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Major Article

Delayed healthcare and secondary infections following freshwater stingray injuries: risk factors for a poorly understood health issue in the Amazon

Jacqueline de Almeida Gonçalves Sachett[1],[2], *Vanderson Souza Sampaio*[1],[3], *Iran Mendonça Silva*[1],[2], *Akemi Shibuya*[4], *Fábio Francesconi Vale*[1],[5], *Fabiano Peixoto Costa*[2], *Pedro Pereira de Oliveira Pardal*[6], *Marcus Vinícius Guimarães Lacerda*[1],[2],[7] *and Wuelton Marcelo Monteiro*[1],[2]

[1]. Diretoria de Ensino e Pesquisa, Fundação de Medicina Tropical Doutor Heitor Vieira Dourado, Manaus, AM, Brasil.
[2]. Escola Superior de Ciências da Saúde, Universidade do Estado do Amazonas, Manaus, AM, Brasil.
[3]. Sala de Análise de Situação em Saúde, Fundação de Vigilância em Saúde do Amazonas, Manaus, AM, Brasil.
[4]. Coordenação de Biodiversidade, Instituto Nacional de Pesquisas da Amazônia, Manaus, AM, Brasil.
[5]. Faculdade de Medicina, Universidade Federal do Amazonas, Manaus, AM, Brasil.
[6]. Centro de Ciências da Saúde, Universidade Federal do Pará, Belém, PA, Brasil.
[7]. Instituto de Pesquisas Leônidas & Maria Deane, Fundação Oswaldo Cruz, Manaus, AM, Brasil.

Abstract

Introduction: This study aimed to describe the profile of freshwater stingray injuries in the State of Amazonas, Brazilian Amazon, and to identify the associated risk factors for secondary infections. **Methods**: This cross-sectional study used surveillance data from 2007 to 2014 to identify factors associated with secondary infections from stingray injuries. **Results**: A total of 476 freshwater stingray injuries were recorded, with an incidence rate of 1.7 cases/100,000 person/year. The majority of injuries were reported from rural areas (73.8%) and 26.1% were related to work activities. A total of 74.5% of patients received medical assistance within the first 3 hours of injury. Secondary infections and necrosis were observed in 8.9% and 3.8%, respectively. Work-related injuries [odds ratio (OR) 4.1, confidence interval (CI); 1.87-9.13] and >24 hours from a sting until receiving medical care (OR; 15.5, CI; 6.77-35.40) were independently associated with the risk of secondary bacterial infection. **Conclusions:** In this study, work-related injuries and >24 hours from being stung until receiving medical care were independently and significantly associated with the risk of secondary infection. The frequency of infection following sting injuries was 9%. The major factor associated with the risk of secondary bacterial infection was a time period of >24 hours from being stung until receiving medical care.

Keywords: Wound infection. Epidemiology. Risk factors. Venomous animals.

INTRODUCTION

Both traumatic and toxic components are involved in stingray injuries from marine or freshwater stingray punctures, and are common in coastal regions globally[1,2]. In Brazil, from 2007 to 2013, most of the 4,118 injuries from aquatic animals were due to stingrays (69%) in marine and freshwater environments[3]. Freshwater stingray injuries are common in the Brazilian Amazon, representing 88.4% of aquatic animal injuries reported from this region[3]. The number of cases detected officially in the Amazon is likely to be considerably lower than the actual number, as a result of underreporting, given the difficulties faced by riverine and indigenous populations living in remote areas to reach health centers. One survey from the State of Acre, in Brazil, found stingray injuries were common, with 18% of rubber tappers and 23% of Amerindians affected at least once in their lifetime[4]. Moreover, delays in patient care, along with the use of homemade remedies may impair healing of the wound site and lead to a high frequency of local complications, such as secondary bacterial infections[5,6]. To assess the health burden due to stingray injuries through population- and hospital-based field studies it is essential to understand the extent of complications following this poorly understood health problem[6].

