
Prevalence of serum IgE to storage mites in a southwestern Ohio population

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Background: Storage mites of the families Chortoglyphidae, Glycyphagidae, Echimyopodidae, and Acaridae are commonly found in agricultural and nonagricultural environments. The prevalence of sensitization to these storage mites in the general population in the United States is unknown.

Objective: To determine the prevalence of serum IgE to the storage mites *Lepidoglyphus destructor* and *Acarus siro* and to evaluate the degree of co-sensitization to various other astigmatid mites in a population in southwestern Ohio.

Methods: Serum samples from 600 people randomly selected from a 1-day submission of approximately 3,000 samples to a clinical diagnostic laboratory were screened for IgE to allergens of *L destructor* and *A siro*. Proteins in the extracts of each mite were electrophoretically separated, transferred to membranes, and incubated in the serum samples, and the slot blots were probed for IgE binding using radiolabeled anti-human IgE and autoradiography.

Results: Thirty-two (5.3%) of the 600 serum samples screened had IgE to allergens from at least 1 of the 2 mite species; 14 (2.3%) and 20 (3.3%) had serum IgE to proteins of the mites *A siro* and *L destructor*, respectively. Additional analysis revealed that most serum samples also had IgE that bound to proteins in extracts prepared from a variety of other astigmatid mite species, but IgE binding profiles suggested little cross-reactivity.

Conclusions: Sensitization to the mites *L destructor* and *A siro* is significant in this region of southwestern Ohio. Further studies are needed to determine the importance of these and other storage mites in occupationally exposed and urban populations of the United States.

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INTRODUCTION

Storage mites of the families Chortoglyphidae, Glycyphagidae, Echimyopodidae, and Acaridae are Acari that belong to the suborder Astigmata, which also includes several species of the allergy-causing house dust mites of the family Pyroglyphidae. Storage mites are common in hay, straw, livestock food, barn dust, grains, and some processed foods (eg, baking mixes, flour, cheese, and meat). Common species of storage mites include *Tyrophagus putrescentiae* and *Acarus siro* in the family Acaridae, *Lepidoglyphus destructor* and *Glycyphagus domesticus* in the family Glycyphagidae, and *Chortoglyphus arcuatus* in the family Chortoglyphidae.

Allergy to storage mites in human and canine populations has gained interest in recent years. A high prevalence of sensitization to storage mites in dogs with atopic dermatitis has been reported in the United States and Europe.¹ Studies in Europe and Latin America report a significant prevalence of sensitization to storage mites in rural and urban human populations, especially among individuals who are occupationally ex-

posed.^{2–12} Allergens from storage mites induce bronchial asthma and allergic rhinitis and possibly atopic dermatitis. In rural populations, storage mite allergies may be more important than allergies from pollens and animal dander.¹³ There are also reported cases of anaphylactic reactions after patients consumed mite-contaminated cooked foods.^{14–21}

Despite these data from other countries, the prevalence of sensitization to several common species of storage mites is not known in the US population. Because these mites are common in stored grain, the mites or their products can contaminate cereals, flour, and baking products.²² Storage mites can also contaminate occupational and nonoccupational environments, so it is likely that a significant portion of the US population may be sensitized to these mites. A recent survey²³ found that 6.5% of a population in southwestern Ohio has serum IgE to the storage mite *T putrescentiae*. The key reason for the lack of storage mite sensitization data is that there are no commercially available extracts for skin and serum testing. However, these mites may be a risk factor for allergic disease in the US population, but they are not usually considered by allergists in making a diagnosis of and prescribing treatment for allergic disease.²⁴ Therefore, the purpose of this study was to determine the prevalence of serum IgE to the storage mites *L destructor* and *A siro* and to evaluate the degree of co-sensitization to various other astigmatid mites in a population in southwestern Ohio.

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METHODS

Serum Samples

The serum samples used in this study were contributed by CompuNet Clinical Laboratories of Dayton, OH, a clinical diagnostic laboratory serving a 7-county region of southwestern Ohio. This region includes areas that are urban, suburban, and rural. The 600 serum samples tested were the same as those described previously²³ and were randomly selected from the approximately 3,000 collected on a single day in November 2002.

