



Predictors of *Bothrops jararaca* venom allergy in snake handlers and snake venom handlers[☆]

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Abstract

Since allergic sensitization to snake venom has been reported, anaphylactic reactions to snake venom might be an underestimated factor contributing to fatal snakebites, independently from the toxicity of the venom itself. However, little information is available on the determinants of such reaction. Hence, we studied a group of workers exposed to *Bothrops jararaca* venom (BJV), in order to clarify the factors related with snake venom allergy. The aim of this work was to investigate the prevalence and predictors of venom allergy among workers exposed to BJV and to confirm the involvement of IgE-mediated mechanisms in this condition. Workers exposed to BJV were assessed for venom allergy using questionnaires and immunological tests. The presence of BJV sensitization was determined through quantification of specific IgE. Allergens were studied using the Western blots and inhibition assays. Of the 67 workers evaluated, 7 (10.4%) presented specific IgE antibodies to BJV. Of those, 6 presented typical symptoms of an IgE-mediated allergic reaction when exposed to BJV. Venom sensitization was associated with length of employment ($P = 0.042$), high levels of total IgE ($P = 0.034$), atopy ($P = 0.051$), and specific tasks, primarily the handling of dried venom ($P = 0.014$). Our observations suggest that exposure to BJV can result in allergic sensitization in snake handlers through IgE-mediated mechanisms. The prevalence rate of this condition appears to be high among these workers, and the handling of dried venom, total IgE level above 100 kU/L, length of employment, and probably history of atopy were predictors of its occurrence.

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Keywords: *Bothrops jararaca*; Snake handler; Snake venom; Allergy; Immunoglobulin E.

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1. Introduction

Every year, 5 million envenoming by snakebites occur worldwide (Chippaux, 1998), with tens of thousands of deaths, particularly in tropical and subtropical countries, where they represent an important public health hazard (Gutiérrez et al., 2006). The morbidity and mortality from snakebites is secondary to direct systemic toxicity from the venom. However, since allergic sensitization to snake venom has been reported, anaphylactic reactions to snake venom might be an underestimated factor that may contribute to fatal snakebites in those endemic regions, independently from the toxicity of the venom itself (Parrish and Pollard, 1959; Schmutz and Stahel, 1985).

On the other hand, sporadic cases were reported in the medical literature describing allergic reactions to snake venom after recurrent exposure through bites (Lounsberry, 1934; Parrish and Pollard, 1959; Hogan and Dire, 1990; Ryan and Caravati, 1994; Alonso et al., 1995) and, presumably, through repeated inhalation of (or dermal/mucosal contact with) the venom (Stanic, 1956; Parrish and Pollard, 1959; Mendes et al., 1960; Ellis and Smith, 1965; Kelly and Patterson, 1973; Wade et al., 1987; Brooks and Graeme, 2004; Prescott and Potter, 2005), mainly among amateur and professional snake handlers.

To date, there have not been published studies evaluating the factors contributing to this condition in specific exposure settings, or on the patterns involved in the observed immunological responses. Hence, we studied a group of workers exposed to *Bothrops jararaca* venom (BJV), in order to clarify the factors related with snake venom allergy. We focused on this species because snakes of the genus *Bothrops* are responsible for the vast majority of snakebites in South America, with the species *B. jararaca* being associated with most human envenomations in the Southeastern region of Brazil (França et al., 2003).

The specific aim of this study was to evaluate the prevalence and predictors of allergy among workers exposed to BJV, and to confirm the involvement of IgE-mediated mechanisms in this condition.

2. Methods

2.1. Study design and population

A cross-sectional study of 70 adult laboratory workers, scientists, technicians, and trainees, all

employees of the Biology Museum, the Herpetology Laboratory, and the Production and Technological Development Division of the Butantan Institute, was carried out in November of 2005. Of the 70 subjects initially selected, 3 were excluded from analysis for being unable to participate due to their work obligations. Therefore, the study sample consisted of 67 subjects.

