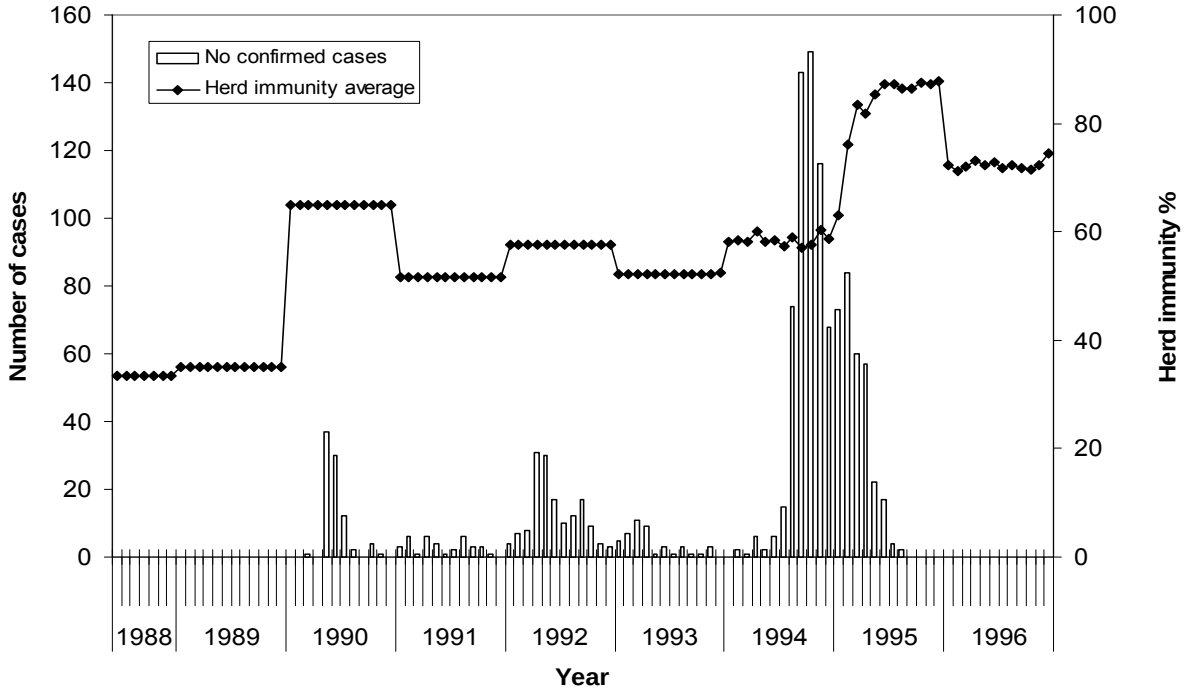

Figure 10 Number of confirmed canine distemper cases and calculated herd immunity (%) among dogs under 2 years of age from 1988-1996 in Finland.

Table 10 Market share (MS) of the high-take canine distemper vaccines (HTV) in Finland, Helsinki metropolitan (HMA), Tampere, Oulu and Turku areas and confirmed cases per area over 1990-1996 (EELA, personal communications T. Illukka, J. Rasi and I. Rastas, Turku, K. Wickström and R. Väyrynen, Oulu).

Year	Overall MS of HTV %	Total no of CD cases	CD in HMA	CD in Tampere	MS of HTV in Oulu %	CD in Oulu	MS of HTV in Turku %	CD in Turku
1990	23.7	87	40	3	69.8	0	M[a]	0
1991	20.3	36	19	0	86.6	0	M	1
1992	13.6	152	60	27	90.4	2	M	5
1993	13.5	45	6	0	50.1	0	M	0
1994	9.9	583	304	48	13.4	0	41.1	7
1995	59.6	318	62	54	67.1	2	95.1	7
1996	75.7	0	0	0	98.8	0	98.6	0

[a] missing information

Table 11 Numbers of confirmed canine distemper cases per county from 1990-1996 in Finland. Helsinki Metropolitan Area abbreviated as HMA.

