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IgE reactivity to milk components in dogs with cutaneous adverse food reactions

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ABSTRACT. We investigated the IgE reactivity to crude and purified milk antigens in the sera of 112 dogs with cutaneous adverse food reactions (CAFRs). Of the 112 dogs, 33 (29%) had specific IgE for crude milk antigens. In the dogs with milk-specific IgE, IgE reactivity to casein, bovine serum albumin (BSA), α-lactalbumin, β-lactoglobulin, and bovine IgG were 81%, 85%, 39%, 27%, and 35%, respectively. Casein and BSA may be important allergens in dogs with CAFRs. Some canine vaccines contain casein hydrolysate as a stabilizer and the pooled serum with anti-casein IgE showed IgE reactivity to the vaccines containing it. Information about IgE reactivity to casein in dogs with CAFRs could be useful for predicting adverse reactions to the vaccines including casein hydrolysate.

KEY WORDS: cow's milk allergen, cutaneous adverse food reaction, dog, IgE, vaccine

Cow milk is one of the common foods that cause cutaneous adverse food reactions (CAFRs) in dogs [13]. CAFRs including food allergy account for 8% in all dermatological diseases in dogs [5]. In humans, food allergy is considered an indicator of the risk for adverse reactions to vaccines [2], and patients with food-allergies demonstrated adverse reactions to vaccines containing the causal food protein [1, 20]. Approximately one-third of dogs with CAFRs have been reported to have milk-specific IgE [3, 8]. However, the IgE reactivity to components of milk has not been elucidated. The prevalence and reactivity of specific IgE for casein, which is present as a stabilizer in vaccines, is important information in veterinary medicine.

Adverse reactions to vaccines have been reported in humans and dogs [5, 12]. Gelatin as a stabilizer in human vaccines has shown to cause anaphylaxis in vaccination [19.21]. A dramatic decrease in the allergic reactions to vaccines was observed immediately after manufacturers marketed gelatin-free vaccines [16]. Furthermore, World Health Organization has set guidelines for the quantity of certain vaccine components per vaccine does such as bovine serum albumin (BSA) [23]. Due to such efforts, human vaccines now contain only trace levels of those components, and the rate of adverse reactions has significantly decreased [11]. Conversely, these components in canine vaccines are much higher in quantity than those in human vaccines [15]. We have previously reported that the rate of adverse reactions to vaccines in dogs is higher than that in humans [12]. Thus, the causes of adverse reactions to vaccines in dogs should be investigated urgently.

Our previous report showed that animal proteins are present in canine vaccines [20]. In particular, proteins derived from cows are often used in the manufacturing process. BSA is a residue of cell cultures used during vaccine manufacturing, and casein from cow milk has been used as a stabilizer in canine vaccines [15]. We have previously demonstrated that BSA in vaccines is an IgE reactive component [14]. However, other components of canine vaccines are not fully recognized as potential risk factors for adverse reactions. In the present study, we evaluated a sample population of dogs for IgE reactivity to milk components and demonstrated that dogs with casein-specific IgE may have a risk for adverse reactions to vaccines containing casein. Measurement of specific-milk IgE in dogs with CAFR will provide important information for safe vaccination.

Commercially available pasteurized cow's milk (Kanagawa, Japan) was used in this study. The protein concentration was measured using Quick Start™ Bradford Protein Assay (Bio-Rad Laboratories, Hercules, CA, USA). Casein, BSA, α-lactalbumin, β-lactoglobulin and bovine IgG (bIgG) as components of cow's milk were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Four vaccines available in Japan were used in this study. Vaccines A and B contained casein hydrolysate (vaccine A: 10.24 mg/dose, vaccine B: 12.5 mg/dose), and vaccines C and D contained no casein. The amount of casein hydrolysate present in

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these vaccines was referenced on the manufacturer's instructions for use. These are combined live vaccines composed of canine parvovirus, canine distemper virus, canine adenovirus type 2, and canine parainfluenza virus. Vaccine B also contained inactivated *Leptospira*.

