

## SOS-7 | Validity of the lymphocyte proliferation test for the diagnosis of canine food allergies with delayed reactions after oral food challenge

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In humans and animals, food allergies (FA) can be caused by an immunoglobulin (Ig)E-mediated mechanism, a cell-mediated pathogenesis, or both (mixed) pathways. Serological or blood tests have historically had little value to predict the diagnosis of FA in dogs, and patch testing and the lymphocyte activation test appear to have the highest concordance with oral food challenge (OFC). Herein, we report the validation of a lymphocyte proliferation test (LPT) for the diagnosis of dogs with FA and delayed reactions (>6h) after OFC. We selected 10 laboratory beagle dogs and 15 healthy dogs without history of digestive or cutaneous signs who came to veterinary clinics for routine procedures. We also evaluated 55 dogs with FA and detailed information on the timing of cutaneous or digestive flares after OFC. For the LPT, blood was collected in EDTA, and peripheral blood mononuclear cells (PBMCs) were separated, counted automatically, and cultured with 5 µg/mL of food allergen extracts before being counted again. Stimulation indices (SI) were then calculated (extract-stimulated cultures/negative controls cultures). Concanavalin A and saline served as positive and negative controls, respectively. Food allergen-specific IgE was quantified using the Pet Allergy Xplorer (PAX) (Nextmune). The SI cut-off point, calculated as the mean of the stimulated cells from beagle dogs +3 SDs was 2.9; SI ≥3.0 was thus considered positive, as defined in humans. The SI of the 10 beagles did not exceed 2.8 during 5 days of coculture. Likewise, a SI ≥3.0 was not seen in any of the 15 healthy dogs tested with 29 food allergen extracts, thus giving a specificity of the LPT of 100%. However, of the 55 dogs with FA, there were 44 (80%) with a flare occurring ≥6h after an OFC. Of these 44, LPT results were available in 28 dogs (64%). All of these dogs had at least one positive stimulation to a food item to which they reacted by OFC; the sensitivity of the LPT for the diagnosis of delayed canine FA was thus 100%. The LPT identified all food ingredients to which the dogs reacted by OFC in 18/28 dogs (64%) and only some of them in the other 10 dogs (36%). Altogether, the LPT identified correctly 51/68 (75%) food ingredients to which the dog had reacted by OFC. The most common food ingredients yielding positive LPT were chicken (50%), turkey (46%), beef (39%), pork (32%), corn (32%) and duck (32%). The PAX was negative in 18 of these 28 dogs (64%) and results overlapped with those of the LPT in three additional dogs (11%) suggesting a mixed FA mechanism in these dogs. In conclusion, delayed food reactions (≥6h) after OFC are the most common subset of FA in dogs, suggesting a cell-mediated pathogenesis that explains the reported poor performance of IgE tests for the diagnosis of canine FA. By contrast, the LPT appears to have excellent accuracy for the diagnosis of these delayed FA in dogs.

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