

Sensitization to Cockroach in Italy: A Multicentric Study

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ABSTRACT

The prevalence of sensitization to cockroach (CR) was evaluated by skin prick test (SPT) in 1299 patients consecutively referred for rhinitis and asthma to five allergy centers in northern, central, and southern Italy. In patients with positive SPT to CR, an SPT with *D. pteronyssinus* extract was made. RAST inhibition was performed with pooled sera of subjects with positive SPT and RAST of at least Class 3 for CR and *D. Pteronyssinus*, to assess the cross-reactivity between these two allergenic sources. The protein content of allergen extracts was assayed by isoelectric focusing (IEF). A total of 170 patients (13%) had a positive SPT with CR extract, and 133 of them (78.2%) were also positive for *D. pteronyssinus*. RAST inhibition showed a cross-reactivity between CR and *D. pteronyssinus*, and IEF detected in the extracts employed a series of bands focusing at the same pI of CR and *D. pteronyssinus* relevant allergens. Therefore, the prevalence of sensitization to CR in the population studied, corresponding to 13%, suggests that CR extract might be added to standard SPT panels for respiratory allergy. However, the cross-reactivity with *D. pteronyssinus* calls for careful evaluation of the clinical importance of this sensitization. (Allergy and Asthma Proc 18:23–28, 1997)

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Discovering new allergenic sources greatly extends our knowledge of atopic diseases and enables us to recognize as allergies clinical conditions otherwise undetermined. This was true for cockroach (CR), especially the most common species *Blattella germanica*, *Periplaneta americana*, and *Blattella orientalis*,¹ reported in recent years as a common cause of respiratory allergy.

Several studies in the United States outlined the importance of CR in causing sensitization and clinical allergy, especially asthma.^{2–4} These findings have been confirmed in other areas such as Asia^{5–10} and Africa.¹¹ By contrast, sensitization to CR seems less frequent in South America¹² and Europe. However, for European countries most reports^{13–15} considered small populations, and only one study¹⁶ dealt with enough patients to achieve epidemiological reliability.

Any assessment of sensitization to CR has to consider the cross-reactivity between CR and *Dermatophagoides spp.* allergens, being reported a close association between CR and house dust mites sensitization.^{6,10}

The aim of this study was to investigate the prevalence of CR sensitization in a population of patients suffering from perennial respiratory disorders recruited in five allergy centers located in northern, central, and southern Italy. To assess the adequacy of the allergenic extracts we used, their components were analyzed by isoelectric focusing (IEF) and the cross-reactivity between CR and *Dermatophagoides* was investigated by RAST-inhibition.

MATERIAL AND METHODS

Patients

The study was carried out in five different allergy centers, two located in northern (Milan and Casatenovo) one in central (Ancona) and two in southern Italy (Naples and Lecce). In the period ranging from October 1990 to December 1992, the centers selected all consecutive patients com-

plaining of respiratory disorders. To be admitted to the study, patients had to have a well-defined history of perennial rhinoconjunctivitis or asthma. Subjects receiving allergen immunotherapy, steroidal, or immunosuppressive drugs were excluded.

In Vivo Methods

Patients were tested by skin prick test (SPT) with an extract of CR whole body (DHS Labs, Spokane, Wash, USA), composed by a mixture of *Blattella germanica*, *Blattella orientalis*, and *Periplaneta americana*. The concentration of extract used for SPT was 20,000 PNU/mL. SPT were performed by 1 mm-tip lancets (Allergy pricker, DHS)¹⁷ in order to standardize prick testing in the different centers. Skin tests were made in duplicate, using glycerococcol solution and histamine dihydrochloride 10 mg/mL as negative and positive controls, and the reaction was considered positive if the wheal was at least 3 mm in diameter, according to the guidelines of the European Academy of Allergy and Clinical Immunology.¹⁸

In patients with positive SPT to CR extract, an SPT with *D. pteronyssinus* extract (DHS Labs) was performed, standardized in activity units by RAST (AUR) at a concentration of 400 AUR/mL. In comparison with the international unit as established in the standardization of allergenic extracts, 1 AUR corresponds to 223 IU.¹⁹ In case of positive reactions to both CR and *D. pteronyssinus* extracts, a blood sample was obtained by venipuncture and centrifuged at 3000 RPM for 10 minutes. Serum was collected and frozen at -20°C until assays were performed.

In Vitro Methods

RAST: Phadebas RAST (Pharmacia, Uppsala, Sweden) was performed on patients' sera in order to detect specific IgE to CR and/or *D. pteronyssinus*. Paper disks activated with cyanogen bromide were coupled with the same CR and *D. pteronyssinus* extracts used for SPT and other *in vitro* methods.

