Tropical Biomedicine 28(2): 223–236 (2011)

Antigenic profile of *Blomia tropicalis*, *Aleuroglyphus ovatus* and *Glycycometus malaysiensis*

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Received 8 October 2010; received in revised form 26 December 2010; accepted 4 January 2011

Abstract. House dust mites and storage mites are well-known causes for allergenic diseases. The aim of this study was to investigate the immunogenic sites of *Blomia tropicalis*, *Aleurogyphus ovatus* and *Glycycometus malaysiensis*. The mites were maintained in a culture medium at 25°C and 75% relative humidity. Mites were harvested either with heat escape or floatation method, purified, homogenized, quantified and used for the production of polyclonal antibody and immunostaining. For each species of mites, five male mice and five male rats were randomly selected and immunized intraperitoneally with respective crude mite extract at two-weekly intervals. *Blomia tropicalis*, *A. ovatus* or *G. malaysiensis* whole mites and paraffin-embedded mite sections were immunostained with the respective polyclonal antibody. The faecal pellets of mites were intensely stained for all the three species in the present study. The legs of sectioned *A. ovatus* were not immunogenic as compared with those of *G. malaysiensis* and *B. tropicalis*. The outer layer (cuticle) of whole mites and the eggs for these species were very immunogenic. Hence, the polyclonal antibodies obtained in this study may serve as potential tools in detecting the eggs and immature mites in environmental samples. Future studies should focus on the antigenic components of eggs since they were relatively abundant in dust and highly antigenic as seen in the present study.

INTRODUCTION

Mites found in human premises can be grouped as house dust mites and storage mites. House dust mites consume human epidermal scales, animal dander, trace nutrients, fungi, bacteria and pollen. They are found in carpets, fabrics, upholstery, pillows and mattresses (Nadchatram, 2005). Their life span is about 4 months under optimal growing conditions at 28°C and 80% relative humidity; it takes around 30 days (3 to 4 weeks) for a mite to grow from an egg to adult stage. Their life cycle includes egg, larva, 3 nymph stages and adult. One female mite can produce 50-80 eggs in its life time (Nadchatram, 2005; Walter *et al.*, 2009).

House dust mites (*Dermatophagoides farinae*, *Dermatophagoides pteronyssinus*, *Euroglyphus maynei* and *Blomia tropicalis*) are well-known causes for allergenic diseases including hay fever, rhinitis, asthma and conjunctivitis (Colloff & Spieksma, 1992; Colloff, 2009b; Thomas *et al.*, 2010). *Blomia tropicalis*, first recognized as storage mites, are now also accepted as house dust mites as they are found extensively in houses (Mariana *et al.*, 2000). *Blomia tropicalis* was found in dust from homes of tropical and subtropical countries, including Spain, India, Taiwan, Brazil, Colombia, the Philippines, Malaysia and Indonesia (Bronswijk *et al.*, 1974; Tsai *et al.*, 1998; Chew *et al.*, 1999; Mariana *et al.*, 2000).

Storage mites include *Glycyphagus domesticus*, *Lepidoglyphus destructor*, *Blomia kulagini*, *Tyrophagus putrescentiae*, *Acarus siro*, *Suidesia pontifica*, *Glycycometus* (*Austroglycyphagus) malaysiensis*, *Aleuroglyphus ovatus* and *Thyreophagus*

entomophagus. They are commonly found in storage facilities for grains such as wheat, corn, oats, barley and hay (Hage-Hamsten & Johansson, 1992; Arlian & Morgan, 2003; Palyvos *et al.*, 2008). They may contaminate or invade and thrive in processed foods made from grains (e.g. flour, cereals and baking mixes) when these products become moist or are stored in humid environments (Arlian & Morgan, 2003). On purchase, 21% of the 571 cereal-based food products from United Kingdom food retail outlets were found to contain mites (Thind & Clarke, 2001). Upon subsequent examination after 6 weeks of storage in the homes of volunteers, mites were present in 38% of the same products. *Acarus siro*, *Tyrophagus putrescentiae*, *L. destructor* and *G. domesticus* were the most common species identified (Thind & Clarke, 2001). Mites and mite allergens may be carried over from the raw grains and contaminate the processed foods, even when they are stored under conditions that prevent mite survival (Arlian & Morgan, 2003).

The distribution of dust mites varies depending on climate, geographical area and socioeconomic conditions. Countries with tropical climate that favours the growth and reproduction of dust mites, such as Malaysia and Singapore, have been reported to have higher mite infestation level (Nadchatram, 2005). Storage mites have been reported as important allergens that cause occupational allergy in farmers, grain-storage workers, bakers, pastry-cooks, millers, storekeepers, shopkeepers, cheese makers, transport workers, zookeepers, upholsterers, miners and so forth (Solarz *et al.*, 2004; Vidal *et al.*, 2004; Storaas *et al.*, 2005; Ruoppi *et al.*, 2005; Koistinen *et al.*, 2006; Szilman *et al.*, 2006; Jeebhay *et al.*, 2007; Stejskal & Hubert, 2008). A total of 5315 mites were isolated and 26 species of astigmatid mites were identified (Szilman *et al.*, 2006). Among them, *Tyrophagus perniciosus* was dominant (57.9%), followed by *T. putrescentiae* (7.7%), *Acarus farris* (5%) and *T. longior* (4.9%). The workers in the Silesian Zoo had sera IgE against *T. putrscentiae* (50%), *L. destrutor* (34.2%) and *A. siro* (34.2%) (Szilman *et al.*, 2006). Therefore, the aim of the present study was to investigate the immunogenic sites of *B. tropicalis* and other storage mites (*A. ovatus* and *Glycycometus malaysiensis*) reactive to polyclonal antibodies produced in experimentally immunised mice and rats.