	Year						
County (major city)	1990	1991	1992	1993	1994	1995	Cumulative no of cases
Uudenmaan (HMA)	52	26	69	10	330	79	566
Turun ja Porin (Turku)	7	1	7	1	24	19	59
Hämeen (Tampere, Lahti)	6	0	42	2	61	108	219
Kymen	6	0	11	21	38	30	106
Vaasan	1	0	6	2	5	12	26
Keski-Suomen (Jyväskylä)	2	0	0	1	62	23	88
Mikkelin	0	0	0	0	13	5	18
Kuopion	0	0	1	0	3	11	15
Pohjois-Karjalan	4	1	1	0	3	2	11
Oulun (Oulu)	0	0	3	0	4	8	15
Lapin	0	0	0	0	1	0	1
Ahvenanmaa	1	0	0	0	0	0	1
Unknown	8	7	12	7	32	23	89
Total	87	35	152	44	576	320	1214

6. DISCUSSION

The overall main theme of the present thesis is the takes of canine distemper vaccines, both from the standpoint of seroconversion (**I, II)**, seroprevalence **(III**) and the endemic and epidemic occurrence of CD in Finland in the 1990s (**IV, V**). The discussion is organised into corresponding sections; both deal at length with the reliability or biological significance of the results and observations.

6.1 Experimental and field takes of the vaccines

6.1.1 Takes of the vaccines

The qualitative presence or absence of antibodies after vaccination is actually more important than the exact quantity, when present. The presence of antibodies after vaccination indicates an active immune response and memory. The considerably varying takes of the distemper vaccines are documented in detail both in experimental settings (**I**, **II**) and in the field (**III, IV,V**). An attempt to account for the varying takes is divided into finding associations between i) the vaccine-specific data (Tables 3 and 4) and ii) host- and usage-dependent data (**II**, **III)**, and the respective takes.

Vaccine-specific data

The formulations of the studied vaccines are different: All CD vaccines for dogs are multivalent, containing one to three other antigens and sometimes an adjuvant in addition to CDV, whereas mink distemper vaccines contain a single antigen without an adjuvant (Tables 3 and 4). Several of the tabulated factors may have a non-spurious association with the take.

The most obvious cause for the differing takes among the CD vaccines is the vaccine strain. In general, Onderstepoort strains are less immunogenic than Rockborn strains (Appel 1987, Chappuis 1995). However, one of the three vaccines in the high-take group, namely Nobivac®, contained the Onderstepoort strain and performed as well as Duramune®, which contained the Rockborn-type strain. Due to the fairly small sample sizes of dogs vaccinated with Nobivac® (n = 158) and especially Duramune® (n = 31), and the fact that all samples were from dogs vaccinated once and very recently, the performance of these vaccines may appear better than it actually is. A marked difference was shown between the two low-take vaccines, Canlan® and Dohyvac®, in the proportion of dogs with detectable antibody levels both in the seroconversion and field studies among dogs less than 2 years of age. The efficacy of an ML vaccine is dependent on the vaccine strain's ability to

replicate in a host. Therefore, the method of antigen production, i.e. the cell line in which the vaccine strain is produced, inevitably influences the take of a vaccine. The antigen for Candur® and Duramune® vaccines was produced in primary and continuous dog kidney cells, for Nobivac® and Canlan® in Vero cells and for Dohyvac® in chicken embryos. The vaccines are in the order of successively decreasing take and it appears that the further the production cell line is phylogenetically from the dog, the lower is the take.

The titre of an antigen in a vaccine dose contributes to the take of the vaccine, provided that the vaccine strain is able to replicate sufficiently in the host. One of the first steps in vaccine development is to establish the dose-response relationship (Dessmetre and Martinoid 1997). In the case of ML vaccines, a balance between efficacy and safety must be sought. The minimum and maximum titre of an antigen in a dose at release must be determined to confirm efficacy and safety throughout the shelf-life of a vaccine. Although the minimum titres of CDV antigen in a dose are not directly comparable among vaccines, Dohyvac® seems to have the lowest titre of antigen per dose. An unexpected observation concerning the group of dogs vaccinated with low-take vaccines (**III**) suggests that the dose-response relationship for these vaccines has not been properly established. A sufficiently immunogenic vaccine would be expected to induce levels of VN titres that would approximately follow a normal distribution curve. This was, for example, the case in dogs less than one year old vaccinated with Candur® (vaccine 1). However, 54% of the dogs of the same age group vaccinated with Dohyvac® had no detectable antibodies, although when titres were detectable their distribution followed that of Candur® with a lower level, giving a satisfactory geometric mean titre of 134. A similar result was obtained with Canlan® (vaccine 2). This observation can also be seen in the results of study **I**.

