

Evaluation of the presence of house dust mites in horse rugs

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Conflict of Interest

No conflicts of interest have been declared.

Abstract

A sample of fourteen horse rugs and two saddle blankets stored in south western Sydney, Australia, an area of known high dust mite prevalence in the human environment, were analysed for the presence of house dust mites. Dust samples from the rugs, blankets and 16 control sites were collected using a vacuum cleaner with a modified attachment and filter. Dust mites were extracted using an adapted floatation technique. Eight rugs and all control samples were positive for mites, which were confirmed to be house dust mites of the genus *Dermatophagoides*. This study confirms that exposure to house dust mites from horse rugs can occur, indicating that house dust mite allergen reactivity on intradermal and serum allergy testing in atopic horses may represent true dust mite hypersensitivity. Nevertheless, quantification studies will be necessary to ensure that there is adequate mite exposure for development of hypersensitivity, and further evaluation of immunological responses, avoidance and provocation, and specific immunotherapy are required to confirm the clinical relevance. To the authors' knowledge, this is the first study to document the presence of house dust mites in the equine environment.

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Introduction

The occurrence of house dust mites in the equine environment has not been extensively studied, and to date there are no conclusive data supporting their presence or absence. One pilot study published as an abstract found house dust mite antigen and mites similar in appearance to house dust mites in a limited number of horse barn dust samples,¹ but this has not been studied further. Despite this, the response to dust mite antigens is fre-

quently assessed by intradermal or serological testing of atopic horses, in a similar fashion to allergy tests in atopic humans, dogs and cats.²⁻⁴ Some atopic horses exhibit positive reactions on intradermal testing with dust mite allergens, although it is controversial whether this represents true allergy to house dust mites, irritant reactions or cross-reactivity with other true allergens.²⁻⁴

Much of what is known about atopy in horses and in particular the role of dust mite allergens has been adapted from human and canine studies. It is well established that exposure and sensitization to house dust mites, most commonly *Dermatophagoides farinae* and/or *Dermatophagoides pteronyssinus*, strongly correlates with the development of allergic disease in both humans and dogs,⁵⁻⁸ and it is proposed that these may be relevant allergens in some atopic horses. *Dermatophagoides farinae* and *D. pteronyssinus* are the most prevalent house dust mite species worldwide, with their relative abundance determined by climatic factors.⁹⁻¹¹

Cross-reactivity between house dust mites and food storage mites has been demonstrated in atopic humans and dogs.^{12,13} Food storage mites are reported in horse feeds^{14,15} and, considering common bulk storage of horse feeds in continuous-use containers, storage mite exposure in the equine environment seems likely. Allergy to storage mites could therefore be responsible for false-positive dust mite reactions in atopic horses, regardless of whether dust mites exist in a typical equine environment.

House dust mites are known to feed primarily on skin scales⁹ and are known to be prevalent in the environment of humans and dogs in bedding, upholstery, carpets and clothing.^{6,8,16,17} *Dermatophagoides* species have been also been grown on equine dander *in vitro*,¹⁸ suggesting they could survive in an equine environment, feeding on shed equine skin scales. We hypothesize that horse rugs and saddle blankets are good sources of equine skin scales for mite nutrition and are likely sites to harbour house dust mites. In locations where horses are housed in enclosed, heated and humidified stables for extended periods, stable floors and bedding may be other potential sites.

The aim of this study was to determine whether house dust mites are present in horse rugs or saddle blankets stored in a region where house dust mites are known to be prevalent in the human environment.

Materials and methods

Sample collection

Dust samples were collected using a household vacuum cleaner (Dyson DC23; Dyson Appliances [Aust] Pty Ltd, Taren Point, NSW, Australia) with a modified polyvinyl chloride (PVC) collection

apparatus (Figure 1) designed to house a conical cloth filter (Figures 2 and 3) to catch suctioned dust.

Sixteen horse rugs were sampled from three locations in south western Sydney, NSW, Australia, a region with high house dust mite



Figure 1. Polyvinyl chloride collection apparatus attached to end of the telescopic rod of the vacuum cleaner for dust sample collection.



