NOTE *Toxicology*

An accidental fatal attack on domestic pigeons by honey bees in Bangladesh

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ABSTRACT. Fatalities among avian species due to multiple bee stings are rare. Sixteen pigeons on a farm in Bangladesh each suffered multiple bee stings. Ten of the pigeons died before treatment, 5 (4–11 stings) died within 12 hr after treatment, and 1 pigeon (only 3 stings) survived. Body temperature, heart rate, respiratory rate, hematocrit, hemoglobin, erythrocytes, thrombocytes, MCV, MCH and MCHC decreased significantly after the incident, but leucocytes, heterophils, basophils, eosinophils, monocytes, ALT, AST, LDH, CK, creatinine, BUN and UA increased markedly. Overall, the hematological and biochemical changes in the bee-stung pigeons were similar to those of mammals; however, avian species may be more sensitive to bee stings than mammals.
KEY WORDS: bee sting, biochemistry, fatal case, hematology, pigeons

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Bangladesh is well known to have some of the most venomous insect populations, especially among *Hymenoptera* species (e.g., bee, hornets), due to its geographical location, environmental characteristics, temperature range and humidity. Thus, bee stings commonly occur among mammals, including humans. Even though modern apiary practices that require the use of standard bee boxes to limit bee-sting exposure are strongly encouraged, the traditional Bangladeshi practice of honey foraging from wild hives persists [8].

Bee venom is an efficient and complex mixture of substances that allows bees to mount a defense against a wide range of predators, from other arthropods to complex vertebrates [9, 11]. The principal components of bee venom are phospholipase A_2, melittin, apamin, peptide 401, histamine, hyaluronidase, norepinephrine, dopamine and serotonin [3, 4, 7, 9, 11, 14]. In mammals, bee stings can produce a wide range of responses, from a local immune response to severe life-threatening systemic reactions that can trigger allergic, anaphylactic or toxic reactions involving skin necrosis, liver injury, cardiac damage, hypotension, shock, respiratory distress syndrome, dyspnoea, asphyxia, pancreatitis, bleeding, hemolysis, thrombocytopenia, rhabdomyolysis and renal failure [3, 4, 6, 7, 9, 11–14, 16, 19]. Consequently, its diverse range of pathophysiological effects on humans and livestock has major medical, veterinary and economic implications [4, 7, 8, 11–13, 17, 20].

Most experimental studies and clinical reports on the toxicity of bee venom have been reported in mammals, such as humans, mice, rats, dogs, cattle and horses [2–4, 7, 9, 11–14, 19–21]. Fatal case reports among pigeons following multiple bee stings are rare. To the best of our knowledge, there have been no previous reports of bee envenomation among pigeons or other avian species. Therefore, herein, we report our pathological, hematological and biochemical findings from a group of domestic pigeons that experienced multiple bee stings in Bangladesh.

Sixteen (9 adults of 8–12 months old and 7 squabs of 3–4 weeks old) semi-scavenging, farm-reared pigeons (*Columba livia*) were attacked by a swarm of honey bees (*Apis mellifera*) around 12 AM (midnight). The next morning, 10 (3 adults and 7 squabs) of the pigeons were dead. Within 6−8 hr after the incident, the remaining 6 live pigeons were taken to Upazila Veterinary Hospital in the Faridpur District of Bangladesh. We removed visible embedded stings from the pigeons and treated them with prednisolone sodium succinate (10 mg/kg, IM), diphenhydramine hydrochloride (5 mg/kg, IM) and sulphadimidine sodium (0.2 mg/kg, IM). Warmed (40°C) 2.5% dextrose saline (10 m*l*/kg, SC) and furosemide (2 mg/kg, PO) were also administered [4, 7, 11, 13, 18]. Before treatment, 4 m*l* of blood was collected from the alar vein of the affected pigeons [10]; additionally, we took the same volume of blood from uninjured age- and weight-matched normal pigeons as normal controls. Hematological changes were measured by MHX-2 hematology analyzer (MH Medical Co., Ltd., Jilin, China),

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Table 1. Changes in body weight, temperature, heart rate and respiratory rate in pigeons who received multiple bee stings

	Body weight (g)	Body temperature (c)	Heart rate (rate per min)	Respiratory rate (rate per min)
Normal	388 ± 7	40.4 ± 0.5	169.7 ± 2.6	39.5 ± 1.4
Attacked	384 ± 7	38.8 ± 0.4*	148.8 ± 2.6***	29.5 ± 1.1**

The data are reported as the mean ± SEM (n=6); *P<0.05, **P<0.01 and ***P<0.001; student's t-test or Mann-Whitney test versus normal pigeons.

