Acta Paediatrica

WILEY

ACTA PÆDIATRICA
NURTURING THE CHILD

ORIGINAL ARTICLE OPEN ACCESS

Negligible Allergen Presence in Hospital Dogs After Washing

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Received: 9 February 2025 | **Revised:** 11 April 2025 | **Accepted:** 15 April 2025

Funding: This work was supported by Samariten Foundation, Gillberg Foundation, Crown Princess Lovisa's Fund, Agria/Swedish Kennel Club's Research Fund, Children's Cancer Fund, ALF Funds and Josephine Neiman Foundation.

Keywords: allergens | animal assisted activity | children | dog therapy | hospital | Hospital Dog

ABSTRACT

Aim: Allergies are a potential risk when introducing dogs in health care. Therefore, I chose to evaluate if allergen levels in Hospital Dogs can be reduced by washing the dogs and thereby develop patient-safe guidelines when implicating Hospital Dogs in Swedish hospitals.

Methods: Samples ($n = 29$) were taken from the dog's fur before and after washing and after the Hospital Dog/child meeting to investigate change in allergens. Further samples were taken when washing with two different shampoos, measuring how long decreased levels of allergens lasted. The dog allergens Can f 1 and Can f 4 were analysed by competitive ELISA.

Results: The mean allergen concentration of Can f 1 decreased from 21.8 ± 11.2 ng/mL before wash to 5.8 ± 3.7 ng/mL ($p < 0.0001$) after wash. Both types of shampoo resulted in a reduction of Can f 1 and Can f 4 and remained low for three days.

Conclusion: By washing the Hospital Dogs with shampoo, we have found a significant reduction of the dog allergen Can f 1 and Can f 4 lasting for at least three days. In the work of introducing certified Hospital Dogs into Swedish hospitals, which so far has not been possible in Sweden, this is an important finding.

1 | Introduction

1.1 | Complementary Treatment for Children in Hospitals

Hospitalisation for children can be a stressful, fearful, and anxiety-provoking experience [1]. Studies have displayed that complementary treatment involving play can reduce stress and anxiety, as well as benefit children when encountering times of illness and hospitalisation [2]. Various methods, such as visits from hospital clowns, music therapy, computer games and watching TV shows, can have anxiety-relieving effects on children [3, 4].

Animal-assisted activity (AAA) is another complementary treatment that has positive effects on children in hospitals, affirmed by several studies in other countries [5–7]. AAA with dogs has a pain-relieving impact on children [5], and children have reported positive effects such as feeling calmer and brighter, gaining better self-confidence and having a more positive experience of the hospital stay when given the opportunity to meet a Hospital Dog [8].

Abbreviations: AAA, Animal-assisted activity; AAII, Animal Assisted Intervention International Standards of Practice.

Summary

- This study was needed to show that it is possible to reduce allergen excretion to even lower levels in hypoallergenic dogs.
- By washing the Hospital Dog with shampoo, we have found a significant reduction of the dog allergens Can f 1 and Can f 4.
- This is an important finding to enable the implication in Sweden of Hospital Dogs, a safe, effective, side effect-free, cost-effective, complementary treatment.

Hospital-based therapy dogs in Canada must meet certain criteria [9]. The Animal Assisted Intervention International Standards of Practice (AAII) are standards developed to benefit and encourage those interested in working with this form of therapy abroad. The AAII includes information on the dog's temperament, training and handling, preparation and socialisation, and also the dog's well-being and health [10]. For health settings in Sweden, Clinical guidelines and Regulations for dogs in healthcare, except hospitals, are published by the National Board of Health and Welfare [11]. This document can benefit the introduction of dogs in those settings. However, it has not been possible to let a dog, now named Hospital Dog (Definition see Figure 1), work inside Swedish hospitals before scientific patient-safe guidelines were produced, of which this study is a part. This research has been necessary because of the strict guidelines in Sweden regarding health hygiene and allergen excretion.

Dogs are a common cause of allergies in the population. The allergic reaction is triggered by various substances, known as allergens [11, 12]. A total of seven allergens in dogs are known, Can f 1–7. These allergens are found in the dog's fur, dander, saliva and urine [13–17]. Allergens are found in all kinds of dogs even such called hypo-allergenic dogs [17]. Allergies in humans are individual, and humans' allergic reactions differ between the different dog allergens Can f 1–7. Can f 1 is a well-researched allergen that commonly causes allergic reactions in humans [13–15].

