

Case report

Immediate hypersensitivity to leaf extracts of *Callisia fragrans* (inch plant) in a dog

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Abstract Contact with members of the plant family Commelinaceae, which includes wandering jew (*Commelina* spp. formerly called *Tradescantia* spp.) and inch plant (*Callisia fragrans*), can cause cell-mediated contact dermatitis in dogs. However, reports of canine IgE-mediated hypersensitivity to these plants have not been published. The purpose of this study was to discover whether IgE antibodies specific for extractable components of *C. fragrans* could be identified in serum from a dog that had anaphylactic shock after exposure to the plant and after skin patch testing with the sap from a leaf of *C. fragrans*. Separate aqueous extracts of leaves and flowers of *C. fragrans* were subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS–PAGE) and Western blotting. Serum from a dog with no history or symptoms of any allergies showed no specific IgE antibodies against the leaf extract. Serum from a dog with clinical symptoms of delayed, but not immediate hypersensitivity to leaf sap from *C. fragrans*, showed only minor IgE recognition of a single 65 k component in sap extracted from leaves harvested in summer but not in winter. However, IgE antibodies to a serum dilution of 1:200 specific for several components of the leaf extract were seen in serum from the dog that had anaphylactic shock after exposure to sap. The molecular weights of these molecules were in the range 51 k to 83 k. The bands on the immunoblots did not match with prominently stained protein bands in the gel, but instead identified molecules in a lightly stained area of the gel with diffuse bands. Testing for glycans indicated that the carbohydrate side chains of glycoproteins contributed significantly to the immunoreactivity of the putative allergens. All three dog sera failed to show any immunoreactivity against the extract from the flowers of *C. fragrans*.

INTRODUCTION

Plants of the family Commelinaceae are monocotyledonous succulent herbs that grow abundantly in tropical and subtropical climates.¹ Of the more than 500 species within this family, many are common house or garden plants. Some members of the family, such as *Commelina cyanea* (formerly *Tradescantia cyanea* with the common name ‘wandering jew’) and *Callisia fragrans* (also called inch or basket plant) (Fig. 1), are creeping, rapidly growing plants that form dense spreading blankets of leaves. They are considered to be weeds in eastern Australia because of their propensity to spread rapidly in natural bushland and agricultural lands.^{2,3} The leaves and stems of these plants snap readily, exuding a mucilaginous sap that can attach to the skin to give prolonged exposure to its components, thereby increasing the chance of sensitization. Confirmed contact allergy in dogs to members of the Commelinaceae family (*Commelina diffusa*, *Murdannia nudiflora* and *Tradescantia fluminensis*) has been reported.^{4,5} Although there have

been no published reports of canine IgE-mediated hypersensitivity towards these plants, an IgE-mediated sensitivity to *Tradescantia albiflora* has been reported in a 32-year-old woman⁶ who reacted with characteristic allergic symptoms immediately after handling indoor *Tradescantia* plants and was found to have strongly positive skin prick tests and specific IgE to extracts of leaves of *T. albiflora*. The purpose of this study was to discover whether or not IgE antibodies specific for extractable components of *C. fragrans* could be identified in the serum from a dog that had anaphylactic shock after exposure to the plant and after routine skin testing with the sap from a leaf of *C. fragrans*.

CASE REPORT

A 3-year-old Staffordshire bull terrier was presented as an urgent dermatological problem. The history included recurrent steroid-responsive papular dermatitis for the past 2 years. A previous episode of acute collapse, injected mucosal vessels, groaning with vomiting and abdominal discomfort that responded to pain relief and intravenous fluids had occurred 4 months earlier. An extensive investigation did not reveal the cause;

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Figure 1. Leaves and flowers of the inch plant, *Callisia fragrans*.



Figure 2. Abdomen and thorax of the case report subject, a 3-year-old Staffordshire bull terrier. Vesicular pustular lesions involving the ventro and ventro-lateral abdomen and thorax are visible.

however, vesicles were noticed on the tongue and lip mucosa and papules on the ventral skin. The day before referral, the dog had been bright, active and well and had been running through large areas of *C. fragrans* with another dog. The other dog was reported to be well and did not develop any clinical signs at the time of contact with the plant or for the next week. Soon after contact with the *C. fragrans*, the dog had again collapsed, was vomiting and was clearly unwell. Overnight, the owner had administered a cephalexin tablet and a cortisone/antibiotic eye ointment. Examination on presentation the next morning revealed a quiet dog



Figure 3. Skin patch test using sap of *Callisia fragrans* applied to a clipped area of unaffected neck skin on the subject Staffordshire bull terrier. At 18 h after application of the sap, the skin was pruritic, erythematous, swollen and exudative.

that was uninterested in its surroundings, reluctant to move and was vomiting. The mucosal vessels had a washed-out muddy dark colour, and refill time was slow. The skin had vesicopustular lesions involving the ventral and ventro-lateral abdomen and thorax (Fig. 2). Similar lesions were present on the lateral face, ventral chin, ears and limbs. Cytology of vesiculopustules predominantly showed neutrophils (some nontoxic) with squames, small numbers of acanthocytes and a few cocci. The dog was hospitalized and placed under close observation. Over a 6-h observation period, there was a gradual improvement. Biopsy of lesional skin was obtained under local anaesthesia, and a patch test with *C. fragrans* was performed by applying the sap of the plant to unaffected neck skin after clipping but without occlusion. The dog was then discharged with instructions to return the next morning to assess the patch test. At presentation 18 h later, the dog had again collapsed, was quiet and had vomited overnight. The skin at the patch test site was pruritic, erythematous, swollen and exudative, with central ulceration and necrosis of the surface and vesiculation around the periphery (Fig. 3). The skin test lesion was biopsied. Immediately thereafter, soluble dexamethasone sodium phosphate and a sterile normal saline fluid drip were administered intravenously and the patient monitored in the hospital. The patient became bright and active over the next hour and appeared normal by the time of discharge 7 h later. At discharge, the patch test exudation was drying up; the patch test and other skin lesions appeared less itchy and were not inducing any self-trauma. Prednisolone at 1 mg kg⁻¹ once a day for 5 days was dispensed for home treatment. Given the history of a severe collapse on two occasions after contact with the suspect plant and the response to application of the plant extract to clipped skin, the confirmed diagnosis was anaphylactic reaction and allergic dermatitis from contact with *C. fragrans*. The yard was cleared of all *C. fragrans* by earth-moving machinery and the area replanted

with grass. The dog has remained in the same house and yard for over 2 years with no return of clinical signs.