The take of a vaccine antigen may also be influenced by other ML or inactivated antigens and the inclusion of an adjuvant in the vaccine. This should already be taken into account when a multiple vaccine is in the developmental stage, and will not be further discussed here. The administration of other vaccines, for example rabies vaccine, simultaneously with the CD vaccine may affect the take.

Host- and usage-dependent factors **(I, II, III**)

In addition to the vaccines themselves, the host animal and the number of vaccinations may have an impact on the take. The factors actually contributing, which will be discussed here, are individual variation, age, breed, the number of vaccinations and the animal species.

No vaccine will induce VN antibodies in every vaccinee. Some individuals are non-responders in which even several vaccinations with a high-take vaccine do not induce VN antibodies. The proportion of these individuals has been estimated to be 1% (Schultz 2006). The present data (**III**) are consistent with this: 9 out of 418 dogs (2%) vaccinated with Candur® at least twice were non-responders. However, a vaccine non-responder may seroconvert after clinical CD, i.e. exposure to a wild CDV (unpublished observation). In the case of low-take vaccines, the reason for the high proportion of animals with no detectable VN antibody could be due to efficient non-specific defence mechanisms that prevent the replication of a vaccine strain in a host early enough so that no specific response occurs. A specific response will then only be seen in individuals that fail to prevent virus replication by non-specific defence. This highlights the importance of properly establishing the previously-discussed dose-response relationship. In studies **I** and **II**, any concurrent infection at the time of vaccination would have been expected to influence all groups evenly. No health problems were observed during these studies. However, in field conditions an unknown number of factors contributes to the take of a vaccine.

Age will influence the immune system both early and late in life, and can therefore be expected to influence the take of a vaccine. The immune system of a young animal may not function as efficiently as that of an adult individual. During ageing, both cellular and humoral immune responses will be impaired (Gerber and Brown 1974, Schultz 1982). Furthermore, during the first weeks or months, maternal antibodies protect newborn animals against infectious diseases and may interfere with ML vaccines, thus preventing the stimulation of active immunity. When the takes of vaccines are assessed in relation to age (Fig. 8), takes can be observed to differ among the age groups of animals vaccinated with low-take vaccines. The higher take among dogs over 2 years of age compared to younger dogs in low-take groups may in fact be explained by the booster effect of wild CDV. The booster effect is difficult to resolve in dogs that already have high enough VN titres (Prydie 1966). High VN titres are able to prevent the replication of CDV (wild or vaccine strain) and therefore the immune system will not be stimulated. Interference by maternal antibodies is considered to be the most important cause of vaccine failure (Fig. 4). None of the beagle puppies had detectable antibodies at first vaccination at the age of 12 weeks (**I**). A total of 1.7% and 2.5% of mink (13 weeks) and silver fox (16 and 14 weeks), respectively, sampled at the time of vaccination had low antibody levels, but nevertheless seroconverted by 1.5–2 months after vaccination (**II**). Based on these results and current vaccination recommendations, which for both dogs and fur animals are designed to minimize this interference, we conclude that maternal antibodies are perhaps a less important cause of

vaccine failure than claimed. Furthermore, they can interfere with vaccinations only during the first weeks or months of life.

Intensive breeding has led to the accumulation of several inherited diseases among dog breeds. Breeding of inherited immunodeficiencies has probably also occurred. The breed of a dog may therefore contribute to the differences in vaccine take. A possible breed effect was controlled in **I** by using pure-bred beagle dogs. On the other hand, the breed effect was diluted due to the presence of several breeds in all groups of vaccinees (**III**). To reliably assess the breed effect on vaccine take, a representative sample of pure-bred dogs of various breeds vaccinated with a single high-take vaccine would be needed.

