Older Age and Time to Medical Assistance Are Associated with Severity and Mortality of Snakebites in the Brazilian Amazon: A Case-Control Study

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Abstract

The Amazon region reports the highest incidence of snakebite envenomings in Brazil. We aimed to describe the epidemiology of snakebites in the state of Amazonas and to investigate factors associated with disease severity and lethality. We used a nested case-control study, in order to identify factors associated with snakebite severity and mortality using official Brazilian reporting systems, from 2007 to 2012. Patients evolving to severity or death were considered cases and those with non-severe bites were included in the control group. During the study period, 9,191 snakebites were recorded, resulting in an incidence rate of 52.8 cases per 100,000 person/years. Snakebites mostly occurred in males (79.0%) and in rural areas (70.2%). The most affected age group was between 16 and 45 years old (54.6%). Fifty five percent of the snakebites were related to work activities. Age ≤15 years [OR=1.26 (95% CI=1.03-1.52); (p=0.018)], age ≥65 years [OR=1.53 (95% CI=1.09-2.13); (p=0.012)], work related bites [OR=1.39 (95% CI=1.17-1.63); (p<0.001)] and time to medical assistance >6 hours [OR=1.73 (95% CI=1.45-2.07); (p<0.001)] were independently associated with the risk of severity. Age ≥65 years [OR=3.19 (95% CI=1.40-7.25); (p=0.006)] and time to medical assistance >6 hours [OR=2.01 (95% CI=1.15-3.50); (p=0.013)] were independently associated with the risk of death. Snakebites represent an occupational health problem for rural populations in the Brazilian Amazon with a wide distribution. These results highlight the need for public health strategies aiming to reduce occupational injuries. Most cases of severe disease occurred in the extremes of age, in those with delays in medical attention and those caused by Micrurus bites. These features of victims of snakebite demand adequate management according to well-defined protocols,
including prompt referral to tertiary centres when necessary, as well as an effective response from surveillance systems and policy makers for these vulnerable groups.

Introduction
Snakebites represent a treatable health problem affecting predominantly tropical low- and middle-income countries [1,2]. The annual number of snakebite cases worldwide is largely unknown and probably underreported with estimates that could reach 5.5 million snakebites annually, with 94,000 estimated deaths [1]. Rural areas in developing countries are the most affected, with varying seasonality usually characterized by peak incidences seen in the rainy and harvesting seasons [3]. The burden of human suffering caused by snakebite has not received the attention it deserves from the public health community, development agencies and governments, and may therefore be appropriately categorized as a neglected tropical disease [1,4]. Most snakebite reporting systems are fragile and underestimate the actual injury burden [5]. Better epidemiological surveillance is necessary to assess the extent of this important public health problem to improve prevention and treatment measures. Most deaths and sequelae from snakebites are preventable by interventions such as making antivenom widely available [6].

In 2013, the Brazilian Ministry of Health reported 27,181 snakebite cases [7]. The highest incidence was observed in the Brazilian Amazon (52.6 cases/100,000 inhabitants) followed by the Midwest (16.4/100,000). These values are expected to be higher in remote areas of the Brazilian Amazon [8] due to considerable underreporting. In this region, adult males living in rural areas [3,9–13] and/or workers exerting farming, hunting and forestry activities [14,15] are the most affected groups, strongly suggesting an occupational risk. A community survey conducted with forest-dwelling Indians and rubber tappers (seringueiros) revealed that 13% of the surveyed population had been bitten during their lifetime [14]. Snakebite incidence correlates with the period of higher rainfalls as increased river levels force snakes to seek new shelters closer to human settlements [3,9,11,12], highlighting the vulnerability of Amazonian communities living in riverbank areas.

Some studies indicate that snakes of the genus Bothrops account for nearly 80% of the snakebites in the Brazilian Amazon region [16–18]. Bothrops atrox and Lachesis muta share the same popular name ‘surucucu’ in certain Amazonian areas, bringing a confounding factor in snake identification by the local population [12]. In the Amazon, clinical data from Bothrops atrox bites show pain as the most frequent local manifestation, followed by swelling, warmth on palpation and necrosis [18,19]. Around 25% of the patients present systemic manifestations, including spontaneous systemic bleeding, ranging from 16–18% [16,18,19] and acute renal failure registered in 10.9% in a case series [19]. A total of 39.0% of patients developed secondary complications, such as cellulitis and abscesses [18]. The clinical picture caused by Lachesis bites is very similar to Bothrops’, except for vagal symptoms that may be present especially in severe Lachesis cases [7,8]. In the Brazilian Amazon, relatively few accidents are caused by coral snakes (Micrurus sp.) [20] or rattlesnakes (Crotalus sp.) [21]. Surveillance systems shows an overall case-fatality rate ranging from 0.4 to 3.9% in the Brazilian Amazon [9,11,12,14,15].