Antigen Extracts

The following species of storage and house dust mites were reared in the laboratory on appropriate growth medium: *C arcuatus*, *L destructor*, *T putrescentiae*, *A siro*, *Dermatophagoides farinae*, *Dermatophagoides pteronyssinus*, and *Euroglyphus maynei*. Live mites were collected as they migrated from cultures, and they were killed by freezing at -20°C . *Blomia tropicalis* mites were a gift from Dr Enrique Fernandez-Caldas of CBF Leti Inc (Tres Cantos, Spain). The *Sarcoptes scabiei* var *canis* were grown on New Zealand white rabbits. Extracts were prepared as previously described^{1,25} by suspending mites in glass-distilled water for 48 hours at 4°C , after which they were ground in a TenBroeck homogenizer. Insoluble material was removed by centrifugation, and the aqueous extract was filtered through a $0.22\text{-}\mu\text{m}$ filter into a sterile vial for storage at 4°C . Protein content was estimated using the method of Bradford,²⁶ with bovine serum albumin as the standard. In parallel, extracts were prepared of the diets used to rear the mites.

Sodium Dodecyl Sulfate–Polyacrylamide Gel Electrophoresis and Immunoblotting

Sodium dodecyl sulfate–polyacrylamide gel electrophoresis and immunoblotting using a slot blot apparatus were used to screen the 600 serum samples for IgE binding to proteins in extracts of *A siro* and *L destructor* according to the methods previously described.^{1,23} Sodium dodecyl sulfate–polyacrylamide gel electrophoresis gels (12% acrylamide Prep-well gels; Bio-Rad Laboratories, Hercules, CA) were loaded with $100\text{ }\mu\text{g}$ of mite extract protein and were electrophoresed at 200 V. Each gel also contained a reference well loaded with a molecular weight standard (Kaleidoscope Standard; Bio-Rad Laboratories). Separated proteins were transferred to ProBlott membranes (Applied Biosystems, Foster City, CA) in buffer according to the manufacturer's instructions. Membranes were washed, blocked, and placed into the slot blot apparatus (Mini-Protean Multiscreen apparatus; Bio-Rad Laboratories), and individual lanes were loaded with serum samples diluted in a ratio of 1:5 ($125\text{ }\mu\text{L}$ of serum + $500\text{ }\mu\text{L}$ of buffer). After overnight incubation, blots were washed, removed from the apparatus, and incubated in $0.7\text{ }\mu\text{Ci}$ of ^{125}I -labeled anti-human IgE (Diamed Inc, Windham, ME) overnight. Blots were washed, dried, and placed on film for autoradiography for varying periods up to 21 days.

Selected serum samples were also used to probe blots containing extracts of all 9 mite species. In this experiment, 10-well 12% gels were loaded with $2\text{ }\mu\text{g}$ of protein of each mite extract along with 1 lane containing the molecular weight standard. After transfer as described in the previous paragraph, each blot was incubated in a single serum sample diluted in a ratio of 1:5 (2 mL of serum + 8 mL of buffer), and IgE binding was visualized as described in the previous paragraph. Twenty-one of these 30 serum samples were also used to probe 3 additional slot blots, each loaded with $10\text{ }\mu\text{g}$ of an extract of 1 of the 3 foods used to grow the mites in this study.

Data Analysis

Autoradiographic films of the various blots were imaged using an EDAS imaging system (Eastman Kodak Co, Rochester, NY). Molecular weights of the various IgE binding proteins on the digital images were determined using Kodak 1-D software. Serum samples were considered to contain IgE to a given mite if 1 or more proteins in the extract of that mite bound IgE that was observed on an autoradiogram exposed for 21 days or less. The absence of observable bands on autoradiograms exposed for 21 days indicated the absence of mite specific IgE.

RESULTS

Sensitization to *A siro* and *L destructor*

Using a slot blot technique, 600 serum samples were initially probed for serum IgE against proteins of the storage mites *A siro* and *L destructor*. Thirty-two (5.3%) of the 600 serum samples had IgE that bound to proteins in the extracts of 1 or both of these storage mite species. Twelve and 18 serum samples had IgE against proteins in the extracts of *A siro* and *L destructor*, respectively, whereas 2 serum samples had IgE to both. Therefore, 14 (2.3%) and 20 (3.3%) of the 600 serum samples had circulating IgE directed at 1 or more proteins in the extracts of *A siro* and *L destructor*, respectively.

