Journal of Pioneering Medical Sciences
Received: January 25, 2025 | Accepted: February 27, 2025 | Published: June 05, 2025
Volume 14, Issue 05, Pages 103-009

DOI https://doi.org/10.47310/jpms2025140516

Modulation of HDM (House Dust Mite)-Specific Immune Response in Mice by Preimmunization with HDM Antigen before *Ascaris lumbricoides* Infection

Fatma H. Galal[1*], Hanan T. Hamza[2], Sherifa H. Ahmed[3], Mervat A.A. EL-Alfy[4] and Nedal M. Fahmy[5]

[1,2,3]Department of Biology, College of Science, Jouf University, Sakaka, 41412, Saudi Arabia
[2]Department of Zoology, Faculty of Science (Girls), Al-Azhar University, Nasr City, Cairo, Egypt
[3]Department of Zoology, Faculty of Science, Port Said University, Port Said, 42521, Egypt
[4]Department of Biology, Faculty of Sciences, University of Bisha, P.O. Box:551, Bisha, 61922, Saudi Arabia
[5]Department of Biology, Faculty of Science, University of Tabuk (UT), Kingdom of Saudi Arabia

*Corresponding author: Fatma H. Galal (e-mail: fhgalal@ju.edu.sa).

Abstract This study's primary goals were to identify and describe the common species of house dust mites that were collected from randomly chosen homes in various Kafr EL-Shekh Governorate locations between 2023 to 2024. Using a vacuum cleaner, samples were taken from the floor, bedding, living areas and bedrooms. Mice were immunized before/after being contamination with *Ascaris lumbricoides* eggs using a house dust mite antigen, from frozen mites. After the experiment, blood was drawn. ELISA method was used to determining total serum IgE, IgG1 and IgG2a as well as HDM (house dust mite)-specific IgE. Mites were found in 22 out of 50 randomly chosen homes (44%). The highest percentage of HDM was found in moldy or damp patches on floors (68.3%), followed by carpets in living rooms (22.3%) and bedroom rugs (9.4%). The infected non-immunized group had a noticeably greater amount of HDM-specific IgE. When the prophylactic group was immunized with HDM-specific antigen before *Ascaris* infection, the OD absorbance value of HDM-specific IgE was at its greatest and drastically decreased. Furthermore, the data showed that HDM-sensitization strongly produced systemic HDM-specific IgG1 and IgG2a. The infected non-immunized group had a significantly higher level of HDM-specific IgG1, but the prophylactic group, which received an HDM-specific antigen vaccination before Ascaris infection, had a significantly lower level of HDM-specific IgG1. Following Ascaris infection, HDM-specific IgG2a levels decreased following immunization with HDM-specific antigen. In conclusion: moist or moldy spots on floor samples had the highest percentage of HDM. Mice are immunized with HDM before Ascaris. By decreasing the IgE-dominated type-2 response, lumbricoides egg infection decreased type 2 immunity.

Key Words House Mite, Immunization, *Ascaris lumbricoides*, TH-2 type Immune Response

INTRODUCTION

One cosmopolitan guest in human habitation is the House Dust Mite (HDM). Almost every home has dust mites. Typically, they can be found in carpets, pillows, mattresses, bed linens and stuff toys. Dust mites feed on organic waste, including human skin flakes and flourish in the stable conditions found in homes. Worldwide, exposure to household dust mites is acknowledged as a primary cause of allergies. In the last 30 years, HDMs have been identified as one of the primary human allergen sources [1,2]. The quantity and concentration of mites can have an impact on how severe allergic reactions are [3]. Generally, one gram of house dust contains 300 mites and under perfect breeding conditions, one gram of mattress dust can contain 5,000 mites. A weight of more than 100 mites per g is considered a risk factor for allergy sensitization. 9. Two main species that cause allergies are *Dermatophagoides pteronyssinus* and *Dermatophagoides farina* the European and American house dust mite, respectively.