Snakebite clinical severity varies by snake species and patient characteristics, but there are few studies identifying the more vulnerable subgroups developing poor outcomes. In India, patient-related factors such as child age, previous health condition and time elapsed until medical assistance can act as risk factors for severity, sequelae and death [22–26]. In the same country, patients admitted with symptoms of neurotoxicity and vomiting [22,24], coagulopathy and...
leukocytosis [23], hypertension, albuminuria, changes in bleeding and prothrombin time [25] were more likely to develop severe outcomes. The delay in antivenom administration was also a risk factor for severity in Nigeria [27]. Capillary leak syndrome, bleeding and respiratory paralysis were risk factors for mortality in patients who received snake antivenom in Nigeria and Korea [28,29].

A better knowledge of severity and mortality due to snakebite would lead to improved management, with expected reduction of sequelae and lethality rates in remote localities in the Brazilian Amazon. The aim of this study is to analyze the profile of snakebites reported in the state of Amazonas, in the Western Brazilian Amazon, and to investigate potential risk factors for severity and lethality.

Materials and Methods

Study area

The State of Amazonas is located in the western Brazilian Amazon, comprising an area of 1,570,946.8 km², with 62 municipalities. The estimated population of the state was 3,807,921 inhabitants in 2010, with 74.2% living in urban zones and 25.8% in rural areas. Approximately 45% of the population lives in the state capital, Manaus. The state has a reduced coverage of highways and roads, with much of the displacement happening via river transportation. The state is densely covered by an evergreen rain forest, standing out the upland forests (terra firme forest), floodplains (várzeas) and flooded areas (igapós).

Data source

Snakebites are compulsorily recorded by the Brazilian Notifiable Diseases Surveillance System ([Sistema de Informação de Agravos de Notificação (SINAN)] based on data report forms used in the investigation and follow-up of cases of venomous animals. The SINAN is a national electronic surveillance system that contains a variety of diseases in an integrated database that also includes snakebites [15]. We included all snakebite cases in the state of Amazonas reported to SINAN from January 1, 2007 to December 31, 2012. Snakebite treatment is provided free of charge only by the Brazilian Government and is not available for purchase in the private sector.

Exposure and outcome definitions

Reporting on snakebites’ severity grading and outcomes (discharge or death) is required by SINAN and it is entered by healthcare providers at the time of case notification (which often happens after discharge). Clinical severity of snakebites in this work was classified according the Brazilian Health Ministry guidelines [17]: i) mild cases: local pain, local swelling and bruising for Bothrops and Lachesis bites or ptosis, slight blurred vision and mild or absent myalgia for Crotalus bites (rarely found in the study area) [21]; ii) moderate cases: local manifestations without necrosis, minor systemic signs (coagulopathy and bleeding, no shock) in Bothrops and Lachesis bites; myalgia and dark urine in may occur in Crotalus bites; iii) severe cases: life-threatening snakebite, with severe bleeding, hypotension/shock and/or acute renal failure for Bothrops and Lachesis bites. In severe Lachesis bites, vagal symptoms may be present in about 14% of cases [30]. Severe Crotalus are those presenting with generalized myalgia, dark urine, oliguria or anuria. It is recommended to consider all confirmed Micrurus cases, although scarcely seen in the Amazonas, as potentially severe due to the risk of respiratory failure. For more details concerning the severity grading and treatment of snakebites used in Brazil see S1 File.
The variables analyzed were sex, age (in years), anatomical region of the bite, area of occurrence (rural or urban), work-related injury (yes or no), time elapsed between the bite and medical assistance (in hours), perpetrating species (Bothrops, Lachesis, Crotalus, Micrurus or non-venomous snakes), severity grading (mild, moderate or severe), outcome (discharge or death), clinical features and antivenom administration data. All variables were checked by two independent researchers before analysis and further investigated for a possible association with severity and lethality as dependent variables.

Due to the potential severity of Lachesis confirmed bites, these few cases are generally considered clinically moderate or severe. However, in areas where there is overlap in the geographical distribution of Bothrops and Lachesis, such as in the Brazilian Amazon, the differential routine diagnosis when the snake has not been captured can be misleading and be associated with misclassification of the causing species.

In order to identify factors associated with snakebite severity and mortality, a nested case control study was used wherein patients evolving to severity or death were classified as cases and those with mild and moderate bites were included as controls.

Spatial distribution
A map was created with the software ArcMap 10.1 in ArcGIS 10.1 (ESRI, USA) using estimates of the mean incidence by municipality. The incidence rate was calculated dividing cases by the population of each municipality [31] by 100 thousand inhabitants. Spatial interpolation of snakebites’ incidence was mapped using data from 62 municipalities by the Inverse Distance Weighting (IDW) method.

Statistical analysis
Only variables presenting completeness higher than 85% were analyzed. Duplicity was solved before data analysis. Data were analyzed using SPSS version 21.0 for Windows (SPSS Inc. Chicago, IL, USA). Proportions of severe cases and deaths were compared by Chi-square test (corrected by Fisher’ test if necessary); differences were considered statistically significant for p<0.05. The crude Odds Ratio (OR) with its respective 95% Confidence Interval (95% CI) was determined considering severity and death as the dependent variables. Logistic regression was used for the multivariable analyses and the adjusted Odds Ratios with 95% CI were also calculated. All variables associated with the outcomes at a significance level of p<0.20 in the univiable analysis were included in the multivariable analysis. Statistical significance was considered if p<0.05 in the Hosmer-Lemeshow goodness-of-fit test.

