Passive protection of dogs against clinical disease due to *Canine parvovirus-2* by specific antibody from chicken egg yolk

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Abstract

The protective effect of immunoglobulins derived from chicken egg yolk (IgY) against infection by *Canine parvovirus 2* (CPV-2) was evaluated in 10 beagle dogs orally challenged with a strain of the virus. The 2-mo-old dogs were divided into 3 groups and treated with powders containing CPV-2 IgY or normal egg yolk for 7 d after the challenge. The 4 dogs receiving normal egg yolk (control group) demonstrated mild symptoms typical of CPV-2 infection, such as vomiting, diarrhea, and weight loss. No symptoms were observed by 16 d after challenge in the 3 dogs receiving 2 g of IgY powder. Of the 3 dogs receiving 0.5 g of IgY powder, 2 had clinical CPV-2 disease; however, the manifestations were less severe than in the control group. Furthermore, the IgY-treated groups had significantly greater weight gain and shorter duration of virus shedding than the control group. These results indicate that IgY is useful in protecting dogs from CPV-2-induced clinical disease.

Résumé

L'effet protecteur d'immunoglobulines provenant du jaune d'œuf de poule (IgY) contre une infection par le parvovirus canin de type 2 (CPV-2) a été évalué chez 10 chiens de race Beagle infectés par voie orale avec une souche du virus. Les chiens âgés de 2 mo ont été répartis en 3 groupes et traités pendant 7 j après l'infection avec de la poudre contenant des IgY dirigés contre CPV-2 ou du jaune d'œuf normal. Les 4 chiens recevant du jaune d'œuf normal (groupe témoin) ont montré des symptômes typiques mineurs d'infection par CPV-2, soit des vomissements, de la diarrhée et une perte de poids. Aucun symptôme n'a été observé jusqu'au jour 16 post-infection chez les 3 chiens recevant 2 g de poudre d'IgY. Parmi les 3 chiens recevant 0,5 g de poudre d'IgY, 2 ont présenté des signes cliniques d'infection par CPV-2; toutefois, les manifestations étaient moins sévères que chez les animaux du groupe témoin. De plus, les groupes traités avec les IgY avaient un meilleur gain de poids et une période d'excrétion du virus plus courte que les animaux du groupe témoin. Ces résultats indiquent que les IgY sont utiles pour protéger les chiens contre une maladie clinique causée par CPV-2.

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Canine parvovirus 2 (CPV-2) infection, a highly contagious disease, is prevalent all over the world, mainly because the virus can survive in harsh environmental conditions for a long time. Natural CPV-2 infection has been reported in domestic dogs, bush dogs, cats, coyotes, bears, and wolves (1,2). The most common clinical signs are pyrexia, vomiting, anorexia, and bloody diarrhea (1). The virus is genetically and antigenically related to *Feline panleukopenia virus*, *Mink enteritis virus*, and *Raccoon parvovirus* (3). Vaccines have been used to prevent CPV-2 infection for many years. However, the vaccines are, in general, ineffective in young puppies owing to the presence of maternal antibodies in the puppies' blood (1,4). As maternal antibody levels wane, the puppies become susceptible to infection by virus in the contaminated environment.

Passive immunization against *Rotavirus* and *Coronavirus* infections in animals by means of oral administration of immune colostrum or immunoglobulins derived from chicken egg yolk (IgY) has had promising results (5–8): feeding animals specific antibodies resulted in significant protection, with increased survival rates and reduced diarrhea and virus shedding. The purpose of this study was to examine whether passive immunization by means of oral administration of IgY specific for CPV-2 could have any protective effect in dogs challenged with the virus.

The CPV-2 strain Cp83016 (9) was used throughout the study. The virus was recovered from infected cells by 3 cycles of freezing and thawing, followed by calcium chloride precipitation. It was then propagated in Crandell feline kidney (CRFK) cell culture (10) and partially purified by centrifugation in an SW40Ti rotor (Beckman Instruments, Palo Alto, California, USA) through a 40% sucrose cushion at 100 000 × *g* for 3 h at 4°C. The viral pellet was suspended in phosphate-buffered saline, and aliquots were stored at −80°C.

Titration for infective virus was performed in the microculture plates as previously described (11). After serial 10-fold dilutions with Eagle's minimum essential medium (MEM) containing 10% fetal bovine serum, 50 μL of each aliquot was transferred to 4 wells per dilution. Then 50 μL of CRFK cell suspension (cell density 2×10^5/mL) in Eagle's MEM was added to each well. The plate was agitated gently and incubated at 37°C for 5 d in a humidified chamber containing 5% CO_2. The growth of CPV-2 was examined by

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hemagglutination assay (11) and the infective titer expressed as the median tissue culture infective dose (TCID50) per milliliter.

To prepare IgY samples, we vaccinated 14-wk-old White Leghorn chickens. Each 1-mL dose of vaccine contained about 10^8 $TCID_{50}$/mL of inactivated CPV-2 mixed with an equal volume of emulsion oil containing 5% (v/v) sorbitan oleate and was injected into the breast muscle. Seven weeks later the chickens were given a booster injection in the same manner. All eggs laid by the vaccinated chickens 2 to 6 wk after the booster were harvested and the egg yolks isolated, pooled, and spray-dried to make IgY powder (12). A control powder was made from the yolk of eggs collected from unvaccinated chickens. We prepared IgY solutions from the egg yolk powders by chloroform extraction (12).