To avoid an infection-preventing host immune response, parasites use a variety of tactics. Even though immune evasion has been designed to promote the

development of parasites within the host, some specific immune evasion techniques may, ironically, also be advantageous to the host. Twelve The main elements of the immunological regulation network that operate during helminth infections have been determined to be regulatory T cells (Tregs), regulatory B cells (Bregs) and alternatively activated macrophages [3-5]. During parasite infection, these immune regulatory cells proliferate and may stop the emergence of immune-driven pathologies that are unrelated, such as autoimmune and allergy disorders [4-6]. The most prevalent intestinal helminth infection in the world, *Ascaris lumbricoides* or roundworm, affects 761.9 million people in underdeveloped regions of Africa, Asia and Central and South America [7,8]. In areas where endemicity exists, Children are frequently infected shortly after birth, continue to be infected throughout their youth and achieve their maximal worm burden by the time they are in preschool and school [9,10]. With 1.46 million disability-adjusted life years (DALYs), Ascariasis is linked to a high degree of worldwide morbidity. This impact is comparable to that of other well-known children's disorders, like meningitis (1.52 million DALYs) [7,11]. According to several researchers, in resource-poor nations, larval Ascaris infection is a significant environmental cause of inflammatory lung illness, asthma symptoms and allergic airway disease [2,9]. According to this epidemiological research, children who have Ascaris-induced allergic airway illness are more likely to have more severe symptoms and to be more sensitive to bystander antigens, such as dust mites in the home [12-14]. The mechanism by which ascariasis causes infants to develop chronic allergic airway illness is unclear, though. Understanding the end-organ damage caused by ascariasis and creating preventative and therapeutic strategies are urgently needed due to the disease's significant impact on morbidity. It has been demonstrated that purified *Dermatophagoides pteronyssinus* protein 2 (Der p 2) is effective as a preventative allergen immunotherapy (AIT) to stop type 2 immunity in mice with asthma [6]. AIT is mostly suggested as a treatment after the disease has been diagnosed and the prognosis for asthma is currently too uncertain to suggest it as a preventive measure. Using the ELISA technique to quantify total serum IgE, IgG1 and IgG2a, we examined whether immunization with HDM would improve or worsen the immune response against an experimental model of Ascaris infection.

METHODS

Collection of House Dust Samples

During the September 2023-February 2024 study period, 50 house dust samples were taken from randomly chosen homes in various Kafr EL-Shekh Governorate locations. The participants were instructed not to clean or vacuum the dust collection area for three days prior to sampling. A vacuum cleaner was used to collect samples from 1 m2 of each sampling location, including bedrooms, floors, bedding and living areas. The bag was cleaned before the sample was collected. After being properly sealed in plastic bags and assigned a numerical label, the samples were delivered to the parasitology section of the Theodor Bilharz Research Institute (TBRI). To prevent growth, samples were kept at 4°C and viewed under a stereomicroscope in the TBRI parasitology section [15].

Examination of dust samples

In a specially designed device adapted from Ibrahim *et al.* [16], mites were extracted from dust samples using modified Berlese-Tullgren funnels. The samples were placed in various stainless-steel containers and mites were allowed to pass through the sieves into plastic ide-mouth bottles that contained either 70% alcohol or distilled water (to detect live mites); a wooden skeleton was constructed with basal holes and covered with a mesh. Following the extraction time, the material was moved to a Petri dish so that it could be viewed via a stereomicroscope.

Preparation of house dust mite Antigen

According to Bartley *et al.* [17], the frozen mites were used to prepare the house dust mite antigen. Using an Ultra Turrex® T 25 D-S2 with an S25N-8G dispersion element (IKA®-Werke GmbH & Co. KG, Germany), one gram of frozen mites was suspended in ten milliliters of ice-cold PBS and homogenized on ice for two 30-second pulses. Centrifugation was used to remove debris and insoluble material for 20 minutes at 4°C at 25,000 g. After being decanted, the soluble material underwent a second centrifugation. The Bradford protein assay kit (Thermo Fisher Scientific, USA) was used to quantify the HDM protein concentration.

SDS-PAGE

Using discontinuous Sodium Dodecyl Sulphate-Polyacrylamid gel electrophoresis (SDS-PAGE) in 12% slab gels (1 mm thick), under reducing conditions (+2-mercaptoethanol) and stained with Coomassie blue (Bio-Rad), the extracted HDM antigen was characterized to determine the molecular weight range.

Experimental model of Ascaris infection

The normally ejected feces of an infected patient who consented to give the parasite to this study were used to collect adult A. lumbricoides worms from our patients at the Department of Parasitology TBRI. With a few adjustments, the embryonation of A. lumbricoides eggs was carried out in accordance with Boes *et al.* [18]. Gently mechanically macerating the eggs from the female uterus, filtering them and cultivating them to embryonation in 0.2 M H2S04 allowed for their purification. In 50 mL tissue culture flasks, the eggs were maintained at a concentration of 25 eggs/µL and maintained at 26°C in the dark.