Figure 2. Conical cloth filter to fit inside PVC collection apparatus to trap the dust sample.



Figure 3. Disassembled PVC collection apparatus with cloth filter in place.

prevalence in the human environment.¹¹ This region is approximately 30–40 km from the east coast of Australia, and has a temperate climate (Table 1). Rugs of varying materials and brands, each a minimum of 1 year old, were sampled.

Sixteen control samples were also collected from three similar locations in the test region to test the reliability of the collection and extraction techniques. The control samples were taken from household areas expected to have high dust mite prevalence: carpets, cloth-upholstered sofas and a mattress, all in use for 5 years or more (Table 2).^{6,12,19} All samples were collected throughout the winter months of June and July 2009.

The test and control sites were vacuumed for a period of 2 min, collecting from the entire inner surface of each horse rug or blanket, and approximately 1 m² of the control sites. The dust samples were transferred to a labelled plastic zip lock bag and stored at room temperature. The dust collection filter was washed in hot water and allowed to dry thoroughly between sampling.

Extraction of mites

The dust samples were processed within 24 h of collection. Mites were extracted using a modified floatation technique.^{20,21} For each dust sample, three subsamples were processed. Approximately 0.1 g of dust was sufficient to enable mites to be found reliably whilst avoiding excessive debris impeding visualization of mites under the microscope. One drop of household dishwashing detergent was pipetted onto the sample, and approximately 6 mL of saturated sodium chloride solution added. The samples were shaken to thoroughly wet and mix the dust, left to settle for a minimum of 30 min and then centrifuged at 220 *g* for 4 min. One drop of 1% crystal violet stain was added with gentle mixing until the majority of the supernatant was coloured (taking care not to disturb the settled dust). The supernatant was poured into a filtering system consisting of a 55 mm diameter Buchnar funnel, a Buchnar flask and a vacuum pump. The Buchnar funnel was lined with one circle of 55 mm Whatman no. 1 filter paper (Whatman International Ltd, Maidstone, UK) that had been marked with parallel track lines 8 mm apart to aid later microscopic examination. The Buchnar funnel sat over the Buchnar flask, and an airtight seal was created using an interface of 'Blu-tack' (Bostick Australia Pty Ltd, Thomastown, VIC, Australia). The Buchnar flask was attached to the vacuum pump by plastic tubing. The vacuum pump was turned on prior to the supernatant being poured into the Buchnar funnel. The filter paper was removed from the funnel after the supernatant had filtered through, and was then examined under a stereo-binocular microscope.

Results

Control samples

All of the control samples were positive for mites. These were similar in appearance to house dust mites. The first eight samples had all three subsamples checked, and all three were positive. For subsequent samples, after the first subsample confirmed mites the following two subsamples were not examined. None of the mites found in any sample was alive, and in the majority of samples the mites had a dehydrated appearance.

Rugs and saddle blankets

Results from the test dust samples are shown in Table 3. Rugs 1–8 were positive in all three subsamples for mites similar in appearance to house dust mites. No live mites were viewed on the filter paper discs, and many of the mites had a dehydrated appearance. Rugs/blankets 9–16 were negative in all three subsamples, although the control samples were positive throughout the time these rugs were tested.

Table 1. Climatic data for sampling locations: mean maximum and minimum temperatures for the year (annual), for June and July, and mean annual rainfall²²

Location	Mean maximum temperature (annual; °C)	Mean minimum temperature (annual; °C)	Mean maximum temperature (June; °C)	Mean minimum temperature (June; °C)	Mean maximum temperature (July; °C)	Mean minimum temperature (July; °C)	Mean rainfall (annual; mm)
Theresa Park and Narellan	23.6	10.2	17.8	4.4	17.2	2.9	767.2
Pheasants Nest, Picton, Thirlmere	23.4	8.8	17.3	3.2	16.8	1.7	803.7