MATERIALS AND METHODS

Experimental subjects

Intravenous blood was obtained from three dogs that were selected for the *in vitro* part of this study: a normal control dog, a second control dog with delayed-type (cell-mediated) hypersensitivity to *C. fragrans* and the subject dog whose clinical response to the sap of *C. fragrans* was the stimulus for this investigation. Both control animals were 4-year-old desexed female Rottweilers. The normal control had no history of any allergies and no adverse response to contact with plants of the Commelinaceae family. The second control had previously developed erythematous vesicular and papular skin lesions on the ventral abdomen, which had been diagnosed as a delayed-type allergic reaction to *C. fragrans*, confirmed by a positive patch test at 48 h, resolution of clinical signs on isolation from the plant and return of lesions on re-exposure to the plant.

Histopathology

Routine histological processing was performed on the skin biopsies collected from the case subject at admission and from the skin patch test site 24 h after application of sap from *C. fragrans*.

Extraction of material from C. fragrans

Approximately 5 g of mature leaf or 2 g of whole flowers were chopped coarsely and placed in a mortar with acid-washed quartz sand (1 g). Liquid nitrogen was added, and the frozen plant material was ground to a fine, chalk-like powder. The powder was allowed to warm to about 4 °C in the mortar, 1 mL of extraction buffer (20 mmol L⁻¹ Tris-Cl pH 8.0, 5 mmol L⁻¹ EDTA, 0.05% SDS) was added and the mixture was ground again. The slurry was transferred to 1.5 mL microfuge tubes, spun at 10 000 g in a microfuge for 10 min and the supernatant collected. The approximate concentration of protein in the supernatant was estimated using the Bio-Rad Protein Assay method (Bio-Rad Lab. Inc., Hercules, CA, USA), with a standard curve set up using bovine serum albumin standards (Sigma, St Louis, MO, USA). The samples of extracted leaves or flowers were stored frozen at -20 °C.

Protein separation by SDS-PAGE

Proteins within the extracted plant material were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) using either 12% or 8% resolving and 4% stacking polyacrylamide gels in a Bio-Rad Mini-PROTEAN II electrophoresis apparatus. Leaf and flower extracts were diluted with 5× SDS-reducing sample buffer (60 mmol L⁻¹ Tris-Cl pH 6.8, 25% glycerol, 2% SDS, 710 mmol L⁻¹ β-mercaptoethanol, 0.1% bromophenol blue) and heated for 5 min at 95 °C before application to the gels. For gels that were

to be stained for protein, the extracts were diluted to give about 10 µg protein per lane. For a full-width gel to be used in subsequent blotting experiments, about 60 µg of protein was applied to a single wide well. Prestained molecular weight standards (either See Blue; Invitrogen, Carlsbad, CA, USA or Prestained Protein Ladder; Fermentas Inc., Hanover, MD, USA) were run in a narrow lane alongside the antigen extracts. Separation of protein bands was effected using constant 200 V and the recommended running buffer (25 mmol L⁻¹ Tris, 192 mmol L⁻¹ glycine, 0.1% SDS). After electrophoresis, the gels were either stained or used for immunoblotting. The gels for staining were fixed (45% methanol, 10% acetic acid) and subsequently stained with either Fast Blue stain (Fisher Biotec, West Perth, WA, Australia) or GelCode Blue Stain Reagent (Pierce, Rockford, IL, USA).

Western blotting

For immunoblotting, the separated proteins were transferred to nitrocellulose membrane (Bio-Rad Trans-Blot Transfer Medium, 0.45 µm) using a Bio-Rad miniblotting apparatus. The transfer conditions were 4 °C, constant 100 V with maximum current of 360 mA, time 2 h, transfer buffer composed of 25 mmol L⁻¹ Tris, 192 mmol L⁻¹ glycine and 20% v/v methanol. After transfer, the membrane was soaked for 2 h at 37 °C in blocking buffer (1% polyvinylpyrrolidone (PVP) (Sigma) in phosphate-buffered saline-Tween (PBS-T) (154 mmol L⁻¹ NaCl, 7.6 mmol L⁻¹ Na₂HPO₄, 2.6 mmol L⁻¹ NaH₂PO₄ and 0.05% Tween-20))⁷ then placed in an immunetics multilane blotting cassette. Blank lanes were filled with 320 µL of PBS-T/PVP. Each test serum was diluted in PBS-T/PVP, then 320 µL of each dilution was pipetted into the appropriate lane of the cassette. The apparatus was incubated at 37 °C for 2 h with intermittent tilting. Movement of tiny air bubbles in the lanes ensured individual mixing of the lane contents each time the plate was tilted. After incubation, the contents of the lanes were sucked out using a pipetter, the lanes were rinsed briefly by flushing cold tap water through all the channels, and then the plate was disassembled and the membrane was transferred to PBS-T and washed four times (8 min each). After draining off the wash solution, secondary antibody (Serotec mouse anticanine IgE, # MCA1895, reported to have no reactivity against dog IgM, IgA, IgG1 or IgG2; Serotec, Oxford, UK) was added (20 µL in 10 mL of PBS-T or approximately 20 µg antibody protein per 25 cm² membrane) and the blot incubated with rocking for 2 h. After washing in PBS-T (4 × 8 min) biotinylated goat antimouse IgG (H+L) (Jackson ImmunoResearch Laboratories, #115-065-146; JIRL Inc., West Grove, PA, USA) was added (20 µL in 10 mL PBS-T) and incubated for 2 h with rocking. This was rinsed off the membrane with 4 × 8-min washes in PBS-T before streptavidin-labelled peroxidase (Jackson ImmunoResearch Laboratories, # 016-030-084) (20 µL in 10 mL PBS-T) was added, and the membrane incubated with rocking for 2 h. After rinsing, the blot in 3 × 4 min PBS-T and 1 × 4 min PBS, Sigma FAST

DAB peroxidase substrate containing 0.03% nickel chloride enhancer was added. Development was terminated by washing the membrane thoroughly in deionized water.

Detection of glycoproteins

A kit (DIG glycan/protein double labelling kit 1500 783, Boehringer Mannheim, Mannheim, Germany) for staining glycosylated proteins by immunoassay was used to distinguish glycans present in the extract of leaves from *C. fragrans*. The extract was separated as usual on a polyacrylamide gel and transferred to nitrocellulose membrane. Mild oxidation with sodium periodate (10 mmol L^{-1} periodate in pH 5.5 sodium acetate) then oxidized the sugar side chain hydroxyl groups to aldehydes, which were coupled to a digoxigenin (DIG) hapten. The introduced haptens were detected with anti-DIG conjugated to horseradish peroxidase, which reacts with a precipitating peroxidase substrate (4-triethylenetrioxyl-naphthol (TETON)/ H_2O_2) to give a blue precipitate.

