CHAPTER THREE

BEE AND WASP VENOM SENSITIZATION IN SCHOOLCHILDREN OF HIGH AND LOW SOCIO-ECONOMIC STATUS LIVING IN AN URBAN AREA OF INDONESIA

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ABSTRACT

Background. There is not much known about venom allergy in tropical regions. Here, we studied the prevalence of specific IgE (sIgE), skin prick test (SPT) reactivity and reported sting-related symptoms, in high- and low socio-economic status (SES) schoolchildren living in urban city of Makassar in Indonesia.
Methods. Children from high- (n=160) and low- (n=165) SES schools were recruited. Standardized questionnaires were used to record information on allergic disorders as well as sting-related symptoms. Parasitic infection, SPT reactivity and sIgE to *Apis mellifera* (bee-venom) as well as *Vespula spp* (wasp-venom) were assessed.
Results. SPT reactivity to bee- and wasp-venom were 14.3% and 12.7%; while the prevalence of sIgE was 26.5% and 28.5%, respectively. When SES was considered, prevalence of SPT to bee- and wasp-venom was higher in high-SES compared to low-SES schoolchildren (bee: 22.8% vs 5.7%, p<0.001; and wasp: 19.6% vs 5.7%, p<0.001). Conversely, sIgE to both venoms was lower in high-SES compared to low-SES (bee: 19% vs 34%, p=0.016; and wasp: 19% vs 38%, p=0.003). Furthermore, among SPT positive subjects, considerable proportion had no detectable sIgE to bee- (65.85%) or wasp-venom (66.67%). Altogether the sensitizations were rarely translated into clinical reaction, as only 1 child reported significant local reaction after being stung. No associations with parasitic infections was found.
Conclusions and Clinical Relevance. Sensitization against bee- or wasp-venom is quite prevalent amongst schoolchildren in Indonesia. The discordance between SPT and sIgE might suggest the direct (non-IgE) effect of venoms in skin reactivity. Recorded sensitizations had poor clinical relevance as they rarely translated into clinical symptoms.

Keywords: venom and insect allergy, epidemiology, pediatrics, urban, developing country

Introduction

The global magnitude of venom allergy is not completely established as the majority of studies have been conducted in temperate or sub-tropical countries. The prevalence of *Hymenoptera* venom sensitization has been reported to vary from 3.66 % to 41.6% [1-5], whereas prevalence of systemic allergic reactions to venoms has been estimated to be between 0.34% to 16% [3, 6-9]. A single *Hymenoptera* sting may induce large local reactions (LLR) and/or systemic reactions (SR) in venom-allergic individuals, and in some instances, SR can be fatal [10]. Altogether, there is not much information on the extent of venom allergy and symptoms in tropical countries.

Indonesia, a tropical country, located across the equator with a warm climate and high humidity, provides favorable conditions for Hymenopterans like bees and wasps. To gather information on venom allergy in Indonesia, we conducted a study amongst schoolchildren of high and low socioeconomic status (SES) living in Makassar, Sulawesi. We investigated the prevalence of reported *Hymenoptera* sting reactions and their correlation with *Hymenoptera* venom-specific IgE and skin reactivity. As the prevalence of parasitic infections can be high in children of low SES [11] and inverse relationship between aeroallergens and helminth infections have been reported [12], we also assessed whether the same is true for venom allergy.

Material and Methods

Study area and design

This study was approved by Health Research Ethics Committee of Faculty of Medicine, Hasanuddin University (Ref: 1504/H04.8.4.5.31/PP36-KOMETIK/2016). Of 500 children (low SES: n= 250; high SES: n=250) invited, 325 children (65%) participated in the study.

The study was conducted in two elementary schools in Makassar, Indonesia. Two elementary schools that were distinct in SES were selected. The low SES school was located near the sea port where majority of parents earned wages by working in fishing or other low-education labor. Students from this school mainly lived in the school neighborhood in a densely populated area which is located near a landfill. The high SES school was situated in the city center and all students were living scattered over the city. The majority of the students' parents worked as a moderate-to-high level civil servants or as professionals in business sector.

After discussion with the school management team and obtaining their agreement to participate in the study, a letter with detailed information on the study was sent to parents of all children in 3rd and 5th grade in both high and low SES schools. The parents could call the study team with any questions regarding the study. We asked the parents' permission to include their children in the study, which was indicated by signing the informed consent letter. Only children who returned the signed letters were enrolled into the study.

Based on availability of sufficient serum samples and considering budgetary restriction, all 70 samples of subjects who were SPT positive to any venom and 130 samples of randomly selected subjects who were SPT negative to any venom, were selected for measurements of specific IgE against *D. pteronyssinus*, bee- and wasp-venom.

Questionnaires

Standardised questionnaire to gather data regarding demographic and socioeconomic status [11] as well as information on *Hymenoptera* sting allergy [13] were administered to the parents. In addition, questions about self-reported asthma, rhinitis, and eczema were asked using a questionnaire which was modified from ISAAC questionnaires when translated into Bahasa Indonesia (Supplementary Questionnaires).

The parents were asked whether the children ever helped their parents with beekeeping or gardening, and some sting-related questions such as, whether their children had ever been stung by bee or wasp, and whether they noticed any response after the sting such as: a large local reaction (LLR), characterized by a swelling defined to exceed 10 cm that lasts for more than 24 hours; and a Systemic Reaction (SR), characterized as generalized hives or angioedema after the sting, or breathing difficulties, or loss of consciousness after the sting [14]. The questionnaires were all translated into Bahasa Indonesia and answered as a Yes or No.