RAST inhibition: This technique was used on pooled sera of the subjects with a RAST to CR and *D. pteronyssinus* at least Class 3, in order to obtain reliable results, according to the method described by Ceska et al.²⁰ Briefly, serial dilutions of CR and *D. pteronyssinus* allergen extracts in phosphate buffer were preincubated overnight with the positive sera pool, and afterward tested in duplicate in the RAST

system utilizing discs coated with CR or *D. pteronyssinus* extracts. As negative control RAST inhibition was performed on the same pool and against the two allergens on discs using a box elder extract, having previously checked that the pool was lacking specific IgE against this allergen. As positive control, the pool was diluted in phosphate buffer alone.

The amount of IgE antibodies bound to the allergen extract on the discs, measured with a ¹²⁵I-anti-IgE in a gamma counter, indicates the ability of the soluble allergen to inhibit the binding to disc-coated allergen of specific IgE in the pooled sera. The percentage of inhibition was calculated with the formula previously reported,²¹ and the inhibition-rate data were plotted on a lin-log paper with dilutions in the log scale (horizontal axis) and inhibition rate in the lin scale (vertical axis). Individual best-fit lines were calculated by linear-regression analysis, using the formula $y = A + B \log(x)$ where $y = \% \text{ inhibition}$, $A = y \text{ axis intercept}$, $B = \text{slope}$, and $x = (\text{total dilution factor})^{-1}$.

Isoelectric focusing (IEF): IEF was performed on CR and *D. pteronyssinus* extracts to separate their proteins by virtue of their isoelectric points (pI) within a stable linear gradient.²² Briefly, 25 µl of each extract and 15 µl of pI reference marker (pH 3.5–9.5 Pharmacia Biotech AB, Uppsala, Sweden) were dispensed on polyacrylamide gel plates (PAG plate kit pH 3.5–9.5 Pharmacia Biotech AB). Power and total time of focusing were set as recommended by the manufacturer. Gels were fixed in trichloroacetic acid solution, stained overnight in Coomassie brilliant blue R 250, destained in an ethanol-acetic acid solution, and dried in the open air.

RESULTS

An overall number of 1299 consecutive patients (618 M, 681 F, mean age 43.4 years, range 10–81) suffering from perennial rhinitis and/or asthma was recruited for the study. Rhinitis was reported by 42%, rhinitis and asthma by 39%, and asthma alone by 19%. A total of 170 subjects (102 M, 68 F, mean age 42.1 years, range 13–77) (13%) had positive SPT for CR. Among them, 37 (21 M, 16 F, mean age 40.8 years, range 15–77) (22.8%) had positive SPT for CR only, whereas 133 subjects (72 M, 61 F, mean age 39, range 13–75) (78.2%) were also SPT-positive for *D. pteronyssinus*. Table I details total numbers and relative percentages of sensitization in the population studied divided by

TABLE I
Sensitization to Cockroach (CR) and *D. pteronyssinus* (Dp) in the Population Studied

Gender	Patients		Patients		Patients		(% on CR)
		(%)	CR+	(% on total)	CR+ Dp+	(% on total)	
Males	618	(48%)	102	(17%)	72	(12%)	(71%)
Females	681	(52%)	68	(10%)	61	(9%)	(90%)
Total	1299	(100%)	170	(13%)	133	(10%)	(78%)

gender, outlining the increased double sensitization in the females toward males.

Figure 1 shows the results of RAST-inhibition between CR and *D. pteronyssinus* extracts and Table II displays the raw data. The inhibition rate if CR was bound to solid phase and *D. pteronyssinus* extract was used as inhibitor was about 70%, whereas 20% inhibition was obtained when *D. pteronyssinus* was bound to solid phase and CR was used as inhibitor.

Figure 2 shows IEF profile of CR and *D. pteronyssinus* extracts. Analyzing CR two bands ranging from pH 4.20 to 5.20, and a single band focused at pH 3.70 were found, corresponding to relevant CR proteic allergens.²³ For *D. pteronyssinus*, multiple bands were observed in the pH zone ranging from 4.00 to 8.10, well known for mite allergens.²⁴

DISCUSSION

CR induces sensitization and clinical symptoms of allergy in town-dwelling and especially multifamily-dwelling populations as reported by a series of studies mainly conducted in the United States,²⁻⁴ but also in other countries.⁵⁻¹¹ In Europe the prevalence of CR sensitization

was reported ranging from 2.7% to 22%,¹³⁻¹⁶ but small populations were considered. A single study dealt with a large number of subjects, namely 1200 children,¹⁶ reporting a prevalence rate of 2.7%, but only patients positive to both SPT and RAST were included.