MATERIALS AND METHODS

Preparation of crude extracts of mites

The mites (*B. tropicalis*, *A. ovatus* and *G. malaysiensis*) were maintained in TetraMin Crisps fish flakes (Tetra GmbH, Germany) at 25°C and 75% relative humidity. Mites were harvested either with heat escape or floatation method, purified, homogenized, quantified and used for the production of polyclonal antibody and immunostaining. The harvested mites (~100 mg) were suspended in phosphate buffered saline (PBS) and homogenized with Microsmash (Tomy, Japan) at 5000 rpm for 30 seconds in the presence of glass beads (1.0 mm in diameter). After that, the suspension was cooled for 3 minutes before being subjected to the subsequent homogenization process. The homogenization process was repeated for 5 cycles. The homogenized crude extracts were then centrifuged at 5000 rpm for 10 minutes. The supernatant was collected and stored at -80°C until further use. The protein concentration of the crude mites extracts was determined using Bio-Rad Protein Assay (Bio-Rad Laboratories, Hercules, CA) (Bradford, 1976).

Immunization of Balb/c Mice and Sprague Dawley Rats

Male Balb/c mice (7 weeks old, 17-23 g) and male Sprague Dawley rats (8 weeks old, 200-350 g) were purchased from University Putra Malaysia (UPM), Malaysia and housed in the animal holding facility at the International Medical University (IMU). The animals were fed with commercially available rodent pellets and water.

For each species of mites, five mice and five rats were randomly selected and immunized intraperitoneally with the respective crude mite extract at two-weekly intervals. Samples of blood were collected from the tail of mice and the saphenous vein of rats before immunization (pre-immunized

blood) and after immunization (post-immunized blood). Each mouse or rat was given the first immunization, which contained 0.1 mL of 1 µg/µL crude mite extract emulsified in 0.1 mL Freund's complete adjuvant (Sigma-Aldrich Corporation, USA). All subsequent booster injections consisted of 0.1 mL of 1 1 µg/µL crude mite extract emulsified in 0.1 mL Freund's incomplete adjuvant (Sigma-Aldrich Corporation, USA). The pre- and post-immunization sera were checked for the presence of antigen specific antibodies using ELISA. When the antibody titres increased to at least two- to three-fold compared with pre-immunization sera, the animals were sacrificed. The mice and rats were anaesthetized with diethyl ether and blood collected via cardiac puncture. The sera containing antigen specific polyclonal antibodies were used for immunostaining throughout the study.

Preparation of Mite Sections for Immunoperoxidase Staining

The mites were fixed in 10% formalin, dehydrated through ascending concentrations of alcohol, cleared with xylene, impregnated and embedded with paraffin wax in an automated Leica TP1020 tissue processor (Leica Microsystems Nussloch GmbH, Germany). The embedded blocks were sectioned with a rotary microtome (Leica Microsystems Nussloch GmbH, Germany) at 3 µm thickness.

Blomia tropicalis, *A. ovatus* or *G. malaysiensis* whole mites were coated with 15 µL of 0.1 µg/mL poly-L-lysine (Sigma-Aldrich Corporation, USA) on a clean glass slide and allowed to dry. The mites were fixed on the slides in cold acetone for 30 minutes.

Immunoperoxidase Staining with Polyclonal Antibody

The paraffin-embedded mite sections were deparaffinized in 3 changes of xylene for 3 minutes each. After that, the sections were rehydrated gradually through descending concentrations of alcohol, and lastly in distilled water for 3 minutes. Both sections of mites and whole mites were then subjected to antigen retrieval procedures in citrate buffer for 15 minutes using a microwave oven. The slides were allowed to cool down in the buffer for about 1.5 hours. The endogenous peroxidase activity was blocked with blocking solution (1: 10; Kirkegaard & Perry Laboratories, Maryland) for 10 minutes. The slides were then rinsed with ultra-pure water for 10 minutes and soaked in PBS for another 10 minutes. The slides were then blocked with diluted swine serum (1: 10) for 10 minutes and were soaked in PBS for another 10 minutes.

Positive and negative controls were included for each staining procedure. The whole and sectioned mites were incubated with the respective species of mite polyclonal antibodies obtained earlier (1: 100) for 2 hours at 37°C in a wet chamber. After the incubation, the slides were washed with PBS for 10 minutes. The slides were then incubated with peroxidase conjugated anti-mouse (1: 200) or anti-rat (1: 1 000) immunoglobulins for 30 minutes at room temperature. After the incubation, the slides were then washed with PBS for 10 minutes prior to incubation with TrueBlue peroxidase substrate (Kirkegaard & Perry Laboratories, Maryland) for 10 minutes. The slides were washed with ultra-pure water for 5 minutes before being counterstained with KPL Orcein for 3 minutes. The slides were then rinsed with ultra-pure water for 5 minutes and allowed to air-dry. Lastly, the slides were mounted with Depex and observed under a compound microscope (Nikon, Japan) at 100 – 1 000X magnification.

RESULTS

The protein concentrations for *A. ovatus*, *B. tropicalis* and *G. malaysiensis* were 1.257 mg/mL, 1.786 mg/mL and 0.971 mg/mL respectively. These antigens were used for the production of polyclonal antibodies in mice and rats. The pre-immunized and post-immunized antibody titres against *A. ovatus*, *B. tropicalis* and *G. malaysiensis* antigens in the sera of immunized mice and rats were determined (Table 1).

Table 1. Optical density (OD) readings of antibody levels in each mouse (M1-M5) and rat (R1-R5) after immunization with *A. ovatus*, *B. tropicalis* or *G. malaysiensis*. Values are expressed as means of duplicate determinations ± 1 SD