All of the subjects in the study sample had been exposed to snake venom or to the snakes themselves, most of which were of the genus *Bothrops*. All participating subjects gave written informed consent. The study protocol was approved by the Ethics in Research Committee of the *Hospital das Clínicas*, University of São Paulo School of Medicine, São Paulo, Brazil (Protocol No. 1039/03).

2.2. Questionnaire

All 67 subjects completed a physician-administered questionnaire containing questions regarding their personal history of allergy, snakes bites, and contact (oral or ocular) with snake venom, as well as their work history (length of employment and specific work tasks) and work-related symptoms.

Subjects were considered positive for a history of allergy if reporting any of the following symptoms: chest tightness; running nose/sneezing; itchy/watery eyes; and itchy skin due to common allergens such as dust, domestic animal dander or airborne fungi. Subjects were considered to have work-related allergic symptoms if they responded affirmatively to the question, “Do you have allergy during working hours, i.e. after contact with a specific snake or snake venom at work?”

The job of snake handler entails specific tasks, including snake cage cleaning, snake feeding, snake venom extraction, the handling of snake venom (liquid/dried), snake room cleaning, snake embalming, the capture/installation of snakes, snake-pit cleaning, and snake dissection. Estimates of the exposure times to snakes or snake venom were derived from the following questions: “How many days per week do you perform [the specific task]?”; and “For how many years have you been performing [the specific task]?” The frequency of exposure to each specific task was reported as a continuous variable, in days per year. To assess the influence of each task at different frequency levels, an exposure time index was calculated by multiplying the years of exposure by the frequency of exposure to each specific task.

2.3. IgE determination

The levels of specific immunoglobulin (Ig) E in serum samples were quantified using the ImmunoCAP[®] system of fluoroimmunoassay (Pharmacia Diagnostics AB, Uppsala, Sweden). The ImmunoCAP[®] is a completely automated immunoassay that consists of a reaction chamber containing a cellulose sponge matrix, which is a flexible hydrophilic polymer to which allergenic components are covalently coupled. Patient serum is added. Following incubation and washing, an enzyme-labeled anti-human IgE antibody is added. After a second incubation and washing, enzyme substrate is added, response is measured, and results are calculated and reported in kU/L (Dolen, 2003). The results were considered positive when binding values were >0.35 kU/L.

The atopic status of each individual was established through the quantification of specific IgE antibodies to a combination of common inhaled allergens: household dust (*Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, *Blomia tropicalis*, and *Blatella germanica*); airborne fungi (*Penicillium notatum*, *Cladosporium herbarum*, *Aspergillus fumigatus*, and *Alternaria alternata*); and animal allergens (cat and dog dander). For the quantification of BJV-specific IgE in the present study, a special ImmunoCAP[®] was developed and used according to the manufacturer specifications (MIAB, Uppsala, Sweden).

Total IgE was measured using the laser nephelometer method (Hamilton and Adkinson, 2003) and was regarded as positive when higher than 100 kU/L.

2.4. Atopy

Subjects were considered atopic if they reported a personal history of allergy and presented at least one positive specific IgE to common inhaled allergens quantified using the ImmunoCAP[®] system of fluoroimmunoassay (Pharmacia Diagnostics AB, Uppsala, Sweden).

2.5. Antigen

Lyophilized crude extract of BJV (Butantan Herpetology Laboratory, Butantan Institute, São Paulo, Brazil) was dissolved in phosphate-buffered saline (PBS), pH 7.4, and stored at -20°C until use.