We chose to assess CR sensitization by SPT because it has long been known that *in vitro* tests are less sensitive than skin tests²⁵; this was recently confirmed by Kang et al.,²⁶ who reported a lesser sensitivity of RAST other than SPT in discriminating asthmatic patients allergic to CR. Moreover, when the present study was performed, no standardized CR extracts were available for *in vitro* tests.

A 13% prevalence rate of sensitization to CR was found, which would make it worth adding CR to the standard panel of aeroallergens tested in patients with suspected respiratory allergy. Our findings should be reliable due to the detection in the extract employed of proteic bands focusing respectively at pH 3.5 and 5.2, that correspond to pI of *Bla g I* and *Bla g II*, the major allergenic components of *Blattella germanica*,²⁷ the most common CR in Europe. A diagnostic extract containing too little of these allergens would result in false negatives in performing *in vivo* or *in vitro* tests.

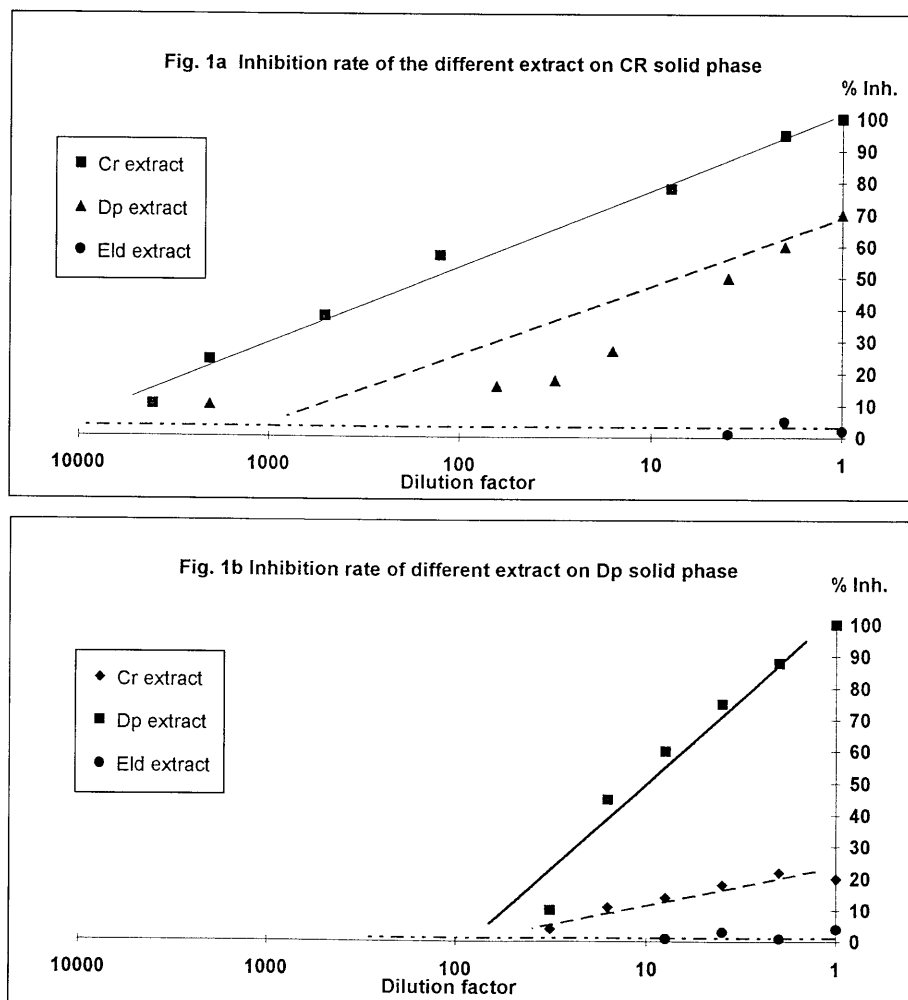


Figure 1. RAST inhibition results. Antigens linked to solid-phase were CR (Fig. 1a) and *D. pteronyssinus* (Fig. 1b). Dp: *D. Pteronyssinus*.

