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Anaphylaxis due to stings of Apis Mellifera Meda (Iranian honeybee) in two mules

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Abstract: Anaphylaxis is a serious allergic reaction that is rapid in onset and can cause death. Honeybee poison is the specific biological product of bees and does not belong to the products originating from the world of plants. It contains apamin, melittin, phospholipase, histamine, and hyaluronidase, and groups which block the nervous system and stimulate the heart and the adrenal glands. Two mules with anaphylaxis were examined in Haki, a village in Urmia, Iran. One mule died before treatment. The other mule received the following treatment: The remaining stingers were removed from the skin and epinephrine, dexamethasone, triplenamine (antihistamine), and cold compresses on the skin were used. Unfortunately, the second mule died 12 hours after the beginning of treatment. According to the comparison of our report with other reports, it can be concluded that mules and dogs exhibit clinical signs much faster than cattle. The clinical signs are also more severe in mules and dogs than in cattle. The exact cause of this difference between species is not clear to us, but it could stem from the differences in the immunological pathways that deal with honeybee's venom, similar to other venoms, and the response of the host's cells to the chemical mediators that are released during anaphylaxis. However, further research is required in this area to test this hypothesis.

Keywords: anaphylaxis / honeybee / Apis Mellifera Meda / mule

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Introduction

Anaphylaxis, as defined by an international multidisciplinary group of experts, is a serious allergic reaction affecting many body systems that is rapid in onset and can cause death (*Sampson* et al. 2006). The diagnosis is based on defined clinical criteria. Hypotension and shock are not necessarily present (*Estelle* et al. 2009). Venom from a stinging insect, food and drugs (especially β-lactam antibiotics) or, less commonly, saliva from a biting insect can induce anaphylaxis (*Freeman* 2004, *Peng* et al. 2004). Most triggers lead to anaphylaxis through a mechanism that involves the cross-linking of immunoglobulin E (IgE) and mast cells and basophils (*Estelle* et al. 2009). The underlying pathogenesis of anaphylaxis commonly involves an immunologic mechanism in which IgE is synthesized in response to allergen exposure and becomes fixed to high affinity receptors for IgE (FceRI receptors) on the surface membranes of mast cells and basophils (*Peavy* et al. 2008). After IgE binds to the receptors, mast cells and basophils release preformed chemical mediators of inflammation, including histamine, tryptase, carboxypeptidase A, and proteoglycans (*Peavy* et al. 2008). The opening of the endothelial barrier in response to chemical mediators has been identified as a critically important process leading to anaphylaxis symptoms in many body organ systems (*Peavy* et al. 2008).

A honey bee that is away from the hive foraging for nectar or pollen will rarely sting, except when stepped on or roughly handled. Honey bees will actively seek out and sting when they perceive the hive to be threatened, often being alerted to this by the release of attack pheromones (*Breed* et al. 2004). The sting's injection of apitoxin into the victim is accompanied by the release of alarm pheromones, a process which is accelerated if the bee is fatally injured (*Pickett* et al. 1982). The release of alarm pheromones near a hive or swarm may attract other bees to the location, where they will similarly exhibit defensive behaviour until there is no longer a threat, typically because the victim has either fled or been killed (*Pickett* et al. 1982, *Hunt* 2007). These pheromones do not dissipate or wash off quickly, and if their target enters water, the bees will resume their attack as soon as it leaves the water.

Beehives in our country (Iran) are transported to flowery areas (such as our province in north-western Iran) in the spring and summer seasons (between March and August) for good quality honey production. In this area, animals graze beside the beehives, but cases of bee attacks against the surrounding animals have not yet been reported (in our country). The reason for this report was the unexplained attack of Iranian honeybees (Apis Mellifera Meda) on two mules that were about ten metres away from the beehives. This distance between the hives and the mules should not stimulate the bees' defensive characteristics under normal conditions. On the other hand, comparison of the results of this report with other reports concerning other species (such as cattle and dogs) suggests that mules and dogs are much more sensitive to the stings of honeybees than cattle; this will be discussed later in the article.

According to the results of our research, it seems that this is the first worldwide report of anaphylaxis due to the stings of honeybees in mules.