The diagnosis of CAFR was performed as described previously by Shimakura *et al.* (2016) [22]. Briefly, dogs with non-seasonal chronic pruritus were clinically diagnosed with canine atopic dermatitis by Prélaud's criteria [18], and the elimination diet trial with or without food provocation tests according to the established method was performed for the definitive diagnosis of CAFRs [6]. Cases of dermatitis without CAFRs, such as parasite infestation or fungal infection diagnosed by routine dermatological examinations and positive clinical evolution upon specific treatment, were excluded from the present study. The negative control sera were obtained from dogs that had always been kept indoors as experimental laboratory animals and had never received cow milk containing foods and any vaccinations.

Specific IgE levels were measured using modified ELISA as described previously by Ohmori *et al.* (2005) [15]. The wells of the microplates (Greiner Bio-One, Kremsmünster, Austria) were coated with cow milk (10 µg/ml) or its components (Casein, BSA, α-lactalbumin, β-lactoglobulin, and bIgG) (each 5 µg/ml) or the vaccines (1,000-fold dilution) at 4°C overnight. After washing, blocking was performed with PBS containing 1% gelatin from cold-water fish (Sigma-Aldrich) for 2 hr. The wells were rewashed and incubated with 10-fold diluted serum in PBS with 1% gelatin and 0.05% Tween 20 (Sigma-Aldrich). When inhibition ELISA was performed, casein (50 µg/ml) was used as an inhibitor and mixed with the serum. After incubation at 4°C overnight, the wells were washed and sequentially incubated with biotinylated goat anti-dog IgE polyclonal antibodies (Bethyl Laboratories, Montgomery, TX, USA), streptavidin-beta galactosidase (Sigma-Aldrich), and 0.1 mM 4-methylumbelliferyl-β-D-galactopyranoside (Sigma-Aldrich) in dilution buffer. The fluorescence intensity was measured as fluorescence units (FU) using a microplate reader (Powerscan MX; DS Pharma Biomedical, Osaka, Japan). The results are representative of three independent experiments. Statistical analysis was performed using the statistical software R. Statistical significance was set at $P<0.05$.

The sera of 112 dogs with CAFR and 20 healthy dogs were measured for specific IgE to crude milk antigens (Fig. 1). The cutoff value, which was the criterion to discriminate between positives and negatives in the assay, was determined using measurements from sera of healthy dogs. Of 112 dogs with CAFRs, 33 (29%) were positive for milk-specific IgE. The positive rate of specific IgE to milk in this study was similar to the previously reported studies from the USA and UK [3, 8]. In humans, the positive rate differs for different countries [4]. This variation may be associated with dietary habit. In dogs from developed countries, dietary habits would have a very small influence on the positive rate of specific IgE because dog food is available to pet owners living in these countries.

IgE reactivity to milk components was evaluated to estimate the allergenic potential of the individual components: casein, BSA, α-lactalbumin, β-lactoglobulin, and bIgG. The sera of 26 dogs with IgE reactivity to milk were measured in ELISA because the sera of six dogs did not have sufficient volume. All 26 dogs with IgE reactivity to crude milk antigens showed positive IgE reactivity for at least one component; the IgE reactive to casein, BSA, α-lactalbumin, β-lactoglobulin, and bIgG were 81%, 85%, 39%, 27%, and 35%, respectively (Fig. 2). Our findings suggested that casein and BSA may be more important allergens for CAFRs in dogs than α-lactalbumin, β-lactoglobulin, and bIgG.

BSA has been reported as an allergenic component for dogs with hypersensitivity to beef, which is one of the most common food allergen sources for dogs [13, 14]. Indeed, IgE cross-reactivity between milk and beef has been reported in dogs [3]. Additionally, BSA was also used as a flavoring agent in food and food supplements, without being mentioned on the label [17]. Furthermore, contamination of beef protein was frequently detected even with strictly monitored diets for the treatment of CAFR [7]. Casein has been used as a stabilizer in canine vaccines [15]. Our study in human vaccines revealed that vaccines containing gelatin as a stabilizer demonstrated anaphylactic reactions on administration [19, 21]. Moreover, the administration of drugs containing casein caused hypersensitivity reactions in milk-allergic children [9, 10]. Manufacturers should remove casein hydrolysate from canine vaccines and use another stabilizer.