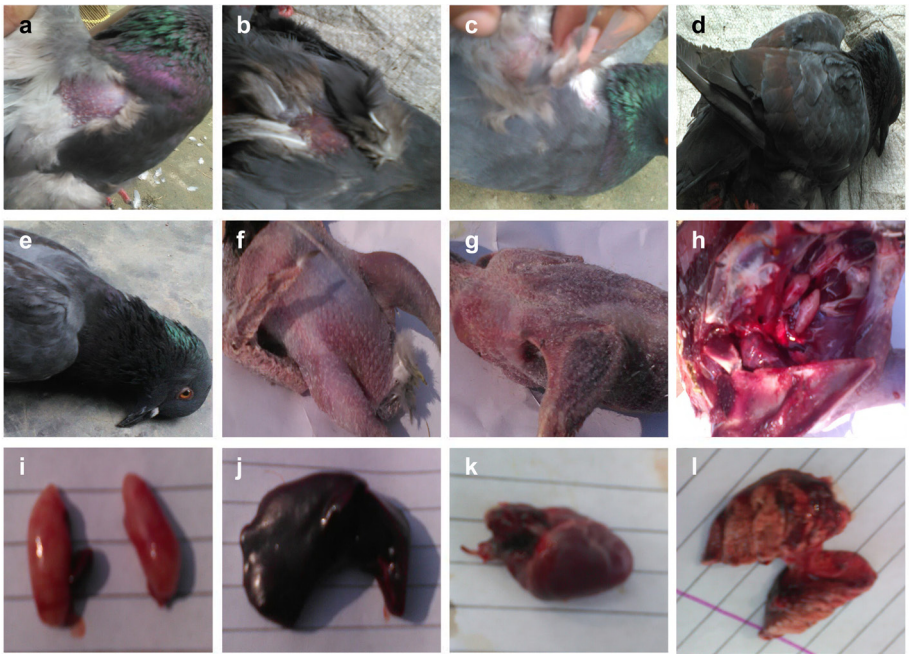

Fig. 1. Clinical signs and postmortem findings of pigeons who received multiple bee stings: (a, b, c) erythema and angioedema; (d) sternal recumbency; (e) cervical rigidity; (f, g) erythema, angioedema and congestion in postmortem; (h) pale discoloration of viscera in abdominal cavity; (i) swelling and congestion in kidney; (j) blackish discoloration and congestion in liver; (k) congestion in heart; (l) congestion in air sacs.

and biochemical differences were measured by Hitachi 911 chemistry analyzer (Hitachi, Tokyo, Japan).

The stung pigeons had significantly lower body temperatures, heart rates and respiratory rates compared to the control pigeons (Table 1). Their cloacal temperatures were taken as a measure of body temperature; respiratory rates were based on sternal movements; and heart rates were recorded using a stethoscope on the left costal area [5]. The stung pigeons showed sternal recumbency and cervical rigidity accompanied by palpebral edema, conjunctival congestion, massive edematous, congestive swelling and erythema around the stung areas (Fig. 1). Angioedema at the sting site is a common symptom in patients who have suffered a massive honey bee attack [4, 7, 11, 13, 14].

Several toxic mechanisms that induce edema formation were identified previously in mammals, including tissue damage (phospholipase A_2, melittin, etc.) and increased cellular and vascular permeability (mast cell degranulation peptides, hyaluronidase, etc.) [11, 14]. Apamin, a neurotoxic peptide found in bee venom, instigates powerful presynaptic inhibition of acetylcholine, which results in neuromuscular blockade of variable severity and progressive flaccid paralysis [2, 4]. Recumbency, bradycardia and bradypnea can result from the effects of envenomation on the vascular endothelium, peripheral nerve endings and myoneural junctions [1, 2]. Melittin, which causes pain, inflammation and systemic toxicity, prevents release of cholinesterase into the neuromuscular and ganglionic synapses, leading to respiratory paralysis and fibrinogen coagulation [4]. In this case, the bee-stung pigeons had abnormal harsh sound, muffled sound and rales in auscultation of the lung and heart.