Case Presentation

Two mules with a history of bee attacks and frequent bee stinging were examined in Haki, a village of Urmia, Iran, on Sep-

tember 2, 2012. Both mules showed anaphylaxis reactions due to the stings, and clinical findings included tachycardia (68 bpm), respiratory distress, severe oedema of the head and throat regions (Fig. 1), and haemoglobinuria (confirmed after centrifugation of urine and blood). The stinging sites, according to the frequency of the stings, included on the skin over the ribs, the fore and hind limbs, the face, and inside the mouth. There were about 100 stingers in the skin of each mule. One of the mules died three hours after the bee attack and before the start of treatment. The other mule received the following treatment: The remaining stingers were removed from the skin (only honey bees leave the stinger and poison gland intact; yellow jackets, wasps and hornets do not), and 5µg/kg epinephrine IV, 1 mg/kg dexamethasone IV (dosage in shock), 1 mg/kg triplenamine IM (antihistamine), and cold compresses on the skin were used. The swelling in the head and throat region decreased significantly one hour after beginning the treatment, but respiratory distress remained, therefore, 2cc/10 kg Bromhexine PO (a bronchodilator drug) was administered. Unfortunately, the mule died 12 hours after the beginning of treatment.

Fig. 1 Severe edema of the eyelid and muzzle in mule after honeybee stinging

Discussion

An important behavioural characteristic of social insects is the defence of the colony upon disturbance by intruders or potential enemies. Alarm pheromone communication among nest mates is critical for effectively organizing such a defence (*Breed* et al. 2004). The defensive behaviour in the honeybee Apis mellifera has been well studied (*Hunt* 2007). Typically, when guard bees are disturbed near the hive, they exhibit a characteristic behaviour in which they raise their abdomen, open the sting chamber and protrude the stinger. Sting pheromone, the most important pheromone in releasing bee defensive behaviour, is secreted by the sting sheath glands and the Koschewnikow gland (*Cassier* et al. 1994, *Lensky* et al. 1995), and it actually consists of more than 20 different components, primarily acetates and alcohols of low molecular weight (*Pickett* et al. 1982). The sting's injection of toxin into the victim is accompanied by the release of alarm pheromones, a process which is accelerated if the bee is fatally injured. Release of alarm pheromones near a hive or swarm may attract other bees to the location, where they will exhibit similar defensive behaviour until there is no longer a threat, typically because the victim has either fled or been killed (*Pickett* et al. 1982, *Hunt* 2007).

Honeybee venom

Honeybee poison is the specific biological product of bees and does not belong to the products originating from the world of plants. It contains apamin, melittin, phospholipase, histamine, and hyaluronidase, and groups which block the nervous system and stimulate the heart and the adrenal glands (*Baracchi* et al. 2011).

The basic composition of the venom, although it has been poorly characterized biochemically, includes a mixture of enzymes, low molecular weight polycationic peptides, biogenic amines, and proteins of high allergenic potential (*Barraviera* 1999, *Santos* et al. 2011). The primary components of the venom include melittin and phospholipase A2, which represent 50 to 75 % of the total venom mass (*Santos* et al. 2011). Apamin, a peptide that causes changes in neurotransmission, comprises only 2 % of the venom dry weight and affects the central and peripheral nervous system by blocking the transmission of some inhibitory impulses (*Cardoso* et al. 2003). Honeybee venom also contains a mast cell degranulating peptide that is responsible for the release of histamine, serotonin, arachidonic acid derivatives, and some factors that act on platelets and eosinophils (*Cardoso* et al. 2003). Histamine and hyaluronidase in the venom are responsible for the diffusion of the poison within the victim by decreasing blood pressure and increasing vascular permeability (*Barraviera* 1999). Melittin, a protein fraction that causes pain and inflammation, is responsible for the overall toxic effect of the venom by preventing the action of cholinesterase at neuromuscular and ganglionic synapses, causing respiratory paralysis and fibrinogen coagulation (*Habermann* 1954). Melittin contains the amino acids leucine, glycine, alanine, isoleucine, threonine, lysine, arginine, and glutamic acid.

