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## Preliminary Observations Using Canine Parvovirus-Specific Transfer Factor in the Prevention of Canine Parvovirus Disease

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**Abstract:** We studied the use of specific transfer factor as a modulator of the immune system, with special respect to its effectiveness in the improvement of antibody production and serum biochemical indexes. A total of 21 adult male Beagle dogs were assigned randomly into 3 test groups. After immunized with a multivalent vaccine, dogs in two groups were fed with different dosages of canine parvovirus-specific transfer factor oral liquid products. Laboratory tests to assess the immune profile of experimental animals were performed till 27 days after initial treatment. Present results showed that dogs in the two groups treated with transfer factor products after vaccination had better antibody responses. Dogs receiving larger amount of transfer factor products exhibited more favorable serum biochemical parameters. Transfer factor could also promote growth significantly. The fact that specific antibody level rose and maintained for longer time in the transfer factor treated groups confirmed the immunomodulating properties of transfer factor. We concluded that transfer factor could enhance immunal response to vaccines, modify biochemical indexes in blood serum, decrease the response caused by stress and improve the health of dogs.

**Key words:** Specific transfer factor, antibody response, serum biochemical indexes

### INTRODUCTION

Canine parvovirus is a highly virulent pathogen that infects domestic dogs worldwide and responsible for clinical problems of neonates and pregnant bitches (Ohshima *et al.*, 2004). The virus may infect the hearts of neonatal puppies causing myocarditis and cause gastroenteritis in older animals with high mortality. Since, it is especially a risk to puppies that are less than 6 months old, initial and follow-up vaccination is always considered critical to prevent CPV.

Transfer Factor (TF), a dialyzable moiety extracted from immune lymphocytes, is an important antiviral agent and has been successfully used for the treatment of many diseases with satisfactory results, such as primary immunodeficiencies, viral infections and tumor diseases (Prasad *et al.*, 1996; Pizza *et al.*, 1996a, b; Neequaye *et al.*, 1990; Tarkkanen *et al.*, 1981; Vera *et al.*, 1979). Although, the mechanism of these phenomenon is not fully elucidated, these observations corroborate the contention that the TF dialysate is a multifaceted activity product. It proves to be an effective therapeutic reagent and can restore the immune functions and achieve clinical improvement.

In this study we investigated the efficacy of CPV-specific transfer factor in increasing the immune response of dogs receiving yearly booster of vaccinations. TF was produced

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following standard methods, i.e., by animal immunization and subsequent replication in tissue culture. Specific antibody and serum biochemical parameters that related to immune response and metabolism were monitored after the initial vaccination. We are convinced that this specific TF-based immunotherapy can enhance antibody responses to vaccine and increased resistance to infection.

## **MATERIALS AND METHODS**

### **Animals and Experimental Design**

A total of 12 male Beagle dogs of 3-5 years old were approved to be used in this study by the Beagle Dog Experiment Base in Yangzhou University, Jiangsu Province, China. All were assessed as healthy before inclusion in the study based on physical examination and blood values within reference ranges for this species. Briefly, blood biochemical indexes included glucose (GLU), alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), creatine kinase (CK), total cholesterol (TCH), triglyceride (TG), total protein (TP), albumin (ALB), globulin (GLOB), serum total bilirubin (Tbil), blood urea nitrogen (BUN) creatinine (CREA) and uric acid (URIC). They were assigned equally into 3 groups and each kept individually in a disinfectant cage (0.8m×1.0×1.0 m), 0.5 m above the cement ground. They were fed with commercial extruded pellets (registered trade marker, Naughty Baby) twice daily with 300 g diets each time, one in the morning and the other in the evening. Water was supplied by automatic drinkers. Canine parvovirus (CPV)-specific TF oral liquid products were provided by Shanghai Academy of Agricultural Sciences. Investigated dogs were immunized with a multivalent vaccine, including live attenuated canine parvovirus, rabies virus, distemper virus, parainfluenza virus, infectious canine hepatitis virus. Dogs in group A were set as a control group. Dogs in group B and group C received 5 and 10 mL TF oral liquid products, respectively, every other day after vaccination.

