

Mite and mite allergen removal during machine washing of laundry

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Background: Few studies have investigated live house dust mite and mite allergen removal from clothing and bedding by washing machines. No studies have investigated the transfer of mites from infested to uninfested clothing and bedding during washing.

Objective: The purpose of this study was to wash different types of clothing and bedding in residential washing machines to determine the live *Dermatophagoides farinae* mite and allergen removal and the mite transfer from mite-infested items to mite-free items.

Methods: Clothing and bedding items were machine washed in 6- and 8-lb loads in warm (36°C-38°C) or cold (22°C-27°C) water with and without recommended concentrations of laundry detergent and sodium hypochlorite bleach. Live mites and allergen present in washed versus unwashed and washed mite-infested versus washed mite-free items were compared.

Results: Washing clothing and bedding in water alone, detergent, or detergent plus bleach removed 60% to 83% of the live mites. Washing removed more mites from some items than from others. When mite-infested items were washed with identical sets of mite-free items, many live mites were transferred to the previously mite-free items. Overall, 84% of Der f 1 was removed from items washed in water alone or in detergent and 98% from items washed in detergent plus bleach.

Conclusions: Washing clothing and bedding in cold or warm water with detergent or detergent plus bleach removed most allergen and a significant ($P < .05$) portion of live mites.

Repeated washing is required to further reduce mite levels.

Live mites were transferred from mite-infested to mite-free items during washing. (J Allergy Clin Immunol 2003;111:1269-73.)

Key words: Dust mites, bleach, sodium hypochlorite, washing, *Dermatophagoides farinae*, allergen, detergent, laundry

House dust mites are among the most important sources of indoor allergens worldwide. These allergens are present perennially and induce allergic rhinitis, atopic dermatitis, and asthma in mite-sensitive individuals. Avoidance of house dust mite allergens can be an effective strategy in reducing allergic asthma and rhinitis for mite-sensitive individuals.¹⁻⁴ Reducing exposure to mites

and their allergens in the indoor environment is recommended to prevent sensitization to house dust mite allergens and the induction of allergic reactions.⁵

Mites and their allergens are found in bedding and on clothing. Mite allergen levels in bedding have been associated with the severity of asthma.⁶ Mites that contaminate clothing might be dispersed, enabling them to contaminate and colonize other new areas.⁷ Likewise, clothing might be a vehicle for transporting and dispersing mite allergens.

Recommendations for killing mites include washing clothing and bedding weekly in hot water (55°C [130°F]).⁵ However, most wash loads in the United States are done in warm or cold water and not in hot water. Laboratory studies that simulated washing by using confined mites showed that bathing mites in warm water containing laundry detergents killed a significant portion of mites.^{8,9} Several studies have shown that acaricides can be added to wash water to kill mites during washing.¹⁰⁻¹² Washing clothing and bedding with detergent at 25°C removes most allergens.^{13,14}

Few studies have investigated mite and allergen removal from clothing and bedding by using washing machines, warm or cold water, and actual clothing and bedding.^{10,11,14} No studies have investigated mite transfer from mite-infested clothing to identical noninfested clothing. Likewise, there have been no studies on the effects that different fibers and clothing types have on mite removal or transfer during washing. Therefore the purpose of this study was to wash different types of clothing and bedding in warm and cold water, with and without detergent and sodium hypochlorite bleach, in residential washing machines to determine the mite and mite allergen removal, as well as the mite transfer from infested to clean clothing.

METHODS

Mite source

The house dust mites used for these experiments were obtained from pure thriving laboratory cultures of *Dermatophagoides farinae* maintained at room temperature (21°C-22°C) and 75% relative humidity.

Test clothing and bedding items

The intention of these experiments was to simulate wash loads that might occur in homes. Often they are mixed loads containing clothing and bedding of varying construction that are made up of various types of fibers (eg, cotton and polyester). Therefore a sufficient number of identical clothing and bedding items were purchased so that within each experiment, 3 identical loads (replicates) containing identical mixtures of clothing and bedding items could be tested for each set of washing parameters. Identical loads were

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also prepared to serve as unwashed control items. All of the items were washed once before testing to remove the sizing. (All new fabrics are treated with starch [sizing]).