Antigen		OD ± SD		
		Sera: Pre-immunized	Sera: After 3rd dose	Sera: After 4th dose
Aleuroglyphus ovatus	M1	0.1405 ± 0.0078	1.5095 ± 0.0318	–
	M2	0.1010 ± 0.0269	2.2155 ± 0.0219	–
	M3	0.2320 ± 0.0424	1.5145 ± 0.0672	–
	M4	0.2060 ± 0.0212	1.8080 ± 0.0594	–
	M5	0.1275 ± 0.0191	1.6335 ± 0.0078	–
	R1	0.0550 ± 0.0071	–	0.3405 ± 0.0361
	R2	0.1445 ± 0.0007	–	0.4320 ± 0.0240
	R3	0.0865 ± 0.0163	–	0.7400 ± 0
	R4	0.1295 ± 0.0035	–	0.7775 ± 0.1237
	R5	0.0630 ± 0.0269	–	0.6040 ± 0.0184
Blomia tropicalis	M1	0.1350 ± 0.0099	0.6240 ± 0.0255	0.6420 ± 0.0354
	M2	0.1345 ± 0.0134	0.9230 ± 0.0509	1.6980 ± 0.0863
	M3	0.1260 ± 0.0099	0.8340 ± 0.0113	1.5370 ± 0.1131
	M4	0.0560 ± 0.0071	0.4110 ± 0.0057	0.5505 ± 0.0233
	M5	0.1270 ± 0.0057	1.0755 ± 0.0205	1.0575 ± 0.0955
	R1	0.5225 ± 0.0290	2.0385 ± 0.0502	2.1775 ± 0.0714
	R2	0.5305 ± 0.0064	1.9375 ± 0.0686	2.7155 ± 0.0021
	R3	0.5285 ± 0.0064	1.6975 ± 0.0092	1.8830 ± 0.0113
	R4	0.1675 ± 0.0346	0.4925 ± 0.262	0.5640 ± 0.0467
	R5	0.6675 ± 0.0035	1.2405 ± 0.0191	1.4510 ± 0.1146
Glycycometus malaysiensis	M1	0.1600 ± 0.0014	1.8255 ± 0.0375	–
	M2	0.1815 ± 0.0530	1.4570 ± 0.0255	–
	M3	0.1885 ± 0.0007	1.7480 ± 0.0820	–
	M4	0.2730 ± 0.0255	1.9175 ± 0.0389	–
	M5	0.2060 ± 0.0354	1.3115 ± 0.0361	–
	R1	0.2225 ± 0.0205	1.3090 ± 0.0608	1.4985 ± 0.0587
	R2	0.2590 ± 0.0042	1.5105 ± 0.0559	1.5400 ± 0.0127
	R3	0.1940 ± 0.0127	1.1085 ± 0.0163	1.2435 ± 0.0050
	R4	0.4135 ± 0.0233	1.7550 ± 0.0339	1.6265 ± 0.0276
	R5	0.2440 ± 0.0028	1.3060 ± 0.0594	1.7950 ± 0.0170

The antibody titres increased 8-22 folds after the 3rd dose of *A. ovatus* immunization in mice. The antibody titres for rats increased after the 4th dose, increasing from 2-10 folds. The mice showed approximately 5-13 folds increase in antibody titres after the 4th dose of *B. tropicalis* injection, whereas the rats only showed 2-5 folds increase in antibody titres after the 4th dose of *B. tropicalis* injection. After the 3rd dose of injection with *G. malaysiensis*, the antibody titres for the mice increased about 6-11 folds. The antibody titres in rats increased slightly only after the 3rd dose and the antibody titres increased 4-7 folds after the 4th dose of *G. malaysiensis* injection. The mice were more responsive than the rats as the antibody titres increased markedly after the 3rd dose compared with the 4th dose for the rats. The animals were anaesthetized using diethyl ether and the blood was collected via cardiac puncture after the 3rd and 4th dose of injections for mice and rats respectively.

Cross-reactivity of rat and mouse polyclonal antibodies against the mites extracts was determined using ELISA

Table 2. Cross-reactivity of rats and mice polyclonal antibodies (Mean OD ± SD) against *A. ovatus*, *B. tropicalis* or *G. malaysiensis*. Values are expressed as means of duplicate determinations ± 1 SD

Type of antigen	**Rats polyclonal antibody against *A. ovatus* (1: 400)**	**Rats polyclonal antibody against *B. tropicalis* (1: 400)**	**Rats polyclonal antibody against *G. malaysiensis* (1: 400)**
A. ovatus	0.9053 ± 0.2363	0.7593 ± 0.1169	0.7988 ± 0.0746
B. tropicalis	0.9667 ± 0.3210	1.0528 ± 0.1180	0.8058 ± 0.0404
G. malaysiensis	0.7180 ± 0.2252	0.5838 ± 0.0872	1.5973 ± 0.2737
Type of antigen	**Mice polyclonal antibody against *A. ovatus* (1: 800)**	**Mice polyclonal antibody against *B. tropicalis* (1: 800)**	**Mice polyclonal antibody against *G. malaysiensis* (1: 800)**
A. ovatus	1.8121 ± 0.2146	0.5298 ± 0.1275	0.2048 ± 0.0267
B. tropicalis	1.5005 ± 0.1864	1.4617 ± 0.3584	0.3561 ± 0.0863
G. malaysiensis	1.0838 ± 0.1703	0.7169 ± 0.2347	1.1885 ± 0.0813

(Table 2). The rat polyclonal antibody against *A. ovatus* showed high and medium cross-reactivity with *B. tropicalis* and *G. malaysiensis*. Medium cross-reactivity between rat polyclonal antibody against *B. tropicalis* with *A. ovatus* as well as *G. malaysiensis* had been detected. Rat polyclonal antibody against *G. malaysiensis* showed medium cross-reactivity with *A. ovatus* and *B. tropicalis*. However, the mouse polyclonal antibody raised against *A. ovatus* showed high cross-reactivity with *B. tropicalis* and *G. malaysiensis*. Medium cross-reactivity of mouse polyclonal antibody against *B. tropicalis* with that of *G. malaysiensis* or *A. ovatus* was seen. Mouse polyclonal antibody raised against *G. malaysiensis* showed very low cross-reactivity with those raised against *A. ovatus* and *B. tropicalis*.

The immunoperoxidase staining with TrueBlue peroxidase substrate (Kirkegaard & Perry Laboratories) was used to visualise the interaction between the polyclonal antibodies and antigens. Polyclonal antibodies against each species reacted positively with the cuticle of whole mites, eggs, faecal pellet and internal structures of the mite sections especially the lining of hind gut (Figs. 1 - 6). The faecal pellet was spherical in shape while the eggs were elongated and cylindrical in shape.