Ethical clearance
This study was approved by the Ethics Review Board (ERB) of the Fundação de Medicina Tropical Dr. Heitor Vieira Dourado (approval number 872.520/2014), as well as by the ERB of the Health Surveillance Foundation of the Amazonas State. All data analyzed were anonymous. Since data were obtained exclusively from surveillance databases, the ERB gave a waiver of informed consent.

Results
Study population
According to the official reporting systems, 9,191 snakebites were recorded in the Amazonas State from 2007 to 2012, resulting in an incidence rate of 52.8 cases per 100,000 person/year. There was a slight variation in the annual incidence rates during the study period (Fig 1).
Incidence was higher in 2009 (1,697 cases; 58.5 per 100,000 inhabitants) and 2012 (1,558 cases; 53.7 per 100,000) and lower in 2011 (1,436 cases; 49.5 per 100,000). All the variables retrieved from the original dataset presented completeness higher than 85% (Table 1). Most of the snakebites occurred in males (7,257 cases; 79.0%). Regarding the area of occurrence, 70.2% were reported in rural areas. The most affected age group was between 16 and 45 years old (5,019 cases; 54.6%). A proportion of 55.0% of the snakebites were related to work activities. Regarding time elapsed from the bite until medical assistance, 68.4% of the cases received treatment within the first six hours after the snakebite, 13.7% within 6–12 hours and 17.9% with more than 12 hours after bite. Most of the snakebites occurred in the feet (58.8%) and legs (27.4%).

Snakebites were caused mainly by Bothrops (73.9%; 35.5 cases per 100,000 person/year) and probably by Lachesis (23.9%; 11.5 cases per 100,000 person/year). Crotalus and Micrurus snakes were responsible for 0.5% (0.2 cases per 100,000 person/year) and 0.4% (0.2 cases per 100,000 person/year) of the bites, respectively. Non-venomous snakebites were recorded in 1.3%.

Most cases were mild (46.1%), followed by moderate (45.8%) and severe (8.1%) cases. There were 55 deaths due to snakebites in the study period, resulting in a 0.6% lethality rate. Lethality rates were 0.7% for Bothrops snakebites and 0.6% for Lachesis snakebites. There were no records of deaths due to Crotalus and Micrurus snakebites in the study period. According to age groups, lethality rate was 0.5% for the ≤15 years group, 0.5% for the 15 to 60 years group and 2.0% for the ≥60 years group.

Time elapsed from bite to antivenom administration at a health facility was 6.2 (SD±7.2) hours for mild cases, 7 (SD±7.1) hours for moderate cases, 9 (SD±8.1) hours for severe cases and 15.5 hours (SD±10.2) for cases evolving to death (Fig 2).

The most frequent local manifestations were pain (97.1%), edema (85.2%), ecchymosis (15.3%) and necrosis (2.3%). Systemic manifestations were present in 19.4% of patients. Of those presenting systemic manifestations, 54.8% presented with hemorrhage, 33.7% with vagal symptoms, 20.5% with myolysis or hemolysis, 19.6% had paralysis and 12.6% had renal injury. Coagulation time was altered in 39.1% of all commers.

Antivenom was provided to 93% of Bothrops bites, 79% of Crotalus bites, 97% of Micrurus bites and 96% of accidents by Lachesis, totalling 94% of the study population. 52.7% of cases
evolving to death did not receive antivenom. One per cent of accidents by Bothrops considered mild received less than the recommended dosage of specific antivenom, 3% of moderate cases received less than recommended and 52% of severe cases received less than expected. A total of 50% of accidents by Lachesis considered moderate received less than the recommended dosage and 81% of severe cases were underdosed. All accidents by Micrurus are reported as severe and 35% of cases received less than the recommended dose of antivenom. Lastly, for Crotalus injuries, 37.8% received less than the recommended dose of antivenom.

Table 1. Clinical and epidemiological features of 9,191 snakebite cases reported in the State of Amazonas, 2007 to 2012.

| Characteristics (n; completeness) | Number | %  |
|-----------------------------------|--------|----|
| **Sex (n = 9,191; 100%)**         |        |    |
| Male                              | 7,257  | 79.0 |
| Female                            | 1,934  | 21.0 |
| **Area of occurrence (n = 9,010; 98.0%)** |        |    |
| Rural                             | 6,323  | 70.2 |
| Urban                             | 2,687  | 29.8 |
| **Age group in years (n = 9,191; 100%)** |        |    |
| 0–15                              | 2,216  | 24.1 |
| 16–45                             | 5,019  | 54.6 |
| 45–65                             | 1,603  | 17.4 |
| >65                               | 353    | 3.9 |
| **Work-related accident (n = 8,200; 89.2%)** |        |    |
| Yes                               | 4,506  | 55.0 |
| No                                | 3,694  | 45.0 |
| **Time-interval to medical assistance (hrs) (n = 8,612; 93.7%)** |        |    |
| 0–6                               | 5,892  | 68.4 |
| 6–12                              | 1,181  | 13.7 |
| >12                               | 1,539  | 17.9 |
| **Anatomical site (n = 9,122; 99.2%)** |        |    |
| Feet                              | 5,372  | 58.8 |
| Legs                              | 2,498  | 27.4 |
| Trunk                             | 37     | 0.4 |
| Upper limbs                       | 1,123  | 12.3 |
| Head                              | 92     | 1.0 |
| **Species (n = 8,366; 91.0%)**     |        |    |
| Bothrops                          | 6,184  | 73.9 |
| Probably Lachesis                 | 1,999  | 23.9 |
| Crotalus                          | 37     | 0.5 |
| Micrurus                          | 36     | 0.4 |
| Non-venomous snakebites           | 110    | 1.3 |
| **Clinical severity of envenoming (n = 8,769; 95.4%)** |        |    |
| Mild                              | 4,040  | 46.1 |
| Moderate                          | 4,019  | 45.8 |
| Severe                            | 710    | 8.1 |
| **Outcome (n = 9,182; 99.9%)**     |        |    |
| Discharged                        | 9,124  | 93.2 |
| Death                             | 55     | 0.6 |
| Death from other causes           | 3      | <0.1 |

