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Feline panleukopenia virus: Its interesting evolution and current problems in immunoprophylaxis against a serious pathogen

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ABSTRACT

Vaccination of cats against feline panleukopenia virus (FPV) has been a routine part of feline medicine for the past 40 or more years, and many of the same vaccines that were first developed in the 1960s are still in routine use today. However, there has been significant evolution of the virus in the last 40 years, in particular the emergence of canine parvovirus (CPV) in dogs in the late 1970s, which was a host range variant of the FPV-like virus, and the world-wide spread of the CPV-derived viruses since 1978. FPV and the various antigenic types of CPV have been isolated from cats, raccoons, and many different wild and captive carnivores. The consequences of these changes in the viral populations have not been investigated, and the effectiveness of the current vaccine protocols have not been reported. Here we review the recent findings about the evolution of the viruses in carnivores including cats, and describe a study that looks at the efficiency of vaccination of kittens using the standard protocols, which shows that many cats are not protected by those approaches.

1. Introduction

Some members of the genus *Parvovirus* are important pathogens in domesticated cats and dogs and particularly in young animals. The viruses are classified in one species *feline panleukopenia virus* that includes feline parvovirus (FPV), canine parvovirus (CPV), mink enteritis virus and raccoon parvovirus (Tijssen et al., 2011). Those viruses are named after their common hosts, domestic cats and dogs, yet it is clear that these viruses also infect many other wild animal hosts in the order Carnivora. In domestic cats and dogs the viruses infect a broad spectrum of tissues and cause a number of diseases, including fetal death after systemic infection in fetuses, ataxia and myocarditis after cerebellar or cardiac infections of neonatal cats and dogs, and hemorrhagic enteritis after infection of the small intestine in animals older than about 5 weeks. The age dependence of the disease is based at least in part on the availability of mitotically active cells in animals of different ages, as the virus requires cell division for its replication.

The parvoviruses are small viruses (20 nm) with a single stranded DNA genome of about 5000 bases, which contains two major open reading frames that express the non-structural (NS) proteins (NS1 and NS2) and capsid (virion) protein (VP), including VP1 and VP2. The viruses replicate using the host cell polymerases and other DNA replication machinery, and the NS1 plays important roles in regulating replication, and in nicking the viral DNA genome and attaching to the genomic 5′-ends during replication. The VP1 and VP2 assemble together to produce the capsid with about 90% VP2 and 10% VP1, where the VP1 unique region contains important functions required for cell infection, including a phospholipase A2 enzyme activity (Zádori et al., 2001). The capsid controls receptor binding and the

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infectious process for cells, and is also the major target of host antibody immunity. The NS2 protein plays roles in the assembly of viruses in cells and in nuclear transport, although the role of that protein in CPV replication is not clear (Parrish, 2010).

Host Range and Host Tropism of the Viruses. The order Carnivora contains over 270 species that are subdivided into two suborders, the Feloidea and Canoidea, as well as several families. The viruses similar to FPV appear to infect members of many of the families in the Carnivora. However, up until the late 1970s domestic dogs and their close relatives (wolves and coyotes) were resistant to infection by FPV-like viruses. In 1978 a new virus variant emerged which was able to efficiently infect dogs, and that virus (CPV) spread worldwide to become well established in dogs, so that today essentially all dogs will be infected by the virus, if they are not vaccinated.

The new canine host range of the CPV was due to only a small number of mutations in the capsid protein gene, which altered residues in the surface of the capsid (Chang et al., 1992). The capsid uses the transferrin receptor type-1 (TfR) to bind and enter cells for infection, and the residues on the surface of the capsids control the interaction with the structure of the apical domain of the TfR (Hüffer et al., 2003; Palermo et al., 2006; Parker et al., 2001). The specific host range for dogs by the virus was associated with a difference in the structure of the canine TfR, as the FPV was unable to bind to either canine cells or to purified canine TfR ectodomain while CPV capsids did bind to the canine TfR (Palermo et al., 2003, 2006). That specific host block to FPV binding was associated with a glycosylation site in the apical domain of the canine TfR, and if the Asn in the canine TfR is mutated to a Lys, as seen in the feline TfR, FPV-like viruses can efficiently bind (Palermo et al., 2006).

As far as is known, the viruses infecting cats have been FPV-like viruses, which all appear to be relatively similar. It was seen a few years after 1978 that CPV were infecting cats, although most of the isolates from cats appeared similar to classical FPV (Mochizuki et al., 1996). A more detailed analysis of viruses from wild animals recently showed that CPV-derived viruses were commonly found in a variety of North American carnivores, including raccoons, bobcats, cougars, and skunks (Allison et al., 2012). The raccoon isolates were particularly interesting, as those contained variants that were like the viruses that were isolated from domestic dogs, as well as a variety of antigenically variant viruses that had what appeared to be uniquely raccoon-specific mutations. Some of the CPV-like raccoon viruses contained groups of mutations that altered the antigenic structure of the capsid so that the virus was recognized by only a few of the 30 monoclonal antibodies produced against different viral capsids (Allison et al., 2012).