Infesting clothing and bedding with mites

For all experiments, clothing and bedding items were equilibrated at approximately 75% relative humidity for 24 hours or longer to humidify the fabrics and to prevent desiccation of the mites after the fabrics were inoculated. Each clothing and bedding item was inoculated with approximately 10,000 live *D farinae* mites (all life stages) plus culture medium. Shirts were inoculated on the inside backs and then buttoned closed, if applicable. Each item was folded loosely and then turned over and patted lightly so that the mites were distributed onto the inside fronts or tops of the items. For every experiment and replicate, each item was folded identically, stacked in the same order inside a hamper, and maintained at approximately 75% relative humidity for 7 days to allow the mites time to disperse throughout the fabrics as if they were in a hamper.

Washing of clothing and bedding items

The transfer of mites between articles and the removal of mites and their allergens were examined by using (1) commercially available powdered detergent (Tide) at the recommended concentration (62.1 g/load), (2) powdered detergent (62.1 g/load) plus liquid sodium hypochlorite (Clorox Bleach) at the recommended concentration (198.2 g/load), and (3) water alone (control). There were also sets of clothing and bedding that were left unwashed for the mite-removal and allergen-removal experiments that served as control experiments.

Two new, identical, residential Kenmore top-loading washing machines (Model no. 110.20892990; Sears, Roebuck and Co, Hoffman Estates, Ill) were used and were connected to the city water supply available at Wright State University. Wash loads alternated between the 2 washing machines.

After the 7-day mite-dispersal period, typical loads of clothing and bedding items were washed in various conditions, as outlined above. Washing was performed according to the recommendations of the American Society for Testing and Materials,¹⁵ except as indicated. All washes consisted of 6- or 8-lb loads (depending on the experiment) in 70 L of water (medium load setting) with a 12-minute wash, 1 rinse, regular agitation speed, and fast spin in warm (36°C-38°C) or cold (22°C-27°C) water (depending on the experiment). A Hobo-Temp temperature data logger (Onset Computer Corp, Pocasset, Mass) capable of monitoring temperature throughout the washing regimen was pinned inside of a clean pillowcase and included in each wash load.

Analysis of live mites

After washing (except for the allergen-removal experiment), clothing and bedding items were hung indoors on a clothesline (≤ 5 hours) until they were vacuumed thoroughly to remove any adherent mites. A portable Hoover Vacuum Cleaner (model no. S7065-060; The Hoover Company, North Canton, Ohio) fitted with a specially designed dust trap was used to vacuum the clothing and bedding. When vacuuming, any mites remaining on the clothing and bedding items were trapped on filters cut from bed sheets (180-thread count). The numbers of live mites were then determined by systematically scanning each filter with a stereomicroscope. The numbers of mites recovered from each clothing and bedding item were determined for each individual replicate.

Mite-removal experiment

The purpose of this experiment was to determine the efficacy of removing live mites from mite-infested clothing and bedding items by means of washing. Identical loads of mite-infested clothing and bedding were washed in 6- and 8-lb loads in cold and warm water

containing (1) powdered detergent, (2) powdered detergent plus liquid chlorine bleach, and (3) water alone. Identical loads were not washed, and these served as positive control loads. For this experiment, a 6-lb load of mite-infested clothing and bedding items consisted of 1 polyester baby blanket, 1 cotton thermal baby blanket, 1 cotton baby throw, 1 cotton men's short-sleeve pullover shirt, 2 gray cotton men's long-sleeve pullover shirts, and 1 green cotton men's long-sleeve pullover shirt. Colors were used only for shirt differentiation. For the 8-lb loads, 3 additional mite-infested items (1 cotton thermal baby blanket, 1 cotton men's short-sleeve pullover shirt, and 1 cotton men's long-sleeve button-type flannel shirt) were included along with the items for a 6-lb load. If necessary, pillowcases (because they are lightweight) were used as ballast to bring each load to the desired load weight. Additional clothing items would have increased the weight to greater than the desired load weights.

The percentages of mites remaining on washed items were determined by comparing values of individual items with the mean values of the matched unwashed control items.