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Spatial distribution

Incidence rates were unevenly distributed across the Amazonas State, although there were snakebites cases reported from all the 62 municipalities. Mapping showed a large area with high incidence rates extending from the Northeast to the Central region of the state, including three hotspots located in São Gabriel da Cachoeira (in the Colombian border), in the Uarini/Alvarães municipalities area and in Novo Airão, where incidence rates were higher than 150 cases per 100,000 inhabitants/year. Smaller areas with high snakebite incidences were located in Rio Preto da Eva, in the vicinity of the capital city, and in Borba, in the upper Southeast region of the state (Fig 3). Incidence rates by municipality are presented in S1 Table.

Risk factors for severity and mortality

From the 9,191 snakebite cases reported in the Amazonas State in the study period, we excluded 102 cases of non-venomous snakebites and 422 cases without severity classification. We used the remaining 8,667 eligible cases to evaluate risk factors for severity and mortality. A total of 708 severe cases and 52 deaths were included as cases and 7,959 snakebites were included as controls (Fig 4).

Table 2 summarizes the results of the univariable and multivariable logistic regression models evaluating factors associated with snakebite severity. Age $\leq$ 15 years [OR = 1.26 (95% CI = 1.03–1.52); (p = 0.018)], age $\geq$ 65 years [OR = 1.53 (95% CI = 1.09–2.13); (p = 0.012)], bites related to work activities [OR = 1.39 (95% CI = 1.17–1.63); (p<0.001)], time elapsed before medical assistance >6 hours [OR = 1.73 (95% CI = 1.45–2.07); (p<0.001)] and Micrurus bites [OR = 5.54 (95% CI = 2.61–12.20); (p<0.001)] were independently associated with the risk of developing a severe illness after snakebites.

Table 3 compares patients evolving to death versus other patients. Age $\geq$65 years [OR = 3.19 (95% CI = 1.40–7.25); (p = 0.006)] and time to medical assistance >6 hours [OR = 2.01 (95% CI = 1.15–3.50); (p = 0.013)] were independently associated with risk of death.
Discussion

Few previous attempts have been made to quantify the burden of envenoming resulting from snakebites in the Brazilian Amazon. Our study confirms previous hospital based reports of a wide geographical distribution of snakebites in the state of Amazonas [9]. We provide a more accurate, up-to-date estimate of the scale of the problem in the Brazilian Amazon by using official surveillance data to estimate the disease burden due to snakebites and to identify independent risk factors for severity and mortality. In this study, systemic manifestations were present in 19.4% of patients, being the most common: hemorrhage, myolysis/hemolysis, paralysis and renal injury, in agreement with previous reports from the Amazon Region, where Bothrops atrox is the main causal agent [18,19,30].

The mean incidence rate in the study area was 52.8 cases per 100,000 person/year, reaching more than 150 cases per 100,000 person/year in several municipalities, comparable to
Fig 4. Flow chart of cases and control selection. The selection of cases and controls was based on the Brazilian Ministry of Health classification. All severe bites were included as cases, and three mild and moderate bites were in the control group for each case.

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Table 2. Factors associated with snakebite severity in the State of Amazonas, 2007 to 2012.