The number of vaccinations had a clear effect on the take only in a single low-take group (Table 7). The low-take of a vaccine was not adequately compensated for by increasing the number of vaccinations or by using several vaccines if all the vaccines were of low-take. Furthermore, the protection provided by vaccination should be attained as soon as possible, ideally after one or two vaccinations. All fur animals were vaccinated only once, which is usually the case in the field. The majority of fur farms do not routinely vaccinate against CD. Emergency vaccination is employed immediately after the detection of CD on a fur farm or when the risk of CD spread from dogs to fur farms is recognised.

As expected, all mink distemper vaccines performed satisfactorily in the target species (**II**). However, an equal performance in other fur animal species cannot be taken for granted. This may, in addition to other factors, explain the apparently variable results among vaccine brands and fur animal species during the 1985-1987 outbreak in Finland (Loikala and Kangas 1988). The geometric mean titre induced in raccoon dog both by Distemink® and Distem®-R-TC vaccines was higher than that in the target species. With raccoon dog, antibody titres higher than with other fur animal species have also been observed against parvovirus (Neuvonen et al. 1982).

6.1.2 Reliability of the vaccine take results

It is inevitable that groups of experimental animals form clusters. This must be taken into account either in the design of the experiment or when analysing the results. In the present case the beagle dogs (**I**) originating from 15 litters were divided into three groups of 25 animals and puppies originating from the same litter were vaccinated with a same vaccine. This clustering was ignored in the experimental design. While the fur animals of publication **II** were housed in separate cages, these were adjacent on two

sides to neighbouring cages. Furthermore, the cages in the same shedhouse shared for example feeding appliances. Even in the field material of publication **III**, clusters most likely occurred in the form of kennels and the clientele of large veterinary clinics. A non-trivial analysis of the dependence of any variable on the independent factors of the experimental design should accommodate clustering effects. This was not done in publications **I** or **II**. The results are, nevertheless, sufficiently reliable because analysis of clustering is considered effective in taking into account the systematic differences in, for example, the genetic, climatic, management or veterinary service factors. All of these were generally constant in the experimental designs. The clustering in publication **III** may have had an impact on the results; however, the associated data were too limited to permit multilevel analysis. The possible clustering effects are considered to be offset at least to some extent by the comparably large sample of dogs (n = 4 627) from around the country.

All three CD vaccines and two of the mink distemper vaccines were commercial products, registered in Finland (Tables 3 and 4). The third mink distemper vaccine was intended to be used in mink and ferret, but not registered in Finland. It is reasonable to assume that although only one batch of each vaccine brand was used in the experiments (**I** and **II**), batch-to-batch variation was within acceptable limits, and therefore the results of the seroconversion studies are representative of each brand of the triple dog vaccines or mink distemper vaccines. In the field, several batches of the three CD vaccine brands were used. Although various combinations of vaccines were available for dogs, triple vaccines containing CDV, CAV-1 or -2 and CPV as antigens were the most popular combinations. Moreover, besides the multitude of other factors, the circulation of a wild CDV in the population probably contributed to the observed take of each vaccine, especially among the older age groups.

The field sample (**III**) is considered to represent the vaccinated urban dog population, firstly because it is relatively large (approximately 10% of the dogs registered annually in Finland), secondly because the use of the three main vaccines (Candur® , Canlan® and Dohyvac®) in the sample corresponded with the contemporary sales proportions, and thirdly because the breed distribution in the sample accorded with the registration statistics of the Finnish Kennel Club.

6.2 Occurrence of CD from 1988-2007 in Finland

CD in dogs reappeared in 1990, after 16 years of absence. Three periods can be distinguished: endemic occurrence from 1990-1993, epidemic occurrence from 1994-1995 and sporadic occurrence from 1996 onwards. The endemic situation is described in publication **V** and the epidemic episode in both publications **IV** and **V**. In both articles, and especially in **V**, the combined impact of vaccination coverage and the takes of the vaccines (in terms of herd immunity) on the proliferation of CD is described in extensive detail. An attempt to extend the explanation for the rise and fall in the epidemic, given especially in **V**, is presented. The situation after 1996 is also briefly reviewed.