The neutralizing activity of the IgY solutions and dog serum samples was determined by assaying the FL74 cell protection activity as previously described (2). Briefly, antibody solutions underwent serial 2-fold dilution in a 96-well flat-bottom microplate in quadruplicate (50 μL/well). The same volume of CPV-2 suspension (2×10^3 $TCID_{50}$/mL) was added to each well; the mixture was agitated and incubated at 37°C for 1 h. Then, 100 μL of uninfected FL74 cells (5×10^4 cells/mL) was added to each well and the mixture incubated at 37°C for 5 d. The virus neutralization titer (NT) was expressed as the reciprocal of the highest dilution of antibody solution that protected the cells from showing cytopathic effects. The NT of the IgY solution was 50 000, whereas that of the control powder solution was less than 10.

Ten 2-mo-old beagle dogs (average weight 3.3 kg) that had been confirmed as seronegative for CPV-2 were divided into 3 groups of 3 or 4 dogs. They were housed in separate cages in an isolation facility throughout the study. Food was withheld for 4 h before the oral challenge with 5×10^6 $TCID_{50}$ of CPV-2. Six hours after the challenge, the dogs were orally administered 2 g of IgY powder (group 1: 3 dogs), 0.5 g of IgY powder plus 1.5 g of control egg powder (group 2: 3 dogs), or 2 g of control egg powder (group 3: 4 dogs). The powders were given at the same doses 3 times a day for the first 5 d and 2 times a day for the next 2 d. Food and water were given to the dogs ad libitum.

The dogs were examined every day until day 16 after challenge for clinical signs, which were scored according to the system shown in Table I. As well, fecal samples were collected daily with the use of sterile cotton swabs and evaluated for virus by a 1-step immunochromatographic test (13), with a Chekman-CPV kit (Adtec, Oita, Japan), performed essentially according to the manufacturer's instructions. Student's *t*-test was used to assess the statistical significance of differences in clinical scores, change in body weight, and total number of days on which virus was detected.

Table II summarizes the clinical response after challenge. The challenge dose had been found to cause clinical disease in 100% of dogs in a previous experiment (data not shown). All 4 dogs in the control group demonstrated typical symptoms, such as vomiting (in all dogs from day 4 after challenge), diarrhea (starting on day 5 in all dogs and lasting 4.5 d on average), and weight loss. None of the 3 dogs in group 1 showed clinical signs of CPV-2 infection during the 16-d observation period, but 2 of the 3 dogs in group 2 (those receiving a lower dose of IgY powder) had vomiting and diarrhea on days 6 and 7. No dog in any of the groups had fever throughout the observation period. The average clinical score was significantly lower in groups 1 and 2 ($P < 0.01$ and 0.05, respectively) than in the control group, as was the average duration of symptoms. The dogs in the control group had lost an average of 0.21 kg of body weight by day 16. The weight gain was significantly higher in groups 1 and 2 than in the control group by day 10 ($P < 0.05$) and day 16 ($P < 0.01$). Groups 1 and 2 excreted CPV-2 for a significantly shorter time ($P < 0.01$) than the control group. All blood samples collected on day 16 showed neutralizing antibody against CPV-2 strain Cp83016, but the average NT was significantly lower in group 1 ($P < 0.05$) than in the other 2 groups.

Table I. System for scoring clinical signs of infection with *Canine parvovirus 2* (CPV-2)

Clinical sign	Score
Temperature (°C)	
≤ 37.3	1
37.4–39.4	0
39.5–39.9	1
40.0–40.5	2
≥ 40.6	3
Mucus in stool	1
Watery stool	2
Bloody stool	3
Anorexia	1
Depression	1
Lethargy	1
Vomiting	1
Coughing	1

These results indicate that sustained oral treatment with specific IgY has a protective clinical effect in dogs experimentally infected with CPV-2 and that the effect is dose-dependent for most evaluation parameters. However, although the dogs in group 1 were clinically protected, they shed CPV-2 in feces for about 1 wk after challenge. This suggests that the protection conferred did not necessarily prevent infection but, rather, limited the infection to subclinical status. Similar results have been reported with experimental *Rotavirus* infection (6,7).

It has been generally accepted that passive immunization against CPV-2 with specific antibodies should be used during the first days of infection to have adequate efficacy (1). In this study, specific IgY was administered soon after virus inoculation. It would be interesting to examine the efficacy of IgY in dogs already demonstrating typical clinical signs. We observed a relatively mild clinical disease after virus inoculation. This suggests that CPV-2 strain Cp83016 may have low virulence after passage in cell culture. A more severe infection model, in which food is withheld for 24 h before virus inoculation and for 48 h after inoculation, should be used to better assess the protective effect of CPV-2 IgY.

To our knowledge this is the first report about the protective effect of IgY in dogs experimentally infected with CPV-2. Our results suggest that passive immunization by means of oral administration of specific IgY may be useful in the treatment of dogs with clinical disease due to CPV-2.

Table II. Comparison of dog groups for clinical response to CPV-2 challenge and treatment with immunoglobulins specific for CPV-2 derived from chicken egg yolk (IgY)

Group	Dose of IgY powder (g)	Clinical signs: Rate[a]	Clinical signs: Duration[b] (d)	Clinical signs: Score	BWG (kg): At 10 d	BWG (kg): At 16 d	Duration of virus shedding (d)	Serum NT[c]
1	2.0	0/3	0[d]	0[d]	0.18[e]	0.32[d]	6.6[d]	1173[e]
2	0.5	2/3	2[e]	3.33[e]	0.18[e]	0.23[d]	7.3[d]	1813
3	—	4/4	4.5	8.5	0.01	−0.21	11.6	1920

[a] Number of dogs with clinical signs/total number of dogs in group

[b] Average number of days on which the dogs showed clinical signs

[c] In blood samples collected on day 16 after challenge

[d] Significantly different from the value for the control group at $P < 0.01$

[e] Significantly different from the value for the control group at $P < 0.05$

BWG — body weight gain after challenge; NT — neutralization titer

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