Mite-transfer experiment

The purpose of this experiment was to determine the live mite transfer from mite-infested clothing and bedding items to identical mite-free clothing and bedding items during washing. Identical 4-lb sets of infested and uninfested items were washed together in the same 8-lb loads in (1) cold water and powdered detergent, (2) powdered detergent plus liquid chlorine bleach, and (3) cold water alone (no detergent or bleach). For this experiment, the identical sets of items included 1 polyester baby blanket, 1 cotton thermal baby blanket, 1 cotton baby throw, 1 cotton men's short-sleeve pullover shirt, and 1 gray cotton men's long-sleeve pullover shirt. As before, pillowcases were used as ballast.

The numbers of mites transferred to clean items were determined and compared with the mean numbers present on the identical mite-infested items.

Allergen-removal experiment

The purpose of this experiment was to determine the mite allergen removal (Der f 1) from mite-inoculated clothing and bedding items after washing in warm water in 8-lb loads. Washing regimens were the same as before. Three sets were not washed and provided the reference allergen levels. The wash loads used for this experiment included 1 polyester baby blanket, 2 cotton thermal baby blankets, 1 cotton baby throw, 2 cotton men's short-sleeve pullover shirts, 2 gray cotton men's long-sleeve pullover shirts, 1 green cotton men's long-sleeve pullover shirt, and 1 cotton men's long-sleeve flannel button-type shirt. If necessary, pillowcases were used as ballast.

Immediately after the wash cycle, each clothing and bedding item was placed (separately) into a Ziploc bag with enough PBS-Tween (Dulbecco PBS + 0.05% Tween 20) to wet the item enough to extract the allergens and then to squeeze extracting buffer from the item. The quantity of water that remained in each item after washing was determined previously by weighing each item before (dry) and after (wet) washing. The total extraction volume included the water that remained in each item plus the buffer added. The clothing and bedding item in each bag was kneaded hourly for approximately 4 hours to extract the allergens. Then a small quantity of buffer was wrung from each item and centrifuged for 10 minutes at 14,000g, and the supernatants were removed and frozen (-80°C) until ELISA analyses were performed. The concentrations of Der f 1 allergen in the supernatants were determined according to the manufacturer's instructions by using ELISA kits obtained from Indoor Biotechnologies (Charlottesville, Va), and the total amount of allergen was calculated. Allergen recovered from the washed items was compared with the mean found in the matched unwashed control items.

TABLE I. Mean $\pm$ SD percentages of live *D farinae* remaining after washing items in 6- and 8-lb loads in 70 L of water for 12 minutes compared with values in matched unwashed control items

Clothing-bedding	Water		Detergent		Detergent and bleach	
	Cold	Warm	Cold	Warm	Cold	Warm
6-lb Loads*						
Polyester blankets	30.0 $\pm$ 11.8	109.4 $\pm$ 50.7	68.0 $\pm$ 30.1	100.0 $\pm$ 11.7	49.6 $\pm$ 23.9	102.1 $\pm$ 10.4
Cotton blankets	8.7 $\pm$ 3.2	30.3 $\pm$ 19.5	28.2 $\pm$ 11.7	27.9 $\pm$ 11.1	22.6 $\pm$ 14.3	25.7 $\pm$ 5.9
Cotton shirts	17.2 $\pm$ 10.4	26.9 $\pm$ 12.5	26.4 $\pm$ 11.0	30.4 $\pm$ 8.9	20.6 $\pm$ 9.7	31.4 $\pm$ 13.7
Mean [†]	16.6 $\pm$ 11.0	39.7 $\pm$ 35.9	32.9 $\pm$ 20.2	39.6 $\pm$ 27.0	25.3 $\pm$ 16.3	39.9 $\pm$ 28.4
8-lb Loads [‡]						
Polyester baby blankets	32.7 $\pm$ 19.8	38.3 $\pm$ 18.8	36.1 $\pm$ 2.4	56.3 $\pm$ 8.1	36.8 $\pm$ 7.5	65.8 $\pm$ 28.4
Cotton blankets	8.4 $\pm$ 2.2	9.6 $\pm$ 4.7	12.3 $\pm$ 2.3	12.5 $\pm$ 4.8	13.3 $\pm$ 3.1	11.6 $\pm$ 6.5
Cotton shirts	27.6 $\pm$ 9.2	20.8 $\pm$ 7.6	20.9 $\pm$ 5.4	26.5 $\pm$ 9.9	29.4 $\pm$ 8.6	25.9 $\pm$ 12.8
Mean [†]	22.4 $\pm$ 12.9	19.2 $\pm$ 11.5	19.8 $\pm$ 8.0	25.3 $\pm$ 14.8	25.3 $\pm$ 10.9	25.6 $\pm$ 19.8

Cold water was 22°C to 27°C, and warm water was 36°C to 38°C.