| Variables                        | Cases (n) | %   | Controls (n) | %   | Crude OR (IC 95%)       | p       | Adjusted OR (IC 95%)    | p       |
|----------------------------------|-----------|-----|--------------|-----|-------------------------|---------|-------------------------|---------|
| **Sex**                          |           |     |              |     |                         |         |                         |         |
| Male                             | 556       | 78.5| 6,302        | 79.2| 0.96 (0.80–1.16)        | 0.680   | ...                     | ...     |
| Female                           | 152       | 21.5| 1,657        | 20.8|                         |         |                         |         |
| **Age (years)**                  |           |     |              |     |                         |         |                         |         |
| ≤15                              | 181       | 25.6| 1,891        | 23.8| 1.10 (0.92–1.31)        | 0.280   | 1.26 (1.03–1.52)        | 0.018   |
| >15                              | 527       | 74.4| 6,068        | 76.2|                         |         |                         |         |
| ≥65                              | 43        | 6.1 | 332          | 4.2 | 1.48 (1.07–2.06)        | 0.017   | 1.53 (1.09–2.13)        | 0.012   |
| <65                              | 665       | 93.9| 7,659        | 95.8|                         |         |                         |         |
| **Anatomical site**              |           |     |              |     |                         |         |                         |         |
| Lower limbs                      | 558       | 78.8| 6,357        | 80.0| 0.93 (0.77–1.12)        | 0.450   | ...                     | ...     |
| Other sites                      | 150       | 21.2| 1,592        | 20.0|                         |         |                         |         |
| **Area of occurrence**           |           |     |              |     |                         |         |                         |         |
| Rural                            | 512       | 72.3| 5,466        | 68.7| 1.19 (1.01–1.41)        | 0.045   | 1.05 (0.89–1.26)        | 0.530   |
| Urban                            | 196       | 27.7| 2,493        | 31.3|                         |         |                         |         |
| **Work-related injury**          |           |     |              |     |                         |         |                         |         |
| Yes                              | 399       | 56.4| 3,934        | 49.4| 1.32 (1.13–1.54)        | <0.001  | 1.39 (1.17–1.63)        | <0.001  |
| No                               | 309       | 43.6| 4,025        | 50.6|                         |         |                         |         |
| **Time-interval to medical assistance (hours)** | | | | | | | | |
| 0–6                              | 297       | 41.9| 2,268        | 28.5| 1.81 (1.55–2.12)        | <0.001  | 1.73 (1.45–2.07)        | <0.001  |
| >6                               | 411       | 58.1| 5,691        | 71.5|                         |         |                         |         |
| **Snakebite type**               |           |     |              |     |                         |         |                         |         |
| Bothrops                         | 494       | 75.6| 5,422        | 74.8| 1                       | ...     | ...                     | ...     |
| Probably Lachesis                | 144       | 22.1| 1,776        | 24.5| 0.89 (0.73–1.08)        | 0.237   | 0.85 (0.69–1.04)        | 0.115   |
| Crotalus                         | 4         | 0.6 | 31           | 0.4 | 1.41 (0.49–4.02)        | 0.512   | 1.20 (0.36–3.95)        | 0.765   |
| Micrurus                         | 11        | 1.7 | 25           | 0.3 | 4.82 (2.36–9.87)        | <0.001  | 4.91 (2.30–10.47)       | <0.001  |

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projections for sub-Saharan Africa and Oceania [1]. In the Brazilian Amazon, reaching health centers is difficult for riverine and indigenous populations as a result of distance and underdevelopment of means of transportation. If we account for a possible underreporting to official surveillance systems [32], the actual incidence of snakebites in this region could be among the highest in the world. We observed a large area with high incidence rates extending from the Northeast to the Central region of the state, where incidence rates were above 150 cases per 100,000 inhabitants/year. This area coincides with municipalities where Amerindian populations predominate, highlighting the vulnerability of these groups. A previous study found an association between snakebite incidence and deforestation [33]. Indeed, the hotspots identified in Novo Airão and Rio Preto da Eva, both in the Manaus region, and in Borba, correspond to areas with intense deforestation, and likely reflect the emergence of economical activities in these municipalities, namely farming and cattle raising.

Most snakebites occurred in males, between 16 and 45 years, living in rural areas and mainly related to work activities. This profile has been reported in other regions in Brazil [13,16,34,35] and in other countries [36,37] and reaffirms the strong relationship between snakebites and rural economical activities in poor regions [38], thus, negatively affecting family economies in the area. Currently, there are no interventions for primary prevention of snakebites as an occupational hazards or to speed access to health services.

More than 30% of patients took more than six hours to receive medical assistance and such delay was an independent risk factor for severity and mortality. A delay to medical care was also identified as a risk factor for poor outcomes in India [23,39], Nepal [36,40] and Nigeria [27,28]. Patients from Amazonian communities depend mainly on river transportation to access health centers.

