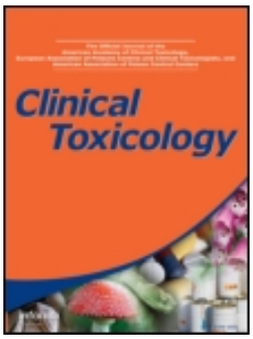

Clinical Toxicology

ISSN: 1556-3650 (Print) 1556-9519 (Online) Journal homepage: informahealthcare.com/journals/ictx20

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To cite this article: Fábio Bucaretchi, Sueli Moreira Mello, Ronan José Vieira, Ronei Luciano Mamoni, Maria HeloíSa Souza Lima Blotta, Edson Antunes & Stephen Hyslop (2008) Systemic envenomation caused by the wandering spider *Phoneutria nigriventer*, with quantification of circulating venom, Clinical Toxicology, 46:9, 885-889, DOI: 10.1080/15563650802258524

To link to this article: https://doi.org/10.1080/15563650802258524

Published online: 02 Dec 2008.

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Clinical Toxicology (2008) **46**, 885–889
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ISSN: 1556-3650 print / 1556-9519 online
DOI: 10.1080/15563650802258524

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CASE REPORT

Systemic envenomation caused by the wandering spider *Phoneutria nigriventer*, with quantification of circulating venom

FÁBIO BUCARETCHI[1], SUELI MOREIRA MELLO[2], RONAN JOSÉ VIEIRA[3], RONEI LUCIANO MAMONI[4], MARIA HELOÍSA SOUZA LIMA BLOTTA[4], EDSON ANTUNES[5], and STEPHEN HYSLOP[5]

[1]*Pediatrics, Faculty of Medical Sciences, State University of Campinas, Campinas, Brazil*
[2]*Poison Control Center, Faculty of Medical Sciences, State University of Campinas, Campinas, Brazil*
[3]*Clinical Medicine, Faculty of Medical Sciences, State University of Campinas, Campinas, Brazil*
[4]*Clinical Pathology, Faculty of Medical Sciences, State University of Campinas, Campinas, Brazil*
[5]*Pharmacology, Faculty of Medical Sciences, State University of Campinas, Campinas, Brazil*

Introduction. Bites by *Phoneutria* spp. spiders are common in Brazil, although only 0.5–1% result in severe envenomation, with most of these occurring in children. Cases of systemic envenomation in adults are very unusual, and no serum venom levels have been previously quantified in these cases. *Case report.* A 52-year-old man was bitten on the neck by an adult female *Phoneutria nigriventer*. Immediately after the bite, there was intense local pain followed by blurred vision, profuse sweating, tremors, and an episode of vomiting; 1–2 h post bite the patient showed agitation and a blood pressure of 200/130 mmHg, and was given captopril and meperidine. Upon admission to our service 4 h post bite (time zero – T0), his blood pressure was 130/80 mmHg with a heart rate of 150 beats/min, mild tachypnea, agitation, cold extremities, profuse sweating, generalized tremors, and priapism. The patient was treated with antivenom, local anesthetic, and fluid replacement. Most of the systemic manifestations disappeared within 1 h after antivenom. Laboratory blood analyses at T0, T1, T6, T24, and T48 detected circulating venom by ELISA only at T0, before antivenom infusion (47.5 ng/mL; cut-off, 17.1 ng/mL); his serum blood sugar was 163 mg/dL at T0. The patient was discharged on the second day with a normal arterial blood pressure and a follow-up evaluation revealed no sequelae. *Conclusion.* This is the first report of confirmed moderate/severe envenoming in an adult caused by *P. nigriventer* with the quantification of circulating venom.

Keywords ELISA; Envenoming; *Phoneutria nigriventer*; Priapism; Spider bite

Introduction

Spiders of the genus *Phoneutria*, popularly known as wandering or banana spiders, live in Central and South America (1,2). Most of the clinically important bites involving this genus occur in Brazil (1–5), where 2,687 cases were reported in 2006 (6). *Phoneutria* species are nocturnal, aggressive spiders that do not construct webs. When molested, *Phoneutria* spiders assume a very characteristic defensive posture and can jump up to 40 cm (1–3). *Phoneutria nigriventer*, which lives in central-western, southeastern, and southern Brazil, is responsible for most bites by this genus in humans (1–5), and its venom is the most studied of the six *Phoneutria* species identified in Brazil (5,7–19).

Most bites by *Phoneutria* spp. cause mild envenoming, with only 0.5–1% being severe, mainly in children (4,5). Despite the medical importance of this genus, there are very few detailed descriptions of systemic envenoming by *Phoneutria* spp. in adults (20,21). In this report, we describe a case of moderate/severe envenoming caused by a large female *P. nigriventer* spider following a bite in the neck region. We also provide a quantification of the serum venom level.