The synergistic action of melittin with phospholipase A2 on phospholipids impairs cell and mitochondrial membrane integrity, alters oxidative phosphorylation, and causes tissue damage (*Cardoso* et al. 2003). Melittin causes the release of lecithin from red blood cells, which is transformed into lysolecithin through the action of phospholipase A2, causing hemolysis (*Habermann* 1954). Phospholipase A2 also acts on tissue respiration and prevents the action of dehydrogenases, in addition to inactivating thromboplastin (*Cardoso* et al. 2003). Furthermore, this enzyme induces the release of prostaglandins that modify vascular permeability, which can even result in anaphylaxis (*Piper* et al. 1969).

The allergenic factors in bee venom consist of hyaluronidases that are responsible for the hydrolysis of hyaluronic acid, in addition to lipases and phosphatases that act on the lysis processes that occur in different tissues and increase the severity of the injury (*Roodt* et al. 2005). The hydrolytic actions of these enzymes are complemented by the actions of other enzymes (lipase, phosphate and esterase) (*Barraviera* 1999). Additionally, the venom contains cardiopep, a non-toxic peptide that has a similar mode of action to that of beta-adrenergic drugs and has anti-arrhythmic properties (*Roodt* et al. 2005).

Honeybee poison effect stimulates cortisone (adrenal gland extract) production; it is the well-known and classic medicine for rheumatic pains and arthritis (*Park* et al. 2004).

Differences between species

Caldas et al. (2013) reported three cases of honeybee stings in cattle in Brazil. The clinical findings in these cases included erythema at the sting sites, marked swelling of subcutaneous tissue and the head, and necrosis of the skin seven days after the attack. These cattle exhibited the signs within the first 48 hours after the attacks.

Eduardo et al. (2007) in a retrospective study on bee stings in dogs, reported a high incidence of death among the dogs after bee stings. Nevertheless, all the dogs get stung by wild honeybees inside the city and the open environments, such as parks, and not by honeybees that are kept in hives for honey production. Since the composition of honeybee venom is similar in all breeds of honeybee, we used the results of this study in our discussion.

According to the comparison of the three cases which occurred in cattle, where each of them had 200 to 300 stingers in their skin, (*Caldas* et al. 2013) with our report and the report of *Eduardo* et al. (2007), it can be concluded that the mules and dogs exhibit clinical signs faster than cattle after being stung by honeybees. The time between being stung and the appearance of clinical signs was much shorter in mules and dogs (within a few minutes), but cattle exhibit the signs within 48 hours after being stung. The clinical signs were also more severe in mules and dogs than in cattle; the cattle did not show signs of anaphylaxis and the signs were only limited to the skin and subcutaneous tissue (Fig. 2), whereas the mules and dogs showed signs of anaphylaxis, including cardiovascular and respiratory failure, and, finally, died.

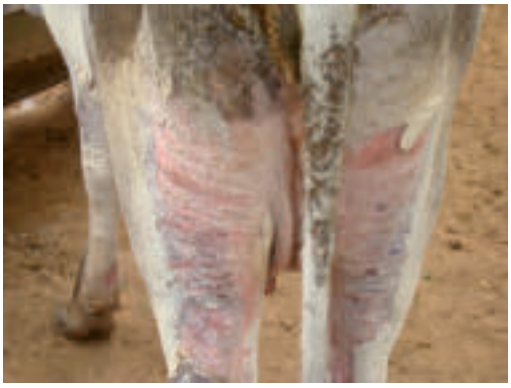

Fig. 2 Retrieved from *Andrade Caldas* et al. (2013): Necrosis and detachment of skin on the hind limbs due to stinging of honeybees (7 days after stinging)

The exact cause of this difference between species is not clear to us, but it could be due to the differences in the immunological pathways which deal with honeybee venom, similar to other venoms, and the response of the host's cells to chemical mediators that are released during anaphylaxis. However, further research is required in this area to test this hypothesis. Since mules and dogs are monogastric animals, it seems that such animals have little ability to deal with honeybee venom (in contrast to cattle), because they showed severe anaphylaxis and, eventually, died even with fewer stings than cattle (about 100 stings each in the mules discussed and fewer in dogs, and about 200 to 300 stings each in the cattle).