In the Central and Western Brazilian Amazon, freshwater stingrays from the Potamotrygonidae family are more frequent, comprising four genera, *Potamotrygon*, *Paratrygon*, *Plesiotrygon,* and *Heliotrygon*, and 28 recognized species. Some species are widely distributed, such as *Paratrygon aiereba*,

Corresponding author: Dr[a] Jacqueline de Almeida Gonçalves Sachett.
e-mail: jacenfermagem@hotmail.com
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Potamotrygon motoro, *P. scobina* and *P. orbignyi*, and others more restricted, such as *Potamotrygon wallacei* and other endemic species[7-13]. The action of a stingray's venom is necrotizing, edematous, proteolytic, neurotoxic, and myotoxic[14-16]. Stingray injuries result in severe local signs and symptoms, causing considerable pain and can result in ulcers that may be eventually complicated with necrosis and bacterial infection[5,6,17-20]. In the Brazilian Amazon, while stingray injuries have been associated with bacterial infection and necrosis, the complication frequency rate is not well known. The most severe cases associated with secondary bacterial infections required antibacterial treatment and a prolonged recovery[20] with long-term disabilities[21], all associated with social and economic losses. Necrotizing fasciitis due to *Vibrio alginolyticus* (marine stingrays) and *Aeromonas hydrophila* (freshwater stingrays)[22,23], tetanus[24], and invasive mycoses[25] secondary to stingray injuries, as well as other severe infections, seem to be less common complications. Small sample sizes and the lack of a standardized clinical protocol for secondary infection definitions limit any precise estimate for these outcomes. Injuries resulting from Amazonian freshwater stingrays have also been reported in non-endemic countries, where these animals are kept as pets, and where physicians are less informed about the management of these types of injuries[26-30].

Improved knowledge of epidemiological aspects of freshwater stingray injuries may likely lead to improved case surveillance of this condition in remote localities in the Brazilian Amazon. The aim of this study was to describe the profile of freshwater stingray injuries reported in the State of Amazonas, in the Western Brazilian Amazon, and to identify potential risk factors for secondary bacterial infections.

METHODS

The State of Amazonas, located in the western Brazilian Amazon, is divided into 62 municipalities and comprises an area of 1,570,946.8km^2, with an estimated population of 3,807,921 inhabitants, of whom more than 25% live in rural areas. The capital, Manaus, comprises approximately 45% of the entire population. Vegetation cover mainly consists of a dense evergreen rain forest. The remaining vegetal cover is primarily composed of dense macrothermic ombrophilous forest. The climate, according to the Köppen classification, is Af (super-humid equatorial), with the rainy season occurring from November to April, with pluviometric precipitations above 2,000mm *per annum* and average temperatures ranging from 26°C to 30°C.

All freshwater stingray injuries in the State of Amazonas that had been reported to the Brazilian Notifiable Diseases Surveillance System [*Sistema de Informação de Agravos de Notificação* (SINAN)] between 2007 and 2014 were included in this study. Case reports were entered by healthcare providers, including primary, secondary, and tertiary care units at the time of case reporting, usually by the physician or nurse involved in managing the patient (mainly after discharge). The variables retrieved were signs and symptoms, sex, age (in years), anatomical region of the injury, area of occurrence (rural or urban), work-related injury (yes or no), schooling (in years of study), ethnic background, the time that had elapsed from the injury until the time of medical assistance (in hours), and outcome (discharge or death). To identify factors associated with secondary infections from stingray injuries, a cross-sectional study was used where development of secondary bacterial infection was classified as the dependent variable.

Data were aggregated according to the municipality and the year of occurrence to highlight changes in the epidemiological profile considering place and time. The mean incidence, calculated as a ratio of the number of cases and the population of each municipality multiplied by 100,000 was used for mapping. The software ArcMap 10.1 in ArcGIS 10.1 (ESRI, USA) was used for this analysis.