The bee-stung pigeons had significantly lower levels of hematocrit (Hct), hemoglobin (Hb), red blood cells (RBC), thrombocytes, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) compared to the control

pigeons; the attacked pigeons also had significantly higher counts of white blood cells (WBC), heterophils, basophils, eosinophils and monocytes (Table 2). The toxicity of bee venom across diverse tissue targets causes an acute inflammatory response [2]. The most important allergen in honey bee venom is phospholipase A_2; it is a glycoprotein with 134 amino acid residues that acts as a cytotoxin and an indirect cytolysin. Phospholipase A_2 comprises 12–15% of the dry weight of bee venom. Hyaluronidase, another major allergen of honey bee venom, shares a 50% sequence identity with vespid venom hyaluronidase [3]. In addition to edema, a rapid release of inflammatory cells (neutrophils, macrophages, monocytes, eosinophils and basophils) and proinflammatory cytokines (IL-1b, TNF-a and IL-6) have been noted in individuals who suffered multiple bee stings [14, 19]. Any number of stings, which can cause a direct toxic reaction, can be associated with a unique hypersensitivity response and can cause a severe systemic allergic reaction [6]. Additionally, leukocytosis is often observed in victims of massive bee envenomation [2, 7, 11]. Eosinophilia and basophilia are the primary vehicles of hypersensitivity to envenomation [4, 6, 13]. Lower levels of RBC, Hb and Hct are indications of hemolysis [14]. Hematotoxin enzyme phospholipase A_2 directly affects the cell membrane and degrades lecithin into lysolecithin [4, 16, 21]. Melittin molecules are able to attach and create transient openings on the surface of red blood cells through which approximately 40 hemoglobin molecules can easily pass out, resulting in lower levels of packed cell volume [4, 16]. Similarly, hemolysis was also found in a dog which was aggressively attacked by bees [13, 21]. Thrombocytopenia may also occur in some cases of envenomation [11, 12].

Table 2. Hematological changes in pigeons who received multiple bee stings

	Normal	Attacked
RBC ($\times 10^6$/m*l*)	4.0 ± 0.1	3.1 ± 0.1**
Hct (%)	51 ± 1	40 ± 1***
Hb (g/100 m*l*)	13.3 ± 0.3	8.8 ± 0.2**
MCV (fl)	50.5 ± 1.0	46.5 ± 0.4*
MCH (pg)	68.8 ± 1.5	58.0 ± 1.7**
MCHC (mmol/l)	36.0 ± 0.5	28.7 ± 0.8***
WBC ($\times 10^3$/m*l*)	14.4 ± 0.3	20.7 ± 1.0**
Heterophils (%)	43.1 ± 1.1	51.0 ± 1.0***
Basophil (%)	0.4 ± 0.1	0.8 ± 0.1*
Eosinophil (%)	0.90 ± 0.13	2.05 ± 0.20**
Lymphocyte (%)	47.3 ± 0.8	63.5 ± 2.3***
Monocyte (%)	1.9 ± 0.2	3.3 ± 0.2**
Thrombocytes ($\times 10^9$/*l*)	38.6 ± 0.8	32.2 ± 1.2*

RBC, erythrocyte; Hct, hematocrit; Hb, hemoglobin; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; WBC, white blood cell. The data are reported as the mean ± SEM (n=6); *P<0.05, **P<0.01 and ***P<0.001; student's t-test or Mann-Whitney test versus normal pigeons.

In the attacked pigeons, there were significantly higher levels of creatinine kinase (CK), lactate dehydrogenase (LDH), aspartate aminotransferase (AST), alanine aminotransferase (ALT), uric acid (UA), creatinine (CRE) and blood urea nitrogen (BUN) compared to the control pigeons (Fig. 2); also, the ratio of AST to ALT was higher. The elevation of these enzymes in serum is a result of injury to the cell membranes of the muscle fibers (e.g. rhabdomyolysis produced by toxins from venom). When these cells are damaged, the cytosolic enzymes, including ALT, AST, LDH and CK, leak out into the circulating blood and exceed normal values [15]. Other studies have shown that, within a few hr of bee envenomation, serum ALT, AST and CK levels are significantly elevated in horses [11] and mice [14], indicating acute liver injury. The increases in serum AST and LDH levels might not only originate from the liver; they might also originate from damage to cardiac tissue, the lungs, skeletal or muscle tissue or red blood cells. Skeletal and muscle necrosis is implicated when there has been a prominent increase in serum CK levels [7, 14, 15]. Additionally, increases in serum CRE, BUN and UA levels are all indicative of kidney toxicity. In the pigeon cases, renal injury might be due to the direct toxic effects of venom and melittin, causing a decrease in the glomerular filtration rate and renal blood flow in response to vasoconstriction. Secondarily, the presence of a large amount of myoglobin and hemoglobin inside the kidney tubules may be due to destruction of the red blood cells and muscle tissue by melittin and phospholipase A_2 [11–13]. Histopathologic and radiographic evidence from many reports has indicated that bee venom toxicity results in necrosis and tissue damage in several organs, including the skin, kidneys, liver, heart and lungs [4, 7, 11, 14, 20].