**Table 3. Factors associated with snakebite mortality in the State of Amazonas, 2007 to 2012.**

| Variables                  | Cases (n) | %  | Controls (n) | %  | Crude OR (IC 95%) | p       | Adjusted OR (IC 95%) | p       |
|----------------------------|-----------|----|--------------|----|------------------|---------|----------------------|---------|
| Sex                        |           |    |              |    |                  |         |                      |         |
| Male                       | 40        | 76.9| 6,818        | 79.1| 0.87 (0.46–1.67) | 0.695   |                      |         |
| Female                     | 12        | 23.1| 1,797        | 20.9|                  |         |                      |         |
| Age (years)                |           |    |              |    |                  |         |                      |         |
| ≤15                        | 10        | 19.2| 2,062        | 23.9| 0.75 (0.38–1.51) | 0.428   | 0.74 (1.35–1.56)     | 0.440   |
| >15                        | 42        | 80.8| 6,553        | 76.1|                  |         |                      |         |
| ≥65                        | 7         | 13.5| 368          | 4.3 | 3.48 (1.56–7.78) | 0.001   | 3.19 (1.40–7.25)     | 0.006   |
| <65                        | 45        | 86.5| 8,247        | 95.7|                  |         |                      |         |
| Anatomical site            |           |    |              |    |                  |         |                      |         |
| Lower limbs                | 43        | 82.7| 6,882        | 79.9| 1.20 (0.58–2.47) | 0.614   |                      |         |
| Other sites                | 9         | 17.3| 1,733        | 20.1|                  |         |                      |         |
| Area of occurrence         |           |    |              |    |                  |         |                      |         |
| Rural                      | 38        | 73.1| 5,940        | 68.9| 1.22 (0.66–2.26) | 0.521   |                      |         |
| Urban                      | 14        | 26.9| 2,675        | 31.1|                  |         |                      |         |
| Work-related injury        |           |    |              |    |                  |         |                      |         |
| Yes                        | 24        | 46.2| 4,309        | 50.0| 0.85 (0.49–1.48) | 0.570   |                      |         |
| No                         | 28        | 53.8| 4,306        | 50.0|                  |         |                      |         |
| Time-interval to medical assistance (hrs) |   |    |              |    |                  |         |                      |         |
| 0–6                        | 24        | 46.2| 2,541        | 29.5| 2.04 (1.18–3.54) | 0.009   | 2.01 (1.15–3.50)     | 0.013   |
| >6                         | 28        | 53.8| 6,074        | 70.5|                  |         |                      |         |
| Snakebite type             |           |    |              |    |                  |         |                      |         |
| Bothrops                   | 38        | 77.6| 5,878        | 75.5| 1                 |         |                      |         |
| Probably Lachesis          | 11        | 22.4| 1,909        | 24.5| 0.94 (0.48–1.83) | 0.86    |                      |         |

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reach hospitals in urban seats, causing a delay in treatment. Moreover, many victims seek medical care after a considerable delay or fail to reach a hospital in time as a result of first seeking treatment from traditional healers [9]. The lethality rate of 0.6% observed in this work is higher than some previous national reports [7,35]. We hypothesize that implementing a rapid transport system for snakebite patients would significantly reduce lethality rates as has been reported in other settings [41].

Interestingly, occupational accidents were more likely to develop severe disease. This relationship between severity and occupational exposure was also observed in India, where rural workers bitten by snakes were more prone to death [37]. We cannot precisely establish a direct association between snakebite severity and occupational exposure, but the distance to be walked from the workplace in farming areas to medical care could explain this risk association.

In India, patients who walked more than 1 km after the snakebite, had a delay in care and a higher risk of death [22]. Reassurance and immobilization of the affected limb with prompt transfer to a medical facility are the cornerstones of the immediate care of snakebites [29,42].

In this work, age less than 15 years old was also an independent risk factor for severity, probably as a result of smaller body volumes [2]. This risk factor was also observed in India [22] and Turkey [43]. One half of deaths from snakebites in California affected children under 5 years of age [44].

Age ≥65 years was a risk factor for severity and mortality. This is relevant as literature scarcely reports elderly as being at a higher risk. In Bothrops and Crotalus snakebites reported in São Paulo, Brazil, patients ≥50 years-old had a higher mortality when compared to younger patients [45]. Taking into account that most snakebite complications, especially by Bothrops, are due to acute renal failure [46–50], the greater severity associated with the elderly may be related to higher prevalences of chronic comorbidities (i.e. hypertension and diabetes) that can lead to a predisposition to evolve to necrosis and acute kidney injury after a bite [51,52]. One speculates that comorbidities leading to vascular disease, such as hypertension and diabetes, commonly found in the elderly, may precipitate life-threatening complications after snakebite envenomings. In a case series from Brazil, eight of 309 patients (2.6%) developed a cerebrovascular event after Bothrops envenoming; five of the eight patients died despite therapy, and the remaining three had irreversible sequelae [53]. Cases of stroke related to Bothrops envenomings have been reported in Brazil in the elderly, including in the Amazon region [54,55]. Population aging will likely affect snakebite epidemiology and its public health impact thus requiring preparation by local health services with a wider distribution of antivenom and life-sustaining supportive therapies besides health care workers’ training in the management of envenoming in this neglected age group.

Our study reports significant underdosing of antivenom in the Brazilian Amazon as has been previously observed [32]. This has several possible explanations: we used an epidemiologic surveillance system to obtain our data where clinical and serum administration dosing information is collected hours or even days after the event, possibly reflecting a difficulty predicting the amount of antivenom needed upon admission; another possible explanation is the lack of appropriate training in antivenom dosing in medical personnel in remote settings; or possibly, unavailability of serum in rural areas where most of accidents occur. Underdosing may play a role in morbidity and mortality associated with snakebites in the region, exemplified by the fact that more than half of deaths lacked antivenom administration; this represents a missed opportunity to improve snakebite outcomes in the region.