Co-sensitization to Multiple Species of Storage, Dust, and Scabies Mites

Kondreddi et al²³ used the slot blot assay method used herein to identify *A siro*- and *L destructor*-reactive serum samples to screen these same 600 serum samples for the presence of IgE reacting to *T putrescentiae* proteins. In that study, we determined that 39 serum samples contained IgE that bound to proteins in the *T putrescentiae* extract. Based on these preliminary slot blot screenings, we selected 30 serum samples that had circulating IgE that bound to proteins in the extracts of 1 or more of the 3 storage mite species (*A siro*, *L destructor*, and *T putrescentiae*) and that had sufficient serum remaining to perform additional analyses. These 30 serum samples were then used to probe blots containing extracts of 9 astigmatid mite species: the storage mites *C arcuatus*, *L destructor*, *B tropicalis*, *T putrescentiae*, and *A siro*; the house dust mites *D farinae*, *D pteronyssinus*, and *E maynei*; and the parasitic mite *S scabiei*. Twenty-one of these 30

serum samples were also tested for IgE binding to proteins in the various mite diets. No IgE binding to any protein in these extracts was observed.

For the 30 serum samples, the number with IgE to each mite species is given in Table 1. Twenty-six, 17, 24, 15, and 16 serum samples had serum IgE that recognized proteins in extracts of the storage mites *C arcuatus*, *L destructor*, *B tropicalis*, *T putrescentiae*, and *A siro*, respectively. Twenty-five, 17, and 19 serum samples had circulating IgE that recognized proteins in extracts of the dust mites *D farinae*, *D pteronyssinus*, and *E maynei*, respectively, whereas 14 serum samples had serum IgE against the parasitic mite *S scabiei*.

All 30 serum samples had IgE directed at proteins in extracts of 1 or more of the storage mite species. Of these 30 serum samples, 10 (33%) had IgE that recognized at least 1 protein in the extracts of all 5 species of storage mites, whereas 27 (90%) had serum IgE that recognized proteins in at least 2 species of storage mite extracts. Twenty-six (87%)

of the 30 serum samples that had IgE for at least 1 storage mite also had IgE for at least 1 of the 3 species of pyroglyphid house dust mites (*D farinae*, *D pteronyssinus*, and *E maynei*). Therefore, 4 serum samples (13%) had IgE against 1 or more of the storage mites but had no IgE that recognized proteins in any species of dust mite. Fourteen serum samples had IgE to the parasitic scabies mite and at least 1 species of storage mite, with all but 1 of these also having IgE to 1 or more of the house dust mites.

IgE Binding to Proteins of Mites From the Superfamily Glycyphagoidea

All except 1 of the 30 serum samples (97%) had IgE that bound to proteins derived from 1 or more of the mites of the Glycyphagoidea superfamily (*C arcuatus*, *L destructor*, and *B tropicalis*), with 13 serum samples (43%) possessing IgE that bound to proteins in all 3 of these extracts (Table 1). Sixteen serum samples (53%) had IgE to *C arcuatus* and *L destructor*,

Table 1. IgE Binding Profiles of the 30 Serum Samples to the 9 Mite Species*

Patient No.	Storage mites					Dust mites			SS	Total mite species
	CA	LD	BT	TP	AS	DF	DP	EM		
292	+	+	+	+	+	+	+	+	+	9
293	+	+	+	+	+	+	+	+	+	9
532	+	+	+	+	+	+	+	+	+	9
546	+	+	+	+	+	+	+	+	+	9
569	+	+	+	+	+	+	+	+	+	9
13	+	+	+	+	+	+	+	+	0	8
100	+	+	+	+	+	+	+	+	0	8
354	+	+	+	+	+	+	+	+	0	8
418	+	+	+	+	+	+	+	+	0	8
281	+	+	+	0	+	+	+	+	0	7
31	+	0	+	+	+	0	0	+	+	6
86	+	0	+	0	0	+	+	+	+	6
162	+	+	+	0	0	+	+	+	0	6
262	+	0	+	0	+	+	+	0	+	6
263	+	0	+	0	+	+	0	+	+	6
350	0	0	0	+	+	+	+	+	+	6
515	+	+	+	+	+	0	0	0	+	6
534	0	0	+	+	+	+	+	+	0	6
244	+	+	+	0	0	+	0	+	0	5
45	+	+	0	+	0	+	0	0	0	4
80	+	+	0	+	0	+	0	0	0	4
130	+	0	+	0	0	+	0	0	+	4
190	+	0	0	0	0	+	+	+	0	4
260	+	0	+	0	0	+	0	0	+	4
531	0	+	0	0	0	+	+	+	0	4
28	0	0	+	0	0	+	0	0	+	3
285	+	0	+	0	0	+	0	0	0	3
9	+	0	+	0	0	0	0	0	0	2
259	+	0	+	0	0	0	0	0	0	2
513	+	+	0	0	0	0	0	0	0	2
Total serum samples	26	17	24	15	16	25	17	19	14	

