The Global Spread and Replacement of Canine Parvovirus Strains

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SUMMARY

Canine parvovirus type 2 (CPV-2) became widespread during 1978 and was reported in many countries during 1978 and 1979. Earlier studies showed that CPV-2 was replaced in the U.S.A. around 1980 by an antigenically and genetically variant virus (CPV-2a). Here we show that CPV-2 was present in the U.S.A., Japan, Belgium and Australia prior to 1980, but that between 1979 and 1982 CPV-2 was replaced by CPV-2a in all of those countries as well as in France and Denmark. Examination of sera collected between 1979 and 1984 from wild coyotes (Canis latrans) in the U.S.A. by an agar gel precipitin assay indicated that the coyotes were originally infected by CPV-2, but that after 1980 the juvenile coyotes were being infected with CPV-2a. The natural global replacement of CPV-2 by CPV-2a over a period of 2 to 3 years indicates that CPV-2a has a strong epidemiological advantage over CPV-2, although the mechanism involved remains to be defined.

Canine parvovirus type 2 (CPV-2) is a recently recognized virus of dogs which was first isolated or described during 1978. Serological studies indicated that CPV-2 was a new virus of dogs, as anti-CPV-2 antibodies could only be demonstrated in dog sera collected after mid-1976. The first evidence of infection by CPV-2 was seen in sera collected in October 1976 in Belgium (Schwers et al., 1979), November 1977 in The Netherlands (Osterhaus et al., 1980), between January and June 1978 in Denmark (Have & Andersen, 1982), May 1978 in Australia (Walker et al., 1980), June 1978 in the U.S.A. (Black et al., 1979; Carmichael et al., 1980), and between July 1978 and January 1979 in Japan (Azetaka et al., 1981; Mohri et al., 1982).

Analysis of sera collected from wild coyotes (Canis latrans) revealed that CPV-2 entered the coyote populations examined during late 1978 or 1979, and that a high proportion of both adult and juvenile animals were infected by 1980 or 1981. Once CPV-2 was enzootic in the coyote population the prevalence of positive serum samples remained high (>90%) (Thomas et al., 1984).

Canine parvovirus type 2 was isolated or the disease described in many countries after 1978 (for reviews, see Carmichael & Binn, 1981; Pollock & Parrish, 1985), and it appears that the virus became globally distributed within a few years of its first recognition. The original virus isolate was called CPV-2 to distinguish it from the previously described, but unrelated, minute
Table 1. Antigenic types of CPV isolates collected in various countries during the years between 1978 and 1984 and tested with monoclonal antibodies

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* Old (CPV-2) isolates had high titres with MAbs D and J and low titres with MAb 1D1. New (CPV-2a) isolates had the opposite reactivity.
† Isolates were tested by HI.
‡ Isolates were tested by ELISA.
§ Virus could not be typed with the MAb used.

virus of canines (CPV-1), although those names have not been formally recognized. Studies revealed that the original CPV-2 was replaced in the dog population of the U.S.A. by an antigenic variant (termed the 'new' CPV) around 1980 or 1981 (Parrish et al., 1985). Here we designate the 'new' CPV as CPV-2a.

To investigate the global spread of CPV-2a we examined isolates collected in various countries since 1978. As domestic dogs in most countries are inoculated with vaccines prepared from CPV-2 strains, we also examined the natural occurrence of CPV-2 and CPV-2a by analysis of sera collected from wild coyotes during the same period.

Virus isolates collected in several countries between 1978 and 1984 were antigenically typed using monoclonal antibodies (MAbs) which have been previously described (Parrish et al., 1982; Parrish & Carmichael, 1983; Parrish et al., 1985). Antibodies used were CPV-2/CPV-2a-specific [not reacting with feline panleukopenia virus (FPV) or mink enteritis virus (MEV) isolates] (MAb 7), CPV-2-specific (MAbs D and J), CPV-2a-specific (MAb 1D1), CPV/FPV/MEV-reacting (MAb F) or FPV-specific (MAb H).

Virus isolates from the U.S.A., France, Belgium, Australia and Japan were tested using the MAbs in haemagglutination inhibition (HI) assays as previously described (Parrish et al., 1982; Senda et al., 1986). Titres were read as the inverse of the last antibody dilution completely inhibiting virus haemagglutination.