Significance of glycans for immunoreactivity

To test whether or not glycoproteins were involved in the immunoreactivity, the transferred proteins on a strip of nitrocellulose membrane were subjected to oxidation to destroy the glycan structures prior to standard immunoblotting procedures. An unblocked nitrocellulose strip of the transferred leaf extract proteins was incubated at room temperature for 2 h in the dark in a solution of 50 mmol L^{-1} sodium periodate made up in 10 mmol L^{-1} sodium acetate buffer pH 5.0. The oxidation reaction was stopped by the addition of sorbitol (final concentration 15 mmol L^{-1}).⁸ The membrane was then washed in PBS-T before being placed in the usual blocking solution (PBST/PVP) and taken through the standard blotting steps. An adjacent strip off the nitrocellulose membrane was not treated with sodium periodate, but was taken through the standard blotting procedures in the same container and solutions as the periodate-treated strip, to ensure that both strips received identical treatment apart from the oxidation step.

RESULTS

Skin histopathology of the case subject

The histopathology of both the natural skin lesions and the patch test were similar but with less acute reaction present in samples of pre-existing papular lesional skin that was obtained at admission. The natural skin lesion had focal superficial coagulative necrosis and elsewhere mild parakeratosis with diffuse eosinophilic spongiosis. The superficial dermis was oedematous with a cellular infiltrate of eosinophils, mast cells and lymphocytes. The superficial vessels had marked eosinophil margination and eosinophils in the vessel wall with occasional thrombi formation. The mid-dermal vessels were dilated with eosinophil margination (Fig. 4a,b). The biopsy of the patch test after 24 h showed eosinophilic necrosis of the upper epidermis,

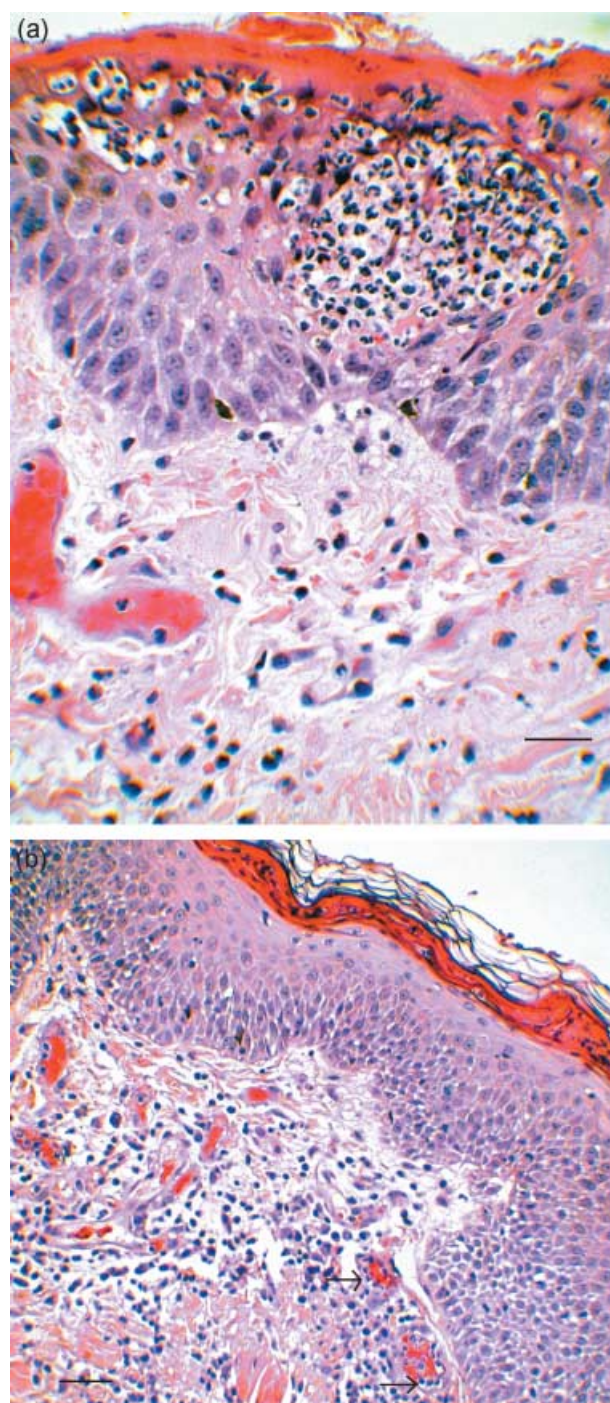


Figure 4. Histology of papule of pre-existing skin lesions on the case subject, taken on admission (a) eosinophilic pustule and (b) surface hyperkeratosis, spongiosis and hyperplasia; arrows indicate eosinophils in the blood vessel wall. Stain: H&E; bar = $50 \mu\text{m}$.

micro pustules of immature eosinophils and mild hyperplasia with spongiosis. The superficial dermis was oedematous with a diffuse cellular infiltration of lymphocytes mast cells and eosinophils. The dermal vessels were dilated with eosinophils marginating and accumulating in the vessel walls with thrombosis. Mid-dermal vessels were mildly dilated with eosinophil margination (Fig. 5). The skin histopathology suggests an acute immunological reaction at the sites of both the natural skin lesions and patch test.

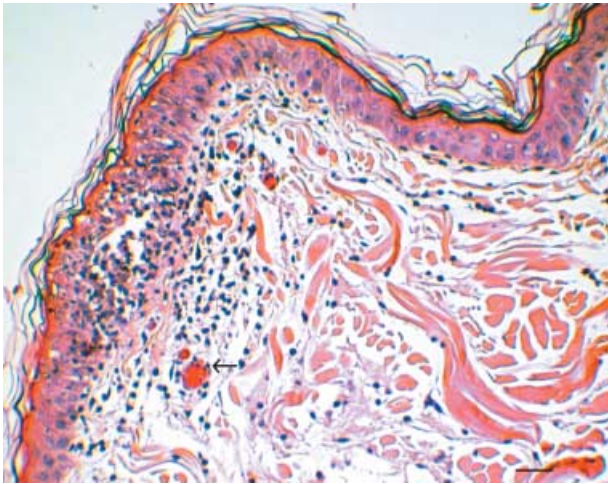


Figure 5. Histology of the patch test site of the case subject, 24 h after application of sap from a leaf of *Callisia fragrans*. Mild hyperplasia, formation of a microabscess with immature eosinophils and lymphocytes. The arrow points to eosinophils migrating into the dermal vessel wall. Stain: H&E; bar = 50 µm.