Abbreviations: AS, *Acarus siro*; BT, *Blomia tropicalis*; CA, *Chortoglyphus arcuatus*; DF, *Dermatophagoides farinae*; DP, *Dermatophagoides pteronyssinus*; EM, *Euroglyphus maynei*; LD, *Lepidoglyphus destructor*; TP, *Tyrophagus putrescentiae*; SS, *Sarcoptes scabiei*.

* Serum samples were scored as positive (+) if IgE binding was observed on 21-day autoradiograms of the blot to any protein in the extract of the mite species.

and 9 of these showed a consistent and distinctive pattern of IgE binding (Fig 1). These 9 serum samples all had IgE that bound to proteins of 102.0 and 72.9 kDa in *C arcuatus* extract and to proteins of 75.5 and 63.9 kDa in *L destructor* extract, although most serum samples had binding to additional proteins in these mite extracts. Although 24 serum samples had IgE that bound to proteins in the *B tropicalis* extract, this binding was generally much weaker than that to proteins of the other 2 species and was directed at higher-molecular-weight proteins (105–120 kDa).

IgE Binding to Proteins of Mites From the Acaridae Family

Eighteen of the 30 serum samples had IgE to proteins from at least 1 of these species, and 13 had IgE to both *T putrescentiae* and *A siro* (Table 1). Immunoreactive proteins in the *T putrescentiae* extract ranged from 40 to more than 300 kDa, with 9 (60%) of the 15 *T putrescentiae*-reactive serum samples containing IgE to a protein of 80.2 kDa (Fig 2). Proteins from *A siro* typically had the weakest binding observed on any given blot.

IgE Binding to Proteins From Pyroglyphid and Scabies Mites

IgE from the serum samples of the 30 patients bound to the widest range of proteins from the pyroglyphid house dust mites *D farinae*, *D pteronyssinus*, and *E maynei*, with proteins of 15 to more than 250 kDa binding antibodies (Fig 3). Sixteen serum samples had IgE to all 3 of these species, and 7 of the 16 showed distinctive binding to an 18-kDa band in the *Dermatophagoides* extracts and to a slightly lower-molecular-weight companion band in the *E maynei* extract.

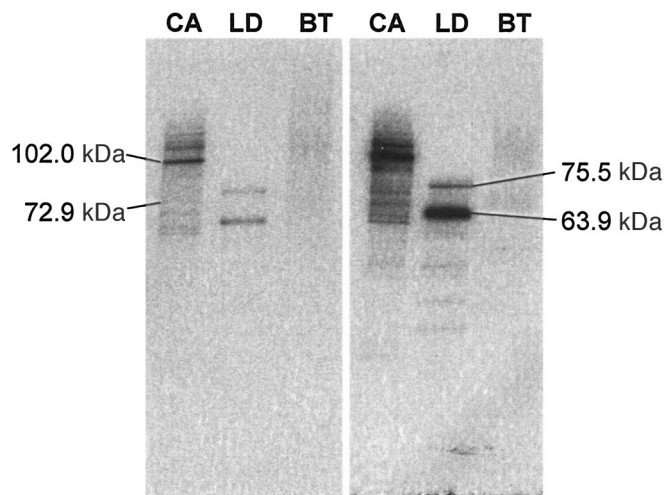


Figure 1. Representative autoradiograms of immunoblots showing binding of human IgE from the serum samples of 2 individuals to allergens of *Chortoglyphus arcuatus* (CA), *Lepidoglyphus destructor* (LD), and *Blomia tropicalis* (BT). Molecular weights of representative proteins are shown.