Monitoring of the database cleaning and analysis was undertaken using the estimates of internal validity (the extent of errors within the system, for example, coding errors) and completeness of data (underreporting of any surveillance variable). A check of both surveillance attributes was undertaken by two independent researchers prior to analysis to minimize a possible observer-expectancy effect during database handling. The non-parametric Spearman's correlation coefficient was used to assess the association between the absolute number of cases and the altimetric river levels. Information in regard to the altimetric river levels was provided from the hydrological information system of the National Water Agency [*Agência Nacional de Água* (ANA)][31]. Secondary bacterial infection rates were compared using a chi-square test (corrected using Fisher's test, if necessary), using individual characteristics from the SINAN database as independent variables. To avoid potential selection bias related to the high frequency of underreporting in the final database, only variables with at least 70% of completeness were considered for this analysis. The crude odds ratio (OR) with its respective 95% confidence interval (CI) was then determined. A backward-stepwise logistic regression was used for the multivariable analyses and the adjusted ORs with 95% CIs were also calculated. All variables associated with outcomes at a significance level of $p < 0.2$ in the univariate analysis were included in the multivariable analysis. A p-value < 0.05 was considered to be statistically significant using the Hosmer-Lemeshow goodness-of-fit test. Statistical analyses were performed using the Stata statistical package version 13 (Stata Corp. 2013).

Ethical considerations

This study was approved by the Ethics Review Board (ERB) (approval number 713.140/2014). Data were handled anonymously; therefore, the ERB waivered the informed consent process. The images presented in the manuscript derive from a prospective project held at the Health Surveillance Foundation of Amazonas, and were approved by the same ERB (approval number 713.140/2014).

RESULTS

A total of 476 freshwater stingray injuries were recorded in the State of Amazonas between 2007 and 2014, resulting in an incidence rate of 1.7 cases per 100,000 person/year.

Regarding seasonality, there was a higher incidence of cases between August and December (**Figure 1**). There was a negative correlation between the absolute number of cases and the altimetric river levels ($p < 0.001$, linear $R_s = -0.420$). Most of the stingray injuries occurred in males (392 patients; 82.4%). The most affected age groups involved patients between 11 and 20 years old (154 patients; 32.4%) and between 21 and 30 years old (80 patients; 16.8%). Regarding the area of occurrence, 73.8% were reported in rural areas. A total of 43.2% of the patients had up to 4 years of schooling. In terms of ethnicity, a mixed ethnicity was most frequently recorded (85.2%). Work activity-related injuries comprised 26.1% of reported injuries. Maintenance and repair services were the most cited formal occupations of patients (56.7%), followed by agricultural and forestry activities (39%). Most of the injuries occurred to the lower limbs (95.6%). Regarding time elapsed from the sting until receipt of medical assistance, 74.5% of the patients received treatment within the first three hours following injury. No deaths from freshwater stingray injuries had been recorded in the period studied. Aside from occupation, all other variables presented data completeness to at least 70% (**Table 1**).

Freshwater stingray injuries were unevenly distributed across the study area, with records obtained from 36 of the 62 (58.1%) municipalities of the state. The regions with the highest incidence rates were Alvarães (77.2 cases/100,000 inhabitants), Uarini (51.5/100,000 inhabitants) and Silves (20.4/100,000 inhabitants).

Table 2 presents local and systemic manifestations observed for freshwater stingray injuries. The most frequent local signs and symptoms observed were pain (99.1%), edema (65.3%), ecchymosis (15.6%), bleeding (12%), and erythema (2.9%). Secondary infections and necrosis were observed in 8.9% and 3.8% cases, respectively. **Figure 2A, Figure 2B, Figure 2C, Figure 2D, Figure 2E, Figure 2F, Figure G** and **Figure 2H** present some of the clinical cases with local manifestations recorded in the study area.

Table 3 summarizes the results of the univariate and multivariate logistic regression analysis evaluating factors associated with secondary infection. Work-related injuries (OR, 4.1; $p < 0.001$) and a time of >24 hours from being stung until receiving medical assistance (OR, 15.5; $p < 0.001$) were independently associated with the risk of secondary bacterial infection.

DISCUSSION

Few previous studies have quantified the burden of stingray injuries in the Brazilian Amazon[3,4]. This study shows that stingray injuries prevailed across all of the study area, with a higher incidence in males living in rural areas. We considered that the lower odds of riverine and indigenous populations reaching health centers most likely resulted in probable underreporting in the region. According to estimates concerning the rate of stingray injuries to people at least once in their lifetime, epidemiological surveillance in rural and indigenous areas would only have a sensitivity of approximately between 5% and 10% in the Western Amazon[4]. Thus, assessing the burden of stingray injuries using population- and hospital-