Clinical signs reported during the major outbreak were typical for CD (**IV**). In a case-control study conducted after the reintroduction of CD in 1990 (Forell 1993, unpublished observation), neurological and integumentary signs were present in 54% and 6% of the cases, respectively, and the case-fatality rate was reported to be 30%. A high prevalence of neurological signs was also observed by Järvinen et al. (1990). During the major outbreak from 1994-1995, neurological and integumentary signs were reported in 7.5% and 45.5% of confirmed cases, respectively (Lounela et al. 1997). Since neurological signs are the major determinant of the prognosis and recovery from CD, the reported mortality rate of 30% during the major outbreak may be an overestimate.

6.2.1 Endemic and epidemic occurrence of CD from 1990-1995

After the appearance of sylvatic rabies in 1988 in Finland, a six-month quarantine for imported dogs was abandoned. Only rabies vaccination was obligatory for dogs before entering the country. This increased both the imports of dogs and the number of Finns travelling with their dogs abroad. Since the majority of the imported dogs are puppies, it is not surprising that CD was reintroduced to the country. Indeed, the first two confirmed cases were detected in imported puppies in March 1990 (Järvinen et al. 1990). In spite of the severe economical recession at the beginning of the 1990s and the high rate of unemployment (over half a million unemployed persons), travelling and imports of dogs further increased when the Soviet Union collapsed and Estonia regained independence in 1991, and the previously practically closed borders to the East and South were opened.

The size of the Finnish dog population increased by roughly 100 000 dogs from 1990-1995, and allowing for the proportion of non-registered dogs, was estimated by the Finnish Kennel Club to be half a million. According to

registrations, the proportion of dogs less than 2 years of age remained above 20% through 1988-1994 (Fig. 9). The size of the registered dog population in 1990 alone exceeded 300 000, which is considered as the minimum population size required to sustain a morbillivirus in circulation in the absence of vaccinations (Black 1991).

CD has probably been introduced to Finland several times since 1990. The symptoms observed after the introduction in 1990 and during 1994-1995 differed with respect to the neurological signs, which may suggest differences in virulence among circulating CDV strains. The high incidence of neurological signs after the reintroduction of CDV could also be a reflection of the poor herd immunity (Böhm et al. 1989). However, only two isolates of CDV from 1994 exist, which prevents, for example, sequence-level comparisons (Ek-Kommonen et al. 2003b). Furthermore, the strain responsible for the epidemic was at least antigenically close enough to the vaccine strains to enable effective prophylaxis.

An independent estimate for the critical level of HI could be calculated using the so-called basic reproduction ratio, R_0 (Nokes and Anderson 1988b). For measles and phocine distemper, an R_0 from 11 – 18 and 2-3 has been estimated, respectively (Woolhouse and Bundy 1997, Swinton et al. 1998). Unfortunately, no numerical estimates for the R_0 of CD have been presented. However, R_0 is very sensitive to heterogeneities created by the spatial structure of populations (Dobson and Foufopoulos 2001), by the age and contact structures in populations, and by a variety of ill-defined management and behavioural factors. A critical level based on R_0 would therefore have to be interpreted with extensive provisions.

Two periods with a steep increase in the HI were apparent (Fig. 10). The first, during 1990, is attributed to the tripling of vaccine sales after re-introduction of CD, and the subsequent increase in vaccine coverage from around 50% to 100% (Table 9). In spite of the steep improvement in HI, CD remained at a low endemic level in the dog population. The second increase in the HI was due to the withdrawal of the low-take vaccine, which had market share of 74%, and the subsequent increase in the market share of high-take vaccines in 1995. When the takes of the vaccines differ significantly, the market share of each vaccine determines the level of HI achieved. This is clear when HI is compared between the years 1990, 1994 and 1995, which all had a vaccine coverage of 100%.

No exact information on the dog population density exists, but it is assumed that there is a fairly constant ratio between the numbers of dogs and the numbers of people. The CD cases appeared to accumulate in the Helsinki

metropolitan area and in some of the biggest cities in the southern and central parts of the country (Table 11). However, there was marked spatial heterogeneity. In the following calculation of the normalized numbers of CD cases per 100 000 citizens, the census figures from 1994 (Ministry of Interior) and the average number of CD cases from 1994-1995 were used. Thus, the normalized numbers were 28.5 in Tampere, 4.3 in Turku, 0.9 in Oulu, 23.2 in Lahti and 39.7 in Jyväskylä (Table 10). Turku and Oulu appeared to form pockets of resistance, especially when compared to the normalized number of 17.3 for the whole country. This can to a large extent be attributed to the substantially higher percentages of high-take vaccines used in these cities than elsewhere in the country (personal communications, R. Väyrynen and K. Wickström, Oulu; T. Illukka, J. Rasi and I. Rastas, Turku; statistics of EELA).