In dogs, casein may be the main allergenic component in milk. We investigated whether casein-specific IgE reacts to vaccines containing casein hydrolysate. For the analysis, sera were pooled from five dogs with strong IgE reactivity to casein. The pooled serum showed IgE reactivity to two vaccines containing casein hydrolysate (Fig. 3A). To determine whether the IgE reactivity to the vaccine was dependent on casein, inhibition ELISA using pooled serum was performed (Fig. 3B). Inhibition ELISA of solid-phased casein as a positive control showed 98% inhibition of IgE reactivity. Next, inhibition ELISA of each solid-phased vaccine showed that the specific IgE reactivity to the vaccine was reduced by the addition of casein.

Our previous study found that IgE in dogs with allergic reactions after vaccination reacted to the vaccine components [15]. In this study, casein-specific IgE in dogs with CAFRs had IgE reactivity to the vaccines, including casein hydrolysate (Fig. 3). Therefore, veterinarians should pay particular attention to

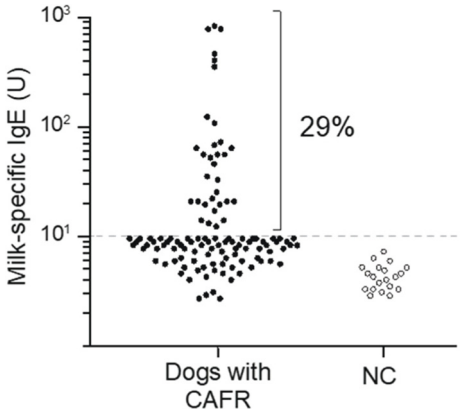

Fig. 1. IgE reactivity to crude milk. The number of dogs with cutaneous adverse food reactions (CAFRs): 112, negative control (NC): 20. The measurements in this ELISA were converted to units (U) because of the correction of measurements of specific IgE among microplates. The cutoff value (dashed line, 10 U) was calculated by the mean plus three times the standard deviation for the specific IgE in NC.