DISCUSSION

The faecal pellets of mites reacted intensely with the respective polyclonal antibodies for all the three species – *B. tropicalis*, *A. ovatus* and *G. malaysiensis* in the present study. The faecal pellets are known to be allergenic (Nadchatram, 2005). In the case of sectioned mites, the internal part of the dust mites was intensely stained especially the lining of the hind gut containing faecal matters. Jeong *et al.* (2002) localized the highest concentration of Der f 2 in the anterior midgut, digestive material and excreted faecal pellets with a monoclonal antibody raised against recombinant Der f 2. Positive immunoreactivity of monoclonal antibody against recombinant Der f 3 were observed in the anterior midgut, posterior midgut and gut content of the lumen and with the strongest immunoreactivity on faecal pellets of both male and female *D. farinae* (Zhan *et al.*, 2010). Der p 1, Der p 2 and Der p 21 of *D. pteronyssinus* were localized in the digestive tract (mount, epithelium and lumen of midgut, and hind-gut) and concentrated in the faecal pellets (Rees *et al.*, 1992; Park *et al.*, 2000; Weghofer *et al.*, 2008).

The legs of sectioned *A. ovatus* were not immunogenic unlike those of *G. malaysiensis* and *B. tropicalis*. This may be due to different antigen/allergen groups in

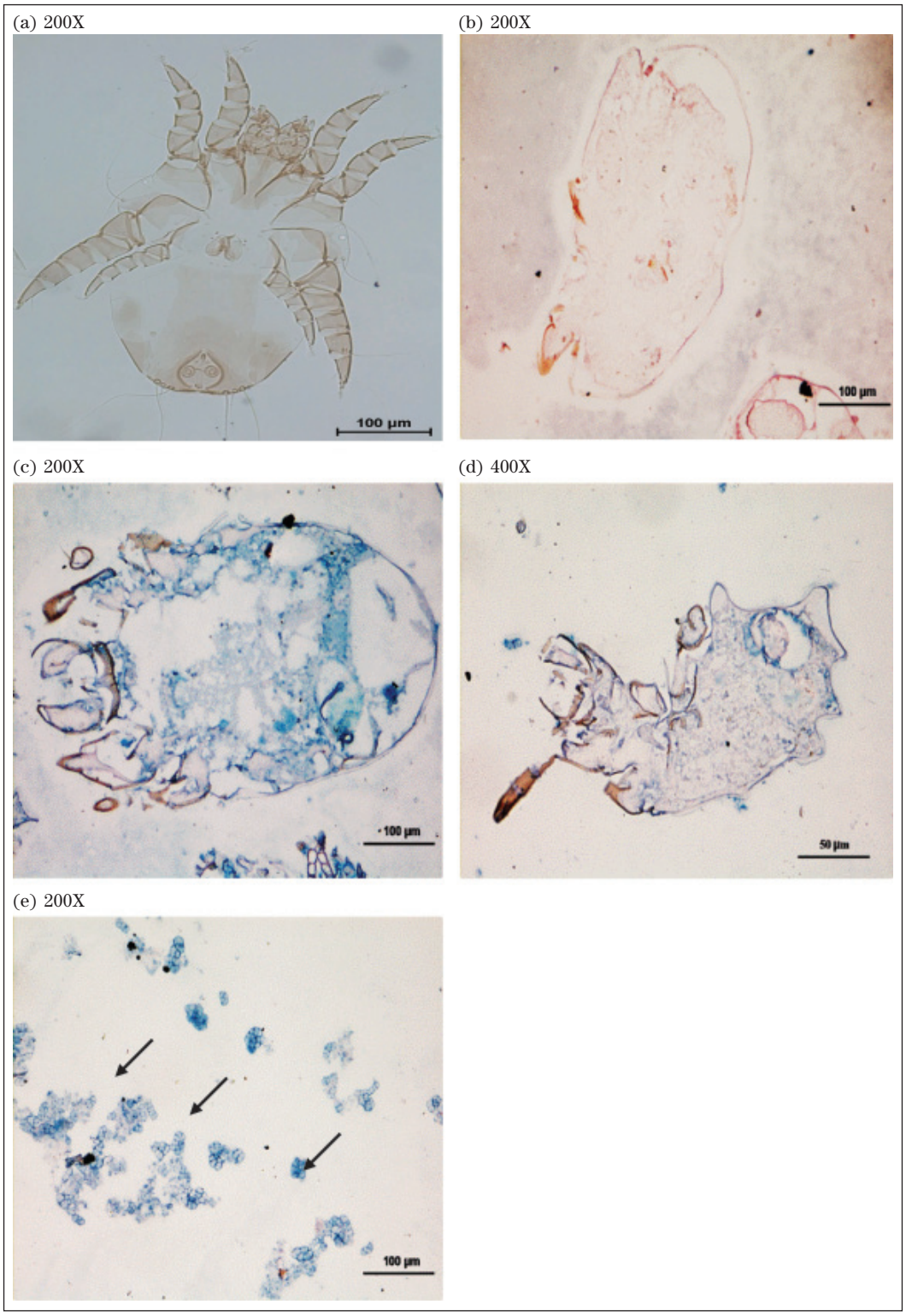

Figure 1. Immunoperoxidase staining pattern of *A. ovatus* sections after incubation with (b) PBS (negative control), (c) mice polyclonal antibody, and (d-e) rat polyclonal antibody. Faecal pellets were shown in thin arrows in (e). Note blue staining of positive reactivity with red counterstain. (a) represents whole mount of a *A. ovatus* mite without immunoperoxidase staining as reference

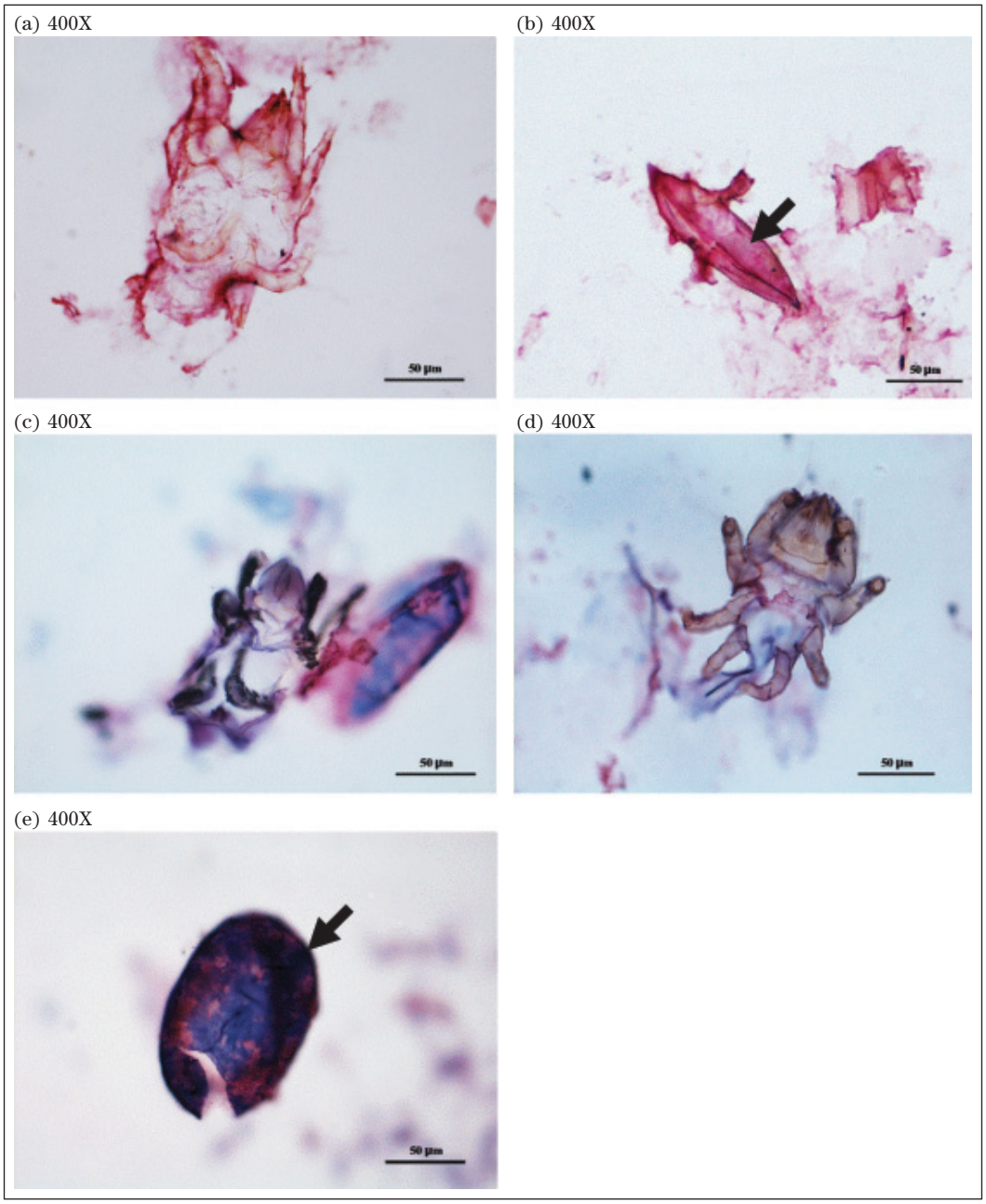

Figure 2. Immunoperoxidase staining pattern of *A. ovatus* whole mites after incubation with (a-b) PBS (negative control), (c) mice polyclonal antibody, and (d-e) rat polyclonal antibody. Eggs were shown in thick arrows in (b) and (e). Note blue staining of positive reactivity with red counterstain

different species. The outer layer (cuticle) of whole mites and the eggs for the three species were very immunogenic. Colloff (1992) reported 75% of the dust sample have eggs and immature mites of *D. pteronyssinus* compared with the adult population of mites. Hence, the polyclonal antibodies obtained in this study may serve as potential tools in detecting the eggs, immature and mature mites in environmental samples. Future studies should also focus on the antigenic components of eggs since they were

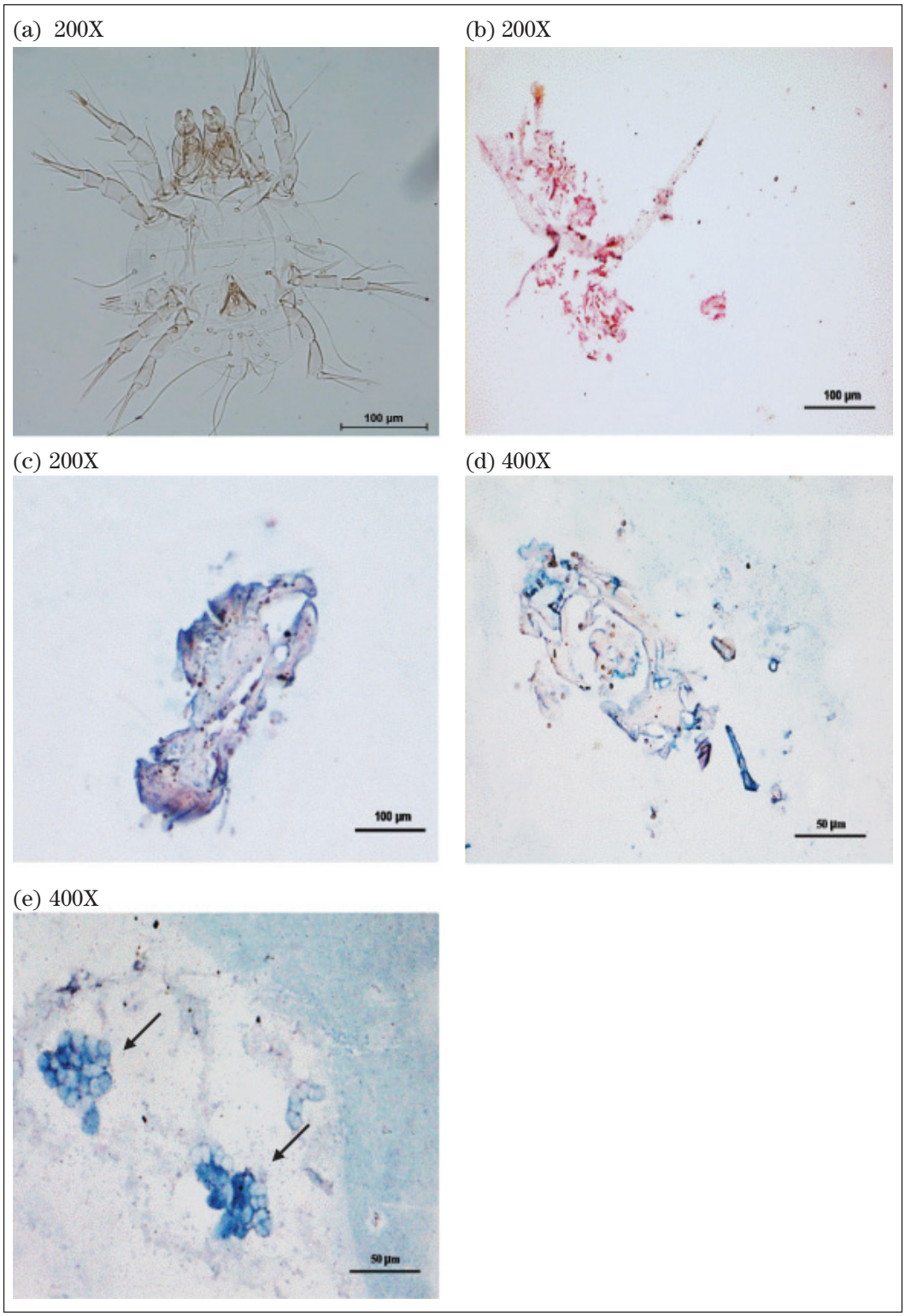

Figure 3. Immunoperoxidase staining pattern of *B. tropicalis* sections after incubation with (b) PBS (negative control), (c) mice polyclonal antibody, and (d-e) rat polyclonal antibody. Faecal pellets were shown in thin arrows in (e). Note blue staining of positive reactivity with red counterstain. (a) represents whole mount of a *B. tropicalis* mite without immunoperoxidase staining as reference

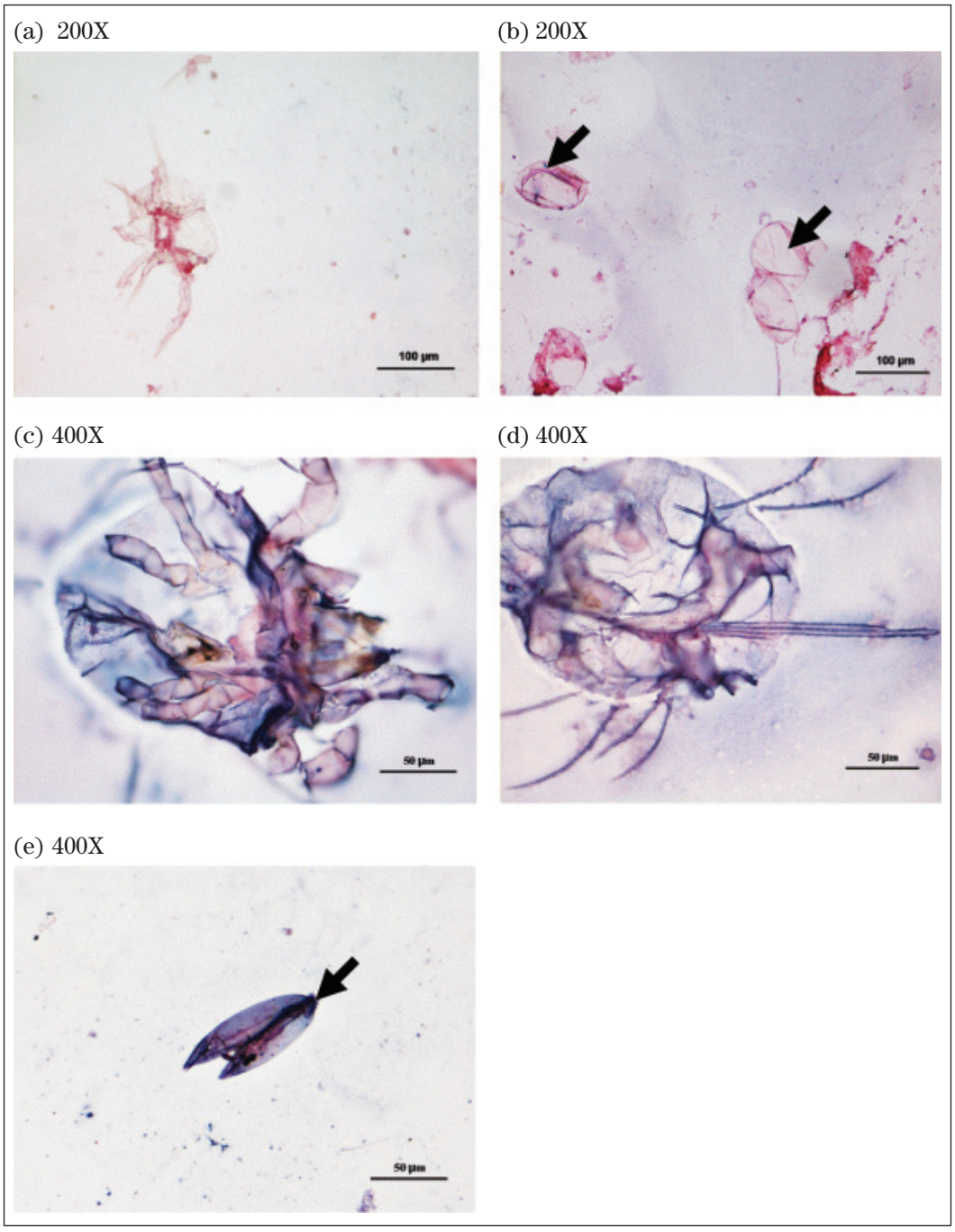

Figure 4. Immunoperoxidase staining pattern of *B. tropicalis* whole mites after incubation with (a-b) PBS (negative control), (c) mice polyclonal antibody, and (d-e) rat polyclonal antibody. Eggs were shown in thick arrows (b) and (e). Note blue staining of positive reactivity with red counterstain

relatively abundant in dust and highly antigenic as seen in the present study.

There are about 30 different proteins in the house dust mite extracts and these proteins are responsible for the sensitization in atopic patients (Colloff, 2009a). Pure mite culture extracts comprise of a mixture of whole mites, nymphs, faecal pellets, eggs and

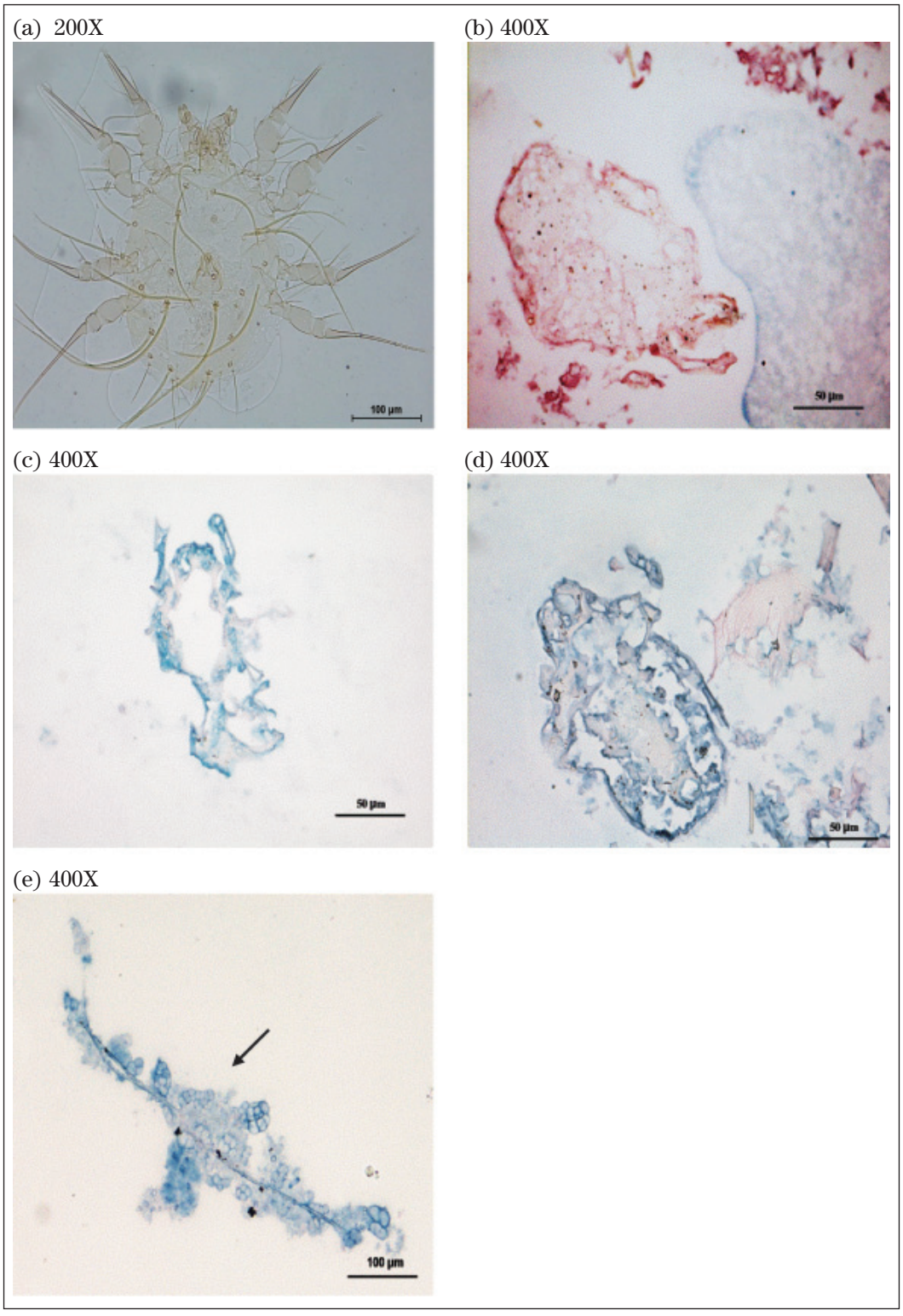

Figure 5. Immunoperoxidase staining pattern of *G. malaysiensis* sections after incubation with (b) PBS (negative control), (c) mice polyclonal antibody, and (d-e) rat polyclonal antibody. Faecal pellets were shown in thin arrow in (e). Note blue staining of positive reactivity with red counterstain. (a) represents whole mount of a *G. malaysiensis* mite without immunoperoxidase staining as reference

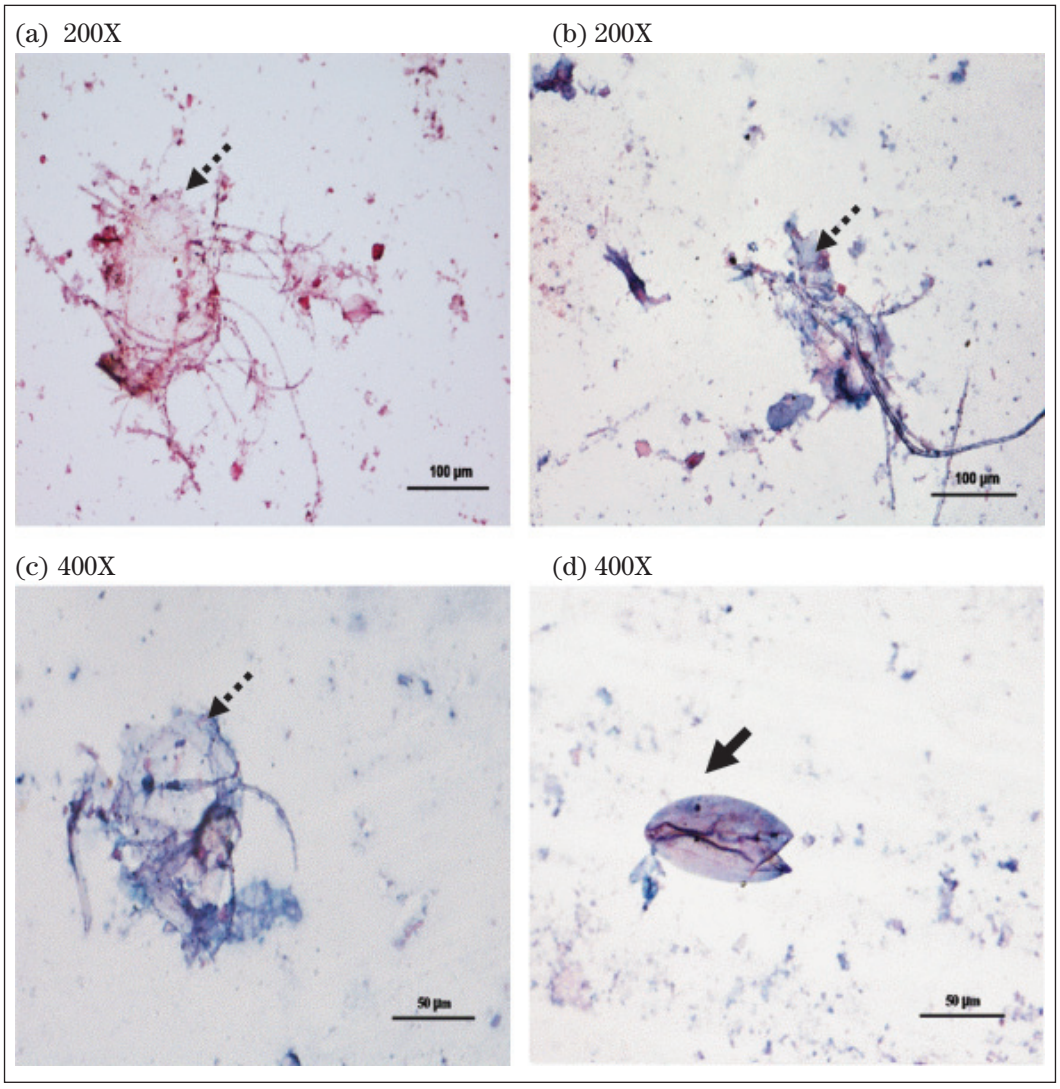

Figure 6. Immunoperoxidase staining pattern of *G. malaysiensis* whole mites (shown in dashed arrows) after incubation with (a) PBS (negative control), (b-c) mice polyclonal antibody, and (d) rat polyclonal antibody. Eggs were shown in thick arrow (d). Note blue staining of positive reactivity with red counterstain

spent culture media. Generally, group 1 and group 2 mite allergens are the most well-known allergen groups involved in allergenic reactions like asthma, allergic rhinitis and atopic dermatitis. Group 1 allergens consist of 25 kDa glycoproteins, and they are primarily found in mite faeces (Thomas *et al.*, 2002). On the other hand, group 2 allergens are approximately 14 kDa proteins, and are found in mite bodies (Thomas *et al.*, 2002). Mite faeces consist of ingested food, debris and enzymes. These enzymes will then break down the food particles in the faecal pellets and continue to nourish the mites. The major allergen, Der p1 (group 1 allergen), was found to cleave the cell adhesion in the lung epithelium, and to destroy the lung defence system which lead to allergic responses or bronchial asthma (Holt *et al.*, 1990; Hewitt *et al.*, 1995).

Blomia tropicalis extracts possess at least 30 different proteins (Caraballo *et al.*, 1996; Tsai *et al.*, 1998; Fonseca & Diaz, 2003; Fernandez-Caldas & Lockey, 2004). Seventy

percent of the sera from patients with positive skin prick test to *B. tropicalis* exhibited IgE response to Blo t 1 (Montealegre *et al.*, 2006). Blo t 5, a 14 kDa allergen, was demonstrated to have highest IgE binding frequency compared with other groups of *B. tropicalis* allergens (Tsai *et al.*, 1998; Montealegre *et al.*, 2006). Blo t 5 has been shown to have 91.8% and 73.5% reactivity with IgE in Taiwanese and Malaysian sera respectively. Blo t 5 specific IgE titres were reported to be significantly higher than those of Der p 5 (Cheong *et al.*, 2003).

No or minimal characterization of *G. malaysiensis* allergens to date has been reported. Approximately 77%, 72% and 85% of 289 patients with asthma and/or allergic rhinitis at 3-5 years, 6-14 years and >14 years in Singapore showed positive skin test reactivity against *G. malaysiensis*. About 26% of those healthy volunteers also showed positive skin test reactivity against *G. malaysiensis* in Singapore. Approximately 69% of the atopic patients produced specific IgE against *G. malaysiensis* as measured by fluorescence allergosorbent test (FAST) (Chew *et al.*, 1999).

Both male and female *A. ovatus* closely resemble each other with stout and pearly white body and red-brown legs. So far, only Ale o 2 allergen was reported for this species (Colloff, 2009a). *A. ovatus* is normally associated with areas where grains are grown, stored and processed. Puerta *et al.* (1993) showed that 79% of individuals with allergic asthma and/or chronic allergic rhinitis in Cartagena, Colombia were sensitized to both *D. pteronyssinus* and *A. ovatus*. Specific IgE to *A. ovatus* was detected in 35 atopic individuals with three to *A. ovatus* only and the remaining to both *A. ovatus* and *D. pteronyssinus*. *A. ovatus* probably contains unique allergens and allergens that cross-react with other cosmopolitan house dust mites. Immunoblot analysis with sera of these patients revealed nine IgE binding bands ranging from 16 to 100 kDa (Silton *et al.*, 1991). Skin rashes on the face, neck, hands and lower arms were reported after handling *A. ovatus* contaminated feedbags in Australia (Geary *et al.*, 2000).

In conclusion, the cuticle (outer layer) of whole mites, eggs, faecal pellet and internal structures of the mite sections were shown to react positively with their respective polyclonal antibody. These polyclonal antibodies may serve as potential pan-specific capturing antibodies for the detection of mite antigens.

Acknowledgements. We are grateful to the Institute for Medical Research (IMR), Kuala Lumpur, Malaysia for providing the dust mites. This research was supported by grants from Ministry of Science, Technology and Innovation Malaysia (02-02-09-SF0007) and International Medical University [Grant number: BMS I02-2008(01)]. We would like to thank Ms Tan Jessie and Ms Ling Suk Jiun for their technical assistance.

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