**Epidemiology and clinical effects of Hump-nosed pit viper (Genus: *Hypnale*) envenoming in Sri Lanka**

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**Key wards**: Envenoming, Sri Lanka, Hump-nosed viper, *Hypnale*, Snake bite

**Abstract**

Hump-nosed pit vipers of Genus *Hypnale* are the commonest cause of snake bite in Sri Lanka. Although there are many reports of local effects, coagulopathy and **acute kidney injury**, it remains unclear how frequent these clinical effects are and therefore the medical importance of this snake genus. The genus has been recently revised to include *H. hypnale* from Sri Lanka and Western Ghats of Southern India, and the two endemic species to Sri Lanka, *H. zara* and *H. nepa.* This was a prospective hospital-based clinical study of definite *Hypnale* spp. bites from July 2008 to July 2010 in six Sri Lankan hospitals. There were 114 patients included and all snakes were correctly identified by hospital staff as *Hypnale* spp. Of these, 93 snakes were identified as *H. hypnale* by an expert, 16 as *H. zara* and five as *H. nepa*. Most bites occurred on the lower limbs in the daytime. There was no difference in the clinical effects between the three species. Pain and fang marks were present in all patients, 101 had local swelling and only 16 (14%) developed extensive local swelling that spread proximally and involved more than half of the bitten limb. Systemic symptoms occurred in 18 patients; four patients had an abnormal 20 minute whole blood clotting test and one patient developed an acute **kidney injury** that required haemodialysis. All patients were discharged alive with a median length of stay of 2 days. This study confirms that hump-nosed viper bites cause only minor effects in most cases. Future studies need to undertake formal coagulation studies and identify important early indicators of renal impairment.

## 1. Introduction

Snake bite is an important medical problem in the tropics with large numbers of envenomings and deaths per year. Although the burden of snake bite is high, the epidemiology remains unclear due to poor reporting of cases (Kasturiratne et al., 2008). In Sri Lanka Hump-nosed pit vipers (HNV) are considered to be the commonest cause of snakebite (Kasturiratne et al., 2005; De Silva and Ranasinghe, 1983). However, the taxonomy of the HNV has been unclear until a recent revision. Anecdotal experience of clinicians suggest that most HNV bites only cause minor envenoming, but there are reports of more serious envenoming and most recently reports of **acute kidney injury** (Kularatne and Ratnatunga, 1999; Ariaratnam et al., 2008; Maduwage et al., 2011a; Herath et al., 2012, **de Silva et al., 1994**).

The *Hypnale* genus was recently revised to contain three nominal species viz: *Hypnale hypnale* (Fig: 1a), *H. nepa* (Fig: 1b) and *H. zara* (Fig 1c) and an un-named new species (*Hypnale* sp. “Amal”), described from a single specimen from Sri Lanka (Maduwage et al., 2009). *H. hypnale* is widespread throughout the lowlands (< 600 meters) of Sri Lanka but also occurs in the Western Ghats that border the west coast of the Indian peninsula. The other two species are restricted to Sri Lanka; *H. nepa* being restricted to the central highlands above ~1,250 m, and *H. zara* being restricted to the rainforests of the island’s south-western lowlands (Maduwage et al., 2009).

Although *H. hypnale* was one of the first snakes in which the pathophysiology of envenoming was studied, (Davy, 1821), the clinical effects **resulting from bites in** humans still remain poorly defined. Coagulopathy, **acute kidney injury** and severe local

effects have been documented following HNV bites in Sri Lanka. However, there is considerable variation in reported effects of *H. hypnale* bites. (de Silva, 1989; Sellahewa and Kumararatne, 1994; Kularatne and Ratnatunga, 1999; Seneviratne et al., 2000; Ariaratnam et al., 2008; Wijewantha and Sellahewa, 2010). The medical importance of *H. hypnale* was also not recognized in India until the first case series of fatal envenoming appeared in literature (Joseph et al., 2007). **There is limited information on the** two *Hypnale* species **endemic to Sri Lanka**. Recent work on the venoms of the three *Hypnale* species suggests that local effects are likely to be the most common and important effects, although the venom has a mild procoagulant effect. (Maduwage et al., 2011b).

The aim of this study is to investigate the clinical effects of envenoming by the three *Hypnale* species, to help determine the clinical importance of hump-nosed vipers.

## 2. Methods

A prospective hospital-based clinical study **of definite bites by *Hypnale* spp. (hump-nosed vipers)** was carried out from July 2008 to July 2010 in six Sri Lankan hospitals representing different climatic zones inhabited by the three species of *Hypnale* (Maduwage et al., 2009) [Figure 2].