Introducing dog therapy in Swedish paediatric hospitals by giving the sick children the opportunity to meet Hospital Dogs would be a very beneficial complementary treatment for the children. Still, it must be done without harming others in paediatric healthcare. Allergies are a potential risk when introducing dogs in healthcare. Few studies have investigated how allergen levels are affected by washing dogs.

Definition of Hospital Dog®

A Hospital Dog® is a dog that meets the following criteria:
Non-shedding: The dog does not shed hair.
Puppy tested: Evaluated for suitability from a young age.
BPH tested: Has completed the Beteende- och Personlighetsbeskrivning Hund (BPH) assessment.
Suitability tested: Assessed for suitability for hospital work.
Regular veterinary checks: Undergoes regular veterinary testing according to a specially designed protocol.
Vaccinated: Basic vaccinations including rabies and leptospirosis.
Allergen tested: Tested for allergens, so-called hypoallergenic dog.
Bacteria cultured: Regular bacterial cultures to exclude multiresistant bacteria.
Well cared for: Maintained in excellent health and condition.
Washed: Washed with schampoo at least once a week.
Trained: Trained to follow specific behaviors, such as not licking.
Guideline adherence: Cared for according to scientifically developed guidelines from a research project.
Certified: Certified by "Sjukhushund i Sverige AB".
Quality assured: Regularly checked by "Sjukhushund I Sverige".
A Hospital Dog® is always accompanied by a well-trained hospital dog instructor.
It is rarely the dog owner who works with the Hospital Dog®, ensuring professional handling and care.

FIGURE 1 | Definition of Hospital Dog.

2 | Aim

The first aim was to investigate whether the presence of allergens Can f 1 in Hospital Dogs can be reduced by washing the dog with a special shampoo. A second aim was to examine whether an ordinary dog shampoo has a similar effect in reducing allergens and assess for how long the level of Can f 1 and Can f 4 remains reduced.

3 | Method

3.1 | Study Design

This study was part of a larger project, 'Effects of Dogs in Paediatric Healthcare', where Hospital Dogs' impact on ill children has been studied.

The allergen study had a descriptive, prospective study with a quantitative design and was performed in two parts. In the first part, allergen samples, so-called brush samples, were taken from a Hospital Dog, Dog 1, before and after washing with a special shampoo and after meeting with a child (Figure 2). The second part was performed with another Hospital Dog, Dog 2, which was washed with two different shampoos. The sampling in part two was performed for one day, before and after the wash, and followed up for 7 days with brush samples.

3.2 | Study Population

The study population in the allergen study was the two Hospital dogs, Dog 1 and Dog 2. In the main study, where the effects of the Hospital Dog on the child were studied, 50 children were given informed consent and included in the meeting with Dog 1. Of these, 29 meetings between dog and child were included in the allergen study. Twenty meetings were excluded because no allergen samples were taken from the dog before meeting these patients, and one was excluded due to an incorrect sample. The study was conducted in a paediatric ward at Uppsala University Hospital. Children aged 3–18 years with various diagnoses participated in treatment sessions with the Hospital Dog, Dog 1. In the second part of the supplement of the study when two different shampoos were tried, the Hospital Dog was Dog 2. The sampling of allergens in study part two did not involve any meetings between the dog and children.

FIGURE 2 | Conducting a brush sample.

3.3 | Procedure

In the first part, levels of Can f 1 were examined, considering it is the main allergen causing allergenic reactions in humans [13] and partly because of the time when the first part of the study was performed, it was the only allergen possible to analyse. The special shampoo, Shampoo A, used in the first part of the study with a Hospital Dog, Dog 1, was developed by researchers in fur animal allergy and allergens, as well as experts on animal skin. The properties of the shampoo are intended to reduce the level of the dog's allergen through the content of promoting ingredients for the dog's skin that have a calming effect on the skin [18]. Allergen samples were taken from the dog before and after washing with the special shampoo, and after each Hospital Dog/child meeting in the study. The dog was washed with the special shampoo before each meeting with the 29 different children in the study. The washing of the dog was done in a specially built dog shower located adjacent to the room in the hospital where the Hospital Dog stays when not working.

The second and supplementary part of the study was conducted once Can f 4 could be measured. A Hospital Dog, Dog 2, was washed with two different shampoos, the special shampoo, Shampoo A, and a regular shampoo, Shampoo B. Allergen sampling for Can f 1 and Can f 4 was carried out for 7 days.

All allergen samples from the Hospital Dog's fur were taken from the neck region using a special brush and a sampling template developed by Medi-Tec Research & Development AB, Stockholm, Sweden. The sampling area was 35 × 30 mm. Brushing of the fur was done along the fur strands, from the hair roots and skin, up to the top of the fur. All layers of fur were brushed through in different directions, and the brush was spun/rotated continuously. A total of 30 brush strokes were performed, 1 stroke per second for 30 s. The brush was then placed in a test tube that is part of the sampling material [19].

3.4 | Analysis Method

Fur samples were prepared by extraction in 1 mL PBS (phosphate-buffered saline) for 1 h. The concentration of the dog's allergen Can f 1 from the sample material was measured using inhibition-ELISA. Monoclonal anti-Can f 1 was added to a 96-well MaxiSorp plate (Thermo Fisher Scientific) coated with goat anti-mouse IgG (Jackson Immunoresearch, Ely, UK). To generate a sensitive assay, the biotinylated allergens were diluted to a suitable concentration. Samples in duplicate and competing biotinylated recombinant Can f 1–2 to 4–6 were added to the wells for two hours. For detection of bound biotin-labelled antigen, the plates were incubated with streptavidin-horseradish peroxidase (Jackson Immuno research) followed by incubation with the substrate 3,3′,5,5′-Tetramethylbenzidine (Sigma–Aldrich). The reaction was stopped with 0.5 M sulfuric acid, and the final absorbance was measured at 450 nm. Concentration was determined against a reference curve obtained with recombinant allergen concentrations ranging from 2.5 to 320 ng/mL. Recombinant (r) Can f 1 and (r) Can f 1–2 to 4–6 with biotinylating labelling were produced as previously described according to Konieczny et al. 1997 and Nilsson et al. 2014 [12, 20].

Monoclonal anti-Can f 1 antibody was a gift from Medi-Tec Research and Development Stockholm AB, Sweden.

3.5 | Statistical Analyses

A descriptive method was used to calculate means, ±standard deviation (SD), and minimum and maximum values. Changes in allergen concentration were calculated with repeated measurements ANOVA. A $p < 0.05$ was considered statistically significant.

4 | Results

The concentration of Can f 1 was analysed in brush samples ($n=29$) before washing, after washing, and after meeting with the patient. The mean concentration of Can f 1 decreased from 21.8 ± 11.2 to 5.8 ± 3.7 ng/mL ($p < 0.0001$) after washing the Hospital Dog, and this reduction remained after the patient meeting at 7.9 ± 4.1 ng/mL, $p < 0.0001$ (Figure 3). There was a variation in the concentration of Can f 1 in the Hospital Dog (Dog 1) during the 29 different study occasions, but all washings of the Hospital Dog showed a reduction in the concentration of Can f 1 (Figures 3 and 4).

In Table 1, Can f 1 levels are compared to the original value before shampoo followed by 7-day follow-up in nanograms/ml and change in % with arrows ↓ showing decrease and arrow ↑ showing increase due to the original value (Table 1) and in Table 2 Allergen levels of Can f4 are compared to the original value before shampoo followed by 7-day follow-up in nanograms/mL and change in % with arrows ↓ showing decrease and arrow ↑ showing increase due to the original value (Table 2).

The concentration of Can f 1 (Figure 5) and Can f 4 (Figure 6) was analysed from brush samples taken before washing and after washing the same Hospital Dog, Dog 2, with different shampoos (A and B). Both types of shampoo resulted in a reduction of Can f 1 (Figure 5) and Can f 4 (Figure 6) and remained low for three days.

5 | Discussion

The first part of this study investigated whether washing with a special shampoo reduces the allergen concentration in the fur of a Hospital Dog, which even before washing has low allergen levels because it is hypoallergenic (Figure 1, Definition of Hospital Dog). Washing was performed before 29 different Hospital Dog/child meetings. A significant reduction ($p < 0.0001$) in the allergen concentration of Can f 1 in the Hospital Dog's fur was revealed after washing with a special shampoo. After the meetings, the reduction persisted; however, in some cases, there was a slight increase in allergen concentration compared to the samples taken immediately after washing. The mean concentration of Can f 1 after washing was 5.76 ng/mL and after meeting with

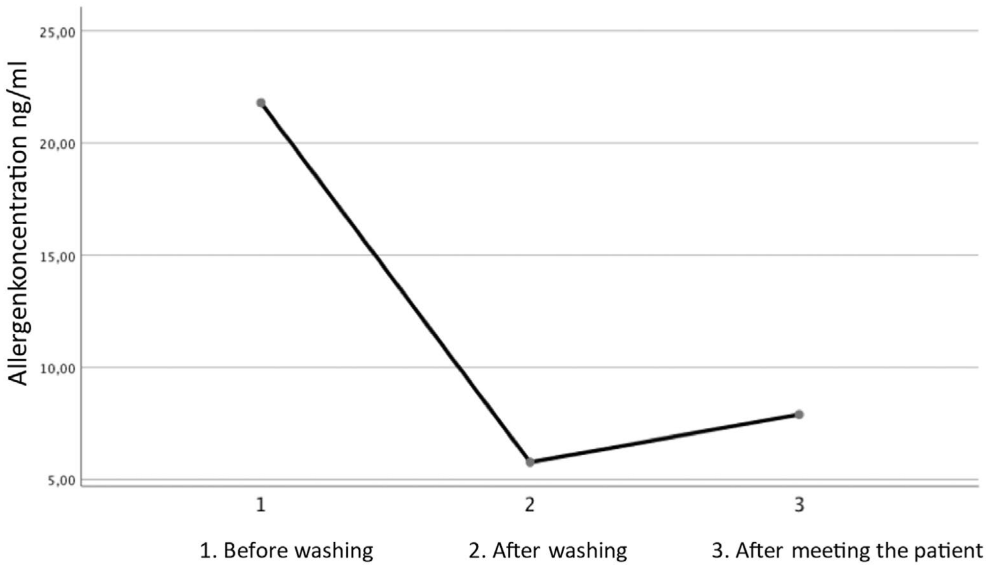

FIGURE 3 | Average change in allergen Can f 1 concentration. The mean values are given in ng/mL.

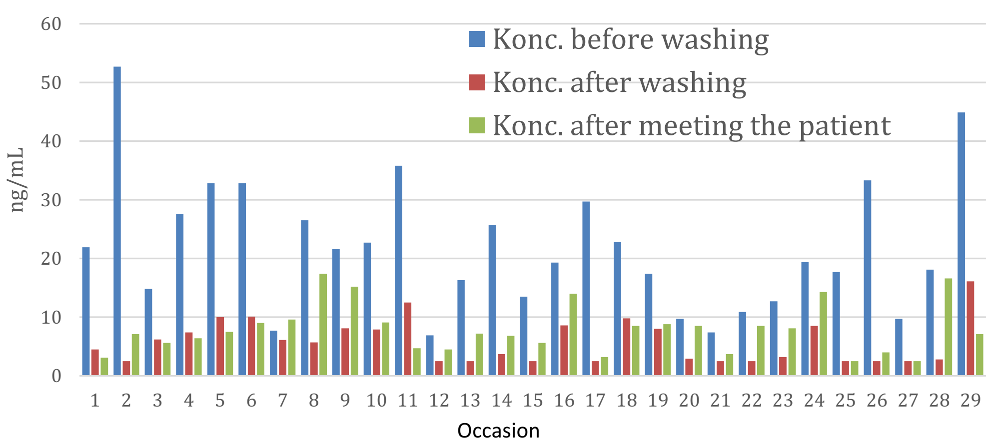

FIGURE 4 | Overview of allergen variation Can f 1 concentration at all 29 study occasions. The mean values are given in ng/mL.

TABLE 1 | The table shows different levels of allergen Can f 1 before wash (PreS) and 1–7 days after wash (PostS) with two different shampoos (A and B). The Hospital dog was unwashed a week before the study started. Mean values are given in ng/mL, and change in % is counted, arrows ↓ showing decrease and arrow ↑ showing increase due to original value.

Can f 1 (2,5–320 ng/mL)	Shampoo A		Shampoo B	
		Change in % from original value		Change in % from original value
Day 0 Pre shampoo original value (PreS)	42		13	
Day 0 day Post shampoo (PostS)	2.5	94% ↓	2.5	81% ↓
Day 1 PostS	7	83% ↓	2.5	81% ↓
Day 2 PostS	24	43% ↓	8	38% ↓
Day 3 PostS	21	50% ↓	30	131% ↑
Day 4 PostS	14	67% ↓	233	1692% ↑
Day 5 PostS	21	50% ↓	22	69% ↑
Day 6 PostS	168	300% ↑	53	308% ↑
Day 7 PostS	34	19% ↓	7	46% ↓

TABLE 2 | The table shows different levels of allergen Can f 4 before wash (PreS) and 1–7 days after wash (PostS) with two different shampoos (A and B). The Hospital Dog was unwashed a week before the study started. Mean values are given in ng/mL and change in % is counted, arrows ↓ indicate a decrease and arrow ↑ indicates an increase due to the original value.

Can f 4 (2,5–320 ng/mL)	Shampoo A		Shampoo B	
		Change in % from original value		Change in % from original value
Day 0 Pre shampoo original value (PreS)	23		38	
Day 0 day post shampoo (PostS)	2.5	81% ↓	2.5	93% ↓
Day 1 PostS	2.5	81% ↓	2.5	93% ↓
Day 2 PostS	2.5	81% ↓	2.5	93% ↓
Day 3 PostS	6	79% ↓	56	47% ↑
Day 4 PostS	9	71% ↓	44	16% ↑
Day 5 PostS	4	3,8% ↓	26	32% ↓
Day 6 PostS	13	131% ↓	43	13% ↑
Day 7 PostS	30	54% ↑	39	9% ↑

the patient 7.9 ng/mL, while it was 21.8 ng/mL before washing. Whether and for how long the reduction of Can f 1 lasts was not investigated in the first part of the study but was studied in the second and supplementary study where two shampoos were tried. The quantity of allergen in the fur was followed up over 7 days. After washing with the special shampoo Allergenius, Can f 1 persisted low over 7 days and Can f 4 over 4 days. After washing with the regular shampoo Pink, Can f 1 persisted low over 5 days and Can f 4 over 3 days.

Our findings are consistent with what has been seen in previous studies where the effect on allergen concentration of washing dogs has been studied [21, 22]. In these studies, the allergen concentration of Can f 1 also decreased after washing. In the study performed by Hodson et al., the change in Can f 1 levels was investigated by washing dogs with shampoo and warm water (40°C–50°C). In the study, the effect of vacuuming the dog was also investigated, which did not have a significant effect on reducing allergen levels. In the same study, 16 dogs were washed with shampoo, and a significant reduction in Can f 1 in the dog's fur was demonstrated. The first day after washing, there was a 98.7% reduction in Can f 1. The effect was followed for 7 days, showing that the concentration of Can f 1 then increased. The shampoo used in this study is not specified [21]. Whiteman et al. used Allergenius Dog, special shampoo, which also was used in our study, to wash 21 dogs. They found an effective reduction in the concentration of Can f 1 [22]. Similarly, in our study, a significant

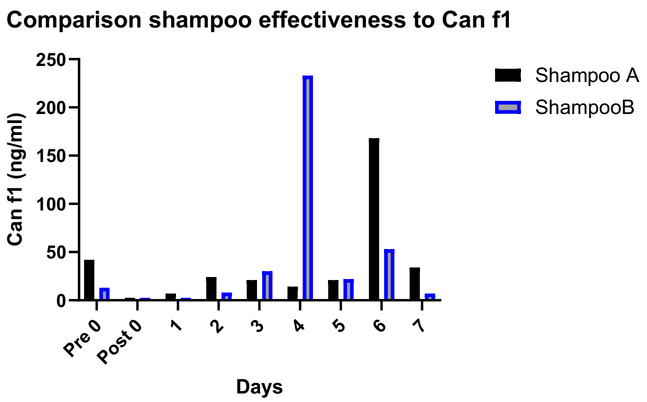

FIGURE 5 | The figure shows different levels of allergen Can f 1 before wash (PreS) and 1–7 days after wash (PostS) with two different shampoos (A and B). The Hospital Dog was unwashed a week before the study started. The mean values are given in ng/mL.

reduction in Can f 1 and Can f 4 concentration appeared after washing the dog with Can f 1 at 94% and Can f 4, 81%.

The allergen analysed in the first part of this study is Can f 1. This allergen was analysed since it is considered the main allergen in dogs, and 50%–70% of those allergic to dogs react to this allergen [14]. Further, when the first of the study was performed, there was no method available to analyse the other

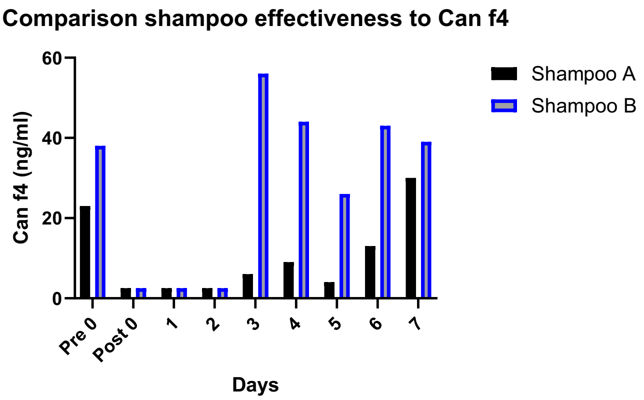

FIGURE 6 | The figure shows different levels of allergen Can f 4 before wash (PreS) and 1–7 days after wash (PostS) with two different shampoos (A and B). The Hospital Dog was unwashed a week before the study started. The mean values are given in ng/mL.

allergens in dogs. In the second and supplemental part of the study, Can f 1 as well as Can f 4 were analysed due to being the most common allergens in the fur. Though the sample in study part two is small, the result indicates that allergen levels in fur can be kept low for days up to almost one week with the special shampoo and that a regular shampoo also reduces allergen levels even if it lasts for fewer days. Wintersand et al. investigated in a small sample how dogs' allergens were airborne. The allergens Can f 1, 2, 3, 4 and 6 were tested. Can f 1, 4 and 6 were all found to be airborne, and Can f 2 and 3 were solely in dogs' saliva and concentrated in the dog's mouth [23].

Considering these results, the risk of environmental contamination with allergens from fur can likely be mitigated by washing the dog. And if the dog is trained not to lick patients, the risk of spreading allergens from the saliva can be further reduced [10, 11]. Can f 5 was not analysed because we only used female dogs and Can f 5 is only found in male dogs [24].

It is important to know the basic levels of Hospital Dog's allergens, and it is a crucial factor to wash the Hospital Dog to reduce levels of allergens since non-allergenic dogs do not exist [17]. Reduction of allergen levels is an important finding in the ongoing efforts to establish dog therapy with Hospital Dogs as a safe complementary treatment for children in Swedish hospitals. The risk of triggering unwanted allergic reactions is reduced when a Hospital Dog is washed with shampoo, and no allergic reactions among the participants, including children, parents, and staff, have been noticed during the study.

In conclusion, our study demonstrates a significant reduction in the dog's allergen Can f 1 and Can f 4 by washing the Hospital Dogs with shampoo at least once a week (Figure 1, Definition of Hospital Dog). Also, the results indicate that the levels of allergens Can f 1 and Can f 4 can be kept low for some days up to one week depending on what shampoo is used.

Author Contributions

C. Wretman: investigation, writing – original draft, writing – review and editing, visualization, data curation, formal analysis. **A. Risberg:** writing – review and editing, writing – original draft, supervision, data curation. **H. Grönlund:** writing – review and editing, methodology, validation, resources. **A. Edner:** conceptualization, funding acquisition, methodology, validation, visualization, writing – review and editing, project administration, resources, supervision.

Acknowledgements

The authors would like to thank the children and adolescents for participating in the entire project and acknowledge the role of the trained Hospital Dogs, the dog instructors, and R.N. Maria Lindström Nilsson.

Ethics Statement

The study is ethically approved by the EPN, Uppsala. Diary number 2014-167.

Conflicts of Interest

The authors declare no conflicts of interest.

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