Immunoglobulin G subclasses level to house dust mites and commensal microbes in healthy dogs and atopic dogs by an in-house ELISA

N. Khantavee¹, C. Chanthick², N. Saelim³, A. Tungtrongchitr³, N. Sookrung⁴, N. Prapasarakul*¹

¹Department of Microbiology, Faculty of Veterinary Science, Chulalongkorn University, Bangkok 10330, Thailand
²Dermatology Unit, Veterinary Teaching Hospital, Faculty of Veterinary Medicine, Kasetsart University, 50 Paholyothin Road, Ladyao, Chatuchak, Bangkok 10900, Thailand
³Department of Parasitology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok 10700, Thailand
⁴Department of Research and Development, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok 10700, Thailand
*Corresponding author: Nuvee.P@chula.ac.th

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Introduction
Atopic dermatitis (AD) is one of common skin diseases in dogs resulting in inflammatory and chronic-relapsing pruritic dermatitis. The common clinical appearance included pruritus and erythema, are often associated with IgE specific to the recent antigen proteins such as mites, ticks, dust, pollen, and microbes (5). Dermatophagoides farinae and Dermatophagoides pteronyssinus are common house dust mites in which to act as causative allergen in atopic dogs (3). Intradermal skin testing (IDST) and Allergen-specific IgE serology testing (ASIS) are the recent tools for specific allergic detection in dog patients. Allergen-specific IgE serology testing (ASIS) is more practical than intradermal testing; no need to concern about withdrawal times of anti-inflammatory drug and risk of anesthetic condition and expertise. Commercial ASIS still have limitation on variation of country specific allergens, time consuming, specificity and lack of some relevant antigens. Malassezia, pachydermatis and Staphylococcus. pseudintermedius can also induce allergen specific-IgE (1,7), but both microbes are not included in commercial ASIS. The level of canine IgG1 subclass are triggered by parasitic infestation, atopic dermatitis and allergen-specific immunotherapy (ASIT) (4). Therefore, a level of allergen-specific IgG subclasses may be able to identify allergen exposure or indicate causative allergens in canine AD. This study aimed to individually determine the different in level of allergen specific-IgG and IgG subclasses against common allergens; D. farinae, D. pteronyssinus, M. pachydermatis and S. pseudintermedius between healthy dogs and atopic dogs using in-housed ELISA assay.

Materials and Methods
Total of 22 sera derived from 11 healthy dogs and 11 atopic dogs that were screened by IDST showing house dust mites positive. All subjects were tentatively diagnosed by the authorized veterinarians using the approve criteria. For house dust mite, protein antigens of D. farinae (DF) and D. pteronyssinus (DP) were separately prepared by sonication and filtration. For microbes, protein antigens of M. pachydermatis (Mp) and S. pseudintermedius (Sp) were separately prepared by a mechanical disruption using glass bead and freeze-thaw assay at least 10 cycles. The protein concentration in supernatant was measured by Bradford assay (2). Each protein antigen was coated to 96 well-plate, 2 µg/well. The micro-well plate was blocking with buffer contained 1% BSA and 0.05% Tween 20 for 1 hour. Then serum samples were diluted 1:500 for house dust mite coating plate, however serum samples were diluted for microbial coating plate. Serum samples were incubated for 1 hour at 37°C. After washing, the plate was reacted to anti-dog IgG1-HRP, anti-dog IgG2-HRP or anti-dog IgG-HRP. ABTS® peroxidase substrate was finally reacted and measured OD intensity at 405nm.

Results and Discussion
In the sera dilution at 1:500, atopic dogs that positive to house dust mite had the significant increase of Df-IgG1 and Dp-IgG1 than those in healthy dogs (fig.1 and fig.2). Whilst, the levels of allergen-specific IgG and IgG2 against house dust mite showed no significant difference between atopic dogs and healthy dogs, except levels of Dg-IgG in atopic dogs (fig.2).
Fig. 1 Levels of *D. farinae*-specific IgG and their subclasses at sera dilution 1:500 between healthy dogs and house dust mite positive atopic dogs. On the other hand, levels of allergen-specific IgG and subclasses in atopic dog sera against *M. pachydermatis* and *S. pseudintermedius* had the significant higher than in healthy at 1:2,000 serum dilution (Fig 3 and 4).

Fig. 2 Levels of *D. pteronyssinus* -specific IgG and their subclasses at sera dilution 1:500 between healthy dogs and house dust mite positive atopic dogs

Fig. 3 Levels of *M. pachydermatis*-specific IgG and their subclasses at sera dilution 1:2,000 between healthy dogs and house dust mite positive atopic dogs

This study presents the difference level of total IgG and each IgG subclass specific to common external allergens; house dust mites and internal allergens; commensal microbes. The results represented the role of IgG subclass to allergic responses as an allergic surrogate instead of IgE. Interestingly, the high levels of house dust mite-specific IgG1 were relevant to house dust mite positive atopic dogs by IDT. Thus, house dust mite-specific IgG1 level could assume the allergic condition and explained immunopathological condition, whereas IgG2 level might not be triggered by external allergens. Our finding confirmed that level of IgG1 was associated with parasitism, atopic dermatitis and allergen-specific immunotherapy (ASIT) (4) which related to T-helper 2 response (8). On the other hand, the levels of total IgG, IgG1 and IgG2 to *M. pachydermatis* and *S. pseudintermedius* were significantly higher in atopic dogs than in healthy. Our in-house ELISA could successfully detect the cut-off value for differentiation between healthy and allergic dogs. It is interesting that IgG to commensal microbiota were also triggered during allergic episode and might play a role in immunological response. In general, IgG2 are a common subclass related to T-helper 1 responses (8) associated furunculosis, otitis externa, hypothyroidism and autoimmune haemolytic anemia (2), and T-helper 1 responses are required for killing microbial infections. By IgG level, our results proved that skin microbiota could induce both T-helper 1 and 2 population during allergic dermatitis. In conclusion, use of the in-house ELISA can measure the level of total IgG and their subclass, which related to IDST. The technique could be applied for specific allergen detection in atopic individual dogs in veterinary field.
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References