

Hymenoptera (wasps, bees and ants) envenomation; a neglected tropical disorder in Sri Lanka

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Abstract

Sri Lanka is a tropical agricultural country in which a huge number of insect species have been reported. Bees, wasps, and ants belong to the order Hymenoptera mostly living in a colony, and colony members attack humans aggressively when they are disturbed. However, Hymenopterans are important in many ways such as pollination service to the agricultural and wild plants, honey production, natural pest control for crops, and being members of the food chains. Therefore, we should help to conserve Hymenopterans while safeguarding ourselves from them.

Unpublished data confirm that thousands of hospital admissions and 15-30 deaths after insect stings in Sri Lanka annually. When Hymenopterans are disturbed, they sting the victims and inject venom as a defense mechanism. Immunological reactions and toxic effects are common clinical presentations after the Hymenoptera sting. Fatal anaphylaxis, rhabdomyolysis, acute kidney injury, acute myocardial infarction (Kounis syndrome), bowel gangrene, cerebral infarction, acute pulmonary oedema, multi organ failure, limb ischaemia and haemolytic anaemia have been reported in Sri Lanka.

Key words: hymenopterans, *Apis dorsata*, *Hymenoptera* sting, anaphylaxis, acute kidney injury, myocardial infarction, cutaneous lesions

This oration describes a clinical and epidemiological study of bee and wasp sting; a series of 322 cases, an analytical study of bee venom of *Apis dorsata* (Bambara), case series of a characteristic cutaneous

lesion over the stinging site of a wasp; *Vespa tropica*, a study of a patient group presented with anaphylaxis after Hymenoptera stings, a rare case of micro-angiopathic haemolytic anaemia due to envenoming by giant Asian honey bee (*Apis dorsata*), a case of severe anaphylaxis after *Ropalidia marginata* (a paper wasp) sting and a case report of giant honey bee (*Apis dorsata*) sting causing acute limb ischaemia.


Introduction

Pharaoh Menes, the first king in the earliest Egyptian civilization who unified Upper and Lower Egypt, died from a wasp or hornet sting in 2600 BC which is thought to be the first reported case of an anaphylactic reaction.¹ There are about 40 deaths due to insect stings in the United States every year.² Except few published observational studies and case reports, accurate data on Hymenoptera sting in Sri Lanka, is unavailable. However, unpublished data from the medical statistics unit of the Registrar General's Office in Sri Lanka confirmed 15-30 deaths per year due to insect stings in this country.

Medically significant stinging bees, wasps, and ants belonging to the order Hymenoptera (Latin for membrane-winged) are broadly categorized into 3 families which are *Apidae* (honeybees and bumblebees), *Vespidae* (yellow jackets, hornets, and wasps), and *Formicidae* (ants).³ Honeybees are small, hair-covered insects with alternating tan and black stripes on their body.³ Domesticated Asian honeybee, *Apis cerana* (Meemessa), feral giant honeybee or giant Asian honeybee, *Apis dorsata* (Bambara) and feral dwarf honeybee, *Apis florea* (Daduvel mee) are

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common stinging social honeybees found in rural and urban Sri Lanka.⁴ Of them, the giant Asian honeybee is the largest (17-20 mm in length) and most aggressive species.⁵ *Vespa affinis* (Linnaeus), *V. tropica* (Linnaeus) and *V. mandarinia* (Smith) are social wasps reported in South East Asia.⁶ *Ropalidia marginata* (old world paper wasp – Kaladuruwa) is the most common paper wasp living in small colonies in the peninsula of India⁷ and Sri Lanka. Huge ant diversity with 341 species is found in Sri Lanka. Of them 82 species are endemic to the country.⁸

Female Hymenopteran has a stinging apparatus called 'stinger' which is a modified ovipositor located at the tip of the abdomen associated with a venom gland and lancet.⁹ If Hymenopterans are disturbed, they sting the victim and inject venom as a defense mechanism. The stinger is detached from the body of the honeybee after a single sting and it dies thereafter, whereas a single wasp is capable of stinging multiple times as the stinger is not separated from the body after a sting. This finding is useful to differentiate honeybees from wasps as wasps do not leave the stinger on the victim's skin.^{5,10,11} Unlike bees or wasps, the ant uses its mandibles to attach itself to the victim's skin and arches its back to inject venom through the stinger. Multiple stings are the rule rather than the exception for fire ant stings.³ Melittin, hyaluronidase, apamin, mast-cell degranulating protein, active amines (serotonin, histamine, tyramine, catecholamines), wasp kinins, histamine-releasing peptides, antigen 5, phospholipase A and B, neurotoxins, acetylcholine, water-insoluble alkaloid, and allergenic proteins are toxic substances found in Hymenopteran venom.^{3,11}

