Antigenic characterization of canine parvovirus isolates from Brazil using specific monoclonal antibodies

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Abstract

Canine parvovirus (CPV) is an emerged pathogen in dogs, first isolated in 1978 in the USA. The original 1978 strain was designated CPV type 2 (CPV-2). However, analysis of CPV isolates in the USA by restriction enzymes and monoclonal antibodies have shown that around the year 1979 a CPV variant strain, designated CPV type 2a (CPV-2a), became widespread. Subsequently, a new antigenic strain, designated CPV type 2b (CPV-2b), was also observed by analysis of CPV isolates from various parts of the world, although the proportion of each strains was different between countries. In this study, the Haemagglutination Inhibition (HI) test with a panel of monoclonal antibodies was used to type canine parvovirus strains in 29 fecal samples collected from symptomatic dogs from 1980 to 1986 and from 1990 to 1995. The results showed a strong predominance of the antigenic type 2a indicating that the CPV epizooty in Brazil followed the same pattern observed in European and Asian countries.

Key words:
Canine parvovirus.
Haemagglutination inhibition.
Antigenic characterization.
Monoclonal antibodies.

Introduction

Since 1978, a dog epizooty of an unknown disease was observed in many parts of the world.[1,2,3,4,5] This disease usually caused death by miocarditis in young dogs or hyperthermia followed by emetic episodes and / or diarrhea in adult canides. The enteritis and pathological traits were very similar to those observed in cats infected by Feline Panleucopenia Virus (FPV). Soon, a strict relationship between this etiological agent and the FPV was recognized. [2,3,6]

The original 1978 strain was designated CPV type 2 (CPV-2) to distinguish it from the previously recognized canine parvovirus known as minute virus of canides.[7] Around 1979, a variant CPV strain, designated CPV 2a (CPV-2a) became widespread and replaced the original strain from 1980 to 1981 in the USA.[8] Analysis of CPV isolates by monoclonal antibodies and restriction enzymes, since 1984 in the USA, identified another antigenic variant, designated CPV type 2b (CPV-2b).[9] The same pattern of spreading of the CPV-2 and the subsequent replacement by new antigenic strains was observed in Germany, Denmark, Japan, France, Australia, and Spain, although the proportion of CPV-2a and CPV-2b was different in those countries.[10,11,12,13,14] The sequential evolution of new antigenic types (antigenic drift) appears uncommon but has been studied extensively using monoclonal antibodies and molecular analysis as a feature of the epidemiology and evolution of canine parvovirus [13,15,16,17,18,19,20,21] and influenza virus isolates [22,23,24,25].

Recently, researches described a new antigenic variant, termed CPV-2c.[26] Although the genetic variants between the isolates of CPV-2c from Vietnam, Taiwan, Italy and Spain differ by only a small number of amino acids, there are also implications

for vaccine efficacy because vaccines based on older variants may not protect against newer variants.[26,27]

In Brazil, CPV-like infections were first observed in 1979 and diffusion of the virus occurred since 1980.[28] Pereira et al.[29] designed a Polymerase Chain Reaction (PCR) assay to type canine parvovirus strains in fecal samples collected from symptomatic dogs from 1980 to 1986 and from 1990 to 1995. Genetic analysis showed that the CPV epizooty in Brazil followed the same pattern observed in the USA: emergence of CPV-2 followed by replacement by the variants CPV-2a and 2b. The predominant strain found in 1980 was CPV-2a, which was substantially replaced by CPV-2b from 1990 to 1995. The same analysis was also applied to show that CPV-2b was the most prevalent type circulating in the State of Rio de Janeiro from 1995 to 2001.[30] Despite the previous genetic characterization of Brazilian CPV strains, there was a lack of data concerning the antigenic characterization of CPV strains in Brazil.

The aim of this study was to characterize antigenically the CPV found in 29 fecal samples from clinically ill dogs from Brazil, from 1980 to 1986 and 1990 to 1995.

Materials and Methods

In this study, a total of 29 fecal samples from clinically ill dogs collected from various areas of Brazil, from 1980 to 1986 and 1990 to 1995, were characterized by the haemagglutination inhibition (HI) test using a panel of monoclonal antibodies (MAb) as previously described.[31] The presence of CPV was previously confirmed by demonstration of haemagglutination (HA), isolation of the virus in tissue culture (CC), electron microscopy (EM), enzyme linked immunosorbant assay (ELISA) or PCR.[29,32] The prototype strains of CPV-2 (CPV-d), CPV-2a (CPV-15) and CPV-2b (CPV-39) and monoclonal antibodies (A4E3, C1D1, C7D6 and B4A2) were kindly provided by Dr. C. R. Parrish (Cornell University, Ithaca, N.Y.).