Experimental Design

Swiss albino CD-1 strain male mice, weighing 20-25 g, were acquired from the Schistosome Biological Supply Center (SBSC) at the Theodor Bilharz Research Institute (TBRI) in Giza, Egypt, through closed random breeding. The animals'

living conditions included a 12-hour light/dark photoperiod, a regulated temperature of 22±3°C and a relative humidity of 50±15%. Water and food were freely available. The Medical Ethical Committee of TBRI, Giza, Egypt, approved this work and the experiments were carried out in compliance with the US National Institutes of Health's Guide for Care and Use of Laboratory Animals (NIH Publication #85-23-1996).

Groups in Experiments A total of 36 mice were split into the following 4 groups at random:

- Group 1: Six mice in a normal, healthy control group
- Group 2: 10 mice inoculated with an immune system. HDM was administered intramuscularly to the right and left tibialis anterior muscles of the mice four, three, two and one week prior to infection
- Group 3: 10 mice infected and immunized. At 1, 2, 3 and 4 weeks after infection, mice were given intramuscular injections of HDM (100 µg/mouse) in the right and left tibialis anterior muscles
- Group 4: In parallel, six healthy, normal mice received an intramuscular injection of HDM (100 µg/mouse) in the right and left tibialis anterior muscles over the same time period of 1, 2, 3 and 4. Eight weeks after infection, the mice were killed and the following metrics were looked at

Determination of HDM-specific IgE

At the conclusion of the experiment, blood was drawn from each of the mouse groups, including the control group. Centrifugation was used to separate the red blood cells from the serum. The following is how ELISA was used to determine the HDM-specific IgE levels. Each well of a polystyrene microtiter plate (Costa, Cambridge, Mass.) received 100 microliters of HDM extract (10 mg/ml in 0.06 mol/L carbonate buffer, pH 9.6), which was then incubated at 4°C for the whole night. To get rid of the HDM-specific IgG, mouse sera were simultaneously treated for four hours at 4°C in 96-well plates covered with anti-mouse whole-molecule IgG antibodies (Sigma, St. Louis, Mo.). After three rounds of washing in 0.05% PBS-Tween solution, the HDM-coated plates were incubated for an entire night at 4°C with IgG-deactivated sera from several mouse groups, including the control group. Following five rounds of washing with 0.05% PBS-Tween buffer, the plates were incubated overnight at 4°C with a specific anti-mouse IgE antibody (Biodesign, Kennebunk, Me). The plates were then rinsed five times with 0.05% PBS-Tween buffer prior to the addition of citric acid-phosphate buffer (pH 5.0) containing 0.15 mg/ml of o-phenylenediamine (Sigma Co., St Louis, Mo.). The color was produced at room temperature and the reaction was stopped with 2.5 mol/L sulfuric acid. At 492 nm, the color (Bio-Rad, Richmond, Calif.) was detected.

ELISA Analysis of Total Serum IgE, IgG1 and IgG2a

Before being quantitatively analyzed for Ig subclasses, isolated serum samples and cardiac blood samples were kept at -70°C. Following the manufacturer's instructions, the mouse ELISA kit (eBio¬science) was used to quantify the quantity of total IgE, IgG1 and IgG2a in mouse serum.

RESULTS

Collection and Microscopical Identification of House Dust Samples

A total of 1200 dust samples were gathered for this investigation between September 2023 and February 2024 from randomly chosen homes spread over the Kafr EL-Shekh Governorate. Mites were found in 22 out of 50 randomly chosen homes (44%). Table 1 demonstrates that the areas with HDM samples and those with negative HDM samples (P<0.001) were those that met the housing criteria (bedrooms, floor, bedding and living rooms by vacuuming from 1 m2 of each sampling place with a vacuum cleaner); the highest percentage of HDM was found in damp or moldy stains on the floor, 819/1200 (68.3%), followed by living room carpets (267/1200 (22.3%) and bedroom rugs 114/1200 (9.4%) (Figure 1).

SDS-PAGE of Extracted HDM Antigen

Profile of Proteins

Figure 2 displays the protein profiles of several antigenic extracts from HDM. The most intense bands at the end of

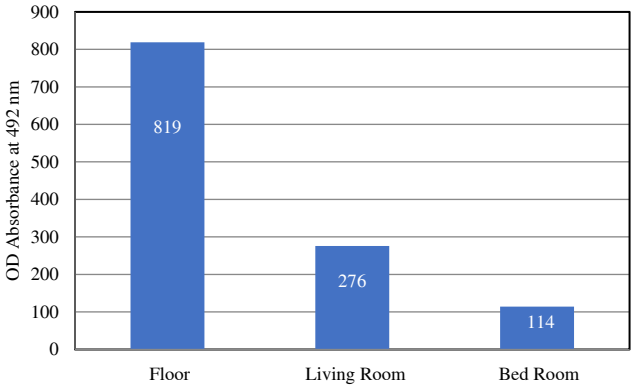

Figure 1: Collection and microscopical identification of house dust samples

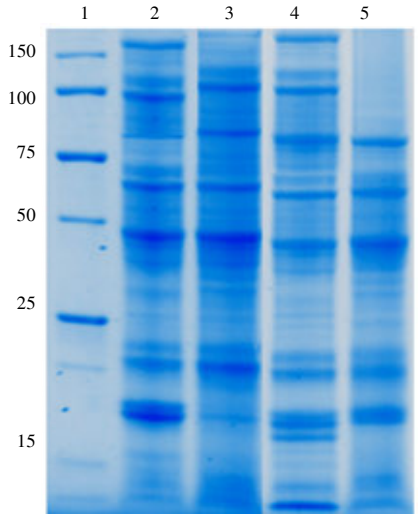

Figure 2: SDS-PAGE of extracted HDM antigen

Lane 1: M.Wt standard, Lane 2: Crude HDM protein, Lane 3: Homogenized HDM protein, Lane 4: Soluble Material of HDM protein, Lane 5: Purified HDM protein

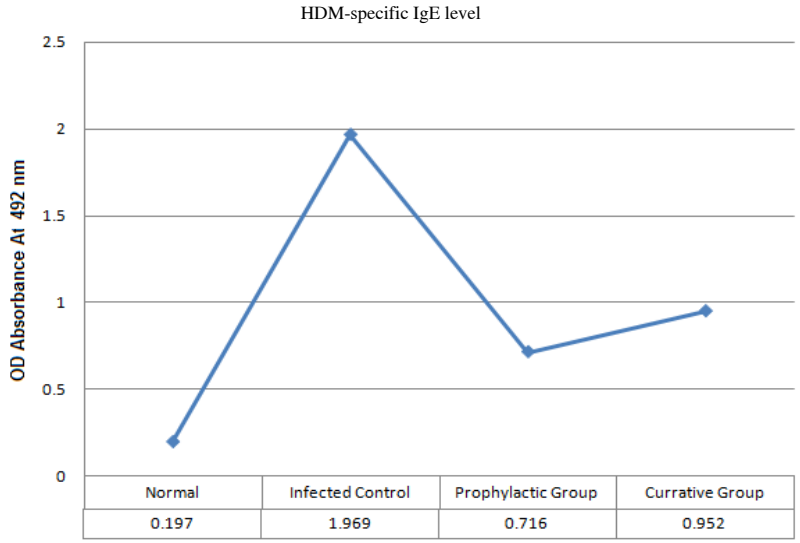

Figure 3: Determination of HDM-specific IgE

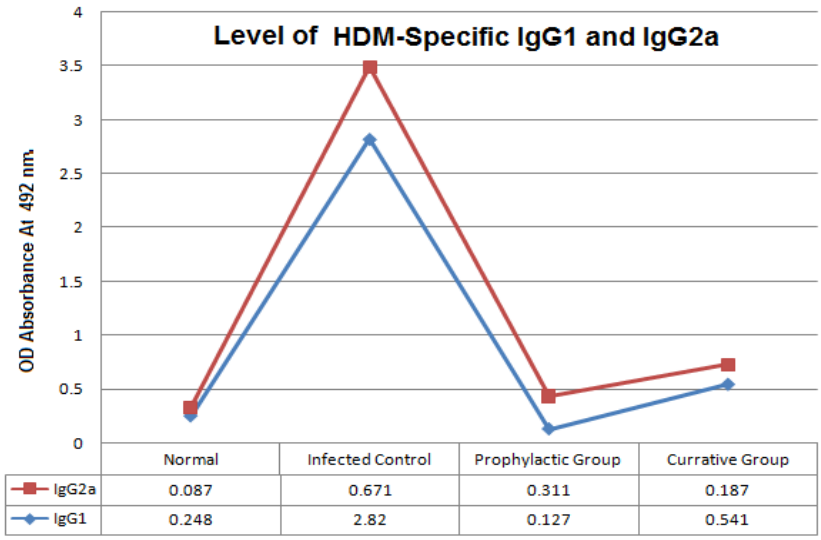

Figure 4: Determination of HDM-specific IgG1 and IgG2a

HDM preparation were at 50 KDa, 22 KDa and 20 KDa. The number and strength of bands in the SDS-PAGE during the various HDM preparation processes were identified. In the final processing step, the HDM protein was more strongly recognized.

Identifying HDM-specific IgE

The generation of IgE specific to allergens is one of the key components of allergic sensitivity. As a result, HDM-specific IgE was measured in each of the groups under study. In the infected non-immunized group, the OD absorbance value of HDM-specific IgE was significantly greater at 1.82, but in the prophylactic group, which received HDM-specific antigen vaccination before *Ascaris* infection, the OD absorbance value of HDM-specific IgE was much lower at 0.72. The OD absorbance value of HDM-specific IgE was considerably reduced to 0.99 following immunization with HDM-specific antigen following Ascaris infection. The HDM-specific IgE responses in immunized mice were almost ten times greater than those in the normal control group (Figure 3).

Determination of HDM-specific IgG1 and IgG2a

The determination of HDM-specific IgG1 and IgG2a was carried out in the various groups under study to comprehend the function of HDM-induced particular antibodies in the allergen-driven type-2 protective immune response to the *Ascaris*. demonstrate that high systemic HDM-specific IgG1 and IgG2a levels were elicited by HDM-sensitization (Figure 4). The infected non-immunized group had a considerably greater OD absorbance value of HDM-specific IgG1 (2.82), whereas the preventive group experienced a significant decrease in OD absorbance value of 0.12 following immunization with HDM-specific antigen before

Ascaris infection. The OD absorbance value of HDM-specific IgG1 was considerably reduced to 0.541 following immunization with HDM-specific antigen following *Ascaris* infection. Following immunization with HDM-specific antigen before *Ascaris* infection (Prophylactic group), the OD absorbance value of HDM-specific IgG2a was highest and considerably decreased by 0.311. The level of IgG2a was much greater in the infected non-immunized group (0.671). The OD absorbance value of HDM-specific IgG2a was considerably reduced by 0.187 following immunization with HDM-specific antigen following *Ascaris* infection. The HDM-specific IgG1 and IgG2a responses in immunized mice were almost five times greater than those in the normal control group.

DISCUSSION

Fifty years have passed since the dust mite was initially recognized as a significant contributor to household dust allergens and, consequently, a major cause of allergic respiratory [3]. One of the primary sources of allergens in human indoor environments, House Dust Mites (HDM) can lead to allergy illnesses like atopic dermatitis, asthma and rhinitis [1,2].

As a result of the increasing number of allergies linked to HDM, research on HDM prevalence has significantly expanded to reduce the possible cause. The incidence of dust mite allergens in the blood and homes of sensitive people has been the subject of research conducted in Egypt over the past ten years. According to several studies [6-8,12,19,20], various mite species have been identified and linked to a wide range of human allergies in locations and at different ages.

The current study aimed to identify House Dust Mites (HDMs) morphologically from dust samples collected from various locations within the Kafr EL-Shekh Governorate between September 2023 and February 2024. Additionally, the study aimed to determine how the prevalence of HDMs was impacted by the quantity of mites in various housing criteria. Dust samples were taken during the six-month study period and the results showed a 44% prevalence rate. Our investigation showed that the samples from the floor had the highest density of HDM, 819/1200 (68.3%), with damp or moldy stains, while the samples from the carpets in the living rooms had the lowest infestation, 267/1200 (22.3%), while the samples from the bedroom rugs had the lowest, 114/1200 (9.4%).

These findings align with those of other Egyptian studies. El-Meligy [13], Ayuso *et al.* [9] and Abou Galalah *et al.* [12], discovered that mites were highly prevalent and dense in Menoufia Governorate areas with high relative humidity and a very poor economic standard. Yahia and Metwally [21] found similar outcomes in Sharkia Governorate. In contrast to this, El-Shazly *et al.* [14].

They found that urban regions (those with higher economic standards) had more dust mites than rural areas in the Dakahlia Governorate. Compared to other governorates, Dakahlia has a greater annual relative humidity, which could account for this. The reproduction rate of mites in the home environment was sustained by household populations that tended to spend extended periods at home, shared bedding areas and left personal belongings in living rooms. Researchers studying mite dispersal in the home environment face a challenging task due to the dearth of reliable data regarding indoor temperature and humidity in various Egyptian [21].

For dust mites, carpet is the ideal habitat [22]. Wilson and Platts-Mills [23] advise minimizing carpets and removing them to be cleaned and let to air dry. Even though we found dust mites more frequently in samples taken from homes where vacuuming is a regular housekeeping procedure, this finding was deemed statistically unimportant. This supported the theories of Boes *et al.* [18] and Sercombe *et al.* [5], who hypothesized that even with contemporary vacuums, it is impossible to remove all dust mite allergens and detritus from a carpet [11]. Using the ELISA technique to detect total blood IgE, IgG1 and IgG2a, trials were conducted to determine if immunization with HDM would improve or worsen the immune response against an experimental model of *Ascaris* infection. Infection with a high number of A is associated with delayed blood eosinophilia and the most significant pulmonary infiltrates and the highest levels of IgE linked to asthmatic reactions happen during the larvae's migration, typically between 10- and 14-days post-infection [4]. It is noteworthy that Cooper [10] demonstrated that individuals from endemic areas who contracted *A. lumbricoides* exhibited a strongly polarized Th2 immune response. This finding implies that a change from a pro-inflammatory response, as seen in murine models and human experimental infections, to a Th2 response may be a characteristic of chronic infections after the tissue migration phase or possibly following adult worm patency in the host lumen. HDM-specific IgE was measured in the several groups under investigation in this study. The infected non-immunized group had a considerably greater OD absorbance value of HDM-specific IgE (1.82), whereas the preventive group experienced a significant drop in OD absorbance value of 0.72 following immunization with HDM-specific antigen before *Ascaris* infection. Following an *Ascaris* infection, the OD absorbance value of HDM-specific IgE was considerably reduced to 0.99 upon immunization with HDM-specific antigen.

Compared to the normal control group, the inoculated mice's HDM-specific IgE responses were nearly ten times greater. Cheng *et al.* [24] observed similar effects, wherein the sensitized B10.RIII mice demonstrated an IgE response and a T-cell response specific to HDM in vitro. Alum-absorbed Der p 1 immunization has been shown to improve the mice's IgE response. Using a mouse model of HDM-induced allergic pulmonary inflammation followed by Ascaris infection, we showed that HDM sensitization alone can produce IgE and IgG1 antibodies that uniquely identify surface-expressed proteins on

Ascaris larvae. We have identified two proteins that *Ascaris* encodes: tropomyosin and enolase.

Tropomyosins are members of a highly conserved protein family that is found in both muscle and non-muscle cells in a variety of isoforms [9]. The HDM-specific IgG1 and IgG2a tests were conducted in the various groups under study in order to comprehend the function of HDM-induced specific antibody determination. Strong systemic HDM-specific IgG1 and IgG2a levels were induced by HDM-sensitization, according to the data. A study conducted by Morsy *et al.* [25] in the Qualyobia Governorate found that dust samples collected from the houses of patients with atopic dermatitis contained three different types of mites. These mites were *Dermatophagoides pteronyssinus*, *Ornithonyssus bacoti* and *Haemogamasus pontiger*. The former species was the most prevalent and produced the strongest allergen. It has been established that home dust mites are one of the etiological factors of atopic dermatitis and that mite genera other than Dermatophagoides may cause allergies.

IgE was shown to be significantly significant in respiratory allergy patients (28) with (5) or without (23) atopic dermatitis, according to Morsy *et al.* [25], who recovered four species of mites from dust collected from the homes of patients with allergic respiratory disorders. They concluded that one of the primary aetiological determinants of allergic respiratory illnesses, with or without atopic dermatitis, is HDM-induced inhalant allergens.

According to Koraiem and Fahmy [26], nine different kinds of mites were found indoors in Greater Cairo. They included *Dermatophagoides pteronyssinus*, *D. farinae*, *Tyrophagous putrescentiae*, *Acarus siro*, *Acheles graciles*, *Cheyletus malaccensis*, *Blomia kulagini*, *Ornithonyssus bacoti* and *Lepidoglyphus destructor* in order of abundance. The primary sources of these mites were Al Wayly (27.6%) and Bolak Al Dakrour (28.8%). Madent Al Salam yielded the fewest species (only three) and the fewest number (1.8%). Domestic birds, commensal rodents and stray and pet dogs and cats were more or less documented in most of the sites that were surveyed. Additionally, 10 out of the 80 homes inspected had patients with a history of allergies of unclear cause (12.5%). House dust mites were a significant contributor to allergy symptoms.

CONCLUSIONS

Moist or moldy spots on floor samples had the highest percentage of HDM. Mice are immunized with HDM before *Ascaris*. By decreasing the IgE-dominated type-2 response, *lumbricoides* egg infection decreased type 2 immunity. This study's data showed that HDM sensitization strongly produced systemic HDM-specific IgG1 and IgG2a. The infected non-immunized group had a significantly higher level of HDM-specific IgG1, but the prophylactic group, which received an HDM-specific antigen vaccination before *Ascaris* infection, had a significantly lower level of HDM-specific IgG1. Following Ascaris infection, HDM-specific IgG2a levels decreased following immunization with HDM-specific antigen.

Conflicts of Interest

The authors declare no conflict of interest.

Ethical Considerations

The Medical Ethical Committee of TBRI, Giza, Egypt, approved this research work and the experiments were carried out in compliance with the US National Institutes of Health's Guide for Care and Use of Laboratory Animals (NIH Publication #85-23-1996).

REFERENCES

[1] Milián, Evelyn, and Ana MarÃa Díaz. "Allergy to house dust mites and asthma." *Puerto Rico Health Sciences Journal,* vol. 23, no. 1, March 2004. https://prhsj.rcm.upr.edu/index.php/prhsj/article/download/419/307.

[2] Pawankar, R. "Allergic rhinitis and asthma: are they manifestations of one syndrome?." *Clinical & Experimental Allergy,* vol. 36, no. 1, December 2005, pp. 1-4. https://onlinelibrary.wiley.com/doi/abs/10.1111/j.1365-2222.2006.02420.x.

[3] Miller, Jeffrey D. "The role of dust mites in allergy." *Clinical Reviews in Allergy & Immunology,* vol. 57, no. 3, June 2018, pp. 312-329. https://link.springer.com/article/10.1007/s12016-018-8693-0.

[4] Phills, James A. *et al.* "Pulmonary infiltrates, asthma and eosinophilia due to Ascaris suum infestation in man." *New England Journal of Medicine,* vol. 286, no. 18, May 1972, pp. 965-970. https://www.nejm.org/doi/abs/10.1056/NEJM197205042861802.

[5] Sercombe, Jason K. *et al.* "The vertical distribution of house dust mite allergen in carpet and the effect of dry vacuum cleaning." *International Journal of Hygiene and Environmental Health,* vol. 210, no. 1, 2007, pp. 43-50. https://www.sciencedirect.com/science/article/pii/S1438463906000721.

[6] Taha, Afaf Abd El-Raouf *et al.* "House dust mites among allergic patients at the Allergy and Immunology Unit, Zagazig University: an immunologic and serologic study." *Journal of Parasitic Diseases,* vol. 42, June 2025, pp. 405-415. https://link.springer.com/article/10.1007/s12639-018-1016-9.

[7] Al Dhduh, M. A. *et al.* "Prevalence and severity of allergic diseases among Egyptian pediatric in different Egyptian areas." *International Journal of Pharmaceutical Sciences Review and Research,* vol. 2, 2015.

[8] Antonios, Sanaa N. *et al.* "House dust mites as a risk factor in chest allergic patients in Gharbia Governorate, Egypt." *The Egyptian Journal of Medical Microbiology,* vol. 21, no. 1, 2012, pp. 51-61.

[9] Ayuso, Rosalia *et al.* "Molecular basis of arthropod cross-reactivity: IgE-binding cross-reactive epitopes of shrimp, house dust mite and cockroach tropomyosins." *International Archives of Allergy and Immunology,* vol. 129, no. 1, September 2002, pp. 38-48. https://karger.com/iaa/article/129/1/38/164475.

[10] Cooper, Philip J. *Immune responses on humans—ascaris* The geohelminths: ascaris, trichuris and hookworm, 2002, https://link.springer.com/chapter/10.1007/0-306-47383-6_6.

[11] de Boer, Rob. "The control of house dust mite allergens in rugs." *Journal of Allergy and Clinical Immunology,* vol. 86, no. 5, November 1990, pp. 808-814. https://www.sciencedirect.com/science/article/abs/pii/S0091674905801873.