Protein separation by SDS-PAGE

The amount of protein extracted into aqueous buffer solution containing 0.05% SDS was greater from *C. fragrans* flowers ($2\text{--}3\text{ }\mu\text{g }\mu\text{L}^{-1}$) than from the leaves (from less than $0.1\text{ }\mu\text{g }\mu\text{L}^{-1}$ up to $0.5\text{ }\mu\text{g }\mu\text{L}^{-1}$). The stained gels of the separated proteins from leaf and flower extracts of *C. fragrans* (Fig. 6) showed multiple light bands in both extracts up to about MW 88 k and a small number of more prominent bands. A band at approximate MW 72 k appeared in both extracts, but other darker staining bands differed in MW between the two extracts. The leaf extracts showed variation in stainable bands after SDS-PAGE, depending on when the leaves were harvested (Fig. 7). Leaves harvested in late summer (lane 1) had the highest concentration of extractable protein and after SDS-PAGE showed bands with MW up to about 98 k and two darkly staining bands at MW 44 k and 46 k. Plants harvested in late autumn (lane 2) had a lower concentration of extractable proteins; lightly staining bands could be seen up to approximate MW 98 k, and a darkly staining band occurred at about 48 k. In the extract from leaves harvested in winter (lane 3), it was difficult to discern any bands above MW 70 k, and the most prominent band was at about MW 56 k. In general, all of the extracts produced many bands that stained poorly and diffusely.

Western blotting

A stained gel and the corresponding immunoblot have been aligned to show the relationship between proteins stained in the gel of *C. fragrans* leaf extract (Fig. 8a) and those that were detected by the dog IgE antibodies (Fig. 8b). The reactive dog serum (lanes 1–3) had IgE antibodies that detected molecules in the MW range 62 k to 80 k, even at a 1:200 dilution (lane 3). In this blot, particularly prominent bands were at approximate MWs of 80, 78, 72, 65 and 62 k. The immunoreactive bands at 62, 72, 78 and 80 k appeared to match stained

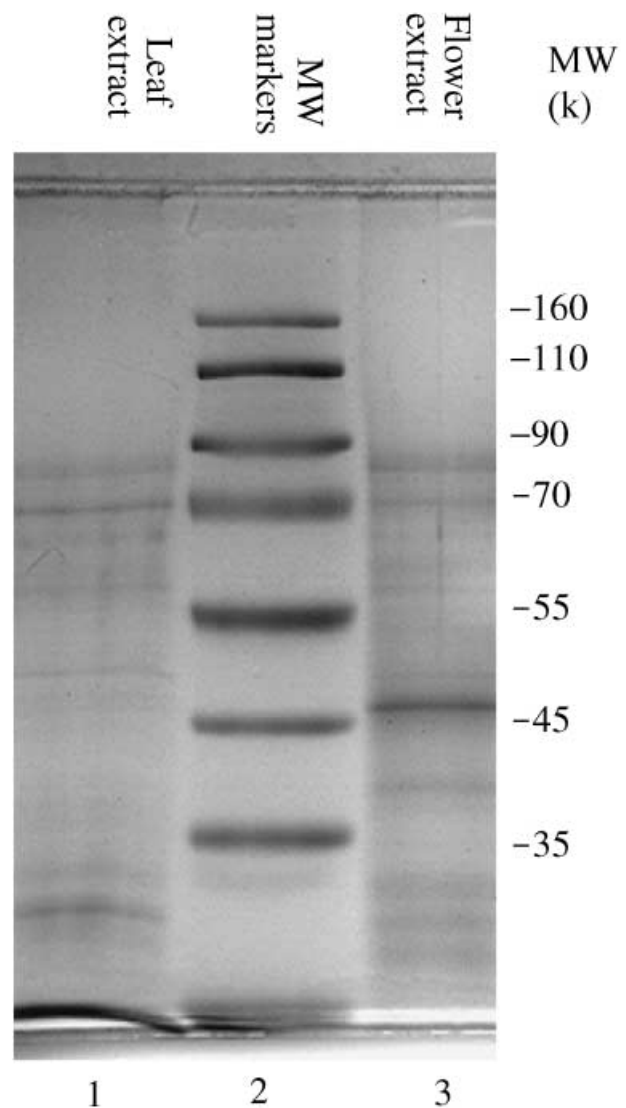


Figure 6. Comparison of protein bands in aqueous extracts of leaves (lane 1) and flowers (lane 3) from the plant *Callisia fragrans*, separated in a polyacrylamide gel. Reducing SDS-PAGE, 8% separating gel, stained with Fast Blue stain (Fisher Biotec, Australia).

protein bands that were relatively prominent in the matching gel, but protein bands at 65, 69 or 76 k were not obvious. Neither one of the other dog sera tested (labelled N and DH) showed any significant reactivity with the *C. fragrans* leaf proteins (normal dog serum lanes 6–9 and serum from a dog known to have delayed hypersensitivity to sap of *C. fragrans* in lanes 11–13) apart from a faint band at MW 65 k in the 1:50 serum dilution of the latter dog (lane 11). Some faint bands occurred in the blots even in the lanes containing no dog serum (e.g. in blank lanes 4, 5, 9 and 10 of Fig. 8 at MW 50 k). These probably reflect the presence of molecules in the plant material that react with one of the solutions used in the blotting procedure, such as streptavidin or peroxidase substrate. Some variation in the MW of the immunoreactive bands occurred, depending on when the leaves used in the gels and blots were harvested. Figure 9 shows clear immunoreactive bands at approximate MWs of 83, 72,

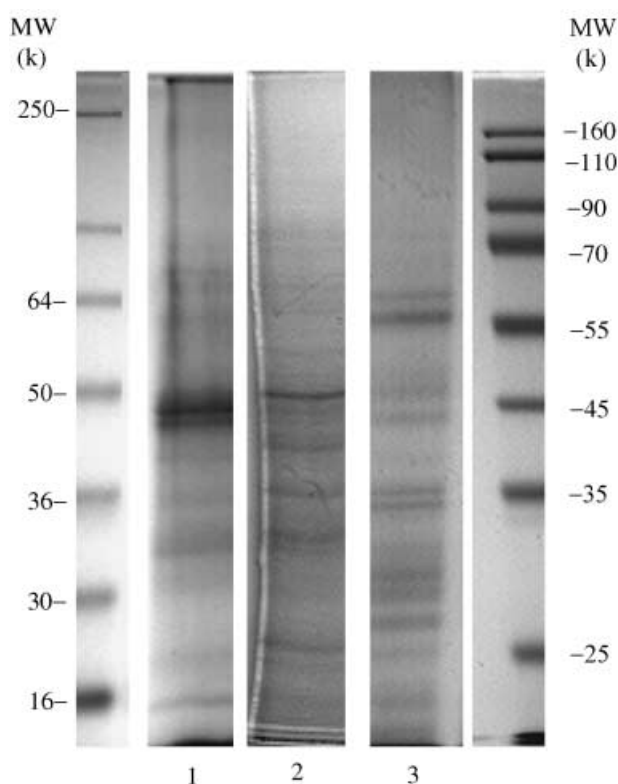


Figure 7. Variation in expression of proteins in aqueous extracts of leaves of *Callisia fragrans* harvested at different times of the year. Reducing SDS-PAGE; 12% separating gel, Fast Blue stain (Fisher Biotech, Australia). MW markers either Invitrogen SeeBlue or Fermentas Prestained Protein Ladder.
Lane 1: late summer leaf extract.
Lane 2: late autumn leaf extract.
Lane 3: mid winter leaf extract.