Skin prick testing (SPT)

SPT reactivity to bee (*Apis mellifera*), and wasp (*Vespula spp.*) venom were tested using allergen extract at concentrations 300 µg/ ml for each venom (Soluprick-SQ, ALK-Abello, Madrid, Spain). SPT reactivity to mould (*Aspergillus spp.*), cockroach (*Blattella germanica*) and house dust mites such as *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae* were also tested (Soluprick-SQ, ALK-Abello, Madrid, Spain). Histamine dihydrochloride (10mg/ml) was used as positive control, meanwhile, allergen diluents as the negative control (Soluprick-SQ, ALK-Abello, Madrid, Spain). This measurement was performed using standard protocol as described previously [15, 11]. SPT was carried out by one trained research staff and measured by another staff for whole study population. Wheal size were assessed at 15 minutes by measuring mean of perpendicular diameter. Wheal size ≥ 3 mm was considered as positive. Anthropometric measurements such as body weight and height were also performed.

Specific and total IgE measurements

Based on availability of sufficient serum samples and considering budgetary restriction, all 70 samples of subjects who were SPT positive to any venom and 130 samples of randomly selected subjects negative for SPT to any venom, were selected for measurements of specific IgE against *D. pteronyssinus*, bee- and wasp-venom.

Total IgE levels in serum were measured using ELISA as previously described with minor modification [16]. For this assay, serum sample was diluted 50 times using PBS containing 0.05% Tween-20. The results were expressed as IU/ml. Allergen specific IgE (sIgE) antibodies against house dust mite *D. pteronyssinus* (d1), bee (i1) and wasp-venom (i3) from serum samples were determined by the ImmunoCAP system according to the manufacturers recommendation (Thermo-Fisher Scientific, Uppsala, Sweden). Specific IgE levels ≥0.35 kU/L was interpreted as positive.

Parasitological examination

Stool samples collected from children were used to assess intestinal helminths and protozoa infection. Kato-Katz method on stool samples was performed to quantify eggs from soil-transmitted helminths such as *Ascaris lumbricoides* and *Trichuris trichiura.* Aliquots of fresh stool samples were preserved in -80°C for further analysis. DNA extraction was performed from frozen stool sample as described elsewhere [17]. PCR was used for detection *of Entamoeba histolytica, Dientamoeba fragilis, Giardia lamblia* and *Cryptosporidium spp.* as has been described in detail previously [18-20].

Statistical analysis

Central distribution of continuous variables was presented as mean ± SD if the data was normally distributed and as median (IQR) if not normally distributed. Total IgE level was log-transformed and the value was presented as geometric mean (95% CI). Categorical variables were presented as proportion. Differences between two groups was tested using student t-test or Mann-Whitney U for continuous data or chi square for categorical data. Logistic regression was used to analyse potential factors that might contribute to the development of sensitization to allergens tested. In multivariate analysis, we included age, sex, zBMI as a priori confounders, as well as other variables that were significant in univariate analysis.

The agreement between skin test and IgE positivity was analysed using Cohen Kappa test while the Spearman rank correlation was used to assess the correlation between skin wheal size and the levels of sIgE [21]. Agreement is defined as having SPT positive with corresponding sIgE positive or having SPT negative with corresponding sIgE negative.

All statistical measurements considered to be significant if p-value <0.05. Statistical analysis was performed using IBM Statistical Package for the Social Sciences (SPSS) Statistics version 25 (IBM-SPSS Inc., Armonk, NY, USA).

Results

Characteristics of the study populations

In total, 325 children were recruited from low (n=165) and high (n=160) SES schools. No differences in terms of age and sex were observed between the two groups (Table 1). Low SES children had significantly lower z-BMI compared to children from high SES school (mean ± SD: -0·96 ± 1·18 vs 0·26 ±1·48; respectively; p-value<0·001). Stool samples were collected from 255 (low SES, n=128; high SES, n=127) children. There was no difference between children who provided stool samples and those who did not in terms of age, sex, and zBMI. Helminths (65.6% vs 1.6%; p-value<0.001) and protozoa infections (72.8% vs 39.2%; p-value<0.001) were more prevalent in low compared to high SES schoolchildren (Table 1). The levels of total IgE were higher in low compared to the high SES schoolchildren (geomean (95% CI): 422.08 (342.52 - 520.12) vs 164.06 (135.38 - 198.81); p-value<0.001)

Sensitization to aeroallergens and venoms

Skin prick tests were performed in 322 children (low SES=164 and high SES=158). After excluding 7 children of the low SES group with a histamine negative response, we found no differences in histamine wheal size between low (n= 157) and high (n=158) SES schoolchildren (mean ± SD, 6.1 ± 0.9 mm vs 6.3 ± 0.9 mm, respectively). Next, when we compared the wheal size of SPT to each *D. pteronyssinus, D. farinae,* cockroach, mould, bee- and wasp-venom allergen, between low and high SES and observed no differences in wheal size of skin response to allergens tested, except for bee-venom which is slightly larger in the low SES compared to the high SES (median (IQR): 4.0 mm (3.25-4.25) and 3.0 mm (3.0-3.5), respectively) (Supplementary Table 1).

The highest percentage of positive SPT was to bee-venom (14.3%), followed by cockroach (13.3%), wasp-venom (12.7%), *D. pteronyssinus* (11.8%), *D. farinae* (7.9%) and mould (5.4%). Strikingly, for all allergens tested the percentage positive SPT was significantly higher in high compared to low SES schoolchildren. Positivity for any SPT was 51.3% vs 22.9%, in high versus low SES group (p-value<0.001) and for specific allergens the values were 22.8% vs 5.7% to bee-venom (p-value<0.001); 17.1% vs 9.6% to cockroach (p-value= 0.049); 19.6 % vs 5.7% to wasp-venom (p-value<0.001); 18.4% vs 5.1% to *D. pteronyssinus* (p-value<0.001); 12.7% vs 3.2% to *D. farinae* (p-value=0.002); and 8.9% vs 1.9% to mould (p-value=0.006).

