Retrospective Serological Analysis of Spontaneous CDV Infection in 192 Dogs

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(Received 16 January 2007/Accepted 26 December 2007)

ABSTRACT. Spontaneous cases of canine distemper virus (CDV) infection were serologically evaluated. The 192 dogs in which CDV antigen was confirmed from tonsil by immunohistological examination were 2- to 4-months old, of various breeds, and unvaccinated. The prognoses were good in 74 dogs with significantly high levels of anti-CDV passive hemolytic aggregation (PHA) titer. In the other 118 dogs with poor prognoses, anti-CDV PHA titer was not detected. Anti-CDV PHA titer had the most significant association with the prognoses of CDV infection, and could be the most reliable and useful indicator for evaluating such prognoses.

KEY WORDS: CDV, immunosuppression, PHA.

Canine distemper virus (CDV) is a negative single-strand RNA the morbillivirus of the family Paramyxoviridae, and is closely related to measles virus [1]. CDV causes multisystemic diseases in dogs, inducing severe immune suppression [1].

CDV infection may sporadically occur despite vaccinations, result in a completely subclinical disease or exhibit several different and distinct clinical and pathologic manifestations. CDV is pantropic in nature, and its antigen has been demonstrated in many different organs and tissues and in a wide variety of cells. In general, lesions are most prominent in the respiratory and alimentary tracts or in the central nervous system [1]. The virus antigen was detected in lymphoid tissues such as the lymph nodes, thymus and tonsils [5, 17]. Some atrophic lymphoid organs were also found to have a severe depletion of CD3 positive T cells and CD21 positive B cells [12]. The degree of lymphocyte depletion correlated with the severity of disease and the persistence of a virus in the lymphoid tissue and central nervous system [12].

For the diagnosis of CDV infection, viral RNA, viral protein and antibody were examined by PCR, immunohistochemistry and serological testing [6, 14].

CDV infections are usually diagnosed in practice by measuring the titer of anti-CDV antibody [15]. However, it is difficult to make a definitive diagnosis from the clinical signs and a serological diagnosis alone, since CDV induces severe immune suppression and lymphocyte depletion. Moreover, only a few studies have been done on the inability to produce antibodies against canine distemper virus [7, 9].

In this study, we retrospectively examined the association between the clinical course of the disease and the serum level of anti-CDV antibody.

A total of 192 spontaneous cases of CDV infection were evaluated retrospectively from 1999 to 2000 in Sapporo, Japan. The dogs were 2- to 4-months old and of various breeds. They had not been vaccinated and showed a range of various clinical signs, including nasal discharge, cough, pneumonia, diarrhea, conjunctivitis, scale, crust, hard pads and neurological symptoms. Some cases had a history of exposure to CDV infected dogs. CDV antigen was confirmed from tonsil in all 192 dogs by immunohistochemical examination.

Serum samples were examined to determine the values of anti-CDV IgG and IgM and passive hemolytic aggregation (PHA) antibody. Paired serum samples were taken from 54 cases at two- to three-week intervals. Anti-CDV IgG, IgM, and PHA antibody levels were determined by ELISA at a commercial diagnostic laboratory (ADTEC Co., Kuma moto, Japan). The significance of difference in the distribution of the anti-CDV antibody titers between 2 groups was investigated by performing Wilcoxon sum rank test (Mann-Whitney U test). And the correlation between the different geometric mean titers was examined by using Mann-Whitney U test for pairwise comparison of each group with different prognosis. A value of <0.01 was considered statistically significant.

Among 192 cases, 48 showed a good prognosis with significantly high levels of IgG and PHA antibody against CDV (Table 1). In these 48 animals, levels of all kinds of anti-CDV antibody were significantly higher than those in the 90 poor prognosis cases (P<0.01), all of which eventually died or were euthanized due to severe clinical signs. In these 90 dogs, no PHA titer was detected, and the levels of anti CDV IgG and IgM were low or undetectable (Table 1). The remaining 54 dogs showed an ambiguous increase of CDV antibody and were reexamined for the CDV titer with paired serum. Twenty-six out of those 54 cases showed increased levels of anti-CDV PHA titer and IgG upon a second examination and fluctuating IgM levels that had declined compared with those at the first examination. Their clinical signs had improved at the second examina-
tion. However, 28 of these 54 cases which showed no change in the PHA titer nor any increase in the anti-CDV IgG titer exhibited progressive clinical signs and poor prognosis (Table 1).