2.6. IgG determination

Enzyme-linked immunosorbent assay (ELISA) was performed in flat-bottomed, 96-well microtiter plates (Nunc F Immunoplates I; Nunc, Roskilde, Denmark). The wells were coated with 100 μL of BJV (10 $\mu\text{g}/\text{mL}$) in sodium carbonate buffer, pH 9.6, according to the technique described by Theakston et al. (1977). Plates were incubated overnight in a humidified chamber at 4°C , after which they were washed three times with PBS-Tween, and the unbound sites were blocked with 200 μL of 3% bovine serum albumin (BSA) for 2 h in a humidified chamber at 37°C . Plates were washed once more and incubated with 100 μL of varying dilutions of serum (in PBS-Tween 1% BSA) for 1 h in a humidified chamber at 37°C . After an additional wash, wells were coated with 100 μL of peroxidase-conjugated goat anti-human IgG (Sigma Chemical Company, St. Louis, MO, USA) for 1 h. After a final wash, an additional 100 μL of peroxidase substrate (OPD) were added to each well, and the reaction was terminated with 50 μL of 30% sulfuric acid. Plates were read using an ELISA reader (Multiskan Spectrophotometer; EFLAB, Helsinki, Finland), and the titers were determined as the reciprocal of the highest dilution which resulted in an absorbance greater than 0.100 at 492 nm, nonspecific reactions being observed below this value.

2.7. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis and Western blots

The BJV proteins were analyzed using sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) with 4% stacking and 12.5% separating gels as described by Laemmli (1970). Prior to electrophoresis, the samples (20 μg each) were mixed with sample buffer (125 mM Tris, pH 6.8, 20% glycerol, 2.5% SDS, and 0.05% bromophenol blue). Pre-stained molecular weight markers (BioRad, Hercules, CA, USA) included myosin (205.73 kDa), β -galactosidase (133.08 kDa), BSA (83.91 kDa), carbonic anhydrase (41.56 kDa), soybean trypsin inhibitor (31.35 kDa), lysozyme (17.26 kDa), and aprotinin (7.01 kDa). One part of each gel was stained with Coomassie blue, and another was transferred to a nitrocellulose membrane at 385 mA for 2 h, as described by Towbin et al. (1979). These latter strips were blocked with 5% skimmed milk for 2 h at room temperature and were

washed in Tris-buffered saline. Subsequently, membrane strips were incubated with a 1:2 dilution of the serum sample for 2 h at room temperature with gentle agitation. Membranes were then washed with Tris-buffered saline and incubated with peroxidase-conjugated goat anti-human IgE (Sigma Chemical Company, St. Louis, MO, USA) for 2 h at room temperature. Color was developed with 0.05% 4-chloro-1-naphthol in 15% methanol, in the presence of 0.03% H₂O₂. The membranes were then washed with water to terminate the reaction. The serum from a volunteer who had not had any previous contact with snakes or snake venom was used as a negative control.

2.8. Inhibition studies

Inhibition assays were performed as described previously (Yunginger et al., 1983; Reimers et al., 2000). In brief, 75 µL of 1:4 dilution of the serum sample was incubated with 225 µL of serial 10-fold dilutions of either venom (BJV and *Crotalus durissus terrificus* venom) from 0.005 to 5000 µg/mL for 1 h at 37 °C. This was followed by an estimation of BJV-specific IgE using the ImmunoCAP[®] system. The inhibition percentage was calculated as follows:

$$\% \text{ inhibition} = (1 - E_s/E_o) \times 100,$$

where E_s is the extinction with the absorbed serum and E_o is the extinction with the unabsorbed serum.

2.9. Statistical analysis

To ascertain whether the values were distributed normally, the Kolmogorov–Smirnov test was used. Since at least one cell of the 2 × 2 contingency tables had an expected frequency of less than 5, Fisher's exact test was used to assess the association between health outcomes and the presence of BJV sensitization. Cramer's V was used as a measure of the degree of association. Analysis of variance (ANOVA) for independent samples was used to study the association between years of occupational exposure (log-transformed), as well as the sensitization to BJV. Correlations between the ImmunoCAP[®] results for specific IgE and the ELISA results for specific IgG were computed using the Spearman rank correlation (r_s). The Mann–Whitney U test for independent samples was used to study the association between different index of exposure to specific

tasks and sensitization to BJV. Values of $P < 0.05$ were considered statistically significant.

All statistical analyses were performed using the Statistical Package for Social Sciences for Windows (version 13.0, 2004; Statistical Products and Service Solutions Inc., Chicago, IL, USA).