[12] Abou Galalah, Doaa I.M. *et al.* "Morphological identification of house dust mite species in Menoufia Governorate and their antigen effect in immunoglobulin E response in allergic patients." *Menoufia Medical Journal,* vol. 32, no. 1, 2019, pp. 88-96. https://www.menoufia-med-j.com/journal/vol32/iss1/14/.

[13] El-Meligy, M. *Study on house dust mites* MD thesis, Menoufia: Medical Parasitology, Faculty of Medicine, Menoufia University 2002.

[14] El-Shazly, Atef M. *et al.* "Present situation of house dust mites in Dakahlia Governorate, Egypt." *Journal of the Egyptian Society of Parasitology,* vol. 36, no. 1, 2006, pp. 113-126. https://europepmc.org/article/med/16605105.

[15] Morsy, Tosson A. *et al.* "Isolation of three species of mites from house dust of atopic dermatitis patients in Qualyobia Governorate, Egypt." *Journal of the Egyptian Society of Parasitology,* vol. 24, no. 2, August 1994, pp. 323-331. https://europepmc.org/article/med/8077751.

[16] Ibrahim, Radwa *et al.* "Species identification and seasonal prevalence of house dust mites in Assiut City, Egypt: A descriptive study in an urban area." *Persian Journal of Acarology,* vol. 11, no. 1, 2022, pp. 83-99. https://www.biotaxa.org/pja/article/view/69666.

[17] Bartley, Kathryn *et al.* "Identification and evaluation of vaccine candidate antigens from the poultry red mite (*Dermanyssus gallinae*)." *International Journal for Parasitology,* vol. 45, no. 13, November 2025, pp. 819-830. https://www.sciencedirect.com/science/article/pii/S0020751915002052.

[18] Boes, J. *et al.* "Embryonation and infectivity of Ascaris suum eggs isolated from worms expelled by pigs treated with albendazole, pyrantel pamoate, ivermectin or piperazine dihydrochloride." *Veterinary Parasitology,* vol. 75, no. 2, 1998, pp. 181-190. https://www.sciencedirect.com/science/article/pii/S0304401797001970.

[19] Yassin, Mohammad K. *et al.* "Study the quality of school environment as new habitat for allergenc mites in Cairo, Egypt." *Egyptian Academic Journal of Biological Sciences, B. Zoology,* vol. 1, no. 1, 2009, pp. 103-116. https://eajbsz.journals.ekb.eg/article_16026_88fc1749bb4564675065d3d570739abe.pdf.

[20] Hossny, Elham *et al.* "Sensitivity to five types of house dust mite in a group of allergic Egyptian children." *Pediatric Allergy, Immunology and Pulmonology,* vol. 27, no. 3, 2014, pp. 133-137. https://www.liebertpub.com/doi/abs/10.1089/ped.2014.0333.

[21] Yahia, Samah H., and Ashraf S. Metwally. "Sensitivity to five types of house dust mite in a group of allergic Egyptian children." *Life Science Journal,* vol. 16, no. 7, 2019, pp. 59-68.

[22] Vojta, Patrick J. *et al.* "Effects of physical interventions on house dust mite allergen levels in carpet, bed, and upholstery dust in low-income, urban homes." *Environmental Health Perspectives,* vol. 109, no. 8, August 2001, pp. 815-819. https://ehp.niehs.nih.gov/doi/abs/10.1289/ehp.01109815.

[23] Wilson, Jeffrey M and Thomas A E Platts-Mills. "Home environmental interventions for house dust mite ." *Journal of Allergy and Clinical Immunology,* vol. 6, no. 1, January 2018, pp. 1-7. https://pubmed.ncbi.nlm.nih.gov/29310755/.

[24] Cheng, Kuang Chuan *et al.* "House dust mite–induced sensitivity in mice." *Journal of Allergy and Clinical Immunology,* vol. 101, no. 1, January 1998, pp. 51-59. https://www.sciencedirect.com/science/article/pii/S0091674998701939.

[25] Morsy, Tosson A. *et al.* "Four species of house dust mites recovered from houses of patients with allergic respiratory diseases." *Journal of the Egyptian Society of Parasitology,* vol. 25, no. 1, April 1995, pp. 195-206. https://europepmc.org/article/med/7602163.

[26] Koraiem, M. K., and I. A. Fahmy. "Studies on house dust mites in Great Cairo, Egypt." *Journal of the Egyptian Society of Parasitology,* vol. 29, no. 1, January 1999, pp. 131-138. https://europepmc.org/article/med/12561892.