In the low SES school, there was no difference in zBMI of children who were positive or negative for allergen SPT (Supplementary Table 2). Moreover, no differences were found in the prevalence of parasitic infection between subjects positive and negative skin test reactivity (Supplementary Table 3).

In contrast to skin test reactivity, analysis of allergen specific IgE revealed that the prevalence of IgE positivity to both venoms was higher in low compared to high SES school (34.0% vs 19.0%, p-value=0.016, for bee-venom; and 38% vs 19%, p-value=0.003, for wasp-venom). The percentage of children with IgE positivity to both bee- and wasp-venom was 17.5% (25% in the low SES and 10% in the high SES, p-value=0.005). There were no differences in the specific IgE positivity to *D. pteronyssinus* between the two groups (Table 1).

Potential factors associated with sIgE positivity and SPT reactivity to any venom

In univariate analysis, skin reactivity was positively associated with high SES (OR, 4.52; 95%CI, 2.28-7.59) while negatively associated with the presence of helminth infection (OR, 0.26; 95%CI, 0.12-0.59). No association with zBMI was observed. In contrast to SPT positivity, sIgE was negatively associated with high SES and high zBMI (OR, 0.45; 95% CI, 0.25-0.81 and OR, 0.77; 95% CI, 0.62-0.96; respectively). No association was observed between sIgE positivity and current helminth infection.

Multivariate analysis adjusted with age, sex, and zBMI revealed that skin reactivity to any venom remained associated with high SES (OR (95%CI), adjusted p-value: 5.15 (2.66-9.97); p.adj<0.001), Similarly, following adjustment with age, sex, and zBMI, the negative association between high SES and sIgE remained intact (0.52 (0.27-0.98); p.adj=0.042)

Reported clinical symptoms

The sting-related questionnaire was assessed in 151 children with completed data on SPT and sIgE to any venom. Of these children, 17 (11.3%) had been stung by Hymenopterans at least once in their lifetime and experienced a local reaction such as sharp burning pain, redness, and slight swelling at the location after being stung and among them, 1 (5.9%) child reported to have a history of LLR while none did report SR after being stung. The child who has reported to have LLR, had a positive skin test but negative sIgE to any venom. Among the remaining 16 children who had been stung but had no reported LLR, 7 were positive for both SPT and sIgE to any venom while 9 children had positive SPT but were negative for sIgE to any venom.

Next, we analyzed the association between sIgE and skin sensitization to *D. pteronyssinus* and self-reported clinical symptoms of allergy among 151 subjects who had completed data. The Venn diagram in Supplementary Figure S1 shows the overlap between sensitization and self-reported and/or ever-diagnosed allergic asthma, rhinitis, or eczema. Among 22 children who reported clinical symptoms of allergy, 16 (72%) were positive in specific IgE to *D. pteronyssinus*, of which, 10 children were also positive for SPT against *D. pteronyssinus*.

Discordance between specific IgE and skin test reactivity

To assess discordance between SPT and sIgE, we selected individuals with positive SPT among 197 subjects, for whom we had both SPT and IgE data. When analysing 41 subjects with positive SPT to bee-venom, we found 27 (65.9%) were sIgE negative. Similarly, among 39 subjects that were SPT positive to wasp-venom, 26 (66.7%) were sIgE negative. However, of 30 children with positive SPT to *D. pteronyssinus*, there were only 5 children (16.7%) that were negative for sIgE to *D. pteronyssinus* (Table 2A and Supplementary Figure S2).

Furthermore, the proportion of SPT negative among sIgE positive to bee- and wasp-venom were, 72.6% and 77.2%, respectively. When we stratified the analysis based on SES, the proportion of discordance was higher in the low SES compare to the high SES and was statistically significant for bee-venom (bee-venom: 84.4% and 52.6%, p=0,01; wasp-venom: 81.6% and 68.4%, p=0.27, respectively). The proportion of SPT negatives among sIgE positive to *D. pteronyssinus* were also assessed. Among 61 children with positive sIgE, 36 children (59.0%) were negative for SPT; with higher discordance in the low SES (77.8%) compared to the high SES (44.1%) (Table 2B and Supplementary Figure S2).

In Figure 1, we` plotted the wheal size of skin reactivity against the specific IgE levels. The results showed a "none to slight" agreement for bee- or wasp-venom sensitization (Kappa = 0.096 (-0.051 – 0.243), p-value = 0.175, Figure 1B and Kappa = 0.047 (-0.092 – 0.186), p = 0.499, Figure 1C, respectively), while a "moderate" agreement (Kappa = 0.434 (0.317-0.550); p-value<0.001, Figure 1A) was found for sensitization to *D. pteronysssinus*

Analysing the correlation between the wheal size and sIgE levels revealed a correlation for sensitization to *D. pteronysssinus* (rho = 0.431, p-value<0.001, Figure 1A) but neither to bee-nor to wasp-venom (rho: 0.055; p-value=0.440, Figure 1B; and rho: 0.071 p-value=0.321, Figure 1C respectively). When socioeconomic status was considered, no difference was found on the agreement analysis for sensitization to *Hymenoptera* venom between high and low SES. We also found no differences on the agreement analysis between helminth-infected vs helminth-uninfected neither between protozoa-infected and protozoa-uninfected as shown in Supplementary Figure S3.