3. Results

3.1. Subject characteristics

Of the 67 subjects evaluated, 37 (55.2%) were female. The mean age was 37.5 ± 12.3 years, and the median age was 38 years (interquartile range 26–46). Subjects reported having been exposed to *B. jararaca* snakes or their venom for periods ranging from 1 to 35 years. The median length of employment was 6 years (interquartile range 2–17). Of the 67 subjects interviewed, 24 (35.8%) were considered atopic, while 31 (46.3%) presented a total IgE level above 100 kU/L. Moreover, 12 subjects (17.9%) presented allergic symptoms when exposed to BJV.

3.2. Venom-sensitized group

Details regarding subjects sensitized to BJV are shown in Table 1. Of the 67 subjects, 7 (10.4%) presented elevated serum BJV-specific IgE levels, as quantified by ImmunoCAP[®], ranging from 0.4 to >100 kU/L. Of those 7 individuals, 6 presented BJV-related allergic symptoms and presented serum-specific IgG titers, as determined by ELISA, ranging from 80 to 1280. The remaining individual reported conjunctivitis associated with exposure to *C. durissus* pit viper venom. An association between positive BJV-specific IgE and BJV-related allergic symptoms was demonstrated using Fisher's exact test ($V = 0.60$, $P < 0.001$). In addition, a positive correlation was established between BJV-specific IgG and BJV-specific IgE ($r_s = 0.70$; $P < 0.001$).

3.3. Nonsensitized vs. sensitized subjects

Table 2 shows the comparison between subjects for whom the ImmunoCAP[®] results for BJV were negative (nonsensitized subjects) and those for whom those results were positive (sensitized subjects).

There was no significant association between gender and positivity for BJV-specific IgE ($V = 0.18$, $P = 0.229$). However, there was a positive association between positivity for BJV-specific IgE

Table 1

Workers sensitized to *Bothrops jararaca* venom: specific symptoms, specific IgG titers (ELISA), and specific IgE results (ImmunoCAP®)

Subject	<i>B. jararaca</i> IgE (kU/L)	<i>B. jararaca</i> IgG (titers ^a)	Allergic <i>B. jararaca</i> venom-related symptoms
1	0.85	160	None
4	12.4	320	Rhinitis
9	0.4	–	Rhinitis, urticaria
18	>100	320	Urticaria
47	8.85	80	Rhinoconjunctivitis, asthma, urticaria
50	>100	1280	Anaphylaxis
67	40.9	160	Anaphylaxis

Of 7 individuals with elevated serum BJV-specific IgE levels, 6 presented BJV-related allergic symptoms. The remaining individual (subject 1) reported conjunctivitis associated with exposure to *Crotalus durissus* pit viper venom.

^aTiter: reciprocal of the highest dilution resulting in an absorbance greater than 0.100 at 492 nm; (–) for values <40.

Table 2

Comparison between nonsensitized and sensitized subjects, as identified through the immunofluorometric assay (ImmunoCAP®) for *Bothrops jararaca* venom specific IgE

Variable	Nonsensitized to <i>B. jararaca</i> venom (n = 60)	Sensitized to <i>B.</i> <i>jararaca</i> venom (n = 7)
Years of employment ^a , median (interquartile range)	5 (2–16)	14 (9–30)
Gender (male/female), n (%)	25/35 (41.7/58.3)	5/2 (71.4/28.6)
Atopy ^b , n (%)	19 (31.6)	5 (71.4)
IgE > 100 kU/L, n (%)	25 (41.7)	6 (85.7)
Work-related symptoms, n (%)	6 (10.0)	6 (85.7)
Rhinitis, n (%)	5 (8.3)	5 (71.4)
Conjunctivitis, n (%)	4 (6.7)	3 (42.9)
Asthma, n (%)	1 (1.7)	3 (42.9)
Urticaria, n (%)	4 (6.7)	5 (71.4)
Snakebites ^c , n (%)	9 (15.0)	2 (28.6)
Mucosal contact with snake venom, n (%)	6 (10.0)	2 (28.6)

Of the 67 subjects evaluated, 7 presented elevated serum BJV-specific IgE levels, as quantified by ImmunoCAP®.