TABLE II

Dilution Factor	CR on Disc			Dp on Disc		
	% Inh. Cr Extract	% Inh. Dp Extract	% Inh. Eld Extract	% Inh. Cr Extract	% Inh. Dp Extract	% Inh. Eld Extract
1	100	70	2	20	100	4
2	95	60	5	22	88	1
4		50	1	18	75	3
8	78			14	60	1
16		27		11	45	
32		18		4	10	
64		16				
128	57					
256						
512	38					
1024						
2048	24	10				
4096	10					
Pearson coefficient: r	0.99	0.87		0.98	0.97	
Correlation significance: P	<0.0001	<0.05		<0.05	<0.05	

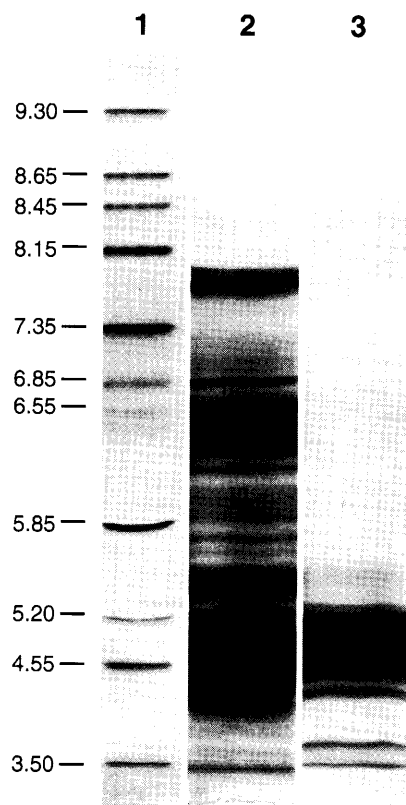


Figure 2. IEF of *D. pteronyssinus* (lane 2) and CR (lane 3) extracts, together with pI markers expressed as pH values (lane 1).

About 80% of CR-positive patients had positive SPT to *D. pteronyssinus* with a significantly higher number of females, perhaps due to the longer permanence in dwellings. This high rate of concomitant sensitivity to CR and mite is comparable to that reported by Tandon et al.,¹⁰ who observed a positivity to CR alone in 8.5% of 200 patients with

perennial respiratory symptoms undergoing SPT with CR and mite extract. RAST inhibition data confirmed an allergenic cross-reactivity, and especially showed the *D. pteronyssinus* ability to inhibit the binding of specific IgE against CR allergens. This fact, together with the IEF finding of different protein content in the two allergens, makes it reasonable to suppose that the cross-reacting allergens common in CR and mites represent almost all of the protein content in the CR, but not in the *Dermatophagoides* species. This could be a valid explanation of why *D. pteronyssinus* is able to inhibit 70% of the CR answer, but not vice versa. This phenomenon led us to study the presence of the different components in the two allergen extracts. IEF shows that *D. pteronyssinus* raw material has many proteic components, focusing at a wide range of pI, responsible for multiple IgE sensitization, whereas CR extract, although representative of the relevant allergens, has a much lower molecular contribution. The lack of inhibition from negative control extract and the good correlation coefficient of regression function, always significant and greater than 0.87, show that RAST inhibition assay we performed is highly specific. The steep slope of the curve of *D. pteronyssinus* extract on itself shows a good biological potency of this extract, higher than the CR one.

The allergenic cross-reactivity we observed was in agreement with several previous reports^{6,15,28} outlining a high incidence of concomitant sensitization to CR and mites. Moreover, Witteman²⁹ recently found a 36 kd allergen with a pI of 5 in a number of arthropods including mites, CR, insects, and shrimps. This component, identified by a monoclonal antibody, was thought to be tropomyosin, which is a protein involved in actin activation during muscle contraction, but its clinical relevance as a cross-reacting allergen has still to be clarified. In fact, another study identified as a *Blattella germanica* species-specific allergen a 36 kd com-

ponent with a different pI from that previously described,²⁷ thus suggesting the presence of two different allergens, or isoallergenic forms, with the same molecular weight.³⁰

In any case, the importance of cross-sensitization to CR and mites cannot be overemphasized, as there are patients showing a positive response in bronchial provocation test exclusively with CR extract.²⁶ In addition, the major allergens of mites show distinctive immunochemical characteristics.³¹ This is indirectly confirmed by the prevalence of mites sensitization in Europe,^{32,33} and even in Italy,³⁴ which is much higher than the 13% we observed for CR. Nonetheless, the CR sensitization rate we found is much lower than CR prevalence reported in the United States.^{35,36} We cannot convincingly explain the difference, because 1) the population we studied lived in urban areas where multifamily dwellings were well represented, and 2) the allergenic activity of the American species *Periplaneta americana* and the European species *Blattella germanica* is similar. Future studies should clarify the clinical importance of CR sensitization in European countries by detecting its presence in dwellings, either directly or by monoclonal antibody-based enzyme immunoassays.³⁷

In conclusion, this study, conducted in Italy on a large town-dwelling population, found a 13% prevalence of CR sensitization. This should make it worth adding CR extract to common standard panels tested in subjects with suspected respiratory allergy. However, the cross-reactivity with *D. pteronyssinus* calls for careful consideration of such a sensitization that should be assessed either by demonstrating its presence in dwellings or by measuring its allergens in house dust samples.

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