70, 62, 58, 53, 51 k and a lower MW band at 35 k for a blot performed on leaves harvested in late winter. The molecular weights of these bands, while in the same general range as the immunoreactive bands detected in the immunoblot shown in Fig. 8, were slightly different in estimated MW, and no reactivity at MW 65 k was detected in serum from the dog with delayed hypersensitivity. The gel strip in the blot in Fig. 9 was cut off one edge of the whole gel prior to the transfer step of immunoblotting and shows only very faintly stained protein bands indicative of a low protein concentration. Despite the low concentration of proteins in the extract, the presence of many specific IgE molecules in the serum is confirmed by the bands that developed in lanes 2 and 3, corresponding to a 1:50 and a 1:100 dilution of the serum from the reactive dog. Serum from the normal dog and the dog susceptible to delayed hypersensitivity to *C. fragrans* again showed no IgE recognition of the separated leaf proteins (lanes 5, 6, 8 and 9 in Fig. 9). A band at apparent MW of 30 k appeared in all lanes, including the blank lanes to which no dog serum was added, was relatively prominent in the stained gel. This band probably corresponds to a protein that reacts with one of the components used in the immunoblotting procedure; its reactivity was no greater in the lanes to which dog serum was added, and it is considered immunologically irrelevant.

Detection of glycoproteins

To test whether any of the immunoreactive bands were glycoproteins, proteins were separated as usual in polyacrylamide gels and transferred to nitrocellulose membrane that was subdivided into several strips. One strip was stained for the presence of glycans. The second strip was subjected to treatment with periodate and to oxidize the carbohydrate side chains, and then this treated strip and a third untreated strip underwent the standard blotting procedure. Figure 10 lane 1 shows that there were three glycans that gave well-resolved bands at approximate MWs of 83, 62 and 58 k. These prominent glycans appeared to correspond with immunoreactive bands, although they matched with the less prominent of those bands detected by the reactive dog's serum IgE antibodies (lanes 3, 4 and 5). Apart from the few well-resolved bands, the glycan stain produced diffuse dark staining above approximate MW 55 k extending up to the top of the gel. As a test for the specificity of this staining method produced appropriate positive and negative control results (i.e. three glycan bands for the glycosylated protein fetuin and no staining of the nonglycosylated protein creatinase) (results not shown), this diffuse staining is believed to indicate the presence of glycans in this region even though individual glycan bands could not be distinguished. The periodate treatment of the transferred proteins (lane 2) substantially reduced their immunoreactivity for most of the bands normally detected in the immunoblots (contrast lanes 2 and 3 in Fig. 10).

The three dog sera were also tested for IgE reactivity against the extract of *C. fragrans* flowers. None of the sera showed any specific recognition of bands in the flower extract.

DISCUSSION

The *in vitro* isolation of specific serum IgE to extracts from leaves of *C. fragrans* supports the clinical diagnosis of anaphylactic collapse from exposure to *C. fragrans*, which occurred when the case subject ran through a large patch of these succulent plants. The reaction to the rudimentary patch test may have been facilitated by lacerations to the epidermis from clipping of the skin site. Unfortunately, the site was not observed for an immediate reaction. It was covered immediately by a bandage as the test was performed late in the day while the owners waited to take the dog home. An intradermal prick test was considered as a means of confirming immediate-type hypersensitivity, but such a test posed considerable and unjustifiable dangers for the patient. Thus, confirmation of *C. fragrans*-specific serum IgE via *in vitro* testing was considered the safest and appropriate method in this dog. That the dog has been free of skin lesions and has not collapsed again for a follow-up period of over 2 years after removal of the plant from its environment argues against an alternative cause of the collapse and skin lesions.