^aTotal working years with snakes or snakes venoms.

^bPersonal history of allergy and at least one positive ImmunoCAP® response to common inhaled allergens.

^cPersonal history of *Bothrops* snakebite.

and years of occupational exposure (log-transformed) using ANOVA for independent samples [$F(1.65) = 4.30$, $P = 0.042$]. In addition, a borderline signifi-

cant association between atopic status and positive BJV-specific IgE was observed ($V = 0.25$, $P = 0.051$). Nevertheless, there was a significant association between total IgE level above 100 kU/L, and positive BJV-specific IgE ($V = 0.27$, $P = 0.034$).

3.4. Work-related symptoms

Positivity for BJV-specific IgE was found to associate positively with rhinitis ($V = 0.54$, $P < 0.001$), urticaria ($V = 0.58$, $P < 0.001$), asthma symptoms ($V = 0.53$, $P = 0.003$), and conjunctivitis ($V = 0.36$, $P = 0.021$).

3.5. Snakebites and mucosal contact with snake venom

Of the 67 subjects, 11 (16.4%) had been bitten by snakes of the genus *Bothrops*, and eight (11.9%) had only limited prior contact (oral or ocular) with snake venom. However, positivity for BJV-specific IgE was not found to be associated with having suffered a *Bothrops* snakebite or with having a history of mucosal contact with BJV ($V = 0.11$, $P = 0.323$ and $V = 0.17$, $P = 0.193$, respectively). A borderline significant association between having suffered a *Bothrops* snakebite and positivity for BJV-specific IgG was observed ($V = 0.27$, $P = 0.051$).

3.6. Exposure index

The Mann–Whitney U test was used to determine whether any of various index of exposure to specific tasks were associated with positivity for BJV-specific IgE (Table 3). Certain specific tasks were found to associate positively with sensitization to BJV: snake cage cleaning ($P = 0.023$), snake venom extraction ($P = 0.049$), and the handling of dried snake venom ($P = 0.014$).

3.7. Allergen identification

To establish the IgE-binding patterns to allergens from BJV, SDS–PAGE was used to separate the proteins from the venom extracts, and Western blots were performed. The BJV proteins detected in the Western blots ranged from ≈ 30 to > 205 kDa. Fig. 1 shows the serum IgE-binding patterns of subjects 18 and 50 (both sensitized to BJV). Components of various molecular weights and IgE-binding patterns were detected in the BJV

Table 3

Associations between different index of exposure to specific tasks and positivity for *Bothrops jararaca* venom-specific IgE using the Mann–Whitney *U* test

Specific task	Index of exposure ^a (interquartile range)		Mann–Whitney <i>U</i> test		
	Sensitized ^b	Nonsensitized ^c	<i>U</i>	<i>Z</i>	<i>P</i>
Snake cage cleaning	1584 (1536–3120)	504 (96–1560)	45.5	2.27	0.023
Snake feeding	192 (132–1296)	96 (24–201)	55.0	1.82	0.068
Snake venom extraction	342 (58–1464)	14 (2–126)	32.0	1.96	0.049
Liquid snake venom handling	60 (48–624)	66 (12–432)	56.0	0.60	0.550
Dried snake venom handling	1104 (456–1616)	46.5 (12–360)	9.5	2.45	0.014
Snake room cleaning	1200 (768–3120)	240 (72–2760)	50.5	1.79	0.073
Snake embalming	12 (12–12)	168 (84–1152)	2.0	1.48	0.140
Capture of snakes	22.5 (14–31)	11 (4–32)	36.0	0.98	0.328
Reception of snakes	1056 (1056–1056)	288 (96–24500)	7.0	0.44	0.663
Snake-pit cleaning	576 (576–576)	144 (48–576)	2.0	0.88	0.378
Snake dissection	384 (384–384)	264 (96–1080)	3.0	0.39	0.697

^aExposure time index calculated by multiplying the years of exposure by the frequency of exposure to each specific task.

