

House dust mite, *Dermatophagoides pteronyssinus*, and its allergens: effects of washing

A. ANDERSEN & J. ROESEN

Institute of Biology and Chemistry, Roskilde University, and Northern Feather Development Centre, Copenhagen, Denmark

The study was undertaken to evaluate the effect washing at various temperatures would have on the house dust mite, *Dermatophagoides pteronyssinus*, and its allergens. Young and old mite cultures underwent a simulated washing programme. To record the number of live mites prior to and after washing a new counting method was applied. The findings demonstrated that although washing reduced the number of live mites, a washing programme at minimum temperature 58°C was required in order to achieve complete extermination of the house dust mites. A distinct protein loss was observed when washing at higher temperature, thus further reducing the total allergen pool without loss of allergen activity as determined by direct RAST.

Key words: allergen avoidance; arachnida; *Dermatophagoides* spp.; mite control; RAST; temperature.

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After discovering that house mites were a major allergy-causing factor (18), various attempts have been made to control and eliminate the mites and their allergens (11, 19).

Some mite-reducing effect has been obtained by using acaricides, hereunder also fungicides (6, 8, 10, 17). However, before recommending a widespread use of these chemicals, it is essential to establish whether their use is harmless, and safe for humans. Moreover, it is a prerequisite to know how they should be applied when combined with other preventive measures.

Several studies found that the use of such preventive measures has had little effect, if any, in controlling house dust mites (3, 4, 7). However, a controlled study by Murray & Ferguson (12) found that if patients' houses were subjected to thorough cleaning measures, a clinical efficacy was clearly demonstrated.

To avoid house dust mites, it is often recommended to exchange pillows, eiderdowns and bed mats with their counterparts produced from

synthetic washable materials (4, 13, 14, 20). Since this advice is obvious, nobody has – as far as we know – investigated what effect washing might have on house dust mites and their allergens.

The purpose of this study was to establish, under laboratory conditions, the effect washing has on the house dust mite, *Dermatophagoides pteronyssinus*, Trouessart, and its allergens.

MATERIAL AND METHODS

House dust mites were reared in the laboratory exactly as described by Andersen (1) and for this study's experiments both young (6-9 weeks) and old (20-30 weeks) cultures were used.

Three 20 mg samples of each culture underwent a simulated washing programme. Prior to washing, the mites in each 20 mg sample were counted under a stereomicroscope. The heat produced by the microscope resulted in increased mite activity and any moving mite was

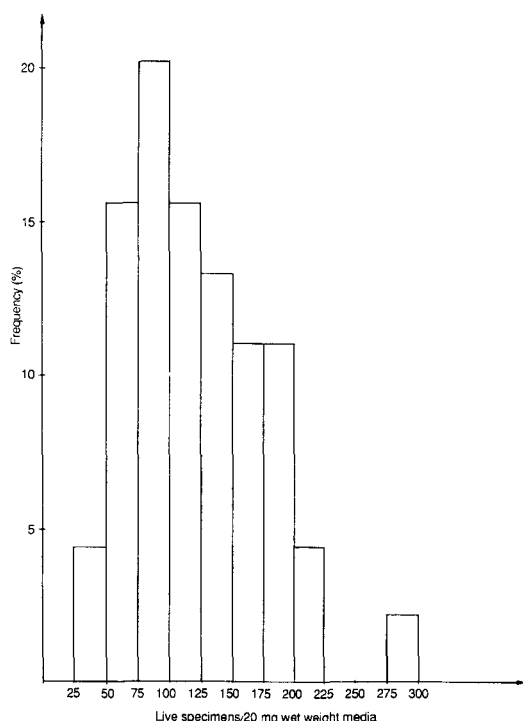


Fig. 1. Histogram showing the distribution of the sample frequencies of live house dust mites recorded prior to the various washing programmes.

recorded as alive. The mites were then put in a dialysis bag which underwent one of the following types of washing: normal, first, and second control. After washing, the contents of the dialysis bag were suction filtered (Whatman filter) and the mites remaining on the filter were counted using the same criteria as mentioned above.