All patients presenting following a suspected snakebite by a hump-nosed viper and who had brought the snake in were entered in the study. Patients were assessed on admission and then every 12 hours until discharge **according to the same protocol at all six hospitals.** Demographic information, details of the bite, clinical effects, laboratory investigations were recorded on a **clinical research form** by the treating doctor or one of the investigators (KM). **The clinical research form included pre-defined clinical information. Twenty minute** whole blood clotting test (WBCT20) was carried out in all patients (Warrell et al., 1977; Sano-Martins et al., 1994) **on admission and repeated in patients every six hours if it was abnormal.**

The severity of local pain was categorized as mild (only evident if patient **was** directly asked about pain), moderate (discomfort to the patient that is controlled by lying still, patient will either be asking for analgesia or happy to accept it if offered) and severe (sleep disturbance and calling for urgent pain relief) (Atkinson et al., 1993). **The presence of secondary infection was based on the treating medical team's diagnosis. Specific systemic effects included coagulopathy, myotoxicity, neurotoxicity and acute kidney injury. Non-specific systemic symptoms included nausea, vomiting,**

**headache, abdominal pain, diarrhoea and generalized diaphoresis which could not be attributed to a specific toxic effect.**

All snake specimens were labeled with patient's name and date of admission. They were provisionally identified in ward and later transported to University of Peradeniya for definitive identification by one of the authors (KM). Dead snakes were transported preserved in 10% formalin while live snakes were transported in special containers for identification and were later released to their natural habitats. All snakes were identified using standard identification keys (Maduwage et al., 2009). Identified specimens were deposited in the Department of Biochemistry, Faculty of Medicine, University of Peradeniya.

## 3. Results

There were 114 snake bite patients who presented with either a live or a dead specimen of a *Hypnale* snake. All snakes were correctly identified as *Hypnale* species by the hospital staff. Of **the 114 snake bite patients**, 93 (81%) were of *H. hypnale*, 16 (14%) of *H. zara* and 5 (4%) of *H. nepa* bites. The median age of patients with all *Hypnale* bites was 39 years (IQR: 20 to 48; Range 4 to 74) and 75 (66%) were male. Most bites occurred in the daytime. The majority of bites were on the lower limb 78 (68%), and the remainder on the upper limb excepting one bite on the upper back. There was no difference in patient characteristics and the effect of bites between the three species, **although there were small numbers of bites by *H. zara* and *H. nepa*.** Most of *H. hypnale* bites were in home gardens whilst all *H. zara* bites occurred in the forests. Most *H. nepa* bites occurred in tea estates.

Fang marks were present in all patients associated with local pain (mild 42%, moderate 46% and severe 12%). One hundred and one (89%) patients had swelling but only 16 (14%) developed extensive local swelling that spread proximally to involve more than half of the bitten limb. **There was no difference in the proportion of patients with swelling or the proportion with more extensive local injury, when comparing those who used a tourniquet or not.** Other local effects **are described in** Table 1.

**Non-specific systemic symptoms** occurred in 18 (16%) of the 114 cases (Table 1). Specific systemic envenoming features occurred only in patients with *H. hypnale* bites. There were four patients with an abnormal WBCT20 and a single case of acute **kidney**

**injury**. No patients had bleeding gums, haematemesis, haemoptysis or ptosis (Table 1). **No patients developed neurotoxicity and myotoxicity.**

Fifty seven percent of the patients received some form of first aid before their admission to hospital. The commonest first aid measures adopted were, washing the bite site and application of a tourniquet above the bite site. The majority of HNV bite victims were admitted to the hospitals within 2 hours of the bite.

All HNV bites were managed symptomatically without the administration of antivenom. The patient with acute **kidney injury** was managed with haemodialysis. Local complications required treatment in 16 patients including, **14 who had wound toilet, one who required finger amputation due to severe local necrosis, and one patient who had a lower leg fasciotomy for regional swelling and local necrosis of the bite site.** All victims recovered and were discharged from the hospital. The median length of hospital stay was 2 days (**Range: 2 to 10 days**).

## 4. Discussion

Development of local swelling and pain were the predominant local signs of the victims in this study. Severe systemic envenoming was present only among the victims of *H. hypnale*. However, non-specific symptoms and mild local envenoming **were** present among the victims of all three *Hypnale* species (Table 1). Hospital admissions of patients envenomed by the three species of HNV in this study **were comparable** with the geographical distribution of the each species as described in Maduwage et al., 2009 (Figure 2). **The study showed that doctors and hospital staff were able to correctly identify HNV to genus level.**

*H. hypnale* prefers more anthropogenic habitats (Maduwage et al., 2009), which may explain the reason for most bites occurring in home gardens during daytime. All the envenoming by *H. zara* in this study occurred in forests which is the most likely reason for the low number of patients envenomed by this species compared to *H. hypnale*. This is consistent with the natural history of the snake as described by Maduwage et al., (2009).

In this study HNV envenoming caused local effects in the majority of patients and less frequently coagulopathy and acute **kidney injury** (Wijewantha and Sellahewa, 2010). Unfortunately more comprehensive coagulation studies were not available to define the frequency and severity of coagulopathy in HNV bites, and further studies should include at least an INR. However, Ariaratnam et al., (2008) reported coagulopathy **as defined by a WBCT20** in 39% and acute **kidney injury** in 10% of the 302 cases of *Hypnale*

envenoming from Sri Lanka. The difference between this study and ours in the frequency of coagulopathy may be due to the use of the WBCT20 to indicate coagulopathy.

Ariaratnam et al., (2008) reported the development of chronic renal failure in 2 patients after *H. hypnale* envenoming. **We were not able to confirm this because follow up of patients after discharge from the ward was not possible in this study.** A recent report of 13 cases of acute kidney injury following a HNV envenoming reported that six developed chronic kidney disease within 1 year of envenoming and histological findings revealed the development of focal segmental glomerular sclerosis, cortical necrosis and interstitial nephritis (Herath et al., 2012). Maduwage et al., (2011a) described a case report of *H. zara* envenoming causing coagulopathy and acute kidney injury. **The mechanism of acute kidney injury in HNV envenoming remains unclear.**

De Silva, (1989) and Ariaratnam et al., (2008) reported two cases of *H. nepa* causing mild local pain and swelling **consistent with this study.** Although severe systemic envenoming has not been observed among the small number *H. nepa* and *H. zara* envenoming cases in this study, these two species **are still likely** to cause severe systemic envenoming **in a small proportion of cases as reported in *H. zara* by Maduwage et al.** (Maduwage et al., 2011a).

Maduwage et al., (2011b), revealed that the three *Hypnale* venoms had similar chromatographic profiles in reverse phase high performance liquid chromatography and SDS-PAGE results. All three *Hypnale* venoms had potent cytotoxicity, mild procoagulant activity, and weak neurotoxic, myotoxic and the phospholopase $A_2$ activity (Maduwage

et al., 2011b). **This is consistent with the clinical findings in this study with mainly local cytotoxic effects and coagulopathy in a small number of cases.**

**There were a number of limitations to the study. There were only a few cases of *H. nepa* and *H. zara* which reflects the frequency of bites by these less common species. This means that larger studies are required to better define severity and frequency of clinical effects in these two species, and whether they are similar to *H. hypnale.* Comprehensive laboratory testing is not routine in Sri Lanka for the management of snake envenoming, and specific tests such as creatinine and creatine kinase are only requested if clinically indicated and if available at the hospital. This lack of laboratory results in all patients and the fact that this study did not include additional blood collection from patients, limits our conclusions on coagulopathy, acute kidney injury and myotoxicity, which are dependent on laboratory investigations. Finally, interventions such as fasciotomy or surgical amputation were determined by the treating doctors and are simply reported in this study.**

This study confirms that HNV bites cause only minor effects in the majority of cases. However, it is important that all HNV bites are observed and have investigations performed to detect the presence of coagulopathy or renal impairment. Future studies will need to define the timing and type of coagulation tests that are required and identify important early indicators of acute **kidney injury**.

**Authors contributions:**

KM, IG, AS designed the study; KM, SB and SM carried out the clinical assessment & identified the species of snakes; KM & IG carried out the analysis and interpretation of

the data; KM, AS, IG and GI drafted the manuscript. All authors read and approved the final manuscript. KM, GI and IG are guarantors of the paper.

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**Ethical approval:** Ethics Committee of the Faculty of medicine, University of Peradeniya, Sri Lanka.

**Table**

**Table 1:** Clinical features in 114 patients with Hump-nosed viper bites.

| **Clinical features** | ***H. hypnale* cases** | ***H. zara* cases** | ***H. nepa* cases** |
|---|---|---|---|
| **Local effects** | | | |
| Local pain | 93 | 16 | 5 |
| Mild | 43 | 4 | 1 |
| Moderate | 39 | 10 | 3 |
| Severe | 11 | 2 | 1 |
| Local swelling | 80 | 16 | 5 |
| Swelling extend more than ½ of limb | 10 | 3 | 3 |
| Local bleeding (>10 minutes) | 5 | 1 | - |
| Fang marks | 93 | 16 | 5 |
| Blistering | 10 | 3 | - |
| Bruising | 21 | 4 | - |
| Lymph node enlargement | 10 | 2 | - |

| | | | |
|---|---|---|---|
| Local necrosis | 12 | 4 | - |
| Secondary infection at the site of bite | 1 | 1 | - |
| **Non specific systemic manifestations** | | | |
| Fever | 10 | 2 | 1 |
| Headache | 13 | 4 | 1 |
| Nausea | 13 | 4 | 1 |
| Vomiting | 9 | 3 | 1 |
| Abdominal pain | 5 | 1 | 1 |
| **Specific systemic manifestations** | | | |
| Haematuria | 1 | - | - |
| Positive WBCT20 | 4 | - | - |
| Acute kidney injury | 1 | - | - |

## Figure legends

**Figure 1:** (a) Merrem's hump-nosed pit viper (*Hypnale hypnale*), Peradeniya (Central province), female 386 mm in total length; (b) High land hump-nosed pit viper (*Hypnale nepa*), Agarapathana, (Central province), male 368 mm in total length; (c) *Hypnale zara*, Galaha (Central province), female 406 mm in total length. *H. hypnale* is distinguished from *H. zara* by having the snout tip not raised (versus distinctly elevated in *H. zara*); 3–5 minute scales between rostral scale and internasals (versus 10–19 minute scales forming a wart-like protuberance at the snout tip in *H. zara*); 5–15 heterogeneous scales on the internasal-prefrontal region (versus 18–39 in *H. zara*); 4–5 scales (versus 3 in *H. zara*) surrounding the maxillary pit; and possessing a lacunal scale which is lacking in *H. zara*. *H. hypnale* is distinguished from *H. nepa* by lacking (versus possessing in H. *nepa*) a wart-like protuberance at the tip of the snout; lacking scales (versus possessing 1–3 scales in *H. nepa*) between the post-foveal and 3rd supralabial; having the lower postocular, 1st lower temporal, 4th and 5th supralabials in contact, with no scale between them (versus separated by a rhomboid scale in *H. nepa*); having the costal scales keeled (versus smooth in *H. nepa*) in mid-dorsal region; possessing 141–158 (versus 122–134 in *H. nepa*) ventrals; and having the lobes of hemipenes smooth, lacking spines (versus hemipenial lobes with spines in *H. nepa*).

**Figure 2:** Distribution of *Hypnale hypnale* (circle), *H. zara* (square) and *H. nepa* (triangle) cases collected in this study.

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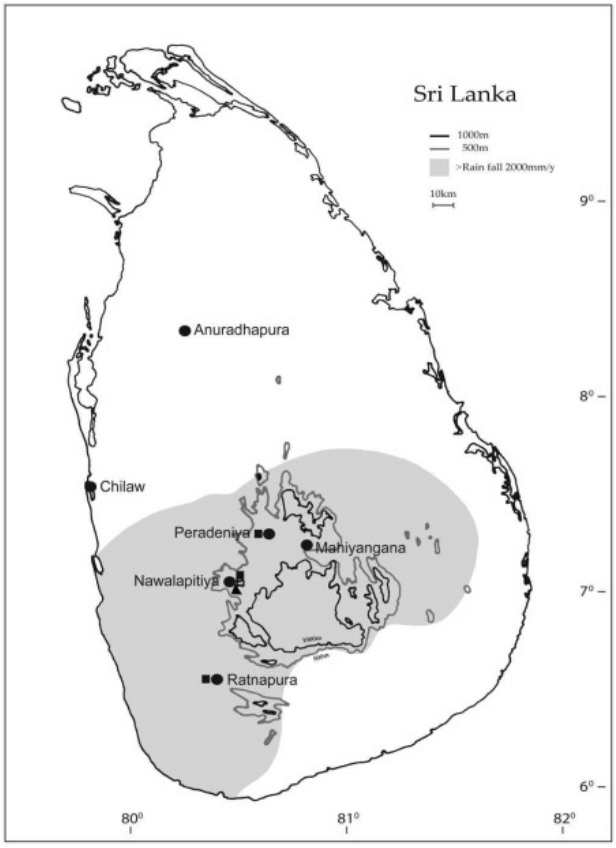
**Figure 1**

**Figure 2:**


Sri Lanka
1000m
500m
>Rain fall 2000mm/y
10km
Anuradhapura
Chilaw
Peradeniya
Mahiyangana
Nawalapitiya
Ratnapura
9°
8°
7°
6°
80°
81°
82°