Viruses collected in Denmark were analysed using an antibody capture ELISA. A capture antibody was prepared from a high titred canine serum, diluted to 10 µg/ml of canine IgG in carbonate buffer (pH 9.6), then adsorbed to microtitre plates for 1 h at 37 °C. The virus isolates in faecal preparations or in tissue culture fluids were diluted 1:4 in phosphate-buffered saline (PBS) with 0.1% (v/v) Tween-20 (PBS–Tween), and incubated on the washed plates for 1 h at 37 °C. Plates were then washed with PBS–Tween. The MAbs in tissue culture fluids were diluted 1:100 in PBS–Tween containing 10% bovine serum and incubated on the plate for 1 h at 37 °C. After washing, the wells were incubated with an appropriate horseradish peroxidase-conjugated anti-species IgG for 30 min at 37 °C, washed, and then incubated with the substrate o-phenylenediamine. Results were expressed as the ratio of the optical density of the MAb/optical density of a polyclonal rabbit antiserum, to control for variations in antigenic mass. Controls included in the assays were known isolates of CPV-2 (CPV-d) or CPV-2a (CPV-39) (Parrish et al., 1985) prepared as tissue culture fluids.

The seven isolates that were collected during 1978 in the U.S.A., Belgium or Australia were all typed as CPV-2. Of the 12 1979 isolates examined, one isolate from Japan and one from the U.S.A. were typed as CPV-2a (Table 1). Between 1980 and 1983 the proportion of isolates of each type changed, and all of the 1983 and 1984 isolates that could be typed were CPV-2a.
Table 2. Cross-protection of dogs immunized with CPV-2 (CPV-a, vaccine strain) and challenged with CPV-2a (CPV-39)

<table>
<thead>
<tr>
<th>Dog number*</th>
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<td>Pre-challenge</td>
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</tr>
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<td>&lt;10</td>
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</tr>
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<td>&lt;10</td>
<td>2560</td>
</tr>
<tr>
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<td>NT†</td>
<td>&lt;10</td>
</tr>
<tr>
<td>84-7C</td>
<td>NT</td>
<td>&lt;10</td>
</tr>
<tr>
<td>83-7C</td>
<td>NT</td>
<td>&lt;10</td>
</tr>
</tbody>
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* V represents vaccinated; C represents non-vaccinated control.
† NT, Not tested.
‡ Clinical signs observed included depression, anorexia, weight loss, lymphopenia between days 2 and 8 after challenge and mucoid or bloody stools.

viruses. Amongst the 322 Danish CPV isolates collected between 1980 and 1982 only seven were of the CPV-2 type (Table 1). These results reveal a global replacement of CPV-2 by CPV-2a in domestic dogs between 1979 and 1983.

To investigate cross-protection between CPV-2 and CPV-2a, five 4-month-old specific pathogen-free (SPF) beagles were inoculated with modified live CPV-2 vaccine, and three other beagles were kept as non-vaccinated controls. All dogs were challenged by oro-nasal inoculation with $10^{6.5} \text{ TCID}_{50}$ of a CPV-2a isolate (CPV-39) and subsequently observed for signs of virus infection (Table 2). The vaccinated animals showed no clinical signs of infection, and shed no virus detectable by haemagglutination (HA) assays (Table 2). In contrast the control animals all developed diseases typical of CPV enteritis and two were sacrificed when severely affected. The surviving control dog shed virus at high HA titres and developed high serum antibody titres. These results indicate that dogs infected with CPV-2 are subsequently protected against re-infection upon challenge by CPV-2a.

The distribution of the two virus types in populations of wild coyotes was examined by testing sera in an agar gel double-diffusion assay. Sera from SPF dogs experimentally infected with CPV-2 or CPV-2a distinguished between purified antigens of each virus type (Fig. 1). Spurs of partial identity allowed the specificity of the sera to be determined.