Table 2. Details of control sites sampled for dust mites and results

Control number	Date collected	Control site
1	08/07/09	Carpet, bedroom 1, P
2	08/07/09	Carpet, living room, P
3	08/07/09	Carpet, gym, N
4	08/07/09	Carpet, study, N
5	11/07/09	Carpet, bedroom 3, N
6	11/07/09	Carpet, family room, N
7	11/07/09	Carpet, bar, N
8	11/07/09	Cloth lounge 1, living room, N
9	11/07/09	Cloth lounge 2, living room, N
10	11/07/09	Mattress, bedroom 4, N
11	11/07/09	Carpet, bedroom 1, N
12	11/07/09	Carpet, bedroom 2, N
13	12/07/09	Carpet, bedroom 1, T
14	12/07/09	Carpet, bedroom 2, T
15	12/07/09	Cloth lounge 1, living room, T
16	12/07/09	Cloth lounge 2, living room, T

N, Narellan; P, Picton; T, Thirlmere.

Table 3. Details of horse rugs and saddle blankets sampled for dust mites and the results

Rug number	Date collected	Location and storage	Dust mite presence
1	29/06/09	TP: cupboard in shed	Positive
2	29/06/09	TP: cupboard in shed	Positive
3	29/06/09	TP: cupboard in shed	Positive
4	29/06/09	TP: cupboard in shed	Positive
5	02/07/09	TP: cupboard in shed	Positive
6	02/07/09	TP: cupboard in shed	Positive
7	02/07/09	TP: cupboard in shed	Positive
8	02/07/09	TP: cupboard in shed	Positive
9	04/07/09	N: cupboard in shed	Negative
10	04/07/09	N: cupboard in shed	Negative
11	04/07/09	N: cupboard in shed	Negative
12	04/07/09	N: left to air outside	Negative
13	09/07/09	PN: on horse in stable	Negative
14	09/07/09	PN: on horse in stable	Negative
15	09/07/09	PN: on horse in stable	Negative
16	09/07/09	PN: cupboard in shed	Negative

TP, Theresa Park; PN, Pheasants Nest; N, Narellan.

Identification of mites

Samples of dust from the control and rug collections were sent to the University of Sydney, Department of Medical Entomology for identification. The mites were confirmed to be *Dermatophagoides* house dust mites.²³

Discussion

This study revealed *Dermatophagoides* dust mites in eight of 16 sampled horse rugs or saddle blankets. To the

authors' knowledge, this is the first study to confirm the presence of dust mites in the equine environment. Although quantitative evaluation was not performed, mites were present in relatively low numbers, and no live mites were detected. Identification to the species level was not readily possible with the mite samples examined. *Dermatophagoides farinae* is a hardier species than *D. pteronyssinus*, surviving higher temperatures and lower humidity,^{7,8} and therefore may be the more dominant species in the outdoor equine environment. Specific immunological techniques should therefore be used in future studies to differentiate the presence of these two species in dust samples.^{7,16,17} The difficulties encountered in the mite retrieval techniques may also have determined the relatively low numbers of mites detected. Numerous dust mite retrieval techniques have been reported, and there is no standardized technique.^{15,20,24–35} The floatation technique used in our study was a modification of earlier methods that resulted in reliable and repeatable dust mite retrieval from the control samples. Other studies have also reported low numbers of mites using vacuum techniques.^{16,17} Capture of live mites from the control samples using adhesive tape was not successful (data not presented). Mite traps may also be used, but a recent study of storage mite contamination of dog food found that this was less effective than floatation.³⁶ Techniques with higher sensitivity, however, though more challenging to perform,³² may be necessary to quantify house dust mite populations accurately in equine samples.

One unexpected finding of this study was the lack of live mites in either the control or test samples, particularly since sample collection was performed in a region of known high dust mite prevalence.¹¹ As mentioned above, this may have been influenced by the sampling in the winter, since mite numbers are highest in humid warm weather and lowest in cool dry weather typical of winter (June/July) in Sydney.²¹ The high suction created by the Dyson vacuum cleaner may also have caused more destruction of the mite bodies.⁷