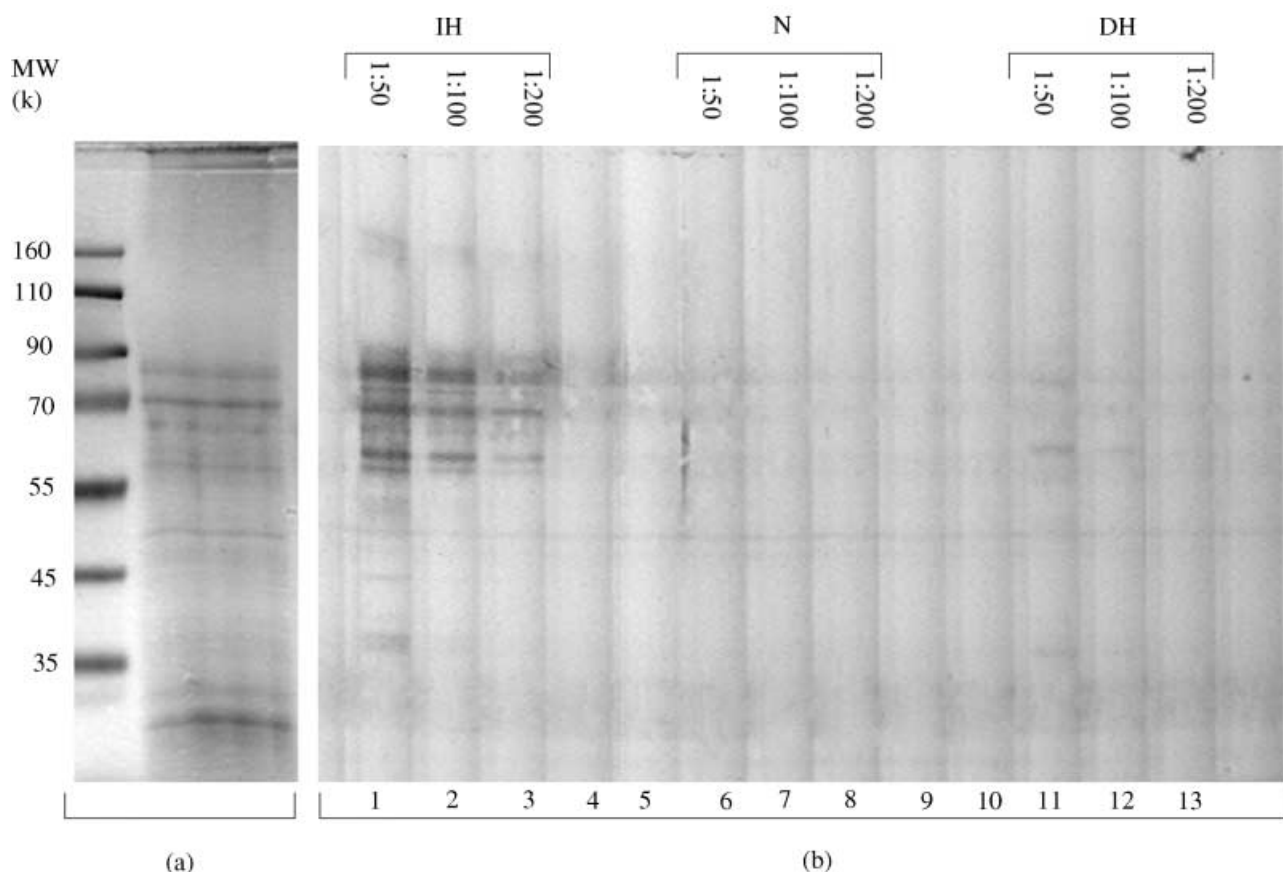


Figure 8. Results of SDS-PAGE (a) and Western blotting (b) of proteins extracted from leaves of *Callisia fragrans* (summer harvest) and tested against sera from three dogs with different clinical histories.

(a) Reducing SDS-PAGE, 8% separating gel, stained with Fast Blue stain (Fisher Biotech, Australia).

(b) Immunoblot: primary antibody IgE in dilutions of dog sera, detected with mouse anticanine IgE, biotinylated goat antimouse IgG, streptavidin-horseradish peroxidase and peroxidase substrate (Sigma FAST DAB with nickel enhancement).

Each dog serum was diluted 1:50, 1:100 and 1:200 for testing.

Lanes 1–3: serum from a dog with apparent immediate hypersensitivity to *Callisia fragrans* (IH).

Lanes 6–9: serum from a dog with no history or symptoms of either immediate or delayed hypersensitivity to any plants or insects (N).

Lanes 11–13: serum from a dog with delayed hypersensitivity to *Callisia fragrans* (DH).

There is a difference in the histopathological description of the disease skin lesions and patch test between the case reported here and the canine with contact allergy to the closely related *T. fluminensis* reported by Kunkle and Gross.⁵ The patch test to *Tradescantia* was reported as a lymphocyte and macrophage reaction with spongiotic vesiculation. Eosinophils were not mentioned. However, there appear to be both eosinophil vessel margination and eosinophils in the dermis around the vessel in the accompanying published photomicrograph. This difference could be explained by a timing difference of the biopsy or that the eosinophils were not considered significant. In the experience of the second author (KVM), eosinophils are prominent in all contact allergy lesions and test sites of animals allergic to plants of the Commelinaceae family, of which *Tradescantia* spp. and *C. fragrans* are members. This case report raises the issue of whether wandering jaw contact allergic reactions are a cellular type IV immune-mediated pathogenesis or a type I IgE-mediated skin contact allergic immunopathogenesis. Further studies of such cases may determine whether the commonly held hypothesis⁹ that these are a type IV immunopatho-

genesis is correct. More recent research has revealed that a transcutaneous type I reaction is possible in the dog.^{10,11} The gross skin lesions and histopathology, including the patch test reaction, are similar to those described in a review of canine atopic dermatitis¹⁰ and in the report by Olivry *et al.*¹¹ Interestingly, the histopathology in the Staffordshire bull terrier in the present report is similar to the three cases of canine contact allergy involving three different members of the family Commelinaceae described by Marsella *et al.*⁴ These authors commented that contact allergic dermatitis with a type IV immunopathogenesis may not be distinct from contact irritant dermatitis. The Staffordshire bull terrier case reported here raises the issue of whether canine contact allergy is distinct from canine atopic dermatitis. Additionally, the control dog, known to exhibit a delayed hypersensitivity reaction after contact with *C. fragrans* plants, was found to have mild serum IgE reactivity against a single component of the sap from leaves harvested in summer. This finding further emphasizes that in practice, types I and IV responses do not occur in isolation. Specific clinical signs may result from a predominantly type I or IV

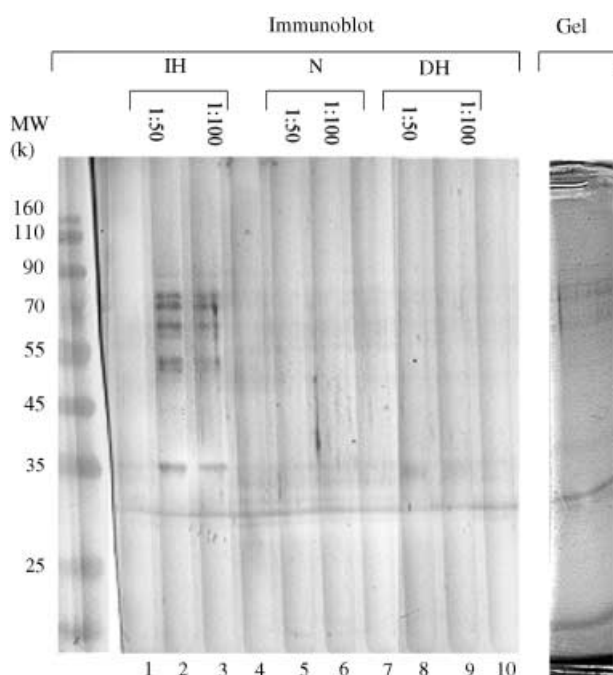


Figure 9. Results of an immunoblot to test for dog serum IgE recognition of components of extracts from *Callisia fragrans* leaves, harvested in late winter. The gel strip shows the extremely small amount of stainable protein present in this extract, but the blot shows distinct bands (lanes 2 and 3).

Lanes 2,3: serum from a dog with apparent immediate hypersensitivity to *Callisia fragrans* (IH).

Lanes 5,6: serum from a dog with no history or symptoms of either immediate or delayed hypersensitivity to any plants or insects (N). Lanes 8,9: serum from a dog with delayed hypersensitivity to *Callisia fragrans* (DH).

Reducing SDS-PAGE, 12% separating gel stained with Fast Blue stain (Fisher Biotec, Australia) and GelCode Stain (Pierce). The gel strip was excised from the large gel prior to the transfer step in the immunoblotting procedure.

Immunoblot details as given in Figure 8 except that the dog sera were diluted 1:50 and 1:100 only.

reaction, but this does not preclude concurrent involvement of both antibody and cell-mediated responses. The finding of low antigen concentrations in winter-growing versus. summer-growing plants could explain the seasonality of allergic reactions observed in clinical practice by the second author (KVM).