### **Sample Collection**

Blood samples (3 mL) were collected from the brachial vein in fasting condition on days 7, 11, 15, 19, 23 and 27 after inoculation. They were kept at 4°C overnight to allow the whole blood to clot and the fibrin to retract. Sera were obtained after centrifugation at 4,000× g for 10 min. They were stored at -70°C and used to evaluate specific antibody response to CPV. The body weight of each dog was also recorded before each blood sampling.

### **Preparation of 1% Suspension of Porcine Red Blood Cells**

The 3.8% sodium citrate was used as the anticoagulant. Fresh porcine whole blood was mixed with twice volume of normal saline and centrifuged at 4000× g for 10 min at room temperature. The supernatant was discarded and precipitated erythrocytes were washed twice with normal saline as described above. Red cells deposition was diluted 1:99 with normal saline.

### **Hemagglutination-Inhibition (HI) Test**

The antigen used in this test was Canine Parvovirus (CPV), donated by Professor Houda Li in College of Veterinary Medicine, Yangzhou University, China. HI test was conducted by the conventional microtiter method as previously described (Mathys *et al.*, 1983) and the  $\beta$  method (i.e., variable antibody titer and constant virus concentration) was used. Equal aliquots of sera were pooled from 7 dogs of each group. Each serum sample was assayed in

triplicate and the titer was expressed as the highest antibody dilution inhibiting 4 hemagglutination units of virus. Briefly, two-fold serial dilution of 25  $\mu$ L sample serum was made with normal saline in U bottom microtiter plate (Nunc). Twenty five microliter CPV antigen suspension, pre-titrated by Hemagglutination (HA) test and contained 4 HA unit virus, was added into each well and allowed to stand at room temperature for 60 min to facilitate antigen antibody reaction. Twenty five microliter of 1% porcine red blood cell suspension was added into each well and again allowed to stand at room temperature for another 30 min. Positive and negative controls were run simultaneously with the test samples to validate the test.

#### Determination of Biochemical Indexes in Serum

Conventional biochemical indexes were determined on days 1, 5, 9, 13, 17 and 21 after inoculation. Plasma glucose (GLU) was determined by the glucose oxidase method (kits for glucose oxidase-peroxidase terminal calorimetric analysis, Shanghai Rongsheng Biotechnology Co., Ltd.). Total Protein (TP) was assayed by biuret calorimetric assay. Conventional serum biochemical markers, concentrations of serum albumin (ALB), globulin (GLOB), total cholesterol (TCH) and triglyceride (TG) were measured by automatic biochemical analyzer (HITACHI 7170S, Japan).

#### Statistical Analysis

Statistical analysis were performed using the one-way ANOVA procedure of SAS (SAS Inst., Inc., Cary, NC). When a statistical significance was detected ( $p < 0.05$ ), comparisons between means were carried out using the Least Significant Difference test.

### RESULTS

#### Evaluation of HI Activity

Compared with vaccinated dogs in group A, animals treated with TF products post immunization elicited a much better antibody response. The dogs in group B and group C always had higher antibody titres to CPV than did animals in group A (Fig. 1 ). At the end

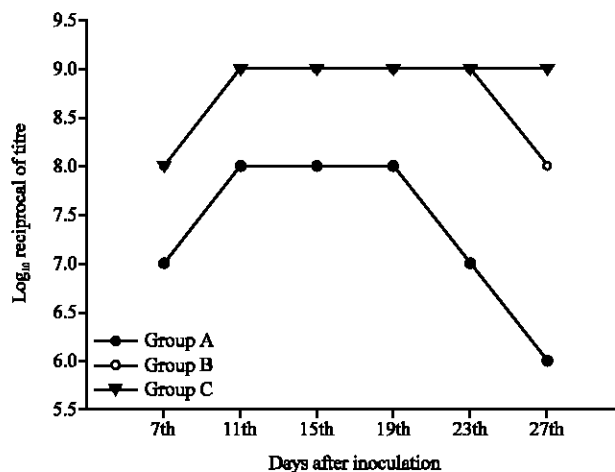


Fig. 1: Changes in antibody titres in immunized dogs

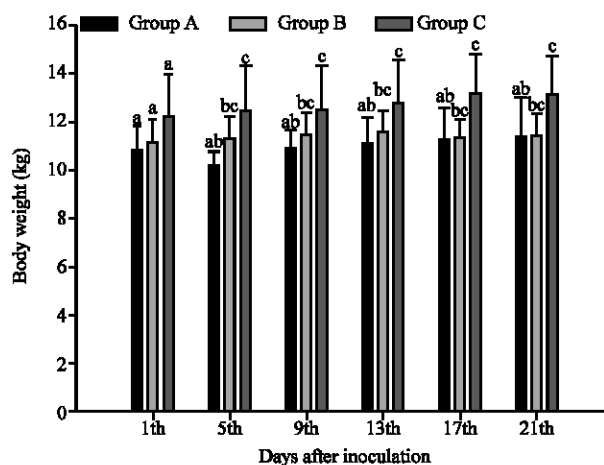


Fig. 2: Comparisons of body weight between dogs in different groups. Columns with different letter on the top had a significant level of  $p < 0.05$

of the experiment, dogs in group C still maintained a higher antibody titre ( $10^9$ ) than animals in group B ( $10^8$ ). Present results indicated that CPV-specific TF oral products, fed to animals after inoculation of vaccines, helped to maintain the antibody titre at a relatively high level and this effect of TF was revealed to be dose related.

#### Effects of TF on the Body Weight

Experimentally immunized dogs in group C, which received immunization in association with large amount of TF oral products, always had significantly higher body weight than animals in other groups (Fig. 2). It was demonstrated that TF had a significant effect on growth promotion and this effect was proportional to the dose, which was consistent with our above mentioned results.

#### Effects of TF on Serum Biochemical Markers

##### Plasma Glucose

A decrease of plasma glucose concentration was observed in all tested animals 5 days after inoculation (Fig. 3). This stage was consistent with the time when antibody was actively synthesized by organism and more nutrients were decomposed. While dogs in group C showed a continual increase in plasma glucose concentration, the second decrease of glucose content was detected in animals from other groups 17 days after inoculation. At this specific stage, a significantly higher glucose concentration was kept by dogs in group C ( $3.44 \pm 0.58 \text{ mmol L}^{-1}$ ) than animals did in group A ( $2.25 \pm 0.91 \text{ mmol L}^{-1}$ ) and group B ( $2.54 \pm 0.29 \text{ mmol L}^{-1}$ ). This higher level of glucose content was maintained till 21 days after inoculation, although, not reaching the statistical significant level. We noticed that when group B was compared with group A, concentrations of plasma glucose followed similar changes with time. However, glucose concentration of group C varied in a different way which indicated a more vigorous metabolism and was evidently induced by intake of more TF oral products.

##### Serum TP, ALB and GLOB

No significant differences were observed in the concentrations of serum TP, ALB and GLOB in all tested animals from different groups. When serum TP and ALB contents were

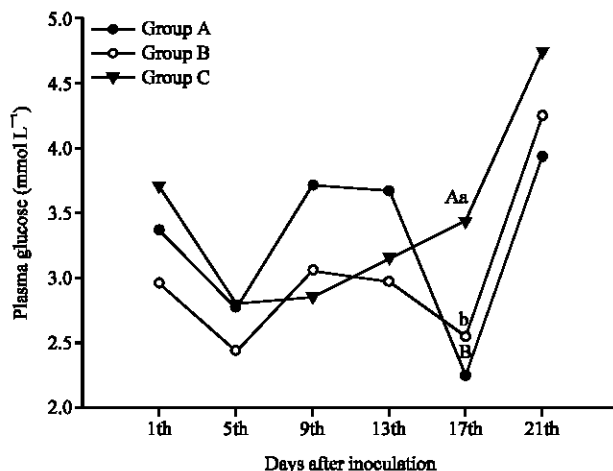


Fig. 3: Variations of average plasma glucose concentration in tested groups. Values with different small and capital letters meant significant difference of 0.05 and 0.01, respectively

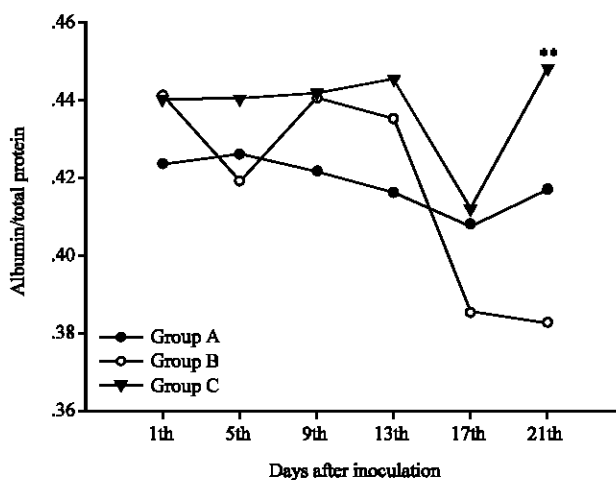


Fig. 4: The ratios of serum albumin/total protein in different groups. Columns with \*\* on the top had a significant level ( $p < 0.01$ )

considered, indexes in group C fluctuated more gently than that in group B and group A. The ranges of variation in group A, B and C were 10.3, 7.38 and 7.00  $\text{g L}^{-1}$  for TP content and 4.07, 4.04 and 2.07  $\text{g L}^{-1}$  for ALB content, respectively. Furthermore, we found out that GLOB concentrations in group C and group B exhibited larger scopes of variation than that in group A (6.87, 6.52 and 6.06  $\text{g L}^{-1}$ , respectively). Since, immunoglobins accounted for the large part of serum globulins, we assumed it to be the result of promoted differentiation of plasmacytes induced by TF oral products. When the ratio of serum ALB/TP were compared among different groups, dogs in group C always had relatively higher values than animals in the other two groups (Fig. 4), which confirmed that TF products did improve the immunity of the organism and this effect could be observed only at a relatively higher TF intake.

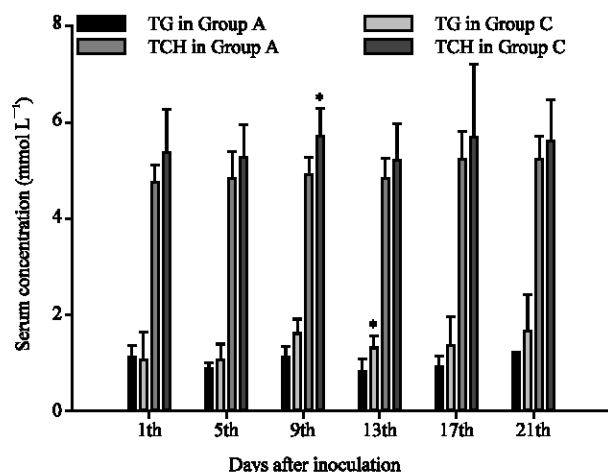


Fig. 5: Comparisons of serum TG and TCH contents in different groups. Columns with \* on the top had a significant level ( $p < 0.05$ )

### Serum TG and TCH

When serum TG and TCH contents were evaluated, dogs in group B had almost the same values as that in group A. However, animals in group C always had relatively higher values than that in group A except the TG content detected on first day after inoculation (Fig. 5). Dogs in group B and C received similar treatments after inoculation. This difference could be attributed only to the different dosage of TF products received. Present results demonstrated that TF promoted the absorption of nutritional elements in the diet. As we know serum albumin played an important role in lipid transportation, the results were consistent with the facts that animals in group C had a relatively higher ratio of ALB/TP.

### DISCUSSION

Canine parvovirus, continuing to evolve and giving rise to new antigenic types and virus mutants that spread through the dog population (Truyen, 2006) is becoming more and more clinically and epidemiologically significant. Since, its first isolation in 1978 (Appel *et al.*, 1979) a number of mutants have been described (Parrish *et al.*, 1985, 1988; Truyen, 1999; Ikeda *et al.*, 2002; Martella *et al.*, 2004). They not only showed antigenic differences but also differed in their reactivity in virus neutralization tests. These differences explained the variation in the minimum protective titre that young animals relied on to defense against virus infection. Up to now, vaccination was successful in transforming the CPV pandemic into a well-controlled situation. They are potent to prevent disease and even infection in most cases. However, most of the currently used vaccines are modified live virus vaccines based on the original virus type. They do not contain the newest antigenic types which imply incomplete protection for the animals. The fact is that there are still many cases of clinical parvovirus in dogs, especially puppies with decreasing level of maternal antibodies. There is a potential need to increase the protective effect of currently used vaccines. In order to increase the potency of traditional vaccine we used CPV-specific TF associated with vaccination to ascertain measurable clinical advantage in the prevention of CPV disease.

Although, the precise chemical nature and the exact mode of action are not fully understood, TF has in many studies proved its efficacy in treating viral infections without

any adverse side effects or drawbacks, even when it is used for long-term treatments and in injectable form (Fujisawa *et al.*, 1984; Corbeel *et al.*, 1984; Neequaye *et al.*, 1990; Pizza *et al.*, 1994; Byston *et al.*, 1996; Levine, 1996; Dvorožnáková *et al.*, 2009). It is an ideal therapeutic agent in increasing the immune response of the organism and reducing the frequency and the severity of diseases.

The present study demonstrated the efficacy of TF in increasing the antibody response. As we know TF, a complex group of many low-molecular-weight proteins, can not only transfer specific immunity from an immune donor to a recipient but also have immune modulating activities (Kirkpatrick, 1996). Present results collaborate with the findings of other investigators. The immunomodulative effect of TF emerged gradually after a stagnation of antibody development. Present study suggested that TF had a positive effect on the host humoral immune response against the infection of CPV, which was beneficial for its resistance against viral pathogen. Meanwhile, a relatively higher body weight was observed in dogs received larger amount of TF, an increase in serum content of glucose was associated with this favorable growth rate. One of the possible reasons was that more nutrients intake were transformed into glucose under the condition of increased digestibility, which facilitated their transportation and further storage in the form of liver glycogen or muscle glycogen and helped to improve the general body defense.

The total protein in serum, coming from synthesis in liver and intestinal absorption, plays an important role in organism growth and development. Since, the main function of ALB is to maintain osmotic pressure in vascular and form the binding protein for material transport, the significantly higher rate of ALB/TP in animals from group C could be used to indicate TF effectiveness in improving animal growth.

TF was traditionally regarded as activators of the cell-mediated immune system and had no significant effects on the B cell-mediated immune function (Alvarez-Thull and Kirkpatrick, 1996). In this study we found out that TF could regulate the status of immunity. The significantly higher antibody titre and wider fluctuation range of serum GLOB content helped the animals received TF treatment to cope with the CPV infection. Although, the immunologic mechanisms underlying the antigen-specific humoral immune effects of TF remain unknown, our observations established beyond doubt the efficacy of specific TF preparations in preventing CPV infection and animals can benefit from these marked improvements.

## **CONCLUSION**

The high fatality rate of CPV has led to a variety of attempts to improve survival by boosting the body's immune defenses in the prevention and control of this disease. A controlled study using TF with specific activity against CPV was thus carried out and demonstrated that antigen-specific TF was efficacious in enhancing the immune response of the organism.

## **ACKNOWLEDGMENT**

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