Type I anaphylactic or immediate hypersensitivity reaction is the most common clinical presentation after *Hymenoptera* sting.³ Toxic effects to venom when injected in large amounts after massive stings (mass envenoming), could lead to life-threatening complications. Fatal anaphylaxis,^{10,12} rhabdomyolysis,¹³ acute kidney injury,¹⁴ acute myocardial infarction (Kounis syndrome),^{10,15,16} bowel gangrene,¹⁰ cerebral infarction,^{17,18} acute pulmonary oedema¹⁹ and multi-organ failure^{13,19} have been reported in Sri Lanka after *Hymenoptera* stings.

Diagnosis of *Hymenoptera* stinging might be made clinically in the emergency department. Stinger should be removed by scraping to prevent further injection of additional venom into the victim.³ Most patients need treatment for symptoms of pain and swelling, allergic reactions, and rarely aggressive supportive care.^{3,20} Subcutaneous immunotherapy is a mode of treatment for patients who has a past history of systemic allergic reaction to *Hymenoptera* venom.⁹ Immunotherapy has

been shown to be 97% effective in reducing anaphylaxis.³ Hymenopterans are important in many ways such as pollination of agricultural and wild plants, honey production, natural pest control for crops, and being members of the food chain. Therefore, we should help to conserve Hymenopterans while safeguarding ourselves from them. To avoid exposure to insect stings, it is advised to refrain from walking outside barefoot and to wear long sleeves, long pants, a head covering, and gloves.⁹

Hymenoptera sting is an underestimated and neglected tropical disorder in Sri Lanka. It is a major cause of hospital admission with a significant impact on the community.^{5,21,22} There is poor medical knowledge among most physicians regarding the mechanism of stinging and the importance of the removal of the stinger, the significance of the number of stingers, and the species identification. Further, the clinical profile after stinging by different species, complications, effects of mass envenoming, components of the *Hymenoptera* venom, and the possibility of immunotherapy was not well studied. Different species have different chemical components especially allergens in their venom. Serological tests could be useful for the diagnosis of unknown cases. National guidelines or protocols, commercially available venom for the diagnosis and the immunotherapy of the *Hymenoptera* envenoming are not currently available in Sri Lanka.

This oration is based on the data from a prospective observational clinical study, an analytical study, 2 case series, and 3 case reports describing the clinical and epidemiological characteristics and venom immunology of *Hymenoptera* envenoming in Sri Lanka. Further, this highlights that *Hymenoptera* envenoming is a neglected tropical disorder in Sri Lanka.

1. Study 1: Bee and wasp stings in Deniyaya; a series of 322 cases⁵

Methods

This study was conducted at the Base Hospital, Deniyaya, Sri Lanka and the medical officers collected data from patients admitted with stings of either bees or wasps during the two-year period from September 2011 to August 2013. Only patients who confidently identified the insect during the incident as honeybees or wasps were included. Specimens of the offending insects were collected for identification. In instances where the insect was not available, the patient was asked to identify the insect from a series of dead specimens of stinging hymenopterans presented by the first author. All patients completed a questionnaire.

Detailed clinical history was obtained and clinical examination was carried out in all patients. Informed written consent was obtained from every individual belonging to the study population. Ethical approval was obtained from the Ethical Review Committee of the Faculty of Medicine and Allied Sciences, Rajarata University of Sri Lanka (ERC/2012/015).

Results and Discussion

A total of 11,254 patients were admitted to medical wards during the study period and 322 of them were stung by wasps or honeybees. There were 173 (53.7%) men and 149 (46.3%) women. The mean age was 42.5 years, (SD=15.1), and 236 (73%) victims were aged 20-60 years. Thirty patients (9.3%) were stung by wasps and 292 (90.7%) were stung by honeybees. Species identification was made in 55 specimens brought by the patients to the zoologist at the Zoology Department, University of Peradeniya. Of them 46 were *A. dorsata*, eight were *V. tropica* and one was *R. marginata* (Figure 1).