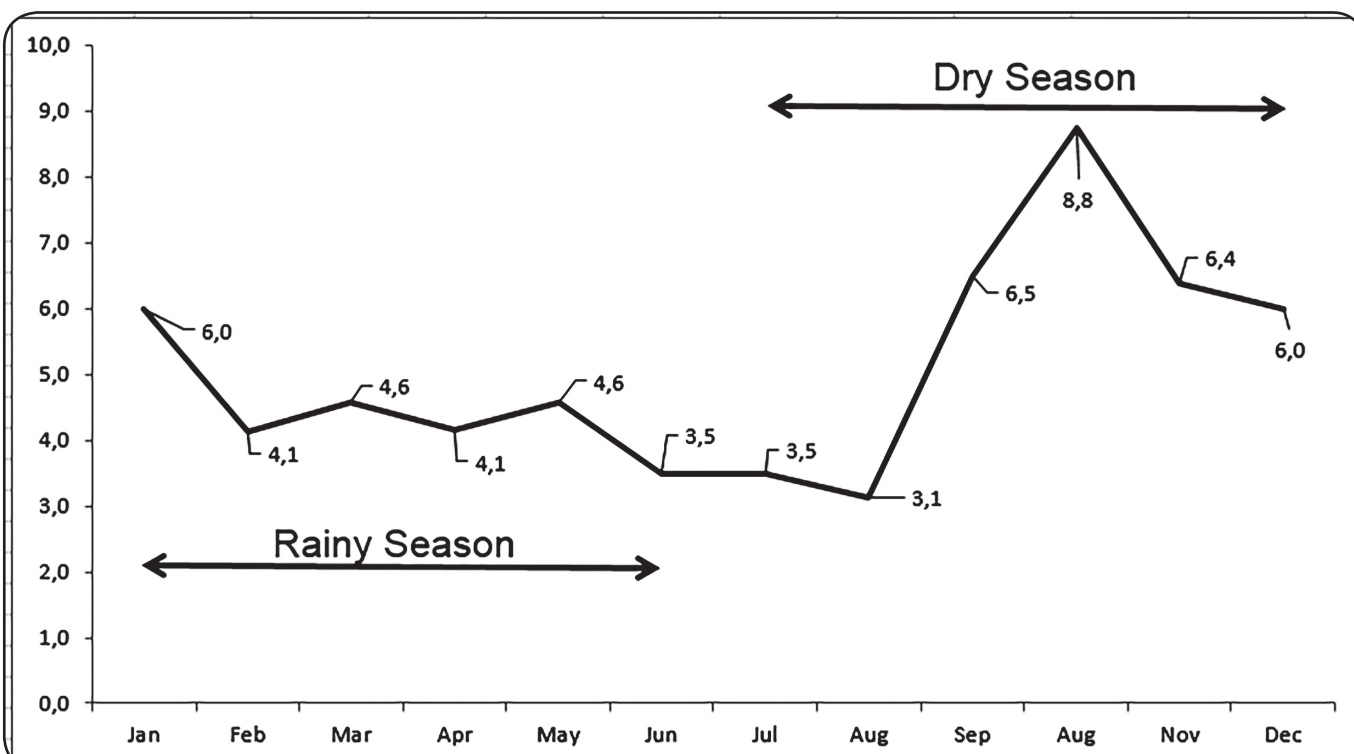

FIGURE 1: Seasonality of stingray injuries in the State of Amazonas, Brazil, between 2007 and 2014.

TABLE 1: Characteristics of the 476 incidents due to freshwater stingray recorded in the State of Amazonas, Brazil, between 2007 and 2014.