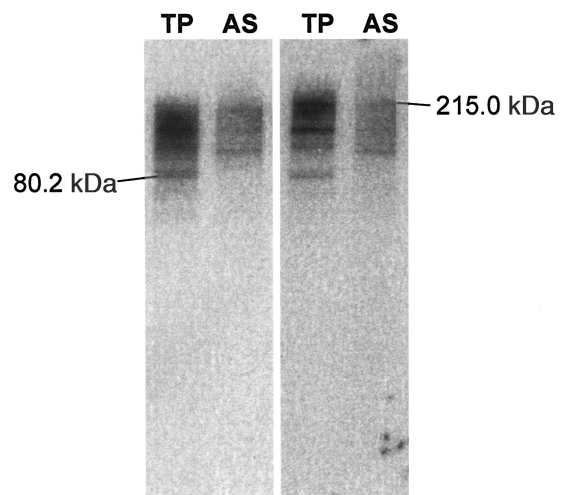


Figure 2. Representative autoradiograms of immunoblots showing binding of human IgE from the serum samples of 2 individuals to allergens of *Tyrophagus putrescentiae* (TP) and *Acarus siro* (AS). Molecular weights of representative proteins are shown.

DISCUSSION

The prevalence of sensitization to stored product mites in the US population is generally unknown. A recent study²³ determined that the prevalence of IgE to the storage mite *T putrescentiae* was 6.5% in a sample of 600 patient serum samples from the Dayton area. In the present study using the same 600 serum samples, we found that 3.3% (n = 20) and 2.3% (n = 14) had IgE to proteins in extracts of the stored product mites *L destructor* and *A siro*, respectively. Therefore, by comparison in this same population using the same methods, the presence of IgE to *L destructor* and *A siro* was approximately half that for the more ubiquitous *T putrescentiae*. This suggests that exposure to these 2 species of storage mites in this population is probably less than it is for the more ubiquitous *T putrescentiae* mite.

The prevalence of serum IgE (sensitization) to *A siro* and *L destructor* in our US population is considerably less than has been reported in surveys in Europe for urban and occupationally exposed populations. In these European studies,^{2,5,9} the reported prevalence of serum IgE to storage mites (*A siro*, *L destructor*, *T putrescentiae*, and *Tyrophagus longior*) ranges from 10% to 22% in nonoccupationally exposed rural and urban populations. Two other studies^{7,12} using skin prick testing reported that sensitization in urban and rural populations in Spain and Iceland to the storage mites *T putrescentiae* and *L destructor* was 6% to 22%. Again, this prevalence is much greater than we found by detecting serum IgE.

Until our studies using these serum samples, the only comparable data for a population from the United States was from a study of 120 farmers in Wisconsin that found that 7.5% had IgE to at least 1 of the storage mites *L destructor*, *T putrescentiae*, *C arcuatus*, and *G domesticus*.²⁷ However, none of the serum samples examined from 14 nonfarmers had

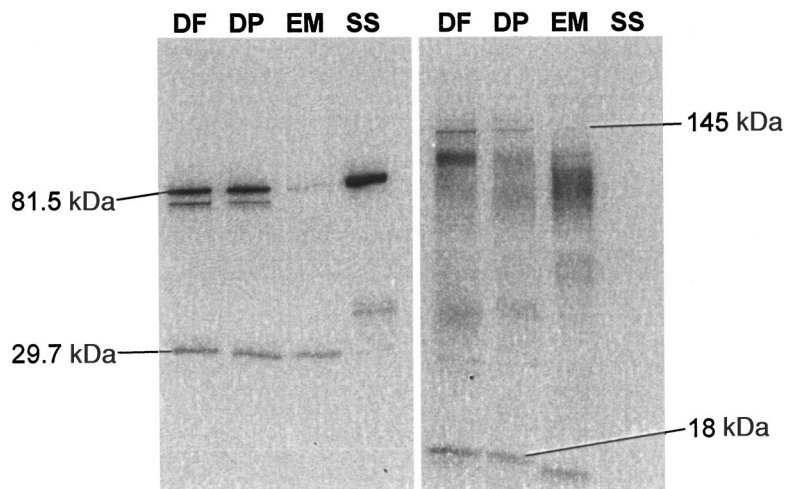


Figure 3. Representative autoradiograms of immunoblots showing binding of human IgE from the serum samples of 2 individuals to allergens of *Dermatophagoides farinae* (DF), *Dermatophagoides pteronyssinus* (DP), *Euroglyphus maynei* (EM), and *Sarcoptes scabiei* (SS). Molecular weights of representative proteins are shown.