^{b,c}Subjects for whom the ImmunoCAP[®] results for BJV were positive (sensitized subjects) and those for whom those results were negative (nonsensitized subjects).

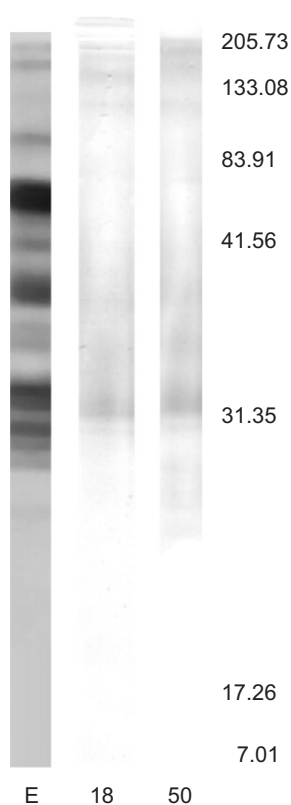


Fig. 1. Western blotting with *Bothrops jararaca* venom proteins for IgE binding with serum samples diluted 1:2 of the subjects 18 and 50 sensitized to *B. jararaca* snake venom; (E) SDS–PAGE (12.5%) of *B. jararaca* venom. Numbers to the left indicate the mobility of molecular mass markers. Components of various molecular weights and IgE-binding patterns were detected in the BJV extract. Strong IgE binding to an ≈ 32 kDa component was observed.

extract. Strong IgE binding to an ≈ 32 kDa component was observed. None of the negative control sera reacted to the BJV extract.

3.8. Inhibition studies

Two individuals (subjects 18 and 50) presented high levels of specific IgE to BJV (ImmunoCAP[®] > 100 kU/L), and inhibition studies were therefore performed. As shown in Fig. 2, inhibition of the ImmunoCAP[®] response was total with BJV and partial with *C. durissus terrificus* venom.

4. Discussion

Allergic sensitization to snake venom was first reported in 1930 (Zozaya and Stadelman, 1930). Since that time, venoms from all major snake families have been implicated in allergic reactions (Zozaya and Stadelman, 1930; Wade et al., 1987). Hypersensitivity to snake venom has been described after recurrent exposure through bites (Lounsberry, 1934; Parrish and Pollard, 1959; Hogan and Dire, 1990; Ryan and Caravati, 1994; Alonso et al., 1995) and, presumably, through repeated inhalation or dermal/mucosal contact with venom (Stanic, 1956; Parrish and Pollard, 1959; Mendes et al., 1960; Ellis and Smith, 1965; Kelly and Patterson, 1973; Wade et al., 1987; Brooks and Graeme, 2004; Prescott and Potter, 2005). Although this condition has been observed in amateur and professional snake handlers, little information is available on the prevalence