Characteristics (data completeness expressed as percentages)	Number	Percentage
Sex (n = 476; 100%)		
male	392	82.4
female	84	17.6
Age group (years; n = 476, 100%)		
0-10	52	10.9
11-20	154	32.4
21-30	80	16.8
31-40	72	15.1
41-50	58	12.2
51-60	41	8.6
>60	19	4.0
Area of occurrence (n = 466, 97.9%)		
rural	344	73.8
urban	122	26.2
Years spent at school (n = 333, 70.0%)		
illiterate	21	6.3
0-4	123	36.9
5-8	136	40.9
>8	53	15.9
Ethnicity (n = 467, 98.1%)		
mixed	398	85.2
European	32	6.9
Indian	27	5.8
African	7	1.5
Asian	3	0.6
Work-related accident (n = 452, 94.96%)		
yes	118	26.1
no	334	73.9
Occupation (n = 323, 67.9%)		
maintenance and repair services	183	56.7
farmer/fisher	126	39.0
trade and services employee	6	1.9
technician	5	1.5
industry employee	3	0.9
Anatomical region of the injury (n = 473; 99.4%)		
lower limb	452	95.6
upper limb	16	3.4
head	4	0.8
body	1	0.2
Time elapsed from sting to medical assistance (hours; n = 462, 97.06%)		
0-3	322	74.5
4-6	55	12.7
7-12	8	1.9
13-24	5	1.2
>24	42	9.7
Outcome (n = 452, 94.96%)		
discharged	452	100.0

TABLE 2: Local and systemic signs and symptoms of freshwater sting injuries reported in the State of Amazonas, Brazil, between 2007 and 2014.

Characteristics (n = 452, complete data, 94.3%)	Yes	Percentage
Acute local manifestations		
pain	445	99.1
edema	293	65.3
bleeding	57	12.0
Chronic local manifestations		
ecchymosis	70	15.6
secondary infection	40	8.9
necrosis	17	3.8
hyperemia	14	2.9
Systemic manifestations		
blurred vision/dizziness	18	4.0
vomiting/diarrhea	3	0.6
sudoresis	1	0.2
fever	1	0.2

FIGURE 2: Local complications of stingray injuries recorded in the study area. **A.** A 16-year-old male presented 12 hours following a stingray injury with a dilacerating, acutely painful, and bleeding injury located within the medial malleolar region of the left foot. **B.** Immediate first aid comprised hot water immersion (45°C) for a boy who presented 2-3 hours following a stingray injury with a dilacerating, edematous, and severely painful wound on the dorsolateral region of the right foot. **C.** A victim presented 2-3 hours following a stingray injury with a dilacerating, edematous, bleeding, and very painful wound located on the lateral aspect of the right foot near to the fifth toe. **D.** A dilacerating and painful stingray injury located on the dorsal aspect of the left foot is shown following irrigation, wound cleansing, surgical exploration, removal of tail fragments, and debridement. **E.** A 30-year-old male, admitted to the health service 12 days after a stingray injury, with an ulcerative, infected, and necrotic wound on the dorsolateral surface of the right foot. **F.** A 35-year-old male presented with secondary infection in the right foot 4 weeks following a stingray injury. Evidence of phlogosis interspersed with regions of ecchymosis was noted. **G.** Two weeks following a freshwater stingray injury located on the dorsal aspect of the right foot, dry necrosis is noted, most likely due to vasculitis associated with secondary bacterial infection, on the distal phalanx of the right hallux. Although different antimicrobial regimens were employed during different stages of the disease, the patient required an amputation of the right hallux. **H.** A female patient presented 3 months following a stingray injury with a poorly healing and necrotic wound located on the dorsolateral aspect of the right foot. The patient still complains of severe pain at the injury site.

TABLE 3: Factors associated with secondary infection due to freshwater sting injuries in the State of Amazonas, Brazil, between 2007 and 2014.