The number of CD cases reflects the area-specific infectious pressure, which can be considered as inverse to the level of herd immunity. The infectious pressure and the number of cases form a kind of vicious cycle: the higher the pressure, the higher the number of cases. When the pressure is low, as for example in the province of Oulu (Table 11), even low-take vaccines can help to keep the disease in check (or at least appear to do so). However, in situations of high infectious pressure only vaccines that induce solid immunity are able to break the cycle. Of course, immunity due to natural infection eventually contributes to the increase in the HI. The observed heterogeneity in the numbers of cases, translated to variability in herd immunity, can on the other hand also lead to pockets of susceptibility and an increased overall risk of infection, as described in the case of measles by van den Hof et al. (2002) and Glass et al. (2004).

Vaccine-induced HI among production animals, such as fur animals, is often a result of the use of a single vaccine brand on a farm, on several farms or even over much larger areas. In the dog population, several vaccine brands normally contribute to HI. The poor performance of one vaccine brand is easily compensated by the protection achieved with better-performing vaccines, at least when none of the brands dominates the market. This, unfortunately, was not the case with the low-take vaccines, and especially the Dohyvac® brand, in the 1990s in Finland. According to Rockborn and Klingeborn (1996), Dohyvac® also enjoyed a market share of 70% in Sweden, and the observed take of the vaccine in Sweden agreed well with the take in Finland (Olson et al. 1997a). In Sweden, CD occurred sporadically from 1986-1996; zero to six cases were reported annually. However, imports of dogs were strictly regulated and, unlike in Finland, canine distemper vaccinations were a condition for import (Olson et al. 1996b).

To conclude: several factors contributed to the reoccurrence of CD in Finland, which culminated in a major outbreak in 1994-1995. Firstly, lifting of the six-month quarantine on imported dogs in the late 1980s severely increased the infectious pressure of CD. Secondly, the size of the dog population, and therefore according to registrations the proportion of young dogs, which are known to be at highest risk of contracting CD, increased rapidly. Thirdly, the HI decreased steadily from 65 to 52% among young dogs between 1990 and in the end of 1993, leading to a situation in which a large proportion of young dogs, despite the fairly high vaccine coverage, remained unprotected.

6.2.2 Reliability of the epidemiological data

Confirmed diagnosis of CD

The representativeness of the sampled CD cases, i.e. how well they represented all diseased dogs during the major outbreak, is a critical issue when making inferences about the actual impact of different vaccines on the situation. Sampling bias may partly explain the obviously low percentage of unvaccinated dogs (4%) compared to that of vaccinated dogs (72%). It may well result from the fact that clinicians considered laboratory confirmation of CD diagnosis unnecessary in the case of unvaccinated dogs, or a reluctance among owners to pay for further testing. The rest, almost one fourth of the cases, had an obscure vaccination history.

Only 24% of all the samples tested by IFA were positive during 1994-1995. In another study, 42% of the cases tested positive with the same method, whereas 74% had IgM antibodies to the virus (Blixenkrone-Møller et al. 1993). The sensitivity of IFA depends on both the quality of the sample and the timing of sampling. It is not possible to detect the virus with IFA if a sample is taken too late in the course of the infection. However, the annual proportions of confirmed cases from 1990-1996 should be comparable and reflect the situation satisfactorily, provided that sampling is carried out at the same frequency among clinically suspected cases from year to year. In general, after the introduction of a disease there will first be underdiagnosis and later overdiagnosis, especially if diagnosis is only based on clinical signs. This is probably reflected in the sampling frequency over the period.