IgE by radioallergosorbent testing to these storage mites. The present study shows a much greater frequency of sensitization in a much larger and more diverse population than was found for the nonfarmers in Wisconsin.

It is not clear how exposure to storage mites occurred in the population we studied. We obtained serum samples from a clinical laboratory that serves 600 physician offices and a large hospital in the Dayton area in southwestern Ohio. Serum samples were sent to this laboratory for a variety of routine tests that were not disclosed to us, and information about the donors was removed. However, this laboratory does not do allergy testing, so the serum samples were not submitted for this specific purpose. The clinical laboratory serves a 7-county region around Dayton, so the samples probably represent people from many environments, including rural, urban, and suburban. Therefore, the serum samples used in this study represent a cross-section of the population in this region.

Storage mites and their allergens can be present in the home and work environments, and they have been found in almost all countries where surveys have been conducted.^{28,29} In addition to environmental exposure, storage mites and their allergens may contaminate foods. Some packaged baking mixes and dry foods are or can become contaminated with mites or mite allergens.^{16,22} Even ham has been reported to be contaminated with *Tyrophagus* mites.³⁰ These data suggest that food may be the source of storage mite allergens and thus that sensitization to these mite allergens may result from ingestion or inhalation of these contaminated foods or cooking materials.

To evaluate sensitization to various other mite species that people commonly encounter, we further determined the presence of IgE to other mite species using 30 serum samples containing IgE to at least 1 of the 3 species (*A siro*, *L*

destructor, or *T putrescentiae*) used in the initial slot blot screening. In addition to these 3 storage mites, this evaluation included extracts of the storage mites *C arcuatus* and *B tropicalis*; the dust mites *D farinae*, *D pteronyssinus*, and *E maynei*; and the scabies mite *S scabiei*.

As has been reported by other researchers, we found that sensitization to storage mites was associated with sensitization to house dust mites. A significant finding from our survey is that 90% of the 30 serum samples had IgE to proteins of at least 2 of the species of storage mites tested and that 87% had IgE to at least 1 species of storage mite and to 1 or more of the pyroglyphid mites. Although house dust and storage mites are the sources of many allergens (possibly >20), they generally share few cross-reactive allergens.³¹⁻³⁵ This suggests that the individuals in this study had exposure to storage and house dust mites in some aspect of their environment. *Acarus siro* and *D pteronyssinus* and *T putrescentiae* and *D pteronyssinus* reportedly share at least 1 epitope on a 25-kDa (group 1) allergen.³² However, the serum samples in our study recognized unique higher-molecular-weight sets of proteins of the storage mites and lower-molecular-weight allergens in the dust mite extracts, suggesting that our patients likely were sensitized to storage mites independently of house dust mites. We cannot directly determine from our data whether some IgE binding to storage and house dust mite extracts was due to allergenic cross-reactivity. A more comprehensive immunobiochemical study is required to confirm this hypothesis. However, 4 of the serum samples had IgE only to storage mites and not to the dust mites, which indicated that some individuals' immune systems recognize storage mite proteins but not house dust mite proteins as allergens. The house dust mites *D farinae* and *D pteronyssinus* occur in most homes in southwestern Ohio; thus, they are

ubiquitous, and it is unlikely that someone in this geographic region is not exposed to allergens of these mites.

In conclusion, our data indicate that sensitization to storage mites occurs in a significant portion of the population in southwestern Ohio. Broader surveys need to be conducted to determine storage mite sensitization in the general US population and the prevalence of mite contamination in foods. Commercial extracts are needed in the United States for allergists to use in the diagnosis and treatment of allergic patients.

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