*Data from 6-lb loads includes 3 replicate loads of 1 polyester baby blanket, 1 cotton thermal baby blanket, 1 cotton baby throw, 1 cotton men's short-sleeve shirt, 2 gray cotton men's long-sleeve shirts, and 1 green cotton men's long-sleeve shirt.

[†]Mean is defined as mean of individual items (n = 21 for 6-lb loads and n = 30 for 8-lb loads).

[‡]Data from 8-lb loads includes 3 replicate loads of 1 polyester baby blanket, 2 cotton thermal baby blankets, 1 cotton baby throw, 2 cotton men's short-sleeve shirts, 2 gray cotton men's long-sleeve shirts, 1 green cotton men's long-sleeve shirt, and 1 cotton men's long-sleeve flannel shirt.

TABLE II. Mean $\pm$ SD (3 replicate loads) live *D farinae* mites remaining on mite-infested items and transferred to identical mite-free items after washing them together in cold (22°C-27°C) water for 12 minutes (8-lb loads and 70 L of water)

Clothing-bedding	Water		Detergent		Detergent and bleach	
	Mite infested	Clean	Mite infested	Clean	Mite infested	Clean
Polyester blankets	1272 $\pm$ 493	1303 $\pm$ 147	954 $\pm$ 98	875 $\pm$ 49	727 $\pm$ 159	575 $\pm$ 273
Cotton blankets	310 $\pm$ 93	567 $\pm$ 231	443 $\pm$ 237	434 $\pm$ 158	450 $\pm$ 229	398 $\pm$ 107
Cotton shirts	2113 $\pm$ 785	423 $\pm$ 74	1867 $\pm$ 864	618 $\pm$ 171	2098 $\pm$ 903	565 $\pm$ 148
Mean*	1223 $\pm$ 977	657 $\pm$ 374	1115 $\pm$ 854	596 $\pm$ 218	1164 $\pm$ 973	500 $\pm$ 173

Each load included 1 infested and 1 clean item as follows: polyester blanket, cotton thermal baby blanket, cotton baby throw, cotton men's short-sleeve shirt, and gray cotton men's long-sleeve shirt.

*Mean is defined as the mean of individual items (n = 15).

Statistical analysis

Statistical analyses of the data were conducted by the Wright State University Statistical Consulting Center by using SPSS software (version 11.0) to perform an appropriate ANOVA on each of the 3 sets of data. Only comparisons with *P* values of less than .05 were considered significantly different.

RESULTS

Mite-removal experiment

After cold or warm washing with water alone, water with detergent, or water with detergent plus bleach in 6- and 8-lb loads, overall 60% to 83% of the live mites were removed from the clothing and bedding items after washing compared with the numbers of live mites recovered from unwashed sets (Table I). Neither washing with powdered detergent nor detergent plus liquid chlorine bleach significantly enhanced removal of live mites over washing with water alone. Warm washes, regardless of the laundry additive used, consistently removed significantly (*P* < .05) higher mean percentages of live mites from items washed in 8-lb loads (74%-80%) compared with items washed in 6-lb loads (60%). Cold washing in water

alone removed significantly (*P* < .05) more mites than any other washing regimen for 6-lb loads. This was not the pattern for 8-lb loads washed in cold and warm water.

Some clothing and bedding items retained more mites during washing than others (Table I). For 8-lb loads, the cotton baby blankets consistently retained fewer mites compared with cotton shirts and polyester blankets. The polyester blankets retained much higher percentages of mites than the cotton blankets and cotton shirts in 6-lb loads.