In this case, 5 pigeons (average 7.8 stings; 4, 5, 9, 10 and 11 stings, respectively) died within 12 hr after treatment, and 1 pigeon (who only received 3 stings) survived. We found swelling, hemorrhage, congestion and discoloration of the skin, liver, kidneys, air sacs and heart in all affected pigeons (Fig. 1). These effects are consistent with common clinical observations, which appear to be major complications among bee-stung mammals, experienced with generalized hemolysis, rhabdomyolysis, development of acute multiorgan dysfunction and marked alterations in circulating blood parameters, resulting in the victim's eventual death [2, 7, 11, 13, 14]. Even though a single sting can potentially be fatal in a mammal probably due to anaphylaxis within 1–2 hr [6, 11, 17], the estimated mean lethal dose (LD_{50}) for most mammals is about 20 stings/kg due to venom toxicity [2, 11, 13]. Indeed, many victims with 15 stings/kg have survived with medical treatment, and it is recommended to keep patients with more than 50 stings/kg in hospital for at least 24 hr with close monitoring of laboratory and clinical parameters as there is still potential for systemic toxicity [7]. In this case, the lowest sting number of the dead birds was 4, and the average number having 100% probability of causing death (LD_{100}) was 7.8. The average body weight of the birds was 388 ± 7 g. Thus, the 10 stings/kg might lead to death of pigeon with intensive treatment, and the estimated LD_{100} of pigeons was 20 stings/kg.

In conclusion, the hematological and biochemical changes experienced by pigeons who received multiple bee stings

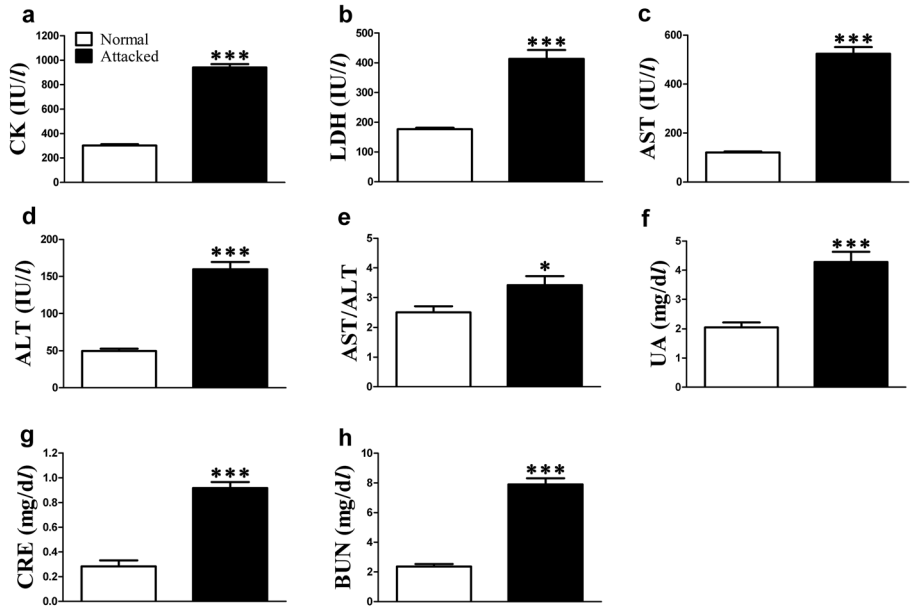

Fig. 2. Biochemical changes of pigeons who received multiple bee stings. (a) CK, creatinine kinase; (b) LDH, lactate dehydrogenase; (c) AST, aspartate aminotransferase; (d) ALT, alanine aminotransferase; (e) the ratio of AST per ALT; (f) UA, uric acid; (g) CRE, creatinine; (h) BUN, blood urea nitrogen. The data are reported as the mean ± SEM (n=6 for normal, n=6 for attacked pigeons); **P*<0.05, ***P*<0.01 and ****P*<0.001; student's *t*-test or Mann-Whitney test versus normal pigeons.

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