No difference related to vaccine usage was observed among confirmed CD cases from 1990-1993. However, the number of cases was small and information on vaccinations scarce. The significantly higher proportion of Dohyvac®-vaccinated dogs among the cases from 1994-1995 may be attributed to several factors: 1) sampling bias towards vaccinated dogs, 2) the presence of the majority of cases in the Helsinki metropolitan area,

where Dohyvac® was widely used, 3) the large clinics situated in areas with high infectious pressure, 4) the possibly greater willingness of large clinics to take samples. Any outbreak also involves vaccinated individuals: the higher the infectious pressure the higher the number of cases among vaccinees. Although the observation of a significantly higher proportion of Dohyvac®-vaccinated dogs among the diseased can not be blindly relied upon, evidence from the low-take of Dohyvac® (**III**) and the low incidence of cases in geographical areas where high-take vaccines were market leaders (**V**), both suggest that this observation is tenable.

Data used to calculate the size and structure of the dog population, vaccine coverage and HI

Vaccine coverage was calculated by using the sales statistics of the CD vaccine brands and the annual number of registered dogs, and by taking into account the current CD vaccination recommendations. The official sales statistics are comprehensive and accurate up to 1996, because all animal vaccines were distributed via EELA. The numbers of registered dogs obtained from the Finnish Kennel Club are also considered accurate. Two estimates, the proportion of non-registered dogs and the life-expectancy of ten years for each dog, were used to calculate the size of the total dog population, which contributed to the number of vaccines needed annually. The local estimate for a life-expectancy of ten years is supported by British and German surveys, where average ages were 11 years 1 month and 10 years, respectively (Michell 1999, Eichelberg and Seine 1996). Both the registered and non-registered dogs were assumed to be similarly vaccinated. The only available information supporting this assumption came from study **III**, which showed that mixed-breed (non-registered) dogs in the field sample tend to be vaccinated largely in the same manner as pure-bred dogs. However, the vaccine coverage calculated here is in fact a best-case scenario, i.e. it shows what could have been achieved with the ideal distribution of vaccines. Because the immunity against CD achieved by ML vaccines is known to last for years (Olson et al. 1997b, Schultz 2006), in our calculation each dog was assumed to be vaccinated six times during its ten-year life. In reality, vaccinations tend to accumulate in the same individuals, and therefore the number of vaccines used is not a direct measure of the coverage.

6.2.3 Sporadic occurrence of CD from 1996 – 2007

Only sporadic cases of CD among dogs have been observed since 1995 (Table 7). Practically all confirmed cases have been associated with imports of dogs, and the continuous low infectious pressure is caused by these

imports. The pattern of occurrence of CD in Finland during the last ten years has resembled that in Sweden from 1985-1996. IFA has mainly been used to confirm clinical CD diagnoses, but the use of RT-PCR methods is increasing due to their higher sensitivity and other advantages. As illustrated in Figure 9, Finland is in the middle of a `puppy boom´ with young dogs making up over 20% of the registered population. Based on our earlier experience, this can be regarded as a high risk period with respect to CD. However, with the currently-available vaccines (Nobivac® and Duramune®) and assuming 100% vaccine coverage, a herd immunity of around 90% should be attainable regardless of their relative market shares. If the average take of the available vaccines is a non-exacting 80%, the above-mentioned critical level corresponds to a vaccine coverage of some 94%. This is consistent with the 90-95% vaccine coverage needed to control measles (Nokes and Anderson 1988a).

7. CONCLUSIONS

1. The development of vaccine-induced immunity in an individual is a multifactorial process, partly depending on the properties of the vaccine but also, and especially in the case of low-take vaccines, on host-specific factors such as age, animal species and breed, and on other factors having an influence at the time of vaccination.

2. The development of vaccine-induced immunity in a population, i.e. herd immunity, depends on the process occurring in individuals; however, while prevention of the clinical manifestation of the disease is sufficient on the individual level, on the population level it is crucial to halt the circulation of the virus. To achieve this, the proportion of individuals with sufficient immunity should be maximized, which imposes higher demands on the performance of vaccines than the mere prevention of clinical signs.

3. The ultimate test and criterion for a vaccine is its contribution to herd immunity. This was clearly demonstrated in the course of the occurrence of canine distemper in Finland in the 1990s.

4. The spatial heterogeneity in the population and vaccine usage may have been a more definitive factor in the epidemiology of canine distemper in Finland than previously thought.

5. Differences in performance among canine distemper vaccines in the field are often difficult to resolve without extensive associative spatial and other information. In Finland, low-take vaccines had a major contribution to the vaccine induced immunity, which made this resolution possible.

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