Mite-transfer experiment

When identical sets of mite-free and mite-infested clothing and bedding articles were washed together, many live mites were transferred onto the clean clothing (Table II). Neither the detergent nor the detergent plus bleach significantly reduced the transfer of mites onto clean clothes compared with the numbers of live mites that were transferred when washed in cold water alone. Some initially mite-free clothing and bedding items acquired larger numbers of live mites during washing than others. In some instances previously mite-free items had larger numbers of live mites after washing than were retained by the matched initially mite-infested items after washing.

TABLE III. Mean $\pm$ SD percentage (3 replicate loads) of Der f 1 removed after washing in warm (36°C-38°C) water for 12 minutes (8-lb loads and 70 L of water)

Clothing-bedding	n	Mean Der f 1 (μ g) extracted per item	Reduction (%)		
		Unwashed control	Water	Detergent	Detergent and bleach
Polyester blanket	3	182.2 $\pm$ 252.1	90.5 $\pm$ 5.8	88.8 $\pm$ 7.6	96.5 $\pm$ 2.5
Cotton thermal blanket	6	169.4 $\pm$ 103.3	95.7 $\pm$ 0.9	97.7 $\pm$ 0.8	100.0 $\pm$ 0
Cotton throw	3	59.5 $\pm$ 103.1	73.3 $\pm$ 7.5	50.6 $\pm$ 57.4	100.0 $\pm$ 0
Cotton short-sleeve shirt	6	129.3 $\pm$ 78.0	93.2 $\pm$ 1.7	91.8 $\pm$ 5.4	97.3 $\pm$ 1.6
Gray cotton long-sleeve shirt	6	81.9 $\pm$ 78.7	88.0 $\pm$ 3.2	92.2 $\pm$ 3.7	99.4 $\pm$ 1.4
Green cotton long-sleeve shirt	3	15.9 $\pm$ 27.6	46.8 $\pm$ 5.3	45.6 $\pm$ 15.7	100.0 $\pm$ 0
Cotton long-sleeve flannel shirt	3	59.6 $\pm$ 66.6	80.5 $\pm$ 7.1	88.6 $\pm$ 1.5	94.5 $\pm$ 4.9
Mean	30	107.8 $\pm$ 111.3	84.5 $\pm$ 15.0	83.7 $\pm$ 24.4	98.5 $\pm$ 2.5

Allergen-removal experiment

Washing with warm water alone, water plus detergent, and water with detergent plus bleach removed an average of 84%, 84%, and 98% of the Der f 1 allergen, respectively, from clothing and blankets (Table III). Washing with detergent did not significantly change allergen removal compared with washing in water alone. The wash loads containing detergent plus liquid bleach removed significantly ($P < .05$) more allergen than the water alone or the water plus detergent.

Der f 1 allergen was removed from some items more easily than from others (Table III). The amount of allergen removed from individual sets of items ranged from 46% to 100%. The mean percentages of Der f 1 removed from the cotton thermal blankets were consistently higher (similar to the mite-removal experiment), and the percentages removed from the green cotton long-sleeved shirts were consistently lower than for the other items when they were washed with detergent and with water alone.

DISCUSSION

In the current study various types of mite-infested clothing and bedding items were washed in residential washing machines by using varied washing parameters (temperature, load size, detergent, and bleach), and live mite and allergen removal was determined. Altering the washing parameters only slightly affected the percentages of mites removed from a particular type of clothing item. Washing clothes and bedding in warm and cold water alone, detergent, or detergent plus bleach in 6- or 8-lb loads substantially reduced the mean numbers (60%-83%) of live *D farinae* mites in these items compared with the numbers of mites in the unwashed clothing and bedding items.

It was previously shown in simulated washing experiments that immersing *D farinae*, *Dermatophagoides pteronyssinus*, and *Euroglyphus maynei* mites for 12 minutes or 1 hour in warm water at 35°C with various laundry detergents or bleach (laundry concentration) was ineffective for killing dust mites.⁹ Therefore the reduction in the number of live mites observed in the present study during the standard 12-minute washing cycle in warm and

cold water was likely due primarily to the physical removal of mites and probably not the acaricidal effects of the water containing detergent or detergent and bleach.

The results of our mite-removal experiments using detergent or detergent and bleach are similar to those of a previous study by McDonald and Tovey¹³ using *D pteronyssinus*. They reported that most (73%) of the live mites were removed from mite-infested duvets, blankets, and sleeping bags after washing together for 9 minutes in cold water with laundry powder. This group also found that when these items were washed in cold water alone, only 22% of the mites were removed. In contrast, our study found no difference in the percentage of mites removed when washing in water alone, detergent, or detergent and bleach. This suggests that it might be more difficult to remove mites from duvets and sleeping bags than from clothing and blankets when they are washed without laundry detergent or that other factors in the 2 studies were different, such as load sizes, action of the washing machines, and methods for determining live mite numbers.

Our study showed that not only did some *D farinae* mites survive and remain on clothing and bedding items during washing, but live mites were also transferred onto clean uninfested clothing. These findings have some important implications. Individuals with mite allergy might be exposed to mite allergens in freshly washed clothes. Also, mites carried on freshly washed clothing might be transferred to locations outside of the home (eg, other homes, workplaces, schools, and automobiles).

Wash loads in a home normally contain various types of clothing and bedding items. Therefore to simulate real-life wash loads, we washed standard items with varying constructions and physical properties. Our results showed that mites were removed more easily from some items than from others. Some individual items retained most of the infesting mite population, whereas others retained few mites. The reasons for this are unknown. Clearly, the different physical properties of clothing and bedding items influence how efficiently live mites are removed during washing.

Reducing mite allergen exposure is critical in the management of mite allergen-induced atopy and asthma. We found that overall 84% of the Der f 1 allergen was removed from mite-infested clothing and bedding items by

washing in warm water alone. However, it is important to note that less than 50% of Der f 1 was removed from one type of shirt. This indicates that although washing removes most allergen from most items, there are exceptions. The addition of laundry detergent did not significantly change the amount of allergen removed during washing. Most allergens are water soluble, and this study confirms that the physical act of washing clothing and bedding in normal wash loads removes most allergen, even though many live mites might remain in the clothing and bedding. Interestingly, the addition of chlorine bleach further increased the amount of allergen that was removed from the clothing to 98% but did not increase mite removal. It is not clear whether the chlorine bleach enhanced removal of the allergen or rather denatured the allergens. A previous study by Chen and Eggleston¹⁶ suggested that sodium hypochlorite might denature mite allergen.

Our allergen-removal results using *D farinae* are similar to the results of other published studies using *D pteronyssinus*. A study by Vanlaar et al¹² reported an approximately 88% reduction in Der p 1 in bedding washed in an acaricidal laundry additive. McDonald and Tovey¹³ found that after cold-water washing of mite-infested bedding items in soapy water and water alone, 5% and 7% of the Der p 1 remained in the fine dust collected from these items. The results of another study by this group that simulated washing (gently shaking water bath) of fine dust containing mite and cat allergens concluded that using detergent solutions at 25°C for 5 minutes was sufficient to extract most Der p 1 (94%) and Fel d 1 (80%) allergens from bed dust.¹⁴ Interestingly, when the bed dust was subjected to simulated washing in water alone, only 34% and 37% of the Der p 1 and Fel d 1, respectively, was removed. Therefore taking the data of our study together with those of previous studies, it appears that washing cycles with detergent or water alone must be longer than 5 minutes to remove most of the mite allergen. Use of bleach significantly improves allergen removal.

In conclusion, washing clothing and bedding in normal wash loads with warm or cold water alone or water containing detergent or detergent and bleach will remove a large portion of the live house dust mites that might infest them. However, 17% to 40% of the contaminating mites remained after one washing. During washing, mites in infested clothing and bedding are transferred to and contaminate previously mite-free clothing. Therefore repeated washings are required to further reduce mite levels in clothing and bedding. This washing regimen would need to be coupled with practices to reduce mite levels in homes to prevent the clothing and bedding from

becoming recontaminated from the indoor environment. Washing clothing and bedding with laundry detergent removes approximately 84% of Der f 1 allergen. Addition of liquid chlorine bleach to laundry detergent results in a 98% reduction in mite allergen but does not influence mite kill or removal.

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