Record keeping may have been influenced by the nature of the surveillance system. Some patients with mild bites in inaccessible areas may not be reported to hospitals and those evolving to severity may die on the way before reaching medical attention. In Brazil, antivenoms are prescribed exclusively by physicians in hospital settings. However, only a few patients bring the
snakes for identification to the health unit, therefore the clinical-epidemiological classification is subject to misclassification, as is mostly based on clinical grounds. For instance, in the absence of the Lachesis or Bothrops-Lachesis antivenoms or in case of snake misclassification, health services often use Bothrops antivenom in the treatment of laquetic accident. However, previous studies in the same region have shown that Bothrops antivenom was ineffective to neutralize the coagulant activity of L. muta venom in humans, hence, contributing to severity [56]. In an uncertain proportion, completion of severity grading may occur retrospectively, according to the number of antivenom vials administered. Since patients are commonly seen initially at the emergency room, case notification mostly occurs after antivenom therapy and sometimes even after patient discharge. As a result, severity classification of snake envenoming by healthcare providers depends on the quality of this retrospective information. As all Micrurus envenomings are considered potentially severe by the Brazilian Ministry of Health guidelines, it was expected for this type of envenoming to be associated with severity. In fact, it was an expected result resulting from an biased association, rather than a real risk factor for poor outcome.

In conclusion: i) the incidence of snakebites in the Western Brazilian Amazon is among the highest in the world; ii) snakebites show a wide distribution in this region; iii) snakebites represent an occupational health problem for rural populations and public heath measures are needed to prevent occupational snakebites; iv) age ≤15 years and ≥65 years, work related bites and delayed medical treatment >6 hours were independently associated with the risk of developing severity and mortality; age ≥65 years and time to medical assistance >6 hours were independently associated with mortality. These features highlight the need for public health interventions aiming to decrease time to medical attention in vulnerable groups. In the Amazon, a major concern relates to the failure in antivenom distribution and storage. The lack of an adequate cold chain impairs antivenom distribution to rural areas and may result in loss of material. Moreover, there is a lack of systematic professional training on diagnosis, specific therapy and clinical management of complications [32]. Improvements in antivenom distribution and time to medical attention could have a significant impact in patient outcomes.

Supporting Information

S1 File. Clinical grading of snakebite according to the Brazilian Ministry of Health.  
(DOCX)

S2 File. STROBE Statement: Checklist of items that should be included in reports of case-control studies.  
(DOC)

S1 Table. Annual mean incidence of snakebites by municipality in the State of Amazonas, 2007–2012.  
(DOC)

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Author Contributions
Conceived and designed the experiments: ELF ML WM. Performed the experiments: ELF JLS AMQ IMS. Analyzed the data: ELF VSS JS AAG JS AMS WM. Contributed reagents/materials/analysis tools: JLS LCLF ML WM. Wrote the paper: ELF VSS JLS AMQ IMS AAG JS AMS LCLF MCS ML WM.

References
1. Kasturiratne A, Wickremasinghe R, Silva N, Gunawardena NK, Pathmeswaran A, Premaratna R, et al. (2008) The global burden of snakebite: a literature analysis and modelling based on regional estimates of envenoming and deaths. PLoS Med 5: e218. doi: 10.1371/journal.pmed.0050218 PMID: 18986210
2. Chippaux JP (1998) Snake-bites: appraisal of the global situation. Bull World Health Organ 76: 515–524. PMID: 9868843
3. Feitosa ES, Sampaio V, Sachett J, Castro DB, Noronha MDN, Lozano JLL, et al. (2015) Snakebites in the State of Amazonas: a largely neglected problem. Rev Soc Bras Med Trop. In press.
4. Williams D, Gutiérrez JM, Harrison R, Warrell DA, White J, Winkel KD, et al. (2010) The Global Snake Bite Initiative: an antidote for snake bite. Lancet 375: 89–91. doi: 10.1016/S0140-6736(09)61159-4 PMID: 20109867
5. Fiszon JT, Bochner R (2008) Subnotificação de acidentes por animais peçonhentos registrados pelo SINAN no Estado do Rio de Janeiro no período de 2001 a 2005. Rev Bras Epidemiol 11: 114–127.
6. Gutiérrez JM, Lomonte B, León G, Alape-Girón A, Flores-Díaz M, Sanz L, et al. (2009) Snake venomics and antivenomics: Proteomic tools in the design and control of antivenoms for the treatment of snakebite envenoming. J Proteomics 72: 165–182. doi: 10.1016/j.jprot.2009.01.008 PMID: 19344652
7. Brazilian Ministry of Health (2014) Sistema de Informação de Agravos de Notificação—SINAN. Brasília: Brazilian Ministry of Health.
8. Brazilian Ministry of Health (2009) Guia de Vigilância Epidemiológica—Caderno 14—Acidentes por animais peçonhentos. Brasília—DF. 23 p.
9. Borges CC, Sadahiro M, Santos MC (1999) Aspectos epidemiológicos e clínicos dos acidentes ofídicos ocorridos nos municípios do Estado do Amazonas. Rev Soc Bras Med Trop 32: 637–646. PMID: 10881100
10. Carvalho MA, Nogueira F (1998) Serpentes da área urbana de Cuiabá, Mato Grosso: aspectos ecológicos e acidentes ofídicos associados. Cad Saude Publica 14: 753–763. PMID: 9878908
11. Nascimento SP (2000) Aspectos epidemiológicos dos acidentes ofídicos ocorridos no Estado de Roraima, Brasil, entre 1992 e 1998. Cad Saude Publica 16: 271–276. PMID: 10738175
12. Bernarde PS, Gomes JO (2012) Serpentes peçonhentas e ofidismo em Cruzeiro do Sul, Alto Juruá, Estado do Acre, Brasil. Acta Amaz 42: 65–72.
13. Lima ACSF, Campos CEC, Ribeiro JR (2009) Perfil epidemiológico de acidentes ofídicos do Estado do Amapá. Rev Soc Bras Med Trop 42: 329–335. PMID: 19684984
14. Pierini SV, Warrell DA, Paulo A, Theakston RD (1996) High incidence of bites and stings by snakes and other animals among rubber tappers and Amazonian Indians of the Juruá Valley, Acre State, Brazil. Toxicon 34: 225–236. PMID: 8711756
15. Waldez F, Vogt RC (2009) Aspectos ecológicos e epidemiológicos de acidentes ofídicos em comunidades ribeirinhas do baixo rio Purus, Amazonas, Brasil. Acta Amaz 39: 681–692.
16. Moreno E, Queiroz-Andrade M, Lira-da-Silva RM, Tavares-Neto J (2005) Características clínico-epidemiológicas dos acidentes ofídicos em Rio Branco, Acre. Rev Soc Bras Med Trop 38: 15–21. PMID: 15717089
17. Sá-Neto RP, Dos-Santos M (1995) Aspectos epidemiológicos dos acidentes ofídicos atendidos no Instituto de Medicina Tropical de Manaus (IMTM), 1986–92: estudo retrospectivo. Rev da Soc Bras Med Trop 28 (supl I) 171.
18. Pardal PPO, Souza SM, Monteiro MRCC, Fan HW, Cardoso JLC, França FO, et al. (2004) Clinical trial of two antivenoms for the treatment of Bothrops and Lachesis bites in the north eastern Amazon region of Brazil. Trans R Soc Trop Med Hyg 98: 28–42. PMID: 14702896
19. Souza A (2000) Snakebite by Bothrops atrox (Lin, 1758) in the State of Amazonas—Brazil: Study of 212 cases with identified snake. Rev Patol Trop 31: 259–268.
20. Pardal PPO, Pardal JSO, Gadelha MAC, Rodrigues LS, Feitosa DT, Prudente AL, et al. (2010) Envenomation by Micrurus coral snakes in the Brazilian Amazon region: report of two cases. Rev Inst Med Trop Sao Paulo 52: 333–337. PMID: 21225218
21. Pardal PPO, Silva CLQ, Hoshino SSN, Pinheiro MFR (2007) Acidente por cascavel (Crotalus sp.) em Ponta de Pedras, Ilha do Marajó, Pará—Relato de caso. Rev Para Med 21: 69–73.