Variable	Secondary infection		Crude OR (CI 95%)	p-value	AOR (CI 95%)	p-value
	yes (%)	no (%)				
Sex						
male	37 (9.4)	355 (90.6)	Ref			
female	3 (3.6)	81 (96.4)	0.36 (0.11–1.18)	**0.091**	0.36 (0.08–1.64)	0.187
Age						
Area of occurrence						
urban	6 (4.9)	116 (95.1)	Ref			
rural	33 (9.6)	311 (90.4)	2.05 (0.84–5.02)	**0.116**		
Ethnicity						
white	2 (6.3)	30 (93.8)	Ref			
mixed	34 (8.5)	364 (91.5)	0.98 (0.39–2.43)	0.967	...	...
Indian	4 (14.8)	23 (85.2)	1.95 (0.64–5.95)	**0.240**	2.59 (0.63–10.69)	0.187
Black	0 (0.0)	7 (100.0)	...	...	...	...
Asian	0 (0.0)	3 (100.0)	...	...	...	...
Years spent in school						
illiterate	1 (4.8)	20 (95.2)	Ref			
0-4	15 (12.2)	108 (87.8)	1.94 (0.90–4.18)	**0.089**	1.39 (0.50–3.84)	0.525
5-8	10 (7.4)	126 (92.6)	0.74 (0.33–1.65)	0.467	...	...
>8	3 (1.0)	304 (99.0)	0.59 (0.17–2.01)	0.396	...	...
Work-related accident						
no	18 (5.4)	316 (94.6)	Ref			
yes	21 (17.8)	97 (82.2)	3.80 (1.95–7.42)	**<0.001**	4.13 (1.87–9.13)	**<0.001**
Anatomical region of the injury						
lower limb	36 (8.0)	416 (92.0)	Ref			
head	2 (50.0)	2 (50.0)	11.34(1.55–82.79)	**0.017**	11.64(0.46–292.81)	0.136
upper limb	2 (12.5)	14 (87.5)	1.57 (0.35–7.19)	0.558	...	...
body	0 (0.0)	1 (100.0)	...	...	...	...
Time elapsed from sting to medical assistance (hours)						
0-3	10 (3.1)	312 (96.9)	Ref			
4-6	5 (9.1)	50 (90.9)	1.12 (0.42–3.00)	0.828	...	...
7-12	2 (25.0)	6 (75.0)	3.82 (0.74–19.68)	**0.109**	4.17 (0.31–56.44)	0.283
13-24	0 (0.0)	5 (100.0)	...	...	...	...
>24	19 (45.2)	23 (54.8)	**18.13** (8.32–39.47)	**<0.001**	**15.48** (6.77–35.40)	**<0.001**

OR: odds ratio; **CI:** confidence interval; **AOR:** adjusted odds ratio.

based field studies in remote areas highlights a large gap in the epidemiology of stingray injuries. Stingray injuries occurred mostly in the younger study population (between 11 and 30 years of age), with a marked seasonality that suggested leisure activities, commonly undertaken in the dry season on beaches that appear along the river banks, were a risk factor for stingray injuries[31]. Our results showed that 26.1% of the stingray injuries were classified as work-related stingray injuries; therefore, there is a need for public health strategies that aim to reduce the incidence of these injuries, especially for rural workers[19,20,31].

Although no deaths or permanent disabilities from freshwater stingray injuries were recorded in the period, severe pain symptoms were commonly observed. Stingray injury victims frequently reported intense pain that seemed out of proportion to the injury[1,32,33]. Moreover, it has been reported that the intensity of such severe pain has led to disorientation in the injured victim[34]. Edema, ecchymosis, and local bleeding were also recorded in this case series, consistent with reported literature[33]. A comparative morphological analysis of the epidermal tissue of the stinger in different marine and freshwater

Brazilian stingrays indicates that, in freshwater species, there is a larger number of protein secretory cells spread over the whole epidermal layer of the stinger while, in marine species, the protein secretory cells are located only around or inside the stinger's ventrolateral grooves[35]. These differences between the stingers of the two groups help explain the more severe complications following accidents with the freshwater species than with the marine species, especially necrosis[19]. Most patient injuries occurred on the lower leg or foot. When a stingray is inadvertently disturbed or stepped on, it reflexively swings its barbed tail upwards, and can inflict deep puncture wounds[19,36,37]. No information regarding the treatment provided to injured patients was available from the official databases. For stingray injuries, immediate first aid treatment involves hot water immersion (45°C) for up to 90 minutes because the venom has a thermolabile nature and, because of the vasodilatory effects of immersion in hot water, symptomatic relief is provided while the limb is immersed. However, pain often returns once the limb is removed from the hot water[19,35,36,37]. It has been suggested that, in the absence of evidence from controlled clinical trials, when hot water immersion is insufficient in terms of pain relief, oral analgesia and titrated intravenous opioids should be administered while arranging local anesthesia at the wound site or administering a regional nerve block[33]. All penetrating injuries require irrigation and cleansing, and larger wounds or those containing debris require surgical exploration to extract any remaining imbedded tail fragments as well as wound debridement[19,33]. In general, our findings showed that systemic signs and symptoms were benign, a finding similar to previous studies[5,38].