Immunity, Antibody Recognition and Vaccination. Feline panleukopenia-like disease has been long recognized in cats, and was first reported in the literature during the 1920s and 1930s, and despite the widespread availability of effective vaccines, is still an important infectious disease of domesticated cats. It appears, however, that the prevalence differs regionally and there are clinics that report a high number of clinical cases and clinics that do not report any, although the diagnostic methods appear equivalent.

The clinical picture in cats that results from FPV or CPV infection is not always clear, and may include mild and non-specific signs such as anorexia and fever, up to severe signs that present with diarrhea, and in some cases fetal or neonatal infections are observed that result in cerebellar disease. Panleukopenia is a clinical sign that appears to be consistently seen in infected cats, with all of the leukocyte subtypes being affected.

Diagnosis is made by virus isolation, PCR for viral DNA, hemagglutination, antigen capture ELISA, or electron microscopy of virus particles in feces. FPV strains can be distinguished from CPV by antigenic typing using specific monoclonal antibodies, while DNA sequencing allows the identification of the specific mutations that distinguish the different virus types.

Immunity that protects animals against parvovirus infection is predominantly based on neutralizing antibodies, although the studies of cellular immunity are scarce (Scott et al., 1970). Most female cats are infected with FPV either due to natural infection or after vaccinations, and develop high levels of circulating antibodies which they then deliver as maternal antibodies to the kittens which protect them against virus infection. The maternal antibodies in kittens decline at a regular rate, and when they have dropped below a certain level the kittens become susceptible to infection by wild-type viruses or to vaccines, generally at some point between 7 and 12 weeks of age. The duration of maternal protection is related to the level of immunity in the mother as well as to the efficiency of transfer to the kitten. While antibodies against FPV also protect cats against most FPV and CPV-related viruses, the neutralizing activity of serum from an FPV-vaccinated cat is reduced when tested against CPV-2, and even more against CPV-2a, -2b and -2c (Table 1). This suggests that there will be consequences for the duration of protection in kittens that is related to the antigenic form of the virus that induced the immunity of the mother and the type of virus that challenges the kitten.

Table 1
Cross-neutralization of 10 feline sera derived by immunization using a commercial FPV vaccine (Jakel et al., 2012) when tested against FPV (strain FPV-b), CPV2 (strain CPV-d) and CPV2c (strain 7/97). Crandell feline kidney cells and 100 $TCID_{50}$ of the respective virus were used. Sera were diluted 1:50 and titrated in log2 steps. Titer is expressed as the last dilution that was able to neutralize the virus.

Serum	FPV	CPV-2	CPV-2c
9	1:12,800	1:6400	1:1600
39	1:12,800	1:6400	1:1600
67	1:12,800	1:6400	1:3200
90	1:6400	1:3200	1:1600
140	1:6400	1:6400	1:800
187	1:12,800	1:6400	1:1600
225	1:12,800	1:12,800	1:3200
233	1:12,800	1:12,800	1:6400
242	1:12,800	1:6400	1:1600
245	1:12,800	1:6400	1:1600
262	1:12,800	1:12,800	1:1600
263	1:6400	1:6400	1:1600

The antigenic structure of the FPV and CPV capsids has been investigated using monoclonal antibodies. Those studies examined the binding footprints of 8 different antibodies, and those were seen to contact almost 70% of the viral surface (Hafenstein et al., 2009), yet the epitopes defined by mapping escape mutations showed that there were two specific regions on the capsid that determined most of the antigenic variation of the capsid (Strassheim et al., 1994). During the evolution of CPV in different hosts significant variation in both of those antigenic sites has been seen, which reduce the reactivity with polyclonal antibodies (Pratelli et al., 2001, Table 1).

Antibodies are commonly detected by hemagglutination-inhibition test (HI), or by ELISA tests, but neutralization tests are also widely used and allow the detection of differences in the cross-neutralization ability of sera against different viruses (Pratelli et al., 2001).

Vaccines against FPV are predominantly modified live virus vaccines (MLV) that have been passaged numerous times on feline or mink cell lines. Inactivated vaccines were used extensively in the past but give low levels of relatively short lived protective antibodies, and have been largely replaced by MLVs.

Under controlled conditions with seronegative animals, such as kittens without maternal antibodies, a single vaccination with a MLV efficiently results in seroconversion within 7 days, and provides complete and long-lasting protection against disease (Gueguen et al., 2000; Gore et al., 2006; Gaskell, 1989).

Maternal antibodies both protect against infection by wild-type viruses, and also interfere with vaccination, and in the case of MLV they inactivate the virus after vaccination by neutralization in the kitten.