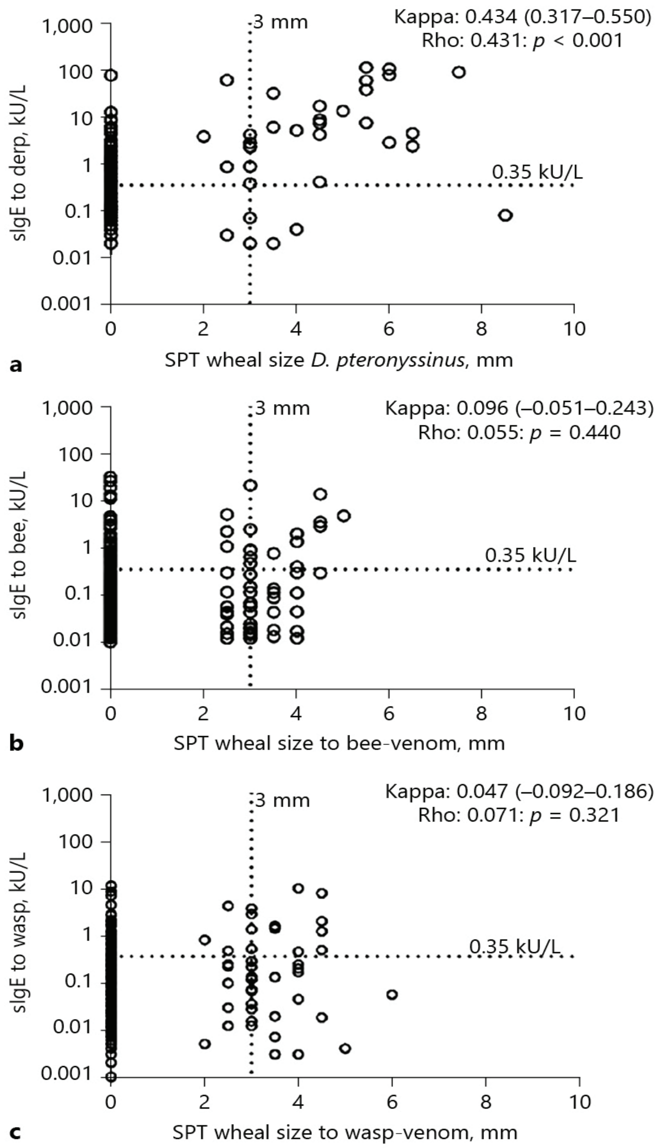

Figure 1. Comparison between SPT wheal size and sIgE to A) *D. pteronysssinus*; b) bee-venom; and C) wasp-venom. Dotted lines show sensitization cut off of 0.35 kU/L for sIgE and 3 mm for SPT wheal size. *Agreement between SPT positivity and sIgE positivity were tested using Kappa Cohen's test and presented as Kappa (95% CI). Correlation between SPT wheal size and level of sIgE to allergen tested presented as Spearman correlation coefficients (rho) and p-values.*

Table 1. Characteristic of study population

Variables	All		Low SES		High SES		*p-value*
	N	Results	N	Results	N	Results	
Age, years, (mean, SD)	325	10.26 ± 0.89	165	10.21 ± 1.08	160	10.32 ± 0.64	0.259
Sex, N, n%	325						
Male	144	44.31	73	44.2	71	44.4	0.981
Female	181	55.69	92	55.8	89	55.6	
z-BMI, mean ± SD	325	-0.36 ± 1.47	165	-0.96 ± 1.18	160	0.26 ± 1.48	<0.001
Parasites infection, N, n%							
Any intestinal parasites	234	150 (64.1)	114	101 (88.6)	120	49 (40.8)	<0.001
Any helminths	255	86 (33.7)	128	84 (65.6)	127	2 (1.6)	<0.001
Ascaris lumbricoides	255	59 (23.1)	128	59 (46.1)	127	0	<0.001
Trichuris trichiura	255	54 (21.2)	128	52 (40.6)	127	2 (1.6)	<0.001
Hymenolepis diminuta	255	2 (0.8)	128	2 (1.6)	127	0	
Any protozoa	234	110 (47.0)	114	83 (72.8)	120	47 (39.2)	<0.001
Entamoeba histolytica	234	18 (7.7)	114	16 (14.0)	120	2 (1.7)	<0.001
Dientamoeba fragilis	234	66 (28.2)	114	41 (36.0)	120	25 (20.8)	0.010
Giardia lamblia	234	87 (37.2)	114	59 (51.8)	120	28 (23.3)	<0.001
Cryptospridium parvum	234	3 (1.3)	114	3 (2.6)	120	0	
Skin prick test reactivity							
Any skin prick test reactivity	315	117 (37.1)	157	36 (22.9)	158	81 (51.3)	<0.001
Any venom	315	70 (22.2)	157	17 (10.8)	158	53 (33.5)	<0.001
Apis mellifera	315	45 (14.3)	157	9 (5.7)	158	36 (22.8)	<0.001

Vespula spp.	315	40 (12.7)	157	9 (5.7)	158	31 (19.6)	<0.001
Any aeroallergen	315	76 (24.1)	157	23 (14.6)	158	53 (33.5)	<0.001
House dust mite (HDM)	315	46 (14.6)	157	12 (7.6)	158	34 (21.5)	<0.001
Dermatophagoides pteronyssinus	315	37 (11.8)	157	8 (5.1)	158	29 (18.4)	<0.001
D. farinae	315	25 (7.9)	157	5 (3.2)	158	20 (12.7)	0.002
Blattella germanica	315	42 (13.3)	157	15 (9.6)	158	27 (17.1)	0.049
Aspergillus spp.	315	17 (5.4)	157	3 (1.9)	158	14 (8.9)	0.006
sIgE (kU_A/L), median (IQR)							
A. mellifera	200	0.08 (0.03 - 0.37)	100	0.15 (0.04 - 0.67)	100	0.05 (0.02 - 0.22)	<0.001
Vespula spp.	200	0.10 (0.01 - 0.45)	100	0.19 (0.04 - 0.70)	100	0.04 (0.01 - 0.26)	<0.001
D. pteronyssinus	200	0.09 (0.04 - 0.66)	100	0.10 (0.05-0.39)	100	0.07 (0.03-1.48)	0.282
sIgE (cut off ≥0.35 kU_A/L)							
Any venom	200	75 (37.5)	100	47 (47.0)	100	28 (28.0)	0.006
A. mellifera	200	53 (26.5)	100	34 (34.0)	100	19 (19.0)	0.016
Vespula spp.	200	57 (28.5)	100	38 (38.0)	100	19 (19.0)	0.003
D. pteronyssinus	200	61 (30.5)	100	27 (27.0)	100	34 (34.0)	0.285
Total IgE (IU/ml), geomean (95%CI)	311	269.21 (231.40 - 313.19)	163	422.08 (342.52 - 520.12)	148	164.06 (135.38 - 198.81)	<0.001

The number of positives (n) of the total population examined (N). SD: standard deviation. z-BMI: z-score of body mass index. CI: Confidential intervals

Table 2. Proportion of tests with discordant results between sIgE and skin reactivity to *D. pteronyssinus*, bee- and wasp-venom in low- and high- SES schoolchildren.

(A) Proportion of sIgE negative among SPT positive schoolchildren			
	D. pteronyssinus	**Bee-venom**	**Wasp-venom**
All, n, N (%)	5/30 (16.7)	27/41 (65.9)	26/39 (66.7)
low-SES, n, N (%)	1/7 (14.3)	4/9 (44.4)	2/9 (22.2)
high-SES, n, N (%)	4/23 (17.4)	23/32 (71.9)	24/30 (80.0)
(B) Proportion of SPT negative among sIgE positive schoolchildren			
	D. pteronyssinus	**Bee-venom**	**Wasp-venom**
All, n, N (%)	36/61 (59.0)	37/51 (72.6)	44/57 (77.2)
low-SES, n, N (%)	21/27 (77.8)	27/32 (84.4)	31/38 (81.6)
high-SES, n, N (%)	15/34 (44.1)	10/19 (52.6)	13/19 (68.4)

Data presented as **(A)** percentage of sIgE negative (n) among sIgE positive schoolchildren (N) and **(B)** percentage of SPT negative (n) among sIgE positive (N) schoolchildren

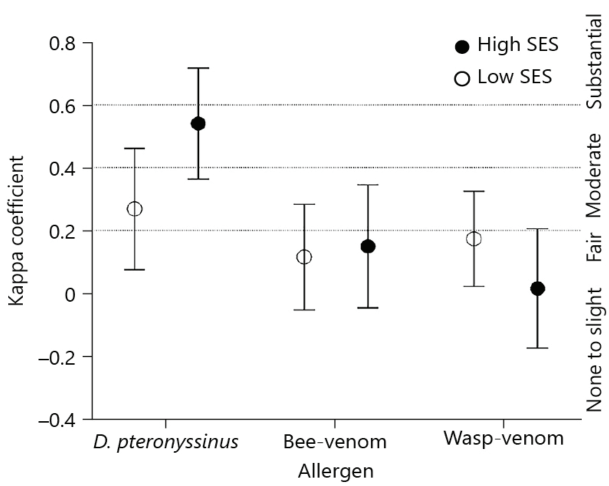

Figure 2. Agreement between SPT and sIgE to *D. pteronyssinus*, bee- and wasp-venom in high and low SES schoolchildren. *Kappa coefficient: ≤0.20 (none to slight agreement); 0.21-0.40 (fair agreement); 0.41-0.60 (moderate agreement); 0.61-0.80 (substantial agreement).*

Regarding *D. pteronyssinus* sensitization, the kappa agreement was stronger in the high compared to low SES ("moderate", 0.541 (0.364-0.717) vs "fair", 0.269 (0.076 – 0.461) for high and low SES, respectively) (Figure 2). Similarly, when we stratify the population into helminth infected and uninfected, as shown in Supplementary Figure S3, weaker agreement also observed in the helminth-infected (fair agreement: 0.228 (-0.041-0.497) compared to helminth-uninfected group (moderate agreement: 0.550 (0.383-0.716). However, no differences in the agreement analysis between protozoa infected vs uninfected groups was found.

Discussion

To the best of our knowledge, this is the first report of a study into the prevalence of *Hymenoptera* venom sensitization in schoolchildren living in an urban area of a tropical country. Studies on the *Hymenoptera* venom sensitization so far were performed in temperate or subtropical areas, where exposure to bee and wasp stings are frequent [3, 9] and these studies involved a high-risk population such as beekeepers and their relatives [22].

Here, we found the prevalence of skin reactivity to bee- and wasp-venom was 14.3% and 12.7%, respectively. A cross-sectional study in Italy reported that 2.98% and 1.45% of primary schoolchildren was positive for skin test reactivity [3]. It should be noted that the venom concentration used in the Italian study was three times lower than in our study. In addition, the test material was purchased from ALK-Abello, while the Italian study used material from Lofarma, which might also contribute to the differences in the prevalence of skin reactivity in the two studies. When specific IgE to venom was considered, we found the prevalence of positive sIgE to bee- and wasp-venom were 26.5% and 28.5%, respectively, which are similar to the findings in an adult German cohort that reported 23.1% and 31.7% IgE positivity to bee- and wasp-venom [23].

In our study, majority (88.7%) of children reported no history of having been stung. From 17 children (11.3%) who had a history of a sting, only one child (5.9%) reported LLR but none SSR. The child who reported LLR had a positive skin test, which could suggest a recent exposure to a sting. Proportion of loss of sensitization to Hymenoptera venom in skin tests has been reported to be 12% per year [24]. The prevalence of clinical reaction in our population is much lower compared to a questionnaire-based survey in Turkish children [9], which reported 24.3% and 9.9% for LLR and SSR, respectively. The prevalence of LLR in our study is comparable to a study in Irish children that reported LLR in 5.8% of the participants [7].

Studies in temperate climates have reported that asymptomatic sensitization to bee- and wasp-venom is a common phenomenon in the general population [25, 26]. Therefore, it was concluded that detected sensitization to both venoms are clinically irrelevant as the presence of sensitization to both venoms did not translate into clinical reactions. In our study, all children with positive sIgE to either bee- or wasp-venom reported no LLR nor SR after being stung. This finding is higher compared to 69.3% reported in Denmark [5].

We observed considerable skin reactivity in the absence of specific IgE to bee- and wasp-venom. This might suggest that skin reactivity to *Hymenoptera* venom is not necessarily through IgE but through IgE-independent mechanisms [27, 28]. Bee and wasp [29] venom may contain several substances that could provoke toxic reactions [30] by inducing mast cell degranulation directly resulting in positive skin reaction in some children. The low molecular weight components of venoms often mediate local toxic and non-allergic reactions while components such as hyaluronidases, phospholipase A1 and A2, antigen 5, serine proteases, and acid phosphatases do so through IgE [31].

The absence of skin reactivity despite the presence of sIgE in our population may suggest the presence of IgE with poor biologic activity, which has been described for cross reactive IgE [32]. The cross-reactive IgE might arise through recognition of *Hymenoptera* venom components, such as hyaluronidases [4, 33]. Interestingly, a 44-kD protein similar to hyaluronidase has been shown in mosquito extracts [34, 35] and might be responsible for the high sIgE that we observe to venoms. This is in line with our finding of higher sIgE positivity in the low SES, which live in conditions associated with inadequate sewage and poor sanitation, and therefore increased population of mosquitos. There is also a possibility that higher total IgE might contribute to the overall elevation of sIgE as reported by Dold et al [36].

Similar to our study, the lack of association between sIgE and skin sensitization to venoms has also been reported in a previous study [37]. However, the study was on selected subjects with SR to insect sting which reported that 32% of participants had negative skin test response. Interestingly, among these subjects, 43% had venom specific IgE antibodies. Discordance between RAST and skin test in their study may reflect the different sensitivity of the two tests for diagnosing the venom reactions and it could also be due to differences in the material used in the two tests [37].

The asymptomatic sensitization is not only seen to venom allergens, but also to other allergens as already reported in plethora of studies conducted in developing countries [38-40]. In our study, most of the children with detected sIgE or positive skin reactivity to *D. pteronyssinus* did not report clinical symptoms. When stratified according to SES, we observed discordant results in *D. pteronyssinus* sensitization where the correlation and agreement between SPT and sIgE was weaker in low SES compared to the high SES children in particular when considering the proportion of SPT negative among sIgE positive children. Skin sensitization to aeroallergens including house dust mites were higher in the high SES compared to the low SES, however, no differences were observed in the prevalence of specific IgE to *D. pteronyssinus* between high and low SES group. This finding was similar to our previous study in the same urban area [11]. In a previous study, thin children have been shown to have less skin sensitivity [41], however this is not the case in our study as the zBMI was similar between those positive and negative for SPT to any allergen tested in the low SES group. In line with our study, Keller-Franco et al [42], showed that nutritional status which was measured by BMI did not affect the skin reactivity to either histamine or *D. pteronyssinus*.

Low SES has been reported to be associated with lower prevalence of skin reactivity to house dust mite despite the presence of sIgE [40]. This we have suggested to be due to the down-modulation of skin reactions to allergens by induction of IL-10 [43]. Parasitic infections, in particular helminths are often highly prevalent in the less affluent populations, leading to expansion of regulatory T cells [44]. However, even though the SPT to venom was lower in low SES schoolchildren, when we tested the agreement between SPT and sIgE, we observed “poor” agreement in both low and high SES groups.

One of the limitations of our study is its cross-sectional design which does not allow us to determine causality and time of exposure. It is known that sensitization to venom peaks few weeks after a sting and recedes over time [24, 45]. In this study we studied past reactions to venom and current sensitization instead of following up sensitization and reactions after being stung. Moreover, the questionnaire-based approach data on allergy and sting-related reaction is restricted by recall bias. In addition, the lack of component resolved diagnostic methods in this study may have hampered the evaluation of true sensitization to venom allergy.

Our findings, however, provide evidence that sensitization against bee- or wasp-venom are quite prevalent in Indonesian schoolchildren living in an urban area of a tropical region. Higher prevalence of skin reactivity was observed in high SES compare to the low SES children, in contrast, sIgE-positive was more prevalent in the low SES compare to the high SES. These sensitizations to Hymenoptera venom appear to have poor clinical relevance as they rarely translated into clinical symptoms. Moreover, we also observed discordance between SPT and sIgE to bee- and wasp-venom, in particular the high proportions of positive SPT in absence of sIgE, which suggests the direct (non-IgE) effect of venoms resulting in skin reactivity. However, further studies are needed to determine the possible mechanisms underlying this which could be through component resolved diagnostic methods.

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Statement of Ethics

This study was approved by Health Research Ethics Committee of Faculty of Medicine, Hasanuddin University (Ref: 1504/H04.8.4.5.31/PP36-KOMETIK/2016). Written informed consent was obtained from all parents prior to data collection.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

Conception and design of the experiments: R.R., M.Y., F.H. and E.S. Data collection and experimentation: A.I.A, J.P.R.K., M.M, S.A.V., S.W., F.H., and E.S. Interpretation of data and statistical analysis: A.I.A, J.P.R.K. Writing of this paper: A.I.A, J.P.R.K., M.M, S.A.V, S.W., F.H., E.S., R.R., and M.Y. The corresponding author had full access to all of the data in this study and takes final responsibility for the decision to submit it for publication.

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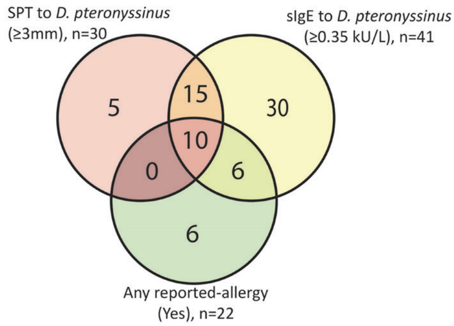
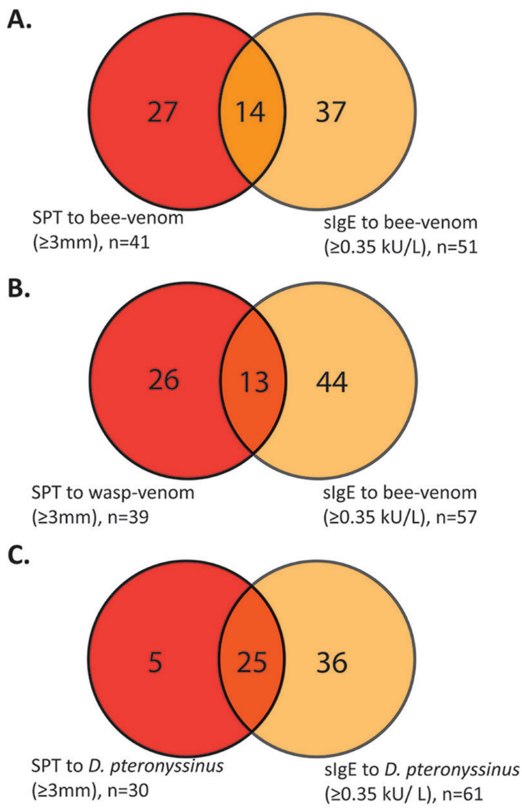
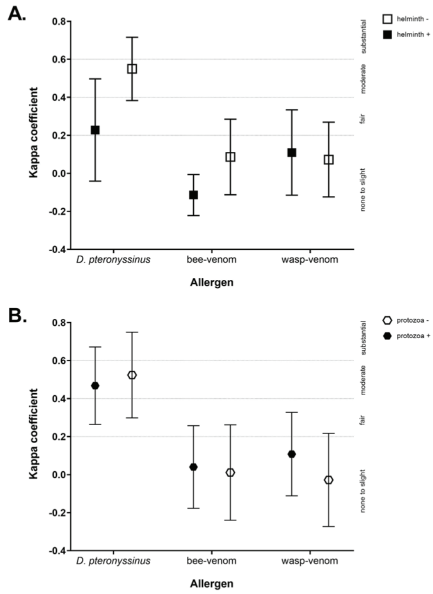
SUPPLEMENTARY FIGURES AND TABLES



Supplementary Figure S1. Overlap between sensitization to *D. pteronyssinus* and self-reported diagnosis of any allergy for subjects (N=151) with completed data of SPT, sIgE and allergy-related questionnaire. SPT: skin SPT: skin prick test. sIgE: specific Immunoglobulin E.



Supplementary Figure S2. Dis-/Concordance of tests between SPT and sIgE to (A) bee-venom, (B) wasp-venom, and (C) *D. pteronyssinus* of all subjects (n=197) with match SPT and sIgE data.



Supplementary Figure S3. Agreement between SPT and sIgE to *D. pteronyssinus*, bee-venom and wasp-venom in A. helminth infected and uninfected schoolchildren, and B. protozoa infected and uninfected schoolchildren. Kappa coefficient: 0.01-0.20 (none to slight agreement); 0.21-0.40 (fair agreement); 0.41-0.60 (moderate agreement); 0.61-0.80 (substantial agreement).

Supplementary Table 1. Wheal size of skin prick test (SPT) among children with positive SPT to each allergen tested

Allergen	Wheal size in mm						*p-value*
	All		**Low SES**		**High SES**		
	N	**median (IQR)**	**N**	**median (IQR)**	**N**	**median (IQR)**	
Aeroallergens							
D. pteronyssinus	37	4.50 (3.50-6.00)	8	4.25 (3.25-6.25)	29	4.50 (3.50-6.00)	0.71
D. farinae	25	5.00 (4.00-6.00)	5	4.00 (3.25-5.00)	20	5.00 (4.00-6.00)	0.11
B. germanica	42	4.00 (3.50-4.50)	15	4.00 (3.50-4.50)	27	4.5 (3.50-5.00)	0.69
Aspergillus spp.	17	3.50 (3.00-4.50)	3	4.50 (4.50-4.50)	14	3.25 (3.00-4.12)	0.54
Venoms							
A. mellifera	45	3.50 (3.00-4.00)	9	4.00 (3.25-4.25)	36	3.00 (3.00-3.50)	0.03
Vespula spp.	40	3.50 (3.00-4.00)	9	3.50 (3.00-4.50)	31	3.50 (3.00-4.00)	0.31

N: total population. IQR: interquartile range. *p-value* derived by Mann-Whitney U test

Supplementary Table 2. Body mass index (zBMI) of SPT positive and SPT negative children living in the low SES

Allergen	zBMI				*p-value*
	SPT positive		**SPT Negative**		
	N	**mean ± SD**	**N**	**mean ± SD**	
Any aeroallergen	23	-1.13±1.19	134	-0.93±1.21	0.45
House dust mite	12	-1.09±1.46	145	-0.95±1.18	0.69
Any venom	17	-0.74±1.80	140	-0.98±1.11	0.60

zBMI: z-score of Body Mass Index. *p-value* derived from student t-test

Supplementary Table 3. Comparison of intestinal parasitic infection prevalence between SPT positive and SPT negative among low SES schoolchildren

Parasites infection n/ N (%)	Any allergen			Any aeroallergen			Any venom		
	SPT positive	**SPT negative**	***p-value***	**SPT positive**	**SPT negative**	***p-value***	**SPT positive**	**SPT negative**	***p-value***
Any intestinal parasites	22/24 (91.7)	73/84 (86.9)	0.53	14/14 (100)	81/94 (86.2)	0.14	11/13 (84.6)	84/95 (88.4)	0.69
Any helminths	16/26 (61.5)	62/95 (65.3)	0.72	10/16 (62.5)	68/105 (64.8)	0.86	7/14 (50)	71/107 (66.4)	0.23
Any protozoa	18/24 (75)	59/84 (70.2)	0.65	12/14 (85.7)	65/94 (69.1)	0.20	9/13 (69.2)	68/95 (71.6)	0.86

Total infected (n) among total population (N) in each group. SPT: skin prick test

SUPPLEMENTARY QUESTIONNAIRES

A. **Core questionnaires for Asthma, Rhinitis and Eczema (The questionnaire was modified from The International Study of Asthma and Allergies in Childhood (ISAAC) core questionnaires)**

1. **Questionnaire for wheezing and asthma**

No	Question	Answer
Q1	Has your child ever had asthma (asma)? Diagnosed by doctor	[ ] Yes [ ] No
Q2	Has your child had wheezing or whistling (mengik/poso/asma) in the chest in the past 12 months?	[ ] Yes [ ] No If no skip to Questionnaire about Rhinitis
Q3	How many attacks of wheezing (mengik/poso/asma) has your child had in the past 12 months?	[ ] None [ ] 4-12 [ ] 1-3 [ ] > 12
Q4	In the past 12 months, how often, on average, has your child's sleep been disturbed due to wheezing (mengik/poso/asma)?	[ ] Never woken with wheezing (mengik/ poso/asma) [ ] Less than one night per week [ ] One or more nights per week
Q5	In the past 12 months, has wheezing (mengik/poso/asma) ever been severe enough to limit your child's speech to only one or two words at a time between breaths?	[ ] Yes [ ] No
Q6	In the past 12 months, has your child's chest sounded wheezy (mengik/poso/asma) during or after exercise?	[ ] Yes [ ] No
Q7	In the past 12 months, has your child had a dry cough at night, apart from a cough associated with a cold or chest infection?	[ ] Yes [ ] No

2. **Questionnaire for allergic rhinitis**

No	Question	Answer
Q1	Has your child ever had problem with sneezing or a runny or blocked nose (nose problem) without cold or the flu?	[ ] Yes [ ] No If no skip to Q6
Q2	In the past 12 months, has your child had a problem with sneezing, or a runny, or blocked nose when you DID NOT have a cold or the flu?	[ ] Yes [ ] No If no skip to Q6

Q3	In the past 12 months, have itchy-watery eyes accompanied this nose problem?	[ ] Yes [ ] No
Q4	In which of the past 12 months did this nose problem occur? (Please tick any which apply)	[ ] Rainy season [ ] Dry season [ ] Anytime [ ] No idea
Q5	In the past 12 months, how much did this nose problem interfere with your child's daily activities?	[ ] Not at all [ ] A Moderate [ ] A little [ ] A lot
Q6	Has your child ever been diagnosed by doctor to had allergic rhinitis?	[ ] Yes [ ] No If no, STOP here

3. **Questionnaire for eczema (allergic dermatitis) (show the pictures to the subject)**

No	Question	Answer
Q1	Has your child ever had an itchy rash (like in the picture) which was coming and going for at least six months?	[ ] Yes [ ] No If no skip to Q6
Q2	Have your child had this itchy rash at any time in the past 12 months?	[ ] Yes [ ] No If no skip to Q6
Q3	Has this itchy rash at any time affected any of the following places? The folds of the elbows, behind the knees, in front of ankles, under the buttocks or around the neck, ears or eyes?	[ ] Yes [ ] No
Q4	Has this rash completely disappear at any time during the past 12 months?	[ ] Yes [ ] No
Q5	In the past 12 months, how often, on average, have your child been kept awake by this itchy rash?	[ ] Never in the past 12 months [ ] Less than one night per week [ ] One or more nights per week
Q6	Has your child ever been diagnosed by doctor to had allergic dermatitis?	[ ] Yes [ ] No

B. **Sting-related Questionnaire**

Questionnaire for venom allergy (show the pictures to the subject).

No	Question	Answer
Q1	Has your child, currently or within the last 5 years occupationally or as a hobby done any of the following?	1. Bee-keeping 2. Farming 3. Gardening 4. None
Q2	Has your child ever been stung?	1. Yes 2. No
Q3	Being stung by an insect, did you notice that your child had a swelling at the site of the sting?	1. Yes 2. No
Q4	Being stung by an insect, did you notice that your child had a swelling with diameter of more than 10 cm, lasting over 24 hours in certain part of his/her body?	1. Yes 2. No
Q5	Being stung by an insect, did you notice that your child had an urticarial reaction on the whole body?	1. Yes 2. No
Q6	Being stung by an insect, did you notice that your child had swelling of the face, tongue or lips?	1. Yes 2. No
Q7	Being stung by an insect, did you notice that your child had an experience with difficulties of breathing, dyspnoea?	1. Yes 2. No
Q8	Being stung by an insect, have you child ever lost consciousness?	1. Yes 2. No