Two hundred (62%) were stung by honeybees or wasps while working in the tea plantations and 88 were stung while walking along roads or foot paths. All the patients developed pain over the stinging site and 79.2% had swelling over the site of the sting. Facial puffiness was reported in 37.3% and nausea or vomiting in 16.5%. Bronchospasms (2.5%), change of voice (0.6%), throat pain (0.9%) and abdominal pain (2.1%) were reported in a lesser number of patients. The majority (78.9%) had localized painful self-limiting swelling without systemic features. Fifteen (4.6%) developed anaphylactic shock after exposure to *Hymenoptera* envenoming but none of them died. The majority of people were stung (62%) when they were

working in tea plantations. Most (98.2%) were stung when victims were engaged in outdoor activities.

Our study showed that 3% of medical ward admissions sought emergency treatment after wasp or bee stings at the Base Hospital, Deniyaya. The reason may be that Deniyaya has tea plantations and forests with plenty of nesting sites for insects. The majority of victims of bee and wasp stings were between the ages of 20-60 years which consists of the working population. Many people were stung (62%) when they were working in tea plantations. All cases were reported during day time from 6.00 am to 7.00 pm indicating that bees and wasps in this area were active during the daytime. The highest number of patients (44.1%) presented during the period of August to September whereas the lowest number of patients were reported from January to March. The reason for this seasonal variation is not clear though it may be related to periods of the pollination of the flowers. The rainfalls and other human activities too could have contributed to this pattern.

Clinical features varied from simple erythema to severe forms of anaphylaxis. Our study confirmed that 4.6% of victims after a bee or wasp sting developed anaphylactic shock whereas in a similar study conducted in Anuradhapura District, 6.4% developed anaphylaxis after *V. affinis* (wasp) stings.²¹ Another study of 80 patients stung by bees or wasps in a hill country of Sri Lanka reported no patients with anaphylaxis.²² Even though several species of honeybees and wasps have been reported in Sri Lanka, this study identified 3 medically important stinging Hymenopterans in Deniyaya namely the Asian giant honeybee (*A. dorsata*), grater banded hornet (*V. tropica*) and paper wasp (*R. marginata*).



Figure 1. Stinging Hymenopterans reported in Deniyaya. (a). *A. dorsata*, (b). *R. marginata*, (c). *V. tropica*.

2. Study 2: IgE cross-reactivity of phospholipase A2 and hyaluronidase of *A. dorsata* (Giant Asian Honeybee) and *A. mellifera* (Western Honeybee) venom: Possible use of *A. mellifera* venom for diagnosis of patients allergic to *A. dorsata* venom²³

Methods

The patients who developed anaphylaxis following a sting by *A. dorsata*, and were treated at Base Hospital, Deniyaya and District Hospital, Bandarawela were recruited for this study. The stinging insect was identified when it was brought to the hospital or in instances where the insect was not available, patient was asked to identify the insect from a series of dead specimens of stinging hymenopterans. A questionnaire was filled obtaining relevant information from the patients and 5 ml of blood was taken from each patient and 5 ml of blood samples were collected from 4 healthy individuals. To collect the venom from *A. dorsata*, electrical stimulation was performed without killing the insects which were collected from the wild. Permission to extract venom from *A. dorsata* was obtained from the Department of Wildlife Conservation of Sri Lanka. The crude venom of *A. mellifera* and three pure venom components: PLA2, apamin, and melittin were purchased. The standard biotechnology (HPLC, SDS-PAGE, and immunoblot, Comparison of hyaluronidase enzyme activity in venom of *A. dorsata* and *A. mellifera*, allergen-specific IgE to honey bee (*A. mellifera*) venom, Identification of IgE reactivity to hyaluronidase, Immunoblot inhibition assay) was performed to detect venom components and their activities at the Medical Research Institute (MRI) and Institute of Biochemistry, Molecular Biology and Biotechnology (IBMBB), Colombo. Ethical approval for this study was obtained from Ethics Review Committee, Medical Research Institute, Colombo, Sri Lanka (No: 46/ 2013). Informed written consent was obtained from all patients and healthy individuals.

Results and Discussion

A total of 30 patients with anaphylaxis due to *A. dorsata* stings were studied. HPLC profiles were obtained with a total of 7 similar peaks for both species. Using commercially available PLA2, apamin, and melittin standards (of *A. mellifera*), three of the peaks were identified as PLA2, apamin, and melittin in the venom sample of *A. dorsata*. Melittin gave the highest peak for both *A. dorsata* and *A. mellifera*.