Sera were collected from wild coyotes trapped in the U.S.A. (in Texas, Idaho or Utah) between 1976 and 1984. The collection of serum and the distribution of CPV antibodies revealed by HI testing has been previously described (Thomas et al., 1984). Sera from juvenile coyotes between 1979 and 1984, as well as sera collected from adults in 1979 and 1980 were examined. Sera from juvenile coyotes were collected during the autumn of each year or the spring of the year following birth, when the pups were either 6 or 12 months old, and antibodies to CPV would therefore represent infections which had occurred within the previous 12 months. As CPV first entered the canine population in 1978 or 1979, anti-CPV antibodies in adult sera collected in 1979 or 1980 would be from infections of the same or the previous year (Thomas et al., 1984).

In contrast to the testing of the experimental sera a proportion of the coyote antibody responses to CPV could not be typed in the agar gel immunodiffusion assay, as they showed no spurs of partial identity against either virus type. However, of the sera which formed virus-specific reactions, the majority of infections in 1979 and 1980 were by CPV-2, while sera collected after that year showed reactions specific for CPV-2a (Fig. 2), suggesting a replacement of CPV-2 by CPV-2a between 1980 and 1981 in the coyote populations examined.

These results revealed a number of facts about the epidemiology of CPV. First, both CPV-2 and CPV-2a are highly transmissible and spread rapidly around the world. The circumstances prevailing in each case would have been different. The initial global spread of CPV-2 in the
Fig. 1. Agar gel precipitin reactions between post-infection antisera (As) raised in SPF beagles against either CPV-2 (O) or CPV-2a (N) isolates (CPV-b and CPV-15 respectively). Spurs of partial identity are present where antisera raised against one virus type reacted with the two different virus antigens. Antigens in the centre row of wells are those of either CPV-2 or CPV-2a (CPV-d and CPV-15 respectively). Antigens were concentrated by ultracentrifugation, banded on sucrose gradients, then dialysed against PBS prior to use. Antisera were used directly without further treatment. Reactions were in borate-buffered agarose gels, incubated at room temperature for 48 h. Viruses and methods used have been previously described (Parrish et al., 1982; Parrish et al., 1985).

previously uninfected canine population occurred within approximately 2 years of the first evidence of the virus in Europe in late 1976 (Schwers et al., 1979). As infection by CPV-2 protects dogs against infection by CPV-2a (Table 2), CPV-2a would have spread within a canine population which was largely immune. However, these results indicate that the spread of CPV-2a and replacement of CPV-2 occurred within a period similar to that of the original spread of CPV-2.

Comparisons of the capsid protein gene sequences of various CPV isolates have indicated that CPV-2 and CPV-2a isolated in the U.S.A. differ in a minimum of six nucleotide and predicted amino acid sequence positions. Also, comparison of sequences with FPV or MEV isolates indicated that CPV-2 and CPV-2a probably diverged from a common ancestor prior to 1978 (C.R. Parrish, unpublished results). Though we have not examined the DNA sequences of CPV isolates from other countries, several CPV-2 and CPV-2a isolates from the U.S.A. and Australia had the same characteristic antigenic types when examined with our complete panel of 30 MAbs (results not shown), and it appears likely that CPV-2a was derived from one source, possibly prior to 1978, and subsequently spread around the world.

These data also indicate that CPV-2a was not spread in any live CPV vaccine, as vaccines of that type were not used in Australia, Europe or Japan during 1979 or 1980. The rapidity with which the original CPV-2 spread around the world in the late 1970s, and the speed at which CPV-2a was subsequently able to spread is perhaps explained by the high titres of virus shed in the faeces of infected animals and the resistance of the virus to inactivation, permitting the
viruses to be carried readily into countries with strict quarantine regulations for dogs. The rapid spread of CPV-2 among the wild coyotes and its apparent replacement by CPV-2a indicates both that the viruses are naturally able to spread rapidly within populations and that the selective advantage of CPV-2a was a viral property and not due to any human intervention.

The emergence and global spread of CPV-2 between 1976 and 1978, and its subsequent replacement by CPV-2a between 1979 and 1982 are events with few obvious precedents. Further studies are required to determine whether the advantage of CPV-2a is due to antigenic selection, to some further adaptation by CPV-2a to dogs enabling it to replicate or spread more efficiently, or perhaps to some combination of those factors.
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REFERENCES


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