Review of the common treatment methods

Extraction of the stings is the most essential and important step in the treatment of a honeybee stinging attack (*Snodgrass* 1956). The longer the sting remains in the skin, the more poison passes into the victim's body. When a honeybee stings a person or animal (stinging causes the death of the honeybee within a couple of days), it instinctively attempts to fly away. However, the barb of the sting catches firmly in the skin and the stinging apparatus is torn off with both the poison glands and the poison sac, and the ganglion of the abdominal nerve chain, which ensures its automatic innervations and contraction outside the bee's body (*Ghulam* et al. 2000). As a result, venom continues to enter the body of the victim (*Snodgrass* 1956, *Ghulam* et al. 2000).Therefore, the sting should be pulled out of the skin as soon as possible.

The principal therapeutic agent is epinephrine, which is a potent sympathetic stimulant. Epinephrine should be administered intramuscularly (10 to 20 µg/kg, equivalent to 5 to 10 ml of 1:1000 dilution of epinephrine for a horse weighing 450 kg) if dyspnea or hypotension is mild. Epinephrine should not be administered subcutaneously because its potent vasoconstriction can lead to poor absorption and tissue necrosis. If dyspnea or hypotension is severe, epinephrine should be administered intravenously or endotracheally if no venous access is available (3 to 5 µg/kg or 1.5 to 2.25 ml of 1:1000 dilution of epinephrine for a horse weighing 450 kg). Epinephrine doses can be repeated every 15 to 20 minutes until the hypotension improves. The side-effects of epinephrine therapy are tachyarrhythmias and myocardial ischemia, which in themselves can be life-threatening (*Paul* et al. 2004).

Other therapies include the use of glucocorticoids to prevent the release of chemical mediators and combat them (because of their effects on the immune system) (*Pickett* et al. 1982). Antihistamines may have beneficial effects because the honeybee venom contains histamine (*Baracchi* 2011).

Bee venom is acidic as it contains the highly acidic peptide melittin, and these interventions are often recommended to neutralize the venom; however, neutralizing a sting is unlikely to be effective as the venom is injected under the skin and deep into the tissues where a topically applied alkali is unable to reach, and so neutralization is unlikely to occur (*Glaser* 2007).

Conclusion

Since the occurrence of anaphylaxis is extremely fast, and given the fact that there is no specific treatment for anaphylaxis even in humans, it seems that the most appropriate way to reduce the incidence of death due to honeybee stings is to prevent the stinging. In order to achieve this purpose, animals should be kept away from beehives, especially because animals are attracted to the beehives out of curiosity which will, eventually, stimulate the defensive behaviour of the honeybees.

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Anaphylaxie nach Stichen von Apis Mellifera Meda (Iranische Honigbiene) bei zwei Maultieren

Die Anaphylaxie ist eine ernste allergische Reaktion, die plötzlich auftritt und oft zum Tod führt. Das Gift der Honigbiene ist ein spezifisches biologisches Produkt der Bienen und zählt nicht zu den Produkten aus der Pflanzenwelt. Das Gift enthält u.a. Apamin, Melittin, Phospholipase, Histamine und Hyaluronidase sowie Wirkstoffe, die das Nervensystem blockieren und Herz und Nebennieren stimulieren. In Haki, einem Dorf in Urmia, Iran, wurden zwei Maultiere mit Anaphylaxie untersucht. Eines der Tiere starb noch vor Beginn einer Behandlung. Das zweite Tier wurde wie folgt therapiert: Noch an der Haut haftende Bienen wurden entfernt, Adrenalin, Dexamethason und ein Antihistaminikum wurden injiziert und die betroffenen Hautpartien mit kalten Kompressen behandelt. Auch das zweite Maultiuer starb 12 Stunden nach Beginn der Behandlung. In Übereinstimmung mit anderen Berichten kann festgestellt werden, dass Maultere und Hunde nach Bienenstichen sehr viel schneller klinische Symptome entwickeln als Rinder. Die Reaktionen sind auch wesentlich ausgeprägter. Der Grund für die unterschiedliche Reaktion ist unklar, kann aber in den unterschiedlichen immunologischen Wirkungsverläufen des Honigbienengifts begründet sein, ähnlich dem anderer Toxine und der Reaktion der Empfängerzellen auf die chemischen Mediatoren, die durch die Anaphylaxie freigesetzt werden. Zur Abklärung dieser Hypothese sind weitere Untersuchung notwendig.

Schlüsselwörter: Anaphylaxie / Honigbiene / Apis Mellifera Meda / Maukltier / Bienenstich / Toxiologie