The relative abundance of melittin, apamin, and PLA2 of both *A. dorsata* and *A. mellifera* venom was calculated using the peak areas of the venom components; the calculated percentages were 67%, 3%, and 16% for *A. dorsata* venom and 66%, 3% and

14% for *A. mellifera* venom respectively. Similar SDS-PAGE profiles were obtained for both *A. dorsata* and *A. mellifera* venom. Twelve of 30 patients (40%) had serum IgE reacting with a 39 kDa band in both *A. dorsata* and *A. mellifera* crude venom. Twenty five of 30 (83.3%) patients had positive venom specific IgE to *A. mellifera* venom. Both *A. dorsata* and *A. mellifera* venom have shown similar hyaluronidase enzyme activity. Healthy serum samples did not show reactivity with either venom.

This study is the first to describe the IgE reactivity of allergenic components of *A. dorsata* venom. The similarity of some venom components such as PLA2 of *A. dorsata* and *A. mellifera* has been identified in a previous study in Thailand and our study has confirmed these findings.²⁴ The present study is also the first to compare the venom profiles of *A. mellifera* and *A. dorsata* by HPLC, and IgE reactivity to both venoms using patient sera following stings by *A. dorsata*.

This study has clearly demonstrated the presence of two allergenic components PLA2 and hyaluronidase in *A. dorsata* venom. Further, the observed similarity of the venoms of *A. mellifera* and *A. dorsata* opens up the possibility of using *A. mellifera* venom in the diagnosis and venom immunotherapy of patients who develop allergic reactions to *A. dorsata*.

3. Case series 1: A characteristic cutaneous lesion over the stinging site of the wasp, *V. tropica*²⁵

We reported four patients who developed a characteristic skin lesion over the stinging site of *V. tropica* (Debara) as the first reported case series.²⁵ They were admitted to the Base Hospital, Deniyaya, during the period of September 2012 to January 2013. One day after the incident a characteristic lesion appeared over the stinging site of the patients. All were oval or annular lesions of about 1-2 cm in diameter. At the center there was a small yellowish brown vesicle on dark brown necrotic tissue which was slightly depressed from the surrounding skin. The necrotic tissue was surrounded by an oedematous and reddish inflammatory area which was tender (Figure 2). The ulcerated lesion was painful during the first few days and there after it became itchy and then healed with scar formation.

The cutaneous manifestations of wasp stings are caused by allergic and inflammatory reactions secondary to toxic substances in the venom. This is believed to be due to the presence of acetylcholine and serotonin in wasp venom.²⁵ The characteristic lesion over the stinging site of *V. tropica* may help the physician to identify the offending insect.



Figure 2. The characteristic skin lesion over the stinging site of *V. tropica*.

4. Case series 2: A study of a patient group presented with anaphylaxis after Hymenoptera stings in Deniyaya, Sri Lanka²⁶

Sixteen patients who presented to Base Hospital, Deniyaya from 2011-2013 following Hymenopterans sting and developed anaphylaxis were described in this case series. Four species were identified up to the species level and they were *A. dorsata*, *R. marginata*, *V. tropica* and *Odontomachus simillimus* (Dala Kadiya, Figure 3). This case series highlights that different Hymenoptera species are responsible for anaphylaxis. In 2011, Ratnatilaka et al reported three patients with ant stings who developed severe anaphylaxis. The offending ants were *O. simillimus* ('Dala Kadiya') or *Tetraponera rufonigra* ('Hathpolaya').²⁷



Figure 3. *O. simillimus* (Dala Kadiya).

5. Case report 1: A rare case of Micro-Angiopathic Hemolytic Anaemia Due to Envenoming by Giant Asian Honey Bee (*A. dorsata*).²⁸

This patient was also admitted to Base Hospital Deniyaya, following mass envenoming from more than 1000 stingers and subsequently developed hematologic features suggestive of thrombotic microangiopathy-related hemolytic anaemia. The transient acute kidney injury and acute hepatic failure seen in the patient were also considered to be secondary to thrombotic microangiopathy. A normal clotting profile ruled out disseminated intravascular coagulation. The place of therapeutic plasma exchange for venom-associated thrombotic microangiopathy is controversial. The patient recovered with symptomatic treatment and meticulous fluid management, without needing therapeutic plasma exchange as an adjunct treatment. This case report highlights the need to add microangiopathic hemolytic anaemia to the growing list of probable life-threatening complications associated with mass envenoming due to the giant Asian honey bee sting.

6. Case report 2: A case of severe anaphylaxis after *R. marginata* (a paper wasp) sting in Sri Lanka²⁹

A 12-year-old schoolboy presented with severe anaphylaxis after *R. marginata* (Kaladuruwa) stings to the Base Hospital, Deniyaya. He was stung by a few paper wasps while observing road construction activities near a bridge. The child recovered fully with the initial resuscitation. This is the first reported case of anaphylaxis after *R. marginata* (Kaladuruwa) stings in Sri Lanka.

7. Case report 3: Giant honey bee (*A. dorsata*) sting and acute limb ischemia: a case report and review of the literature³⁰

A 65-year-old man with hypertension and hyperlipidaemia presented with envenoming from an attack of a swarm of *A. dorsata*. He subsequently developed acute limb ischaemia following a left femoral artery thrombus and made a complete recovery with anticoagulation and surgical embolectomy. *A. dorsata* sting can lead to variable degrees of thrombotic events^{17,18} which can be life-threatening. Therefore, it is of extreme importance that practicing clinicians be aware of thrombotic tendencies associated with *A. dorsata* sting to diagnose and treat the condition promptly.

Discussion

Hymenoptera sting is an underestimated and neglected tropical disorder in Sri Lanka that cause

devastating health, social and economic consequences mostly in the rural population. The pathophysiology and clinical consequences, stinging mechanism and different Hymenopterans, risk factors for the sting, epidemiology, components in the venom, diagnostic methods, and treatment modalities have not been extensively studied and the knowledge on insect sting among medical professionals is poor in Sri Lanka. The two studies,^{5,23} 2 case series and 3 case reports,^{25,26,28,29,30} described above, give good scientific knowledge about Hymenoptera sting in Sri Lanka and help physicians for best clinical approach to their patients.

Our first study on 'clinical-epidemiological study on bees and wasps stings in Deniyaya, Sri Lanka'¹⁵ concluded that a significant number of patients admitted after Hymenoptera stings (3% of medical admissions) in rural hospitals and 4.6% developed anaphylaxis. So, the primary healthcare workers at peripheral hospitals located in high-risk areas of Hymenoptera sting should have proper training with access to emergency medications.

The study on 'IgE cross-reactivity of phospholipase A2 and hyaluronidase of *A. dorsata* (Giant Asian Honeybee) and *A. mellifera* (Western Honeybee) venom: Possible use of *A. mellifera* venom for diagnosis of patients allergic to *A. dorsata* venom'²³ clearly observed the presence of two allergenic components PLA2 (15 and 16 kDa doublet) and hyaluronidase (39 kDa) in *A. dorsata* venom and the similarity of the venoms of *A. mellifera* and *A. dorsata* opens up the possibility of using *A. mellifera* venom in the diagnosis and venom immunotherapy of patients allergic to *A. dorsata*. A larger clinical study to evaluate *A. mellifera* venom for in vivo diagnosis followed by a trial of desensitization would be the next step forward.

A characteristic cutaneous lesion over the stinging site of the wasp, *V. tropica* was the first reported case series.²⁵ As most victims of wasp stings present to health care centers with pain and swelling over the sting site, the lesion we describe may help physicians to identify the offending wasp species as *V. tropica*. A study of a patient group presented with anaphylaxis after Hymenoptera stings in Deniyaya, Sri Lanka concluded four species were responsible for anaphylaxis. They were *A. dorsata*, *R. marginata*, *V. tropica* and *O. simillimus* (Dala Kadiya).²⁶

We were able to publish two rare complications after the giant Asian honey bee (*A. dorsata*) envenoming in the medical literature. Those reports were 'A rare case of micro-angiopathic hemolytic anaemia due to envenoming by Giant Asian Honey Bee' (*A. dorsata*)²⁸

and 'Giant honey bee (*A. dorsata*) stings and acute limb ischemia: a case report and review of the literature'.³⁰ Therefore, it is of extreme importance that practicing clinicians be aware of hemolytic anemia and thrombotic tendencies associated with *A. dorsata* stinging to diagnose and treat them promptly.

Conclusions

With this scientific evidence, the paper concludes that the Hymenoptera sting is an underestimated and neglected tropical disorder in Sri Lanka which causes devastating health, social and economic consequences mostly in rural populations. Further, our studies will encourage researchers to conduct more scientific studies on epidemiology and diagnostic tests for insect stings and clinical trials on immunotherapy.

Author declarations

Ethics approval

Ethical approvals were obtained for both studies from the Ethical Review Committee of the Faculty of Medicine and Allied Sciences, Rajarata University of Sri Lanka (ERC/2012/015) and from the Ethics Review Committee, Medical Research Institute, Colombo, Sri Lanka (No: 46/ 2013). Informed written consents were obtained from all the participants of studies, case series, and case reports.

Consent for publication

All authors consented to the publication.

Competing interests

No conflict of the interest.

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