Case report

A previously healthy 52-year-old man with no history of arterial hypertension (last measured 4 months earlier) was cleaning a public garden when he leaned against a tree and was bitten on the right side of the neck by an 8-cm-long female *P. nigriventer* spider that was captured and brought for

Received 19 September 2007; accepted 9 June 2008.
Address correspondence to Fábio Bucaretchi, Faculty of Medical Sciences, State University of Campinas, Pediatrics and Poison Control Center, Centro de Controle de Intoxicações – HC/UNICAMP, Campinas, CEP 13081-970, Brazil. E-mail: bucaret@fcm.unicamp.br

Fig. 1. Female *P. nigriventer* responsible for the bite.

identification (Fig. 1). Immediately after the bite, there was intense, non-radiating pain followed by blurred yellow vision, profuse sweating, tremors, and an episode of vomiting.

At the first medical facility, 1–2 h after the bite, the patient showed agitation and a blood pressure of 200/130 mmHg, and was treated with sublingual captopril (25 mg) and intravenous meperidine (50 mg). Upon admission to our service 4 h after the bite (time 0 – T0), the blood pressure was 130/80 mmHg with a heart rate of 150 beats/min, mild tachypnea, agitation, cold extremities, profuse sweating, generalized tremors, priapism, and local erythema at the bite site. The case was classified as moderate/severe (3) and the patient received five vials of undiluted antiarachnid antivenom [Instituto Butantan, São Paulo, Brazil; $F(ab')_2$, 5 mL/vial, equine origin, 1 mL neutralizes 7.5 minimum lethal doses of *P. nigriventer* reference venom in guinea-pigs] infused intravenously over 15–20 min without pretreatment with antihistamines or corticosteroids; local anesthesia with 2% lidocaine (to control pain) and an intravenous infusion of Ringer lactate (500 mL) were also initiated. There were no early reactions to the antivenom and most of the systemic manifestations of envenomation disappeared within 1 h after antivenom; residual local pain was treated with one additional injection of lidocaine.

Blood analyses at T0, T1, T6, T24, and T48 revealed an elevated glucose level at T0 (163 mg/dL; reference value, 70–100 mg/dL), but with normal serum potassium, sodium, creatinine, and urea levels. Serum nitric oxide (NO), measured as nitrite using Griess reagent after the reduction of nitrate to nitrite (commercial kit; Cayman Chemical Co., Michigan, USA), was elevated at T0 (54.6 nmol/mL) but had decreased to 24.8 nmol/mL at T1, with no marked fluctuations thereafter. Circulating venom [measured by enzyme-linked immunosorbent assay (ELISA)] was detected only at T0 before antivenom infusion (47.5 ng/mL; the values for T1, T6, T24, and T48 were below the detection limit of 17.1 ng/mL of the assay). Arterial blood pressure was 130/80 mmHg at T0, with a slight elevation (160/90 mmHg) at T8 that normalized (120/80 mmHg) by the time the patient was discharged on the second day. A follow-up evaluation 3 days after discharge revealed a normal arterial blood pressure and no local or systemic sequelae.

Sandwich ELISA for the quantification of *P. nigriventer* venom

ELISA

The sandwich ELISA for the quantification of *P. nigriventer* venom was adapted from Chávez-Olórtegui et al. (22). *Phoneutria nigriventer* $F(ab')_2$ antibodies were purified from commercial arachnid antivenom (Instituto Butantan) by immunoaffinity chromatography using *P. nigriventer* venom immobilized on a CNBr–Sepharose column. Ninety-six-well plates (high protein binding; Corning, NY, USA) were coated overnight at 4°C with 100 µL of *P. nigriventer* $F(ab')_2$ antibodies (20 µg/mL in 0.1 M sodium carbonate, pH 9.6) and then washed three times with washing buffer (WB, 0.9% NaCl containing 0.05% Tween-20; Sigma, Uppsala, Sweden). After blocking with 2% bovine casein (Sigma) in phosphate-buffered saline (PBS) for 1 h at room temperature and washing again, the serum samples, standards, and controls (100 µL) diluted at 1:1 in incubation buffer [PBS, containing 0.05% Tween-20, 0.02% normal horse serum (Sigma), and 0.25% bovine casein] were added followed by incubation for 1 h at room temperature. The plates were subsequently washed with WB and incubated with a *P. nigriventer* $F(ab')_2$–peroxidase conjugate (1:250, in incubation buffer). After further washes, the plates were incubated with substrate (100 µL of 0.2 mg of *o*-phenylenediamine/mL and 0.05% H_2O_2 in 0.15 M citrate buffer, pH 5.0) for up to 15 min in the dark at room temperature. The reactions were stopped by adding 50 µL of 5% H_2SO_4 and the final absorbances were read at 492 nm in a Multiskan MS multiwell plate reader (Labsystems, Helsinki, Finland). The data were fitted by linear regression using GraphPad Prism software (GraphPad Inc., San Diego, CA, USA). The appropriate antibody concentrations used in this assay and the quality of the conjugate were selected and evaluated essentially as described elsewhere (23). In addition, normal horse serum (0.01%, final concentration) was used to minimize cross-reactivity of the antibodies with non-specific antigens (24).