22. Sankar J, Nabeel R, Sankar MJ, Priyambada L, Mahadevan S (2013) Factors affecting outcome in children with snake envenomation: a prospective observational study. Arch Dis Child 98: 596–601. doi: 10.1136/archdischild-2012-003025 PMID: 23716133

23. Suchithra N, Pappachan JM, Sujathan P (2008) Snakebite envenoming in Kerala, South India: clinical profile and factors involved in adverse outcomes. Emerg Med J 25: 200–204. doi: 10.1136/emj.2007.051136 PMID: 18356348

24. Kalantri S, Singh A, Joshi R, Malamba S, Ho C, Ezoua J, et al. (2006) Clinical predictors of in-hospital mortality in patients with snake bite: a retrospective study from a rural hospital in central India. Trop Med Int Health 11: 22–30. PMID: 16398752

25. Dharod MV, Patil TB, Deshpande AS, Gulhane RV, Patil MB, Bansod YV (2013) Clinical predictors of acute kidney injury following snake bite envenomation. N Am J Med Sci 5: 594–599. doi: 10.4103/1947-2714.120795 PMID: 23559724

26. Saravu K, Shastry A, Somavarapu V, Kumar R (2012) Clinical profile, species-specific severity grading, and outcome determinants of snake envenomation: An Indian tertiary care hospital-based prospective study. Indian J Crit Care Med 16: 187. doi: 10.4103/0972-5229.106499 PMID: 23559724

27. Ogunfowokan O, Jacob DA, Livinus OL (2011) Relationship between bite-to-hospital time and morbidity in victims of carpet viper bite in North-Central Nigeria. West Afr J Med 30: 348–353. PMID: 22752823

28. Habib AG, Abubakar SB (2011) Factors affecting snakebite mortality in north-eastern Nigeria. Int Health 3: 50–55. doi: 10.1016/j.inhe.2010.08.001 PMID: 24038050

29. Kim JS, Yang JW, Kim MS, Han ST, Kim BR, Shin MS, et al. (2008) Coagulopathy in patients who experience snakebite. Korean J Intern Med 23: 94. PMID: 18646512

30. Sá-Neto RP, Dos-Santos M (1995) Aspectos clínicos comparativos do acidente botrópico e laquério. Rev Soc Bras Med Trop 28: 173.

31. Instituto Brasileiro de Geografia e Estatística (2010) Censo Demográfico 2010. Available: http://www.ibge.gov.br/home/estatistica/populacao/censo2010/default.shtm.

32. Wen FH, Monteiro W, Silva AMM, Tambourgi DV, Mendonