NOTE *Internal Medicine*

Prevalence of food-responsive enteropathy among dogs with chronic enteropathy in Japan

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ABSTRACT. There have been limited reports on the prevalence of adverse food reactions among dogs suffering from chronic enteropathy (CE) in Japan. We examined the prevalence and histological features of food-responsive enteropathy (FRE) in a total of 32 dogs with history of CE. Fourteen of 18 cases (56.2%) diagnosed as FRE had lymphocytic-plasmacytic enteritis or eosinophilic enteritis by histopathological examination. Characteristic histopathological changes indicating FRE were not identified in 18 cases, though 4 cases did not show any abnormalities. Results collected from this study provided important information that can help to change the way dogs with CE are treated in the future.

KEY WORDS: chronic enteropathy, dog, food-responsive enteropathy, inflammatory bowel disease

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Chronic enteropathy (CE) in dogs includes antibiotic-responsive diarrhea (ARD) responding to antimicrobial therapy, adverse food reactions (AFRs) responding to diet therapy and inflammatory bowel diseases (IBDs) [3, 8]. Generally, IBD is diagnosed based on the detection of inflammation in the intestines identified by histopathological examination. However, in order to accurately diagnose IBD, it is necessary to completely rule out ARD and AFRs by performing multiple antimicrobial and diet therapies. A previous report indicated that 55.7% (39/70) of dogs with CE responded to dietary changes (food-responsive (FR)), while 30% (21/70) was found to be responsive to steroid treatment (ST) where steroid is needed for the alleviation of clinical symptoms [1]. Even though the author did not find any histopathological differences between dogs in the FR and ST groups, the results suggested that the canine chronic enteropathy activity index (CCECAI) is a good diagnostic indicator for CE, whereas negative prognostic factors for CE include high histopathological scores in the duodenum, and low cobalamin and albumin serum levels [1]. Up-to-date, there are no reports on the prevalence and clinical features of AFRs among dogs diagnosed with CE in Japan.

Specially formulated diets used to eliminate AFRs in dogs suffering from CE are often selected based on information from a list of previously exposed food. Although Allenspach *et al.* used Purina L/A salmon and rice in all the cases to eliminate AFRs [1], it is unclear whether only one diet is adequate for this purpose. This suspicion was raised as it is widely known that selection of food allergens to be removed from diets in cases of food allergy is a particularly difficult task [5]. Nevertheless, it has been recently reported that the Lymphocyte Proliferative Test (LPT) is helpful in selecting the most suitable food allergen that should be eliminated from the diet of dogs with food allergy [2, 6, 9].

The objective of this study is to find out the prevalence of AFRs in dogs diagnosed with CE in Japan. Moreover, histopathological examinations were performed with samples obtained through endoscopy in order to find out if there is a connection between the AFRs and the histopathological diagnosis.

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Information of dogs that visited 10 animal hospitals (Primo Animal Hospital Nerima-Animal Allergy Medical Center, Yuki Animal Hospital, Urayasu Central Animal Hospital, Japan Animal Medical Center, Ogawa Canine & Feline Hospital, Kannai Animal Clinic, AEON Animal Medical Center, Hiratsuka Animal General Medical Center, Tokyo University of Agriculture and Technology, and Yamaguchi University) from August 2006 to February 2013 with the primary complaint of chronic digestive symptoms for over three weeks, including vomiting, diarrhea and weight loss, were compiled. Among all the cases, a total of 32 dogs meet the following requirements as criteria of study inclusion: 1) no immunosuppressive agents used for 2 weeks prior to enrollment into this study; 2) Endoparasitic infections were ruled out by fecal examinations performed by direct smear and zinc sulfate flotation; 3) dogs were treated with antibiotics (metronidazole 15–30 mg/kg BID, ampicillin 10–20 mg/kg BID or fluoroquinolone 5 mg/kg SID) for at least 2 weeks to rule out antibiotic-responsive enteropathy; 4) mucous membrane samples were collected from the stomach and duodenum through post-fast endoscopic examinations for histopathological examinations; and 5) elimination diet trials were performed for at least 10 days. The dogs that improved the gastrointestinal signs were includes in the food-responsive group. Elimination diet was selected individually based on the result of LPT (Animal Allergy Clinical Laboratories Inc., Sagamihara, Japan), which measured the *in vitro* proliferative responsiveness of lymphocytes to the following 18 possible food allergens: beef, pork, chicken, egg white, egg yolk, milk, wheat, soybean, corn, mutton, turkey, duck, salmon, codfish, catfish, capelin, potato and rice. In cases where the lymphocyte proliferative index exceeded 1.2%, the food was considered as a causative allergen and excluded from the diet trial [2]. Glucocorticoid (prednisolone 0.5–2 mg/kg/day) was administered to dogs that showed no clinical improvement by food elimination tests. If clinical improvement was observed after glucocorticoid therapy, the dogs were included in the steroid-treatment group. Furthermore, serum albumin concentration was measured in all cases, and level of 20 g/L or lower was defined as hypoalbuminemia. The severity of clinical presentations was determined with CCECAI [1]. CCECAI is a clinical scoring index, which is the sum of score assessing nine aspects of dogs suffering from CE, such as activity, appetite, vomiting, stool consistency, frequency of bowel movement, weight loss, albumin concentration, ascites fluid, subcutaneous edema and itching.

The correlation between hypoalbuminemia and the food-responsive/ steroid-treatment groups was analyzed statistically with the Fisher's exact test. Statistical processing was performed with GraphPad Prism 5.0 (GraphPad Software Inc., San Diego, CA, U.S.A.). Data were considered as significant different when $P<0.05$.

The clinical characteristics of all 32 dogs are described in Table 1. The average age of the dogs enrolled in the present study was 5.33 years (range: 4 months −13 years), and the breed of dogs in this study included Toy Poodle (n=6), Shiba (n=3), Papillon (n=3), Pomeranian (n=3), Shih Tzu (n=2), Miniature dachshund (n=2), Chihuahua (n=2) and Mongrels (n=2), and one Jack Russell terrier, Yorkshire terrier, Boston terrier, French bulldog, Labrador retriever, Shetland sheepdog, Welsh corgi, English Cocker Spaniel and American Cocker Spaniel. From the LPT results, 31 of 32 cases had positive lymphocyte proliferative response to more than 2 food allergens (Supplementary Table 1), and one dog (case no.1) did not show reactivity to any food allergens.

After the dogs were fed with elimination diets based on the LPT results, 18 of 32 dogs (56.2%) were assigned as food-responsive, while the remaining 14 dogs (43.8%) were only responsive to steroid treatment (Table 1). Elimination diets that improved the clinical signs of dogs in the food-responsive group were: ANALLERGENIC (n=8), Select Protein (D&T) (n=1) (Royal Canin Japon Inc., Tokyo, Japan), z/d ULTRA Allergen-free (n=2), w/d (n=1) (Hill's-Colgate (Japan) Ltd., Tokyo, Japan), Amino Protect Care (n=2) (Nosan Corporation, Yokohama, Japan), D Assist KO Select Protein (n=1), D Assist FP Select Protein (n=1), (Eukanuba, Cincinnati, OH, U.S.A.), and home-made diets (n=2) (Supplementary Table 1). The results in the present study showed that the percentage of dogs diagnosed with CE in Japan that clinically improved after elimination diets is 56.2% (18/32). A previous study conducted in Switzerland [1] suggested that the percentage of dogs suffering from CE that clinically improved after elimination diets stood at 55.7% (39/70).

The results of the histopathological examinations from all the cases were lymphocytic-plasmacytic enteritis (LPE; n=25), eosinophilic enteritis (n=3) and minimal change (n=4) (Table 1). In those cases diagnosed as LPE, 13 (52%) were in the steroid-treatment group, and 12 (48%) were in the food-responsive group. From this result, there are no specific histopathological changes consistent with a diagnosis of food-responsive enteropathy. A previous study on histological evaluation in dogs diagnosed with CE also showed that there was no difference between steroid-responsive and food-responsive diarrhea [1, 7]. Moreover, another study also showed that most of dogs with diet-responsive chronic enteropathy had been diagnosed as a LPE by endoscopic examination [10]. Thus, as supported from our result in this study, food-responsive enteropathy (or AFRs) and steroid-treatment enteropathy (IBD) cannot be discriminated based on histopathological results. Cases of ARD and AFRs that would respond to antibiotics and dietary change might be mistakenly treated with steroid if they are not properly ruled out first. On the other hand, all four cases showing no abnormality in the histopathological examinations were in the food-responsive group. Even though four cases were not enough to make a solid conclusion, it suggests that recommending dietary management first is appropriate if no abnormality was observed in the histopathological examinations. Among the three cases of eosinophilic enteritis, one was in the steroid-treatment group, and two were in the food-responsive group. Clinical importance of eosinophilic infiltration in intestinal mucosa was not clarified well. However, Walker *et al.* showed a tendency for eosinophilic infiltration in food-responsive enteropathy, and the mean density of eosinophils seemed to decrease after treatment [10]. From previous and our studies, the relationship of eosinophilic infiltration and pathogenesis of food-responsive

Table 1. Summary of information and results

Case No.	Age (Y)	Sex	Breed	Hypoalbuminemia (g/*l*)	CCECAI value	Histopathological diagnosis	Elimination diet	Group
1	6	Spayed female	Pomeranian	No	7	Nil	Select Protein (D&T)	Food-responsive
2	5	Castrated male	Mongrel	No	11	Nil	D Assist KO Select Protein	Food-responsive
3	5	Castrated male	Chihuahua	No	6	Nil	ANALLERGENIC	Food-responsive
4	7	Castrated male	Toy poodle	No	11	Nil	ANALLERGENIC	Food-responsive
5	5	Castrated male	English Cocker Spaniel	No	7	Lymphocytic-plasmacytic enteritis	ANALLERGENIC	Food-responsive
6	13	Intact male	Mongrel	No	8	Lymphocytic-plasmacytic enteritis	Home-made food (ostrich meat, pumpkin)	Food-responsive
7	0.3	Intact female	Shih Tzu	No	4	Lymphocytic-plasmacytic enteritis	ANALLERGENIC	Food-responsive
8	6	Spayed female	Labrador retriever	No	6	Lymphocytic-plasmacytic enteritis	ANALLERGENIC	Food-responsive
9	0.7	Castrated male	Papillon	No	5	Lymphocytic-plasmacytic enteritis	Amino Protect Care	Food-responsive
10	8	Intact male	Shih Tzu	No	6	Lymphocytic-plasmacytic enteritis	Home-made foods (chicken, Somen)	Food-responsive
11	5	Spayed female	Toy poodle	No	1	Lymphocytic-plasmacytic enteritis	ANALLERGENIC	Food-responsive
12	11	Spayed female	Toy poodle	Yes (15)	5	Lymphocytic-plasmacytic enteritis	z/d ULTRA Allergen-free	Food-responsive
13	2	Spayed female	Welsh corgi	No	10	Lymphocytic-plasmacytic enteritis	w/d	Food-responsive
14	5	Spayed female	Jack Russell terrier	No	4	Lymphocytic-plasmacytic enteritis	ANALLERGENIC	Food-responsive
15	3	Spayed female	Shiba	No	5	Lymphocytic-plasmacytic enteritis	Amino Protect Care	Food-responsive
16	8	Spayed female	Papillon	No	3	Lymphocytic-plasmacytic enteritis	ANALLERGENIC	Food-responsive
17	12	Castrated male	Papillon	Yes (17)	10	Eosinophilic enteritis	z/d ULTRA Allergen-free	Food-responsive
18	4	Castrated male	Shiba	No	7	Eosinophilic enteritis	D Assist FP Select Protein	Food-responsive
19	1	Spayed female	Toy poodle	No	4	Eosinophilic enteritis	ANALLERGENIC	Steroid-treatment
20	6	Spayed female	Pomeranian	No	13	Lymphocytic-plasmacytic enteritis	d/d salmon & potato	Steroid-treatment
21	1	Intact female	Shiba	Yes (15)	8	Lymphocytic-plasmacytic enteritis	Amino Protect Care	Steroid-treatment
22	2	Intact female	Toy poodle	Yes (13)	9	Lymphocytic-plasmacytic enteritis	Amino Protect Care	Steroid-treatment
23	7	Spayed female	Shetland sheepdog	Yes (17)	3	Lymphocytic-plasmacytic enteritis	D Assist KO Select Protein	Steroid-treatment
24	1	Intact male	Yorkshire terrier	No	12	Lymphocytic-plasmacytic enteritis	ANALLERGENIC	Steroid-treatment
25	9	Intact male	Pomeranian	Yes (15)	13	Lymphocytic-plasmacytic enteritis	z/d ULTRA Allergen-free	Steroid-treatment
26	2	Spayed female	Chihuahua	No	7	Lymphocytic-plasmacytic enteritis	z/d ULTRA Allergen-free	Steroid-treatment
27	9	Intact male	Miniature dachshund	Yes (13)	11	Lymphocytic-plasmacytic enteritis	ANALLERGENIC	Steroid-treatment
28	7	Intact female	Boston terrier	Yes (11)	10	Lymphocytic-plasmacytic enteritis	Amino Protect Care	Steroid-treatment
29	5	Intact male	French bulldog	Yes (10)	8	Lymphocytic-plasmacytic enteritis	ANALLERGENIC	Steroid-treatment
30	6	Intact female	Miniature dachshund	No	11	Lymphocytic-plasmacytic enteritis	D Assist KO Select Protein	Steroid-treatment
31	2	Castrated male	Toy poodle	No	4	Lymphocytic-plasmacytic enteritis	z/d ULTRA Allergen-free	Steroid-treatment
32	8	Spayed female	American Cocker Spaniel	Yes (12)	8	Lymphocytic-plasmacytic enteritis	ANALLERGENIC	Steroid-treatment

enteropathy was not yet clarified.

In the present study, the diets of the dogs were changed based on the LPT results. LPT may be helpful to find the suitable elimination diet for the dogs with food-responsive enteropathy. However, the positivity in LPT did not necessarily indicate that the dog had the food-responsive enteropathy, because 31 of 32 cases had at least one positivity to food allergen by LPT and about half of the dogs did not improve the symptoms after diet changes.

A weak point in this study was that the reproducibility of the clinical results was not confirmed by performing food provocation tests after the improvement of clinical symptoms. Therefore, it remains unclear whether these dietary changes can suppress the inflammatory responses through immunological mechanisms. Furthermore, the role of food allergens as one of the etiologies of CE has not yet been established. It is well known that food allergy in dogs occurs in the presence of two types of pathological conditions, the IgE-mediated and the non IgE-mediated hypersensitivity [4, 6, 9]. In cases of the non IgE-mediated hypersensitivity, it has been indicated that LPT can be useful in identifying the possible causative antigens. Instead of focusing on the pathological mechanism of AFRs or IBD, demonstrating the role of LPT in AFRs seems far more important as LPT can also be used to identify food allergens in treating cases of food-responsive enteropathy.

Ten out of 32 cases showed hypoalbuminemia. Out of the 10 cases, 8 (80.0%) were in the steroid-treatment group, and 2 (20.0%) were in the food-responsive group. Although the percentage of steroid-treatment group among all cases was 43.8%, the percentage of steroid-treatment group among all cases with hypoalbuminemia increased to 80.0%. Sensitivity and specificity of hypoalbuminemia in the steroid-treatment group were 57.1% (95% confidence interval 0.29–0.82) and 88.9% (95% confidence interval 0.65 to 0.99), with likelihood ratios of 5.14. Statistical analysis with Fisher's exact test showed significant correlation between hypoalbuminemia and each of the groups (P=0.0084) and a high odds ratio of 10.67 in cases with hypoalbuminemia compared with those at normal albumin level. These results suggested that immunosuppressive agents, such as steroid, will be required, in addition to dietary management, in cases of CE with hypoalbuminemia.

The CCECAI value is a scoring index for CE in dogs. The average CCECAI in all the cases was 7.4 (range: 1–13) (Table 1). The CCECAI value in the steroid-treatment group (8.6 ± 3.3 (mean ± SD)) was higher than that in the food-responsive group (6.4 ± 2.8 (mean ± SD)), but there were no statistically significant differences (P=0.096). A previous study [7] reported that the steroid-responsive group showed a significantly higher CCECAI value (P=0.05) as compared to the food-responsive group. Mean CCECAI values of food-responsive group in our study and their study were quite similar (6.4 versus 6.0, respectively), but the values in the steroid-treatment group were different (8.6 versus 10.0, respectively). Mean CCECAI value in the steroid-treatment group in our study was lower than the other study, and this possibly contributed to no significance between the two groups in our study. Unfortunately, we were unable to identify the reason behind the difference between the CCECAI values of the studies. Larger studies with more complex analyses might be required in the future.

In conclusion, the present study examined dogs from multiple animal hospitals in Japan that were diagnosed with CE, and the results revealed that 56.2% (18/32) of them had food-responsive enteropathy. In particular, cases where histopathological examinations appeared to be normal and hypoalbuminemia was not observed had a higher probability of being diagnosed with food-responsive enteropathy. Therefore, it is recommended that food allergens to be avoided in the elimination diet should be selected based on the results of LPT and subsequent elimination diet therapy be carried out to prevent any unnecessary use of immunosuppressants.

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