While counting, the microscope heat caused the filter to dry out. Afterwards supplementary food medium was added (100 mg) and the carefully folded filter was placed in a culture flask. To prevent the mites from escaping, the flask was sealed with a piece of dialysis membrane and the rearing process repeated (1). After 9–11 days the contents of the flask were examined and the living mites were counted.

To estimate allergen activity, a serum pool was established by pooling equal volumes of serum from 10 patients showing allergy to house dust mites, i.e. based on skin prick test and RAST scores of 3 to 4. Human sera for

control purposes were obtained from nonatopic individuals chosen among healthy laboratory staff members.

Extracts from old cultures (20–30 weeks) of the house dust mite *D. pteronyssinus* were processed as described in a previous paper (2). The protein content of the extracts obtained was estimated by the Folin-Ciocalteu method (9) using tyrosin as the standard.

The allergen activity of the extracts was determined by the direct RAST method. Activation of paper discs with BrCN and coupling of proteins under increased incubation time were done as described by Ceska et al. (5) and Tovey & Vandenberg (16), except that peroxidase conjugated anti-IgE was chosen instead of isotope-marked anti-IgE.

Dialysis bags containing either cultures with live mites or culture extracts underwent simulated washing. A water basin containing 7 l of water and furnished with thermostat and stirrer served as washing machine. The 14.3 g soap was used for pre-washing and 42.8 g for main washing. The soap was made up of equal portions of soap brands recommended for allergies. The washing programme comprised: pre-washing (3 min), main washing (4 min), rinse (1 min), rinse (2 min), rinse (3 min), and rinse (4 min).

The bags underwent the above imitation of an ordinary washing programme at the following temperatures: 40°C, 50°C, and 58°C. Other identical samples were used as first control, i.e. they underwent the same washing procedure as described above, but soap was omitted. Finally, samples which were neither exposed to the increased temperature nor to soap served as a second control.

Statistical analysis. Since data showed an uneven distribution (Fig. 1) only non-parametric statistics were used (15).

RESULTS AND DISCUSSION

The present study was undertaken to evaluate the effect washing at various temperatures would have on the house dust mite, *Dermatophagoides pteronyssinus*, and its allergens.

All observations of the number of mites recorded prior to washing are presented in the histogram (Fig. 1) and the median value has been calculated to be 113 live mites per 20 mg wet weight medium. Only moving specimens were counted, thus any farate mites or eggs were not recorded.

The survival frequency of the mites when subjected to various washing programmes and

temperatures is shown in Table 1. To establish which factors determine the cause of death, both the young and old mite cultures underwent normal, first, and second control washings. It can be seen that drowning is the major cause of death. However, some mites will survive exposure to water (Table 1). At higher temperatures, the mortality rate increases significantly (Kruskal-Wallis $P < 0.05$, $n_1 = n_2 =$

Table 1

Mean number (with observed extremes) of live specimens/20 mg house dust mite culture and the corresponding survival percentage after various washing programmes with different temperatures

Culture	Washing	Before	After (A)	Later (B)	A	B
Young ($n = 3$)	Blind 10-20°C	76 (56-98)	2 (0-3)	1 (0-3)	2.5 (0-5.4)	1.9 (0-4)
Old ($n = 3$)		166 (78-220)	13 (5-24)		9.5 (2.3-14.1)	
Young ($n = 3$)	Normal 40°C	91 (67-125)	4 (1-9)	1 (0-2)	3.7 (1.5-7.2)	0.9 (0-1.6)
Old ($n = 3$)		135 (88-185)	16 (11-23)	3 (0-8)	12.2 (8.4-15.9)	1.4 (0-4.3)
Young ($n = 3$)	Control 40°C	94 (81-107)	3 (2-4)	2 (1-3)	3.7 (1.9-4.9)	1.8 (0.9-3.2)
Old ($n = 3$)		184 (69-284)	5 (1-10)	0	2.5 (1.4-3.5)	0
Young ($n = 6$)	Normal 50°C	135 (107-176)	0	1 (0-1)	0	0.3 (0-0.9)
Old ($n = 3$)		86 (46-144)	3 (0-8)	1 (0-2)	3.9 (0-11.6)	0.9 (0-1.4)
Young ($n = 3$)	Control 50°C	113 (83-144)	1 (0-1)	1 (0-1)	0.3 (0-0.9)	0.5 (0-0.9)
Old ($n = 3$)		89 (44-130)	0	0 (0-1)	0	0.8 (0-2.3)
Young ($n = 3$)	Normal 58°C	164 (159-169)	0	0	0	0
Old ($n = 6$)		75 (58-88)	0	0	0	0
Young ($n = 3$)	Control 58°C	197 (165-225)	0	0	0	0
Old ($n = 3$)		105 (83-121)	0	0	0	0