It has been experimentally demonstrated that severe or fatal cases of CDV infection had low or undetectable levels of CDV antibody, which would suggest that they were at the early stage or even the fatal [7, 9]. However, there have been no serological reports on natural CDV infection. In our survey, serological examinations did not reflect severe CDV infection, suggesting that severe immunosuppression might have occurred.

Although serology has frequently provided diagnostic and prognostic findings in many infectious diseases [3, 4], it is poorly suited to show a definite prognosis of CDV infection, since CDV virus induces severe immune suppression [7, 9]. There are many reports on such immune suppression caused by CDV infection [8, 10, 12], describing decreased lymphocytes and increased numbers of apoptotic cells. CDV was found to be distributed in the lymphocytes, inducing lymphocytic apoptosis both directly and indirectly, while CDV infection that has reached the acute or subacute phase has been seen to induce severe lymphoid depletion and immunosuppression [8, 12]. In such cases, the detection of CDV antigen was described as a diagnostic method that did not depend a serological examination [13]. In our study, fatally infected dogs could not be diagnosed only by serological examination alone, since they were considered to be immunosuppressed by CDV and to lack CDV antibody, which precluded the detection of antigen.

In the present study, we analyzed the association between the clinical course of CDV infection and the serum anti-CDV IgG and IgM levels and PHA titer. A higher level of anti CDV-PHA titer provided protection against systemic disease due to CDV, strongly correlating with antibody neutralization. This technique of measuring anti-CDV also detects maternal transfer immunoglobulin against CDV. Mean half-lives of the anti CDV maternal antibodies in puppies were estimated at 15.1 days and these puppies had less 1:10 titers at 56 days after birth [2]. Some of cases, which showed good prognosis, had high PHA and IgG titer, despite of low IgM titer. IgM is firstly detected antibody after CDV infection [16]. It was considered that they had maternal transfer antibody. A PHA titer over 40 was high enough to protect against CDV virus infection. Also, a high titer of PHA could indicate recovery from the disease as acquired immunity.

All animals with poor prognoses in our survey showed a low anti-CDV PHA titer and were unable to sustain a significant antiviral antibody response to PHA titer. The data suggested that the anti-CDV PHA titer would be the most important indicator of recovery from the disease, therefore PHA has positive collation to neutralizing antibody.

Waner and others reported that the serum levels of IgM and IgG antibodies to CDV increased 9 days after vaccination, and that the highest average titers were recorded after 12 days [16]. In a previous study, CDV-specific IgM was detected by ELISA one week before the serum neutralizing test showed positive [11]. Though anti-CDV IgM and IgG levels had increased in some dogs with low PHA levels in this study, further samples taken from these dogs after 2–3 weeks revealed high PHA and IgG titers with a low IgM titer. However, the cases with poor prognoses showed no changes in any class of antibody levels. Moreover, IgG levels had decreased in some cases upon a second examination, it was considered transfer maternal antibody and had been consumed.

In this study, anti-CDV IgG and IgM levels were significantly lower in poor prognostic cases than in good ones. However, 8 cases with moderate levels of IgG or IgM titers showed progressive clinical signs, indicating that it was difficult to determine a definitive prognosis of CDV infection by anti-CDV IgG and IgM titers alone. Since a high PHA titer was associated with a good prognosis in CDV infection cases, PHA titer may well be the most reliable and useful indicator for evaluating such prognoses. Antiviral IgG and IgM levels were also reliable in detecting in the middle to late stage of infection.

Table 1. Serum anti CDV antibody titer

<table>
<thead>
<tr>
<th>prognosis</th>
<th>1st</th>
<th>2nd</th>
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<tbody>
<tr>
<td></td>
<td>PHA</td>
<td>IgM</td>
</tr>
<tr>
<td>good cases</td>
<td>GMT(n=46)</td>
<td>867 (25–1600)</td>
</tr>
<tr>
<td>74 cases</td>
<td>range</td>
<td>(10)</td>
</tr>
<tr>
<td>Poor cases</td>
<td>GMT(n=90)</td>
<td>&lt;10 (&lt;10)</td>
</tr>
<tr>
<td>118 cases</td>
<td>range</td>
<td>10 (10)</td>
</tr>
</tbody>
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* Paired serum samples were taken from 54 cases at two to three-weeks intervals. Antibodies titers are described by geometric mean titer.
REFERENCES