Briefly, porcine erythrocytes were collect in Alsever´s solution, and stored at 4 ^{0}C for at least 24 hours before use. Erythrocytes were washed three times in PBSS (0.015 *M* phosphate buffer, 0.9% NaCl, pH 7.0) and were suspended to 1% (v/v) in PBS containing 0.1% bovine serum albumin. Fecal specimens from field animals were suspended in nine parts of parts of ice-cold PBS and agitated vigorously for 1 minute. Suspensions were centrifuged (12.000 g) at 4 ^{0}C for 60 minutes and the supernatant were tested for HA activity. Tests were performed in 96-well plastic V-plates. For HA tests, serial two-fold dilutions (0.05 ml) of fecal solutions were prepared from initial 1:2 dilution. An equal volume of ice-cold porcine RBC suspension was added to each well and plates were incubated at 4 ^{0}C for 2 to 4 hours. For HI testes twofold serial dilution of monoclonal antibodies (MAb) A4E3, C1D1, C7D6 and B4A2 were made in V-bottom plastic plates. To each Mabs dilution was added 0.025 ml of HA antigen that contained 8 HA units. After 1 hour at room temperature, 0.05 ml of the appropriate chilled erythrocyte suspension was added and plates were incubated at 4 ^{0}C for 4 to 16 hours until erythrocyte controls had formed clear buttons. Low or no titer with C1D1 indicates that the virus is type 2. High titered reaction with C1D1 and C7D6 indicates that the virus is either a type-2a or type-2b. Low titer (< 4) with B4A2 indicates that the virus is a type-2b. Reaction with A4E3 indicate that virus is a CPV strain, either type-2, type-2a or type-2b.

Results and Discussion

The epidemiological history of CPV in Brazil resembled in many aspects that seen in other countries, where frequency shifts on antigenic types were reported.[9,11,12,13,33,34] Herein, the results of the antigenic characterization by the HI assay showed that all samples were characterized as CPV-2a, except for one, collected in 1995, characterized as CPV type 2.

It is interesting to note an apparent

disagreement with previous studies using the PCR assay[29, 30] regarding the predominant strain that replaced the original CPV-2 in Brazilian samples.

However, PCR, as well as restriction site mapping analysis, detect point mutations in specific genome regions not necessarily related to phenotypic changes that lead to antigenic variation. On the other hand, nucleotide sequence followed by phylogenetic analyses of large regions of the genome improves molecular characterization, particularly in genome regions under evolutive pressure such as the VP1 and VP2 genes of CPV.[9,34,35,36]

Indeed, analysis of 31 sequences of the VP1/VP2 gene obtained from symptomatic domestic dogs in Brazil, sampled from 1980 to 2000, used to evaluate evolutionary changes in CPV, revealed that CPV type 2a was the predominant antigenic type circulating in Brazil [37], which agrees with our results.

Although these data have restricted epidemiologic meaning, it is important to emphasize the complete lack of information concerning antigenic phenotypic characteristics of CPV in Brazil. Carnivore parvoviruses have continuously evolved since CPV-2a/2b-related strains were isolated in domestic cats as well as a new antigenic type, designated CPV type 2c, was detected in wildcats.[38,39,40] The interspecies transmission seems to be more complex since CPV-2c was recently isolated in domestic dogs [26] and more studies will be required to address this question.

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Caracterização antigênica de isolados de parvovirus canino do Brasil utilizando monoclonais específicos

Resumo

O Parvovírus Canino (CPV) é um patógeno emergente em cães, isolado pela primeira vez em 1978, nos Estados Unidos. A amostra original de 1978 foi designada CPV tipo 2 (CPV-2). Entretanto, análises de isolados de CPV dos Estados Unidos, por enzimas de restrição e anticorpos monoclonais demonstraram que cerca de 1979, uma amostra variante, designada CPV tipo 2a (CPV-2a) tornou-se prevalente. Subseqüentemente, uma nova amostra antigênica, designada CPV tipo 2b (CPV-2b) também foi observada por análises de isolados de CPV de várias partes do mundo, embora a proporção fosse diferente entre os países. Nesse estudo, foi utilizado o teste de Inibição da Hemaglutinação (HI) com um painel de anticorpos monoclonais para a tipagem de 29 amostras fecais de parvovirus canino, coletadas de cães sintomáticos de 1980 a 1986 e de 1990 a 1995. Os resultados indicaram uma forte predominância do tipo antigênico 2a indicando que a epizootia de CPV no Brasil seguiu o mesmo padrão observados na Europa e países Asiáticos.

Palavras-chave:
Parvovirus Canino.
Inibição da Hemaglutinação.
Caracterização antigênica.
Anticorpos A

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