Penetrating stingray wounds may present with delayed healing and secondary infection, with these wounds having worsened due to the dermonecrotic effects of the venom[33,38]. Secondary infection has been reported to be the most important complication of stingray injuries[19,33,36,37], and occurred in 9% of the patients in this study. Given the size, penetrating nature, and slow healing of stingray injuries, there is a significant risk for secondary infection[39]. One study considered secondary infections were more likely to occur in unclean and larger wounds[33], but risk factors for secondary infections are poorly understood. In this study, work-related injuries and a time >24 hours from being stung until obtaining medical assistance were independently and significantly associated with the risk of secondary infection. This association between work-related injuries and secondary infection prevalence was not expected. Since reporting of work-related injuries in Brazil is compulsory, stingray injuries, especially those that progress to complications such as secondary infection, are more likely to be reported than accidents occurring within other groups, and reporting bias may have elevated this association. However, specific hygiene behaviors, including the use of homemade medicines and environmental workplace exposures predisposing to secondary infection, cannot be discarded[21,22,40].

In this study, the rate of secondary infection was 9%. Estimates of secondary infection rates range from rare cases in one observational study[33]. Delayed patient care at a health unit was found to increase the risk of secondary infection up to 15 times in the Brazilian Amazon region. In this region, poor access to health centers is a reality due to long distances and a lack of transportation in remote areas. The implementation of a rapid transport system for stingray injury patients, integrated with providing assistance for other health issues, is likely to significantly reduce morbidity and probable disability rates related to secondary infections. Immediate first aid for stingray injuries is simple, involving hot water immersion and wound cleansing, and this information should be incorporated in travel medicine and occupational health programs within endemic areas.

Medical management of secondary infection from stingray injuries remains controversial, partly because of the limited number of evidence-based management protocols available[6,33,38]. Bacterial strains and their antimicrobial resistance profile in general are not determined for stingray wounds in the Amazon, particularly in remote settings, and the treatment for secondary infection is generally empirical. The most common agents in *P. motoro* stingray mucus are Gram-negative rods, namely *Aeromonas* spp., including B-lactam-resistant bacterial strains with the potential to cause severe secondary infection in wounds acquired during stingray envenoming[40,41]. Trimethoprim/sulfamethoxazole, ciprofloxacin, or tetracycline have been suggested for the treatment of fish wound infections[36]. Treatment failure has previously been reported in Central Brazil for ciprofloxacin[21] and in California, United States, regarding trimethoprim/sulfamethoxazole[42] for patients presenting with stingray wound infections.

In this study, analysis was limited to the existing fields of the SINAN reporting forms. This may have impaired the identification of risk factors for secondary bacterial infections. Information on first aid and management post-injury were not evaluated in this study as this information was not available from SINAN. The heterogeneity of the observers and the incomplete data concerning the natural history of the patients must be considered as limitations when analyzing these data. We highlight that estimates of independent risk factors for complications, using a more complete set of independent variables, should be assessed in future multicentered prospective studies. Moreover, the nature of the surveillance system may have influenced record keeping, for example, patients in remote areas with mild stings may not have reported their injuries to health services. However, the broad population coverage of the official surveillance and the low cost for data collection allowed us to obtain valuable information for the primary care health system due to the large sample size available for analysis. Furthermore, our results indicate a sufficiently strong association to suggest a likely causal connection.

In conclusion, our study showed a wide distribution of stingray injuries, with a higher incidence in males residing in rural areas. The frequency rate for secondary infections following stingray injuries was 9%. If the time from being stung until receiving medical care was >24 hours, this delay in treatment was the major factor associated with the risk of secondary bacterial infection. The possibility of reducing local effects and sequelae through the use of early antibiotic therapy for secondary infection, anti-inflammatory medication, and complementary treatments needs to be further investigated in line with good clinical practice. Cooperative efforts towards the control of this poorly recognized health problem through research and surveillance partnerships, particularly in the Amazon region, are imperative. Moreover, training multidisciplinary teams in stingray injury management, case monitoring, and surveillance is needed in Amazonian health services.

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Conflict of interest

The authors declare that there is no conflict of interest.

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