Nonetheless, small numbers of dead mites were consistently found in control samples and in eight of 16 of the sampled rugs, with all the positive rugs from one location. The lack of mites in eight of 16 rugs may indicate the absence of mites or low population numbers below the sensitivity of our extraction technique. Regardless, the presence of mites did not appear to be associated with variation in rug material or age. The rugs from all three sites were mostly polyester, nylon and/or cotton; each site had rugs from many different brands; and all had been in use for more than 1 year. All locations are within

the same climatic region, although the storage micro-environment is likely to vary considerably. Humidity and, to a lesser degree, temperature are known to be major factors influencing mite survival.^{21,37–39} Further questioning revealed some differences in storage conditions (Table 3). All rugs (eight of eight) from Theresa Park were in a cupboard in a shed; three of four from Narellan were in a cupboard in a shed (one was left outside); whereas only one of four from Pheasants Nest was in a cupboard in a shed (three came directly off stabled horses). Furthermore, all the mite-negative rugs (rugs 9–16) but none of the mite-positive rugs (rugs 1–8) were intermittently washed and purposefully exposed to sunlight. Laundering of bedding and clothing influences human dust mite exposure, with washing above 55 °C and sunlight exposure known to kill mites.^{40,41} It therefore seems likely that laundering and sunlight exposure of horse rugs influenced dust mite retrieval and may be useful in avoidance measures.

Future quantitative studies testing a greater number of rugs are necessary to determine accurate prevalence data, and ultimate relevance of dust mites to equine atopic dermatitis. In humans, 2 µg of group 1 allergen per gram of house dust is proposed as the threshold for sensitization, and lower levels are not associated with development of allergy.⁸ Establishing rug types, locations, storage conditions and management measures that inhibit the build up of dust mite populations would also be of practical use if dust mite hypersensitivity in the horse is confirmed.

This study is the first to demonstrate the presence of *Dermatophagoides* house dust mites in the equine environment, and is a first step in determining the relevance of hypersensitivity of these mites to atopic horses. Demonstrating mite exposure, however, needs to be followed by further studies on specific IgE serology, intradermal tests, lymphocyte proliferation tests, passive transfer, controlled exposure and provocation, avoidance and amelioration, and the response to specific immunotherapy before making definitive statements on the role of *Dermatophagoides* sensitivity in equine atopic dermatitis, inclusion of dust mite allergens in equine test panels, dust mite avoidance measures and immunotherapy.

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Résumé Un échantillon de 14 couvertures de chevaux et deux tapis de selle entreposées en Australie, dans le sud ouest de Sydney, une zone connue pour sa prévalence élevée d'acarien de poussière dans l'environnement humain, a été analysé pour la présence d'acariens de poussière de maison. Des échantillons de poussière des couvertures, des tapis de selle et de 16 sites de contrôle ont été prélevés par un aspirateur équipé d'un sac et d'un filtre spéciaux. Les acariens de poussière ont été extraits par la technique de flottaison. Huit couvertures et tous les échantillons de contrôle étaient positifs pour les acariens, ceux-ci ont été identifiés du genre *Dermatophagoides*. Cette étude confirme que l'exposition aux acariens de la poussière de maison par l'intermédiaire des couvertures de chevaux existe. Ceci indique que les tests intradermiques positifs aux allergènes des acariens de la poussière de maison ou les tests sériques allergiques des chevaux atopiques peuvent correspondre à une réelle hypersensibilité aux acariens ou à une réactivité croisée (par exemple aux acariens de stockage). Cependant, des études quantitatives seront nécessaires pour prouver qu'il y a une exposition suffisante aux acariens pour le développement d'une hypersensibilité et d'autres tests de réponse immunologiques, éviction et provocation, immunothérapie spécifique, sont nécessaires pour confirmer la pertinence clinique. A la connaissance des auteurs, il s'agit de la première étude qui documente la présence d'acariens de poussière de maison dans l'environnement des chevaux.

Resumen Se analizaron muestras de catorce alfombras de caballos y de dos mantas de silla almacenadas en el suroeste de Sidney, Australia, un área de conocida alta prevalencia de ácaros de polvo en el entorno humano, y se analizaron para la presencia de ácaros del polvo. Las muestras de polvo de las alfombras, de las mantas y de 16 lugares control se extrajeron con una aspiradora con un filtro modificado. Los ácaros se extrajeron utilizando una técnica de flotación adaptada. Ocho alfombras y todas las muestras control fueron positivas para ácaros, los cuales fueron identificados como ácaros del polvo del género *Dermatophagoides*. Este estudio confirma que puede producirse exposición a ácaros del polvo en las alfombras de caballos, lo cual indica que la reactividad a los alérgenos de ácaros en pruebas intradérmicas y pruebas alérgicas del suero en caballos atópicos puede representar una verdadera hypersensibilidad a ácaros del polvo o reactividad cruzada (por ejemplo con ácaros de la comida almacenada). Sin embargo, estudios cuantitativos serán necesarios para asegurarse de que hay una exposición suficiente a los ácaros para desarrollar hypersensibilidad; y mayor evaluación de las respuestas inmunológicas, retirada y provocación, e inmunoterapia específica son requerimientos para confirmar la relevancia clínica. A nuestro entender, este es el primer estudio que documenta la presencia de ácaros del polvo en el entorno de equinos.

Zusammenfassung Eine Auswahl von vierzehn Pferdedecken und zwei Satteldecken, die im Südwesten von Sydney, Australien, gelagert waren, einer Gegend, die für die hohe Prävalenz von Hausstaubmilben in der menschlichen Umgebung bekannt ist, wurde auf das Vorkommen von Hausstaubmilben untersucht.

Staubproben von den Pferde- und Satteldecken und von 16 Kontrollstellen wurden mit Hilfe eines Staubsaugers mit modifiziertem Aufsatz und Filter, gesammelt. Die Hausstaubmilben wurden mittels einer adaptierten Flotationsmethode extrahiert. Acht Pferdedecken und alle Kontrollstellen waren positiv für Milben, die als Hausstaubmilben der Gattung *Dermatophagoides* bestätigt werden konnten. Diese Studie bestätigt, dass die Exposition zu Hausstaubmilben durch Pferdedecken erfolgen kann, was darauf hinweist, dass die Reaktivität auf Hausstaubmilben im Intradermaltest und in der Serologie bei atopischen Pferden eine wahre Staubmilbenallergie oder eine Kreuzreaktion (z.B. mit Futtermittelmilben) repräsentieren kann. Dennoch werden quantitative Studien notwendig sein, um sicher zu gehen, dass eine ausreichende Exposition zu Milben besteht, um eine Hypersensibilität zu entwickeln. Eine weitere Untersuchung der Immunreaktionen, Vermeidung und Provokation, und eine spezifische Immuntherapie sind nötig, um die klinische Relevanz zu bestätigen. Nach dem besten Wissen des Autors handelt es sich hierbei um die erste Dokumentation des Vorkommens von Hausstaubmilben in der Umgebung von Pferden.

要約 オーストラリア、シドニーの南西部は人の環境中にコナダニの発生率が高いことで有名な地域であり、ここに保管されていた 14 枚のウマの敷物と 2 枚の鞍用毛布にハウスダストマイトが存在するかどうかを解析した。改良したアタッチメントとフィルタを装着した掃除機を用いてラグ、毛布と 16 のコントロール部位から埃のサンプルを収集した。コナダニは改良した浮遊法を用いて抽出した。8 枚のラグとすべての対照サンプルでダニが陽性であり、これらは *Dermatophagoides* 属のハウスダストマイトと確定された。この調査により、ウマの敷物からハウスダストマイトに暴露する可能性があり、アトピーのウマでハウスダストマイトへの皮内反応や血清アレルギー試験におけるアレルギー反応は、真のチリダニ過敏症あるいは交差反応 (e.g. 食物の貯蔵ダニ) を示す可能性のあることを確認した。それにもかかわらず、定量化試験は過敏症の発生に十分なダニの暴露があるということを保証するのに必要であり、さらなる免疫学的反応、回避法、誘発、特異的免疫療法の評価が臨床的な関連性の裏付けとして必要とされる。筆者の知るところでは、これはハウスダストマイトが馬の環境中に存在するという初めての報告である。