One of the characteristics of plants from the Commelinaceae family is the brittle nature of their stems and leaves, which readily exude a watery sap when damaged. Irritant effects as a result of contact with the sap have been linked to the presence of raphides, which are bundles of narrow, elongated needle-shaped calcium oxalate crystals.¹² In plants of this family, raphides are usually associated with mucilage and fibrillar material in the plants.¹³ Although such crystals might cause an irritant effect after contact with these plants¹⁴ and could facilitate the entry of sap components into the skin from an abrasive effect, calcium oxalate crystals alone could not account for the development of the T-cell-mediated contact dermatitis described in the three dogs after contact with members of the family Commelinaceae.⁴ In this study, a normal control and two

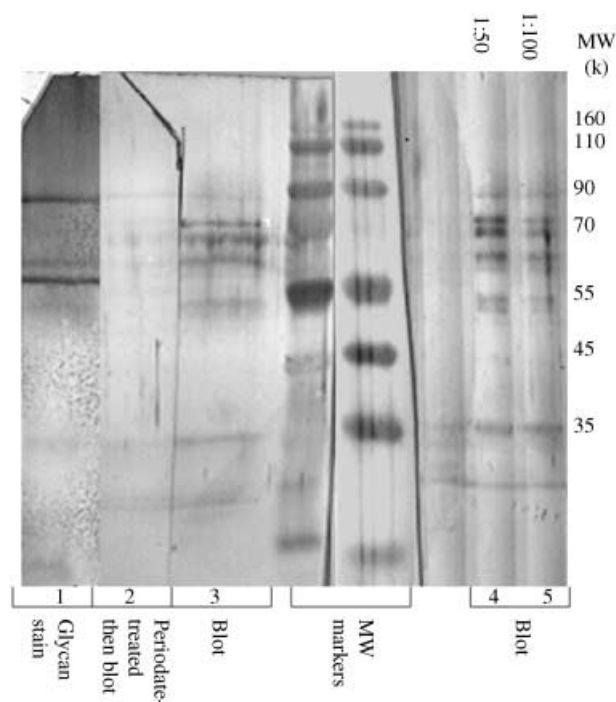


Figure 10. Glycan detection in extracts of leaves of *Callisia fragrans* and evidence of their involvement in immunoreactivity.

The extract was separated in a 12% polyacrylamide gel and transferred to nitrocellulose membrane. One strip of this membrane was tested for the presence of glycans (lane 1). Adjacent strips of this membrane were either used in a standard immunoblot to detect specific IgE antibodies in dog serum (lane 3) (procedure detailed in Figure 8) or first treated with sodium periodate to oxidize the glycans before being taken through an identical blotting procedure (lane 2). The gel and blot were set up at the same time as the blot illustrated in Figure 9, part of which is reproduced here (lanes 4 and 5).

dogs diagnosed with different types of hypersensitivity to *C. fragrans* were also investigated. As expected from the clinical observations, the normal control had no detectable specific IgE antibodies against components of leaf or flower extract of *C. fragrans*, and the dog showing delayed contact dermatitis had only slight IgE reactivity against a leaf sap component. However, serum from the subject dog of this report, which had anaphylactic shock after a rudimentary patch skin test with sap from *C. fragrans*, had IgE antibodies specific for several components of the leaf extract although not for extract of the flowers. Although many plant allergens are known, most of the bands detected in this case were at higher MW than those of the most commonly reported plant allergens that range from 6 k up to about 60 k.¹⁵ The immunoreactive bands detected were quite clear, yet bands of protein at matching MW were generally not readily discerned within the stained gels. The reagents used to stain the gels were Fast Blue stain, which is a modified form of Coomassie Blue R-250, and GelCode Blue Stain, which utilizes Coomassie G-250. Binding by Coomassie stains is reported to be associated with the presence of basic amino acids, primarily lysine, arginine and histidine, and the amount of dye bound is approximately proportional to the number of positive charges found on the protein.^{16,17} One

explanation for the faint bands in the stained gel may be that these proteins have relatively low numbers of basic amino acids. An alternative explanation is that there is indeed a very small amount of protein in the bands and that the important immunoreactive parts of these molecules are not the protein cores but are abundant attached carbohydrate moieties. Several characteristics of the plant extract and its behaviour on gels and blots support the latter suggestion. The mucilaginous nature of the *C. fragrans* leaf sap implies the presence of polysaccharides, and it is known that polysaccharide allergens of mucilaginous components of sap from various plants, including vegetable gums such as arabic, guar and psyllium, induce IgE-mediated hypersensitive reactions.¹⁸ The smeary, unsharp bands after electrophoretic separation, as occurred with the gels of *C. fragrans* leaf extracts, are associated with the presence of glycoproteins with high carbohydrate content.⁸ The variation in MW of detected immunoreactive bands depending on time of leaf harvest implied a change in molecular structure, such as the extent of protein glycosylation, related to season. The synthesis and degradation of many vegetative storage proteins of herbaceous perennials are known to be seasonally regulated¹⁹ with mobilization of nutrients during autumn to persistent subterranean structures, which accumulate low MW and polymeric reserves.²⁰ It is known that variable glycosylation of plant proteins occurs²¹ and that the same plant protein (such as peroxidase) can have distinct isoforms that have different N-linked glycans and consequently different MW.²¹ The 'laddering' effect of bands seen on the immunoblots could have resulted from differently glycosylated forms of the same core protein having slightly different MW. Variable glycosylation could explain the shifts in MW with time of leaf harvest. The significant loss of immunoreactivity on the blots after treatment with periodate supports the suggestion that in this case the carbohydrate side chains were significant participants in the reaction with the IgE antibodies. If the amino acids serine and threonine are important components of the allergen epitopes, periodate treatment could have caused loss of reactivity, but the overall characteristics of the gels do not favour this conclusion. Allergies to plant glycoproteins are well documented, with fucosyl and xylosyl residues on the allergens being particularly important in binding IgE antibodies.^{15,22–25} Complex glycans are also known to have high immunogenicity,^{26,27} and carbohydrate-reactive IgE antibodies may cross-react with glycan structures from other allergen sources.¹⁵ The potential for cross-reactivity may have implications for the management of a dog exhibiting immediate hypersensitivity as in the study reported here.

Pollen is often implicated in allergic responses, but in this case, there was no detectable recognition of proteins extracted from *C. fragrans* flowers. As these plants flower only briefly and with little pollen¹ sensitization to plant components is more likely to occur from contact with the sap released from injured leaves. The stained gels of the flower extract and leaf extract had a

similar MW range of bands, but none of those detected in the flower extract were found to be immunoreactive. Expression of allergens can be confined to specific parts of a plant. For example, an 18 kDa protein of dandelions, with homologies in amino acid composition to several allergenic proteins, is exclusive to the dandelion roots and stem tissue.²⁸ The flowers of *C. fragrans* may either lack the relevant allergens altogether or, if present, may be at too low a concentration or insufficiently glycosylated to be detected by the specific IgE in the dog serum.