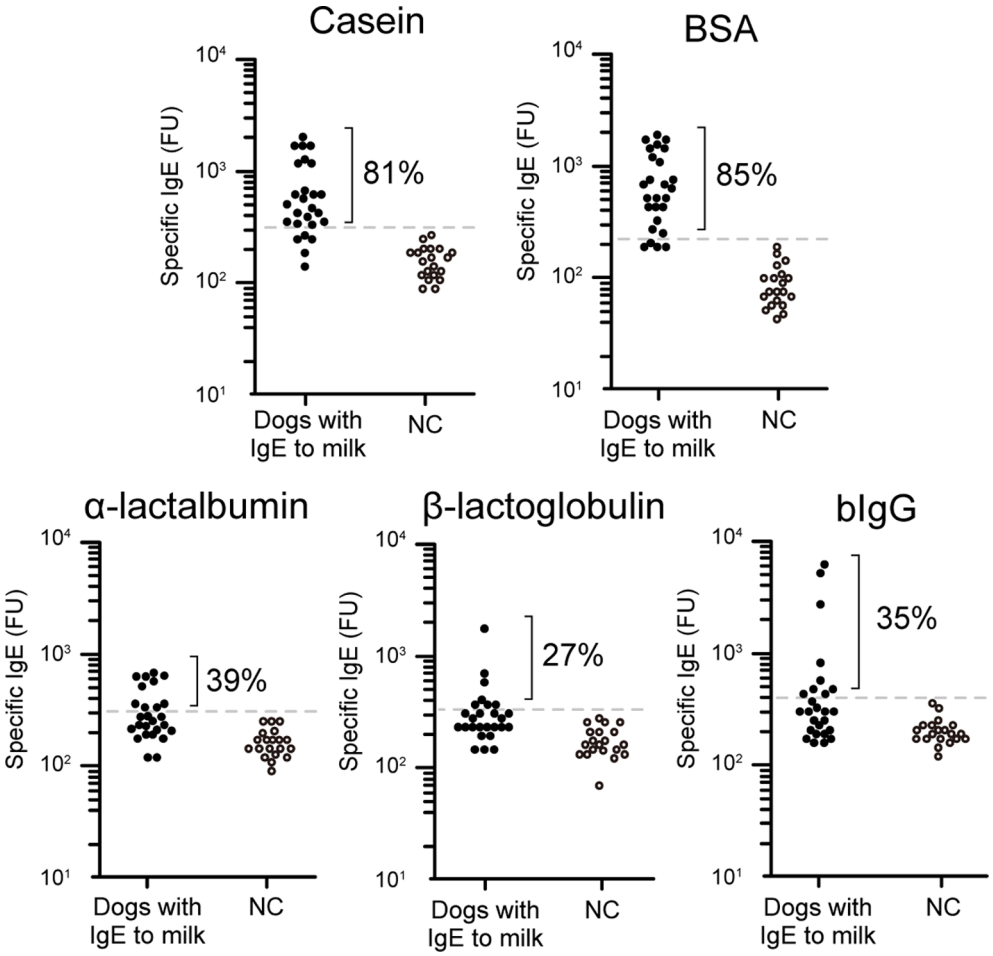

Fig. 2. IgE reactivity to components of milk. The number of dogs with IgE to crude milk: 26, negative control (NC): 20. The dashed lines indicated the cutoff values, which were calculated by the mean plus 3× standard deviation for the specific IgE to each component in NC. The cutoff values for casein, bovine serum albumin (BSA), α-lactalbumin, β-lactoglobulin, and bIgG were set at 323, 236, 304, 327, and 409 fluorescence units (FU), respectively.

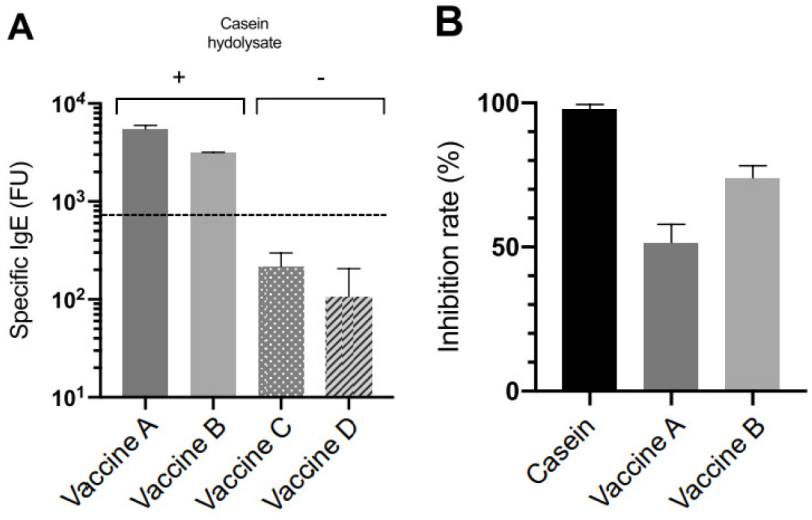

Fig. 3. Cross-reactivity between casein and vaccines containing casein hydrolysate. **(A)** Levels of specific IgE against two vaccines containing casein hydrolysate. Pooled serum with strong IgE reactivity to casein was used in this assay. The dashed lines indicated the cutoff value, which was calculated by the mean plus three times the standard deviation for the specific IgE in NC (738 fluorescence units (FU)). **(B)** Inhibition of the vaccines-specific IgE by casein inhibitor. Inhibition rate represented the reduction of IgE reactivity by the inhibitor.

the anamnesis of CAFRs caused by milk before the administration of the vaccine containing casein hydrolysate. Approximately 30% of dogs with CAFRs showed IgE reactivity to milk. This study suggested that casein and BSA in cow milk might be the most important allergic components in dogs. In addition, information of IgE reactivity to casein could be useful for the prediction of adverse reactions after vaccination.

POTENCIAL CONFLICT OF INTEREST. The authors have nothing to disclose.

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