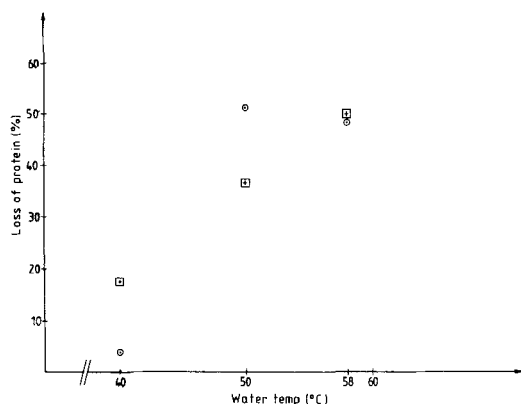


Fig. 2. Protein loss of house dust mite extracts (normal washing □, first control washing i.e. without soap ○) subjected to various water temperatures.

$n_3 = 4$) and complete extermination will occur at 58°C. This is further confirmed by subsequent examination of the cultures. (Table 1).

Errors, such as overlooking specimens when counting, live eggs, etc., are accounted for by comparing, immediately after washing, the number of surviving mites with the number of mites alive 9–11 days after washing. If the highest number of mites is recorded 9–11 days after washing, this figure will be used in the subsequent calculations.

Both young (6–9 weeks) and old (20–30 weeks) cultures were subjected to the various washing programmes, old cultures having been found to contain a high frequency of nymphs (1). These nymphs might be better equipped to withstand unfavourable conditions (1, 19). No significant difference in the survival of young or old mite cultures was observed at the various temperatures with the exception of 58°C, at which temperature all mites are killed (Mann-Whitney U test: $0.75 < P < 0.111$, $n_1 = n_2 = 5$). However, the old cultures showed a better survival tendency.

Prior to and after washing, the protein concentration of the extracts was determined. Fig. 2 illustrates the pronounced protein loss which occurred when increasing the water temperature. Direct RAST determinations of the extracts before and after washing at 58°C (Fig. 3) failed to demonstrate any significant reduction in the allergen activity (Kruskal-Wallis $P >$

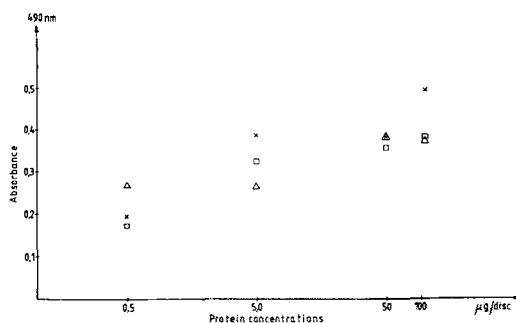


Fig. 3. The allergen activity of house dust mite extract prior to (□) and after normal (×) and first control (Δ) washing as estimated by direct RAST determinations.

0.1, $n_1 = n_2 = n_3 = 4$). However, this observation should be added to the data concerning protein loss at high temperatures. Accordingly, even though the allergens do not lose their activity, the total allergen pool will be reduced and this will be particularly evident at the highest temperature applied.

As a conclusion the present findings show that although washing reduced the number of live mites, a washing programme at 58°C minimum temperature was required in order to achieve complete extermination of the house dust mites.

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Address:

Annette Andersen

Institute I, Life Sciences and Chemistry

Roskilde University

P.O. Box 260

DK-4000 Roskilde

Denmark