In both FPV and CPV vaccines a so-called immunity gap has been identified, and that is the period when the kitten is no longer protected against infection by the wild-type virus which infects through oro-nasal routes, but where the low levels of residual maternal antibodies are sufficient to interfere with a successful vaccination with a MLV. In controlled studies this has been shown to be the case when maternal antibodies are still present with very low titers; $<$1:10 as determined by HI. When all maternal antibodies have waned, the kitten will be fully susceptible for infection and vaccination (Scott et al., 1970). Clinical cases in kittens often result from infection during this period, and it is therefore important to define the best time point for vaccination for each individual cat to minimize the risk of this period. In principle this could be done by determining the titer of the queen or of the littermates and predicting the time at which immunity declines to sufficiently low levels to allow vaccination (Scott et al., 1970; Friedrich and Truyen, 2000). In practice this is difficult and expensive to do, and a more common approach is to repeatedly vaccinate the kittens at intervals of 2–3 weeks, starting at week 8 and finishing around week 16 of age (Anonymous, 2006). While this approach covers most of the kittens, there will be a percentage of kittens that are still not covered, and several studies have reported the occurrence of sero-negative kittens at an age of 15 weeks that had been vaccinated once or twice, and parvovirus disease in some vaccinated kittens suggest that vaccination may not always be successful even using the standard protocol (Dawson et al., 2001; DiGangi et al., 2012; Addie et al., 1998).

2. Example – understanding vaccination failure – a study

In the last years numerous cases of potential vaccine failures (suspected lack of expected efficacy) have been reported in the Norwegian Forest cats, as seen by the pharmacovigilance bureau of the Paul-Ehrlich-Institute, the governmental agency responsible for licensing of vaccines in Germany.

A possible genetic predisposition to an impaired immune function was suggested, and this was therefore examined using a serological study, where kittens of the Norwegian Forest cats were vaccinated with one of three commercial vaccines, and compared to the responses of control domestic short hair cats. The vaccination schedule recommended by the German Standing Veterinary Vaccination Committee was applied, which requires three vaccinations, administered at 8, 12 and 16 weeks of age, where seroconversion was indicative for a successful vaccination. The study was recently published (Jakel et al., 2012).

A total of 64 kittens from 16 litters were vaccinated against FPV at the age of 8, 12 and 16 weeks with one of three commercial polyvalent vaccines. Blood samples were taken before each vaccination and after completion of the vaccination series, at the age of 20 weeks. Sera were tested for antibodies against FPV by HI assay and by serum neutralization assay (N-test); the tests were conducted in parallel by two independent diagnostic laboratories.

The results basically confirmed that maternal antibodies did interfere with vaccination and that there was an "immunological gap" occurring during the decline of maternal antibody where there was interference with the modified live vaccines that we were testing. The inter-laboratory difference was minimal, and it was obvious that the neutralization test was more sensitive for the detection of the maternal antibodies than the HI assay, and sera that were negative in HI tests were often positive in N-test.

One unexpected finding from this study was that in a substantial proportion (37%) of kittens that appeared seronegative by our tests showed no seroconversion, even after three vaccinations including a final dose at 16 weeks, and these results were seen in both the NFC and the control groups of kittens. No differences were seen between the groups, and although the design was not designed to directly compare the efficacy of the vaccines used, there did appear to be a difference in the efficacy of induction of seroconversion in kittens with some titer of maternal antibodies.

Conclusion relative to FPV vaccination protocols. This study shows that there may be problems with the vaccination of cats with the commercially available vaccines using the standard protocols. Although the dossiers associated with the vaccines clearly demonstrate a very good efficacy in seronegative cats, in the field the situation appears to be different. A larger survey is needed to define the extent of this problem.

These results suggest that a vaccination later in the first year of life, well after the 16 weeks where the standard protocol ends, may be recommended to minimize the number of cats that are challenged by the wildtype field viruses. Having the booster vaccine after 6 months instead of 12 months, as generally recommended in the vaccination guidelines, may also further decrease the risk for kittens to develop disease after FPV infection. The general decrease in the susceptibility of older animals to disease after infection makes the balance between time of vaccination and risk to the animal complex, and worthy of further study.

The emergence of FPV and CPV-like viruses with altered antigenic structures may also change the susceptibility of the kittens to different field viruses. While the complete loss of cross-reactivity between vaccines and field viruses seems unlikely, the differences may manifest themselves in the differences in the time of susceptibility of the kittens to wildtype viruses, depending on the specific antigenic form of the virus that immunizes the mother to generate the maternal immunity, and the form of the virus that either challenges the kitten or that is present in the vaccine. It appears likely that changes in the time of susceptibility of kittens to wildtype viruses and to vaccination may occur; the implications of those differences are still poorly understood.

The emergence in cats and other carnivores of variants of CPV also has potential to alter the disease seen in cats, and monitoring of the viruses that are commonly found should be used to anticipate any future issues that may arise.

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