Assay parameters

The background absorbance values and detection limit for the ELISA were determined using sera samples from 23 healthy adult donors with no previous history of spider bites (or bites/stings by other venomous animals) and who had not previously been treated with antivenom for such accidents. The donors were recruited from persons attending the university hospital where the patient was treated and were from the

same social (working) class as the patient. The sera were obtained after the patients had provided written consent and after approval by the Committee for Ethics in Human Research of the Faculty of Medical Sciences, UNICAMP. The absorbances of these samples ranged from 0.092 to 0.185 (mean ± SD = 0.116 ± 0.029).

The assay cut-off value was defined as the mean absorbance of the 23 donor sera plus two standard deviations [cut-off absorbance = 0.116 + (2 × 0.029) = 0.174], and the limit of detection (LOD) was the venom concentration that corresponded to this cut-off absorbance (LOD = 17.1 ng/mL) (25), as determined from a calibration curve with standards covering the concentration range of 0–100 ng/mL (0, 1, 2, 5, 10, 20, 50, 100 ng/mL, diluted in a pool of sera from healthy donors). At these concentrations, the standard curve was linear, with a high coefficient of determination (R^2 = 0.9919) (Fig. 2). For each ELISA, a complete (new) standard curve was run concomitantly to allow accurate sample quantification. All standards and samples were assayed in duplicate.

Quality controls (20, 40, and 80 ng/mL) were used to spike samples and to determine the assay coefficients of variation (CV). The accuracy and intra- and inter-assay precision were estimated at low (20 ng/mL, which was close to the LOD), medium (40 ng/mL), and high (80 ng/mL) concentrations of *P. nigriventer* venom diluted in a pool of sera from healthy donors. The intra-assay CV for each venom concentration was calculated from six determinations done simultaneously, and the inter-assay CV was calculated from three determinations done independently. The accuracy was calculated as the mean venom concentration that was detected with respect to the expected venom concentration, expressed as a percentage. As shown in Table 1, the assay showed high accuracy and good intra- and inter-assay precision (CV < 10%). The cross-reactivity of this ELISA with venoms from other arthropods, including the scorpion *Tityus serrulatus* and the spider *Loxosceles gaucho*, which, together with *P. nigriventer* venom, are used to produce the arachnid antivenom, was not examined here because the spider responsible for the bite was positively identified as *P. nigriventer*. The venom used in the standard curves was milked by electrical stimulation from the same spider that caused the accident.

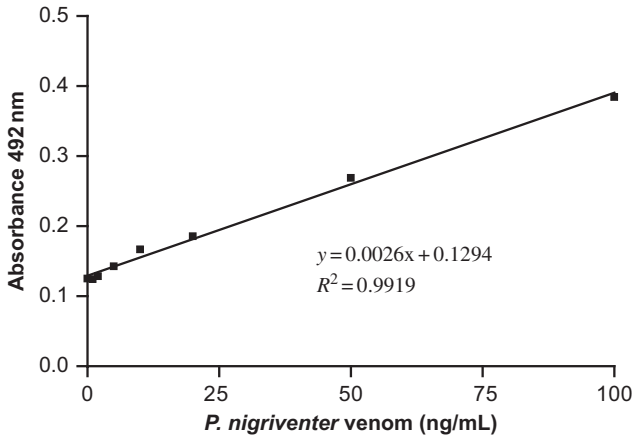

Fig. 2. Standard curve for the ELISA used to quantify *P. nigriventer* venom. The points represent the means of duplicate determinations that differed from each other by < 5% in each assay. Similar results were obtained in four additional assays.

Discussion

Phoneutria nigriventer bites on the head and neck are very unusual but potentially serious. The quick onset and the severity of the clinical manifestations seen in our case probably resulted from rapid venom absorption from the bite site, close to major neck vessels.

Intense local pain is the major symptom reported after most *P. nigriventer* bites (3–5) and was also observed in our case. Experimental studies have indicated that this hyperalgesia involves peripheral (tachykinin NK_1 and NK_2, and glutamate receptors) and central (spinal) mechanisms (neurokinins, excitatory amino acids, NO, proinflammatory cytokines, and prostanoids), all probably acting in synergism (16,17,19).