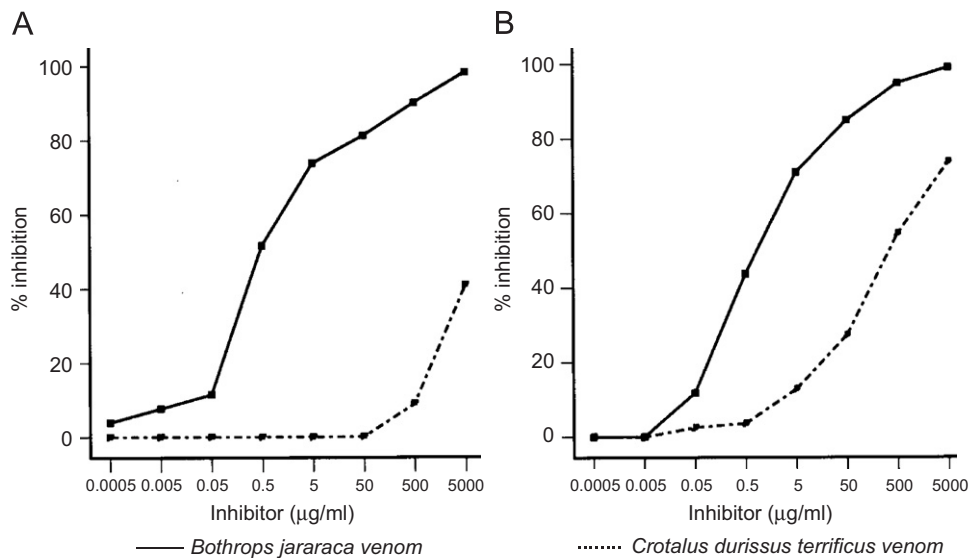


Fig. 2. Inhibition of immunofluorometric assay (ImmunoCAP®) specific to *Bothrops jararaca* venom by absorption of sera from subjects 18 (A) and 50 (B) with *Bothrops jararaca* venom and *Crotalus durissus terrificus* venom. Inhibition of the ImmunoCAP® response was total with BJV and partial with *Crotalus durissus terrificus* venom.

of the condition among such workers, its determinants, the extent of cross-reactivities between different snake venoms, and the molecular nature of these allergens.

In individuals sensitized to snake venom, the symptoms observed (urticaria, rhinoconjunctivitis, and asthma symptoms) can be triggered by minute doses of venom (Wadee et al., 1987). Systemic anaphylaxis has also been described, even in individuals not previously bitten (Prescott and Potter, 2005). Studies have provided evidence that these reactions are IgE-mediated, particularly when the exposure is recurrent (Kelly and Patterson, 1973; Wadee et al., 1987; Kopp et al., 1993; Alonso et al., 1995; Reimers et al., 2000).

Two criteria are employed to define snake venom hypersensitivity: a clinical history that definitively establishes a temporal association between contact with snake venom and an allergic reaction; and the detection of venom-specific IgE antibody through a 'confirmatory' skin test or serological assay. Although the use of a skin test has been described in the medical literature (Hogan and Dire, 1990; Kopp et al., 1993; Reimers et al., 2000), historically being considered the most sensitive and specific confirmatory test in clinical practice (Hamilton, 2004), no such test was used in the present study, due to the potential for anaphylaxis in response to an inherently toxic antigen not commonly used in clinical practice. The ImmunoCAP®

system was chosen because it is one of the most accurate specific IgE tests available and is considered the standard for serologic testing (Williams et al., 2000).

Since our investigation was a cross-sectional study, the prevalence of positivity for BJV-specific IgE (10.4%) might represent an underestimation of the actual risk, since workers with severe symptoms might have changed occupations.

The fact that 1 of the 7 sensitized workers presented allergic symptoms only when exposed to *C. durissus* venom suggests that, in addition to the common allergen responsible for the cross-reaction, there are antigens unique to each of the venoms, as observed by Mendes et al. (1960). Indeed, a positive association was found between BJV-related allergic symptoms and BJV-specific IgE detected through the immunofluorometric assay (ImmunoCAP® system) method ($P < 0.001$), indicating that ImmunoCAP®, a widely available and highly reproducible assay, is useful for the routine testing and monitoring of exposed individuals.

Sensitivity to BJV was found to be significantly associated with urticaria, rhinitis, conjunctivitis, and asthma symptoms, although the association with conjunctivitis was weak in comparison to the others. Some conjunctivitis-related symptoms were probably provoked by the local inflammatory effects of BJV (França et al., 2003) and not by IgE-mediated mechanisms.

Our results show an association between BJV sensitization and length of employment ($P = 0.042$). Therefore, total working years can be considered a simple indicator of the cumulative exposure level. Previous studies have shown that exposure level is a strong predictor of sensitization to other occupational allergens (Suarthana et al., 2005). Sensitivity to BJV was also found to be associated with high total IgE level ($P = 0.034$) and atopy ($P = 0.051$), both of which are considered predictive of sensitization to other occupational allergens, including high-molecular-weight proteins (Suarthana et al., 2005).

This is the first study that describe an association between venom sensitization and levels of exposure to snake venom. Sensitivity to BJV was significantly associated with snake cage cleaning ($P = 0.023$), snake venom extraction ($P = 0.049$), and, especially, the handling of dried snake venom ($P = 0.014$). These data are in agreement with those of case reports describing snake venom sensitization through contact with dried snake venom (Stanic, 1956; Parrish and Pollard, 1959; Mendes et al., 1960; Ellis and Smith, 1965; Kelly and Patterson, 1973; Wadee et al., 1987). Snakebites and mucosal contact with snake venom have been sporadically cited as risk factors for venom sensitization (Lounsberry, 1934; Parrish and Pollard, 1959; Hogan and Dire, 1990; Ryan and Caravati, 1994; Alonso et al., 1995; Reimers et al., 2000; Brooks and Graeme, 2004; Prescott and Potter, 2005), although no such associations were observed in the present study ($P = 0.323$ and 0.193 , respectively).

The application of ELISA has permitted reliable identification and sensitive quantification of venom antibody (IgG) in snakebite victims (Theakston et al., 1977; Theakston, 1983). Venom antibodies have been detected in the sera of patients within 1 week of envenomation. Antibody titers peak at approximately 1 year, drop to minimum levels after 3 years, and can still be detectable 40 years after the bite (Reid and Theakston, 1983). However, in the present study, only a borderline significant association between *Bothrops* snakebites and positivity for BJV-specific IgG was observed ($P = 0.051$). The high specific IgG levels might therefore be attributable to inhalation of or repeated mucosal contact with the venom. In the present study, BJV-IgG antibody was observed in 6 of the 7 BJV-sensitized workers, and the correlation was statistically significant. Other authors have also reported high specific IgG levels in snake venom sensitized

individuals (Wadee et al., 1987; Kopp et al., 1993; Alonso et al., 1995).

Although the ≈ 32 -kDa fraction of strongly bound IgE identified in two of the subjects might be the major allergen present in the BJV, further studies are needed in order to confirm this hypothesis.

We observed that BJV definitively inhibited the BJV-specific ImmunoCAP[®] response, thereby confirming the specificity of the positive ImmunoCAP[®] reaction, whereas the *C. durissus terrificus* venom produced partial inhibition. These findings suggest that there are allergens common to both venoms, although cross-reactivities to various snake venoms have been described. Mendes et al. (1960) observed that, among patients exposed to *Bothrops* or *Crotalus* venom, some presented positive reactions to intracutaneous or passive transfer tests involving venoms with which they had not had any previous contact, suggesting that the two venoms possess a common allergen. Alonso et al. (1995), using radioallergosorbent testing, also suggested the existence of common epitopes between the venoms of *Bothrops alternata* and *C. durissus terrificus*.

Although it is well documented that venom-specific immunotherapy is the only approach by which tolerance to *Hymenoptera* sting allergy can be achieved (Jutel et al., 2005), snake venom immunotherapy is not available to our knowledge. Therefore, management of occupational snake venom allergy involves preventive measures and medical surveillance. Levels of BJV-specific IgE can be readily quantified. Workers with a known allergy to snake venom should have a self-injectable epinephrine kit (Brooks and Graeme, 2004) and be trained in how to use it for emergency situations. Authors have been recommended that such individuals should have no further contact with snakes or snake venom (Kopp et al., 1993), but we believe that each case must be considered individually before taking this decision.

In conclusion, our results suggest that BJV is a potential source of allergens that can result in snake venom allergy among snake handlers and snake venom handlers. Apparently, the prevalence of this condition is high in this population, and IgE-mediated mechanisms are implicated in its development. The handling of dried venom, total IgE level above 100 kU/L, length of employment, and probably history of atopy were predictors of sensitization. In order to identify additional risk factors, further studies are warranted. Ideally, such studies should be prospective and focalized on the

development of specific IgE sensitization in snake handlers and snake venom handlers following exposure to venom.

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