This study is the first published report of a dog with immediate type hypersensitivity to components of sap of a plant from the family Commelinaceae. The specific serum IgE from this dog detected several molecules with a range of MW from about 51 to 83 k, and the carbohydrate moieties on most of these molecules appeared to be significant in this immunoreactivity. The likelihood that IgE antibodies directed against glycans may cross-react with glycan structures from other allergen sources has implications for the clinical management. Investigations are continuing to learn more about the molecules being detected by this serum. The demonstration of immediate hypersensitivity to *C. fragrans* also suggests that clinicians should consider the possibility of both types I and IV reactions to plants from the family Commelinaceae.

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Résumé Un contact avec une plante de la famille des Commelinaceae, qui inclut le wandering jew (*Commelina* spp. Auparavant appelée *Tradescantia* spp.) et l'Inch (*Callisia fragrans*), peut provoquer une dermatite de contact à médiation cellulaire chez le chien. Cependant, des descriptions d'hypersensibilité médiée par les IgE à ces plantes n'ont pas été publiées. Le but de cette étude était de déterminer s'il était possible de démontrer des IgE spécifiques à des composants de *C. fragrans* dans le sérum de chiens ayant présenté un choc anaphylactique après exposition à la plante, et après test épicutané à un extrait de *C. fragrans*. Des extraits séparés de feuilles et de fleurs de *C. fragrans* ont été analysés par SDS-PAGE et Western blotting. Le sérum d'un chien sans histoire ou signe d'allergie n'a pas montré d'IgE contre les extraits de feuilles. Le sérum d'un chien avec des signes cliniques de réaction retardée, mais pas de réaction immédiate, à un extrait de feuille de *C. fragrans* a montré seulement une réaction mineure par les IgE à un composant de 65k retrouvé dans les extraits obtenus en été mais pas en hiver. Toutefois, des anticorps IgE ont été retrouvés à une dilution sérique de 1:200 pour différents composants des feuilles, pour tous les chiens ayant eu des réactions anaphylactiques. Les poids moléculaires de ces molécules variaient de 51k à 83k. Les bandes de l'immunoblot ne coïncidaient pas avec les bandes de protéines colorées dans le gel, mais plutôt avec des molécules identifiées dans une zone peu marquée du gel. En testant pour les glycans, la chaîne carbohydrate des glycoprotéines contribuait de façon importante à l'immunoréactivité des allergènes suspects. Les trois séras des chiens n'ont pas montré de réactivité aux extraits de fleurs.

Resumen El contacto directo con plantas de la familia *Commelinaceae*, que incluye el judío errante (*Commelina* spp. previamente llamada *Tradescantia* spp.) y *Callisia fragrans* pueden causar dermatitis de contacto en perros. Sin embargo, casos de hipersensibilidad a estas plantas mediada por inmunoglobulina E (IgE) no han sido publicados. El propósito de este estudio fue descubrir si IgEs específicas para extractos de *C. fragrans* podían identificarse en el suero de un perro que tuvo un choque anafilático tras ser expuesto a la planta y tras una prueba de parche en la piel con la savia de la hoja de *C. fragrans*. Extractos acuosos separados de la hoja y de las flores de *C. fragrans* fueron sometidos a la prueba de SDS-PAGE (electroforesis en gel de poliacrilamida con tampón SDS) y a Western blotting. El suero de un perro sin historia ni signos de alergia no presentó inmunoglobulinas E específicas contra el extracto de las hojas. El suero de un perro con signos clínicos de hipersensibilidad retardada, pero no inmediata, a la savia de la hoja de *C. fragrans* demostró solo mínimo reconocimiento con IgE específica para un componente de 65kD de peso molecular, en savia extraída de hojas en verano, pero no en invierno. Sin embargo, en el perro con choque anafilático se observaron anticuerpos IgE a una dilución de suero de 1/200 específicos para varios componentes de la savia de la hoja. Los pesos moleculares de esas moléculas estuvieron

entre 51kD y 83kD. Las bandas tras la transferencia a la membrana no correspondieron con bandas de proteína teñidas con intensidad en el gel, sino que identificaron moléculas escasamente teñidas en el área del gel con bandas difusas. La prueba de glicosilación indicó que las cadenas laterales de carbohidratos de las glicoproteínas contribuían significativamente a la inmunoreactividad de los posibles alérgenos. Los sueros de estos tres perros no presentaron inmunoreactividad al extracto de las flores de *C. fragrans*.

Zusammenfassung Der Kontakt mit Mitgliedern der Pflanzenfamilie der Commelinaceae, zu der die Tagblume (*Commelina* spp, vormalig *Tradescantia* spp. genannt) und die Callisie (*Callisia fragrans*) gehören, können beim Hund eine zellvermittelte Kontaktdermatitis verursachen. Berichte über eine canine IgE-medierte Hypersensibilität zu diesen Pflanzen ist jedoch noch nicht publiziert worden. Das Ziel dieser Studie war es festzustellen, ob IgE Antikörper spezifisch auf extrahierbare Komponenten von *C. fragrans* im Serum eines Hundes bestimmt werden konnten, welcher einen anaphylaktischen Schock erlitten hatte, nachdem er der Pflanze ausgesetzt war und nach einem Haut Patch Test mit dem Saft eines Blattes von *C. fragrans*. Einzelne wasserlösliche Extrakte von Blättern und Blumen von *C. fragrans* wurden SDS-PAGE und Western Blotting unterworfen. Das Serum von einem Hund ohne der Anamnese oder Symptomen von irgendwelchen Allergien zeigte keine IgE Antikörper, die spezifisch auf den Blattextrakt waren. Im Serum eines Hundes mit klinischen Symptomen einer Überempfindlichkeitsreaktion vom verzögerten, jedoch nicht vom Sofort-Typ auf Blattsaft von *C. fragrans*, erkannten IgE Antikörper schwach eine einzige 65k Komponente im Saftextrakt von Blättern, die im Sommer geerntet worden waren, aber nicht von Blättern, die im Winter geerntet worden waren. Allerdings wurden IgE Antikörper auf einige Komponenten des Blattextraktes bis zu einer Serumverdünnung von 1:200 im Serum jenes Hundes gesehen, welcher einen anaphylaktischen Schock gezeigt hatte, nachdem er dem Pflanzensaft ausgesetzt worden war. Die Molekulargewichte dieser Bestandteile waren im Bereich von 51k bis 83k. Die Banden der Immunoblots korrelierten nicht mit den deutlich gefärbten Proteinbanden des Gels, sondern identifizierten Moleküle an einer schwach gefärbten Stelle des Gels mit diffusen Banden. Die Untersuchung auf Glykane ergab, dass die Kohlenhydrat-Seitenketten der Glykoproteine signifikant zur Immunreaktion der vermeintlichen Allergene beitrugen. Keines der drei Hundeseren zeigte eine Immunreaktion auf den Blumenextrakt von *C. fragrans*.