Peptides present in *P. nigriventer* venom cause vascular smooth muscle contracture and increased skin vascular permeability by activating the tissue kallikrein system and tachykinin NK_1 receptors (9–12). The intraperitoneal injection of *P. nigriventer* toxin T×2–5 in adult mice produces priapism, hypersalivation, and death by pulmonary edema and respiratory distress (18). These effects are abolished by pretreatment with 7-nitroindazole, a selective neuronal NO synthase inhibitor, indicating a role for NO in these responses (18). Increased NO formation and the resulting relaxation of corpus cavernosum smooth muscle (12,14,18) could possibly explain the long-lasting

Table 1. Accuracy and intra- and inter-assay variation for the ELISA used to quantify *P. nigriventer* venom

Venom concentration (ng/mL)		Accuracy	Intra-assay CV	Inter-assay CV
Theoretical	Measured	(%) (n = 6)	(%) (n = 6)	(%) (n = 3)
20	19.5 ± 0.9	97.5	4.1	4.7
40	41.1 ± 0.9	102.7	5.8	2.2
80	75.0 ± 1.6	93.7	2.9	2.1

Venom concentrations expressed as means ± SD (n = 6). CV, coefficient of variation.

priapism (1–2 h) seen in the present case. Although priapism has been described in children with systemic envenoming by *Phoneutria* spp. (4,5,26), this phenomenon has not previously been reported in adults.

The severe arterial hypertension recorded 1–2 h after the bite and that persisted up to T8 was consistent with studies in laboratory animals (13,15) (the normal blood pressure seen at T0 was probably related to the captopril administered at the first medical service). In anesthetized rats, the intravenous injection of *P. nigriventer* venom produces a biphasic response in arterial blood pressure characterized by short-lasting hypotension followed by sustained hypertension. The hemodynamic changes caused by *P. nigriventer* venom in rabbits involve central and peripheral components (13,15). The central component is mediated by the activation of cardiovascular centers that increase the sympathetic outflow to the periphery whereas the peripheral component involves either the direct activation of vascular α_1-adrenergic receptors or catecholamine release from sympathetic nerve endings (15). The tachycardia, profuse sweating, cold extremities, and transient hyperglycemia seen here suggest that the venom possibly increased sympathetic activity. However, we were unable to quantify the serum catecholamine levels to confirm this activation.

Antivenom has been used to treat envenoming by *Phoneutria* spp. in Brazil since 1925 (20). Current guidelines of the Brazilian Ministry of Health recommend that antivenom be given only to patients who develop important systemic clinical manifestations such as severe arterial hypertension, diaphoresis, convulsions, priapism, pulmonary edema, and shock (3); these manifestations occur in less than 3.3% of cases, including children (4,5). The clinical state of our patient improved rapidly following antivenom administration (within 1–2 h after infusion), in agreement with the outcome of early cases (20,21).

There are no clinical reports on the circulating levels of *P. nigriventer* venom, although Chávez-Olórtegui et al. (22) found levels of 11 and 26 ng/mL in two patients envenomed by *P. nigriventer*. However, these authors provided no clinical details of their patients and gave no indication of whether antivenom was administered in the two cases, so direct comparison with the findings for the case reported here is difficult. Using the ELISA described here, we have also measured a similar venom level (67.8 ng/mL) in a fatal case of a 4-year-old boy bitten by a *Phoneutria* spider in the southern Brazilian state of Santa Catarina (unpublished case attended at the State Poison Control Center of Santa Catarina, in Florianópolis, SC, Brazil). Although the venom level observed in our case was higher than those reported by Chávez-Olórtegui et al. (22), it was nevertheless compatible with that reported for other arachnids, particularly scorpions such as *Androctonus mauretanicus mauretanicus* and *Buthus occitanus* (venom levels 15.9–49.7 ng/mL) (27), *Androctonus australis* (venom levels 17–29 ng/mL) (28), and *Tityus serrulatus* (venom levels 4.0–50.0 ng/mL) (29).

No venom was detected by ELISA at various times after antivenom. It is unclear whether this lack of detection reflected the inability of the assay to detect very low venom concentrations after neutralization by antivenom (30) or the rapid clearance of the venom from the circulation (independently of the presence of antivenom). In mice, *P. nigriventer* venom levels are highest during the first 30 min following subcutaneous inoculation with no venom being detected beyond 4 h post injection (22).

Conclusion

Bites by *P. nigriventer* in the neck region can produce moderate/severe envenoming in adults. The early onset of clinical manifestations following such bites may be facilitated by the rapid absorption of venom in this region and result in circulating venom that can be quantified by ELISA. However, further studies, including a large prospective case series of patients bitten by these spiders, are needed to adequately assess the contribution of circulating venom, NO, catecholamines, and other mediators, such as cytokines, to the range and severity of clinical manifestations seen after envenoming by *Phoneutria* spp.

Acknowledgments

The authors thank Thomaz A. A. Rocha e Silva for photographing and milking the spider. E.A., M.H.S.L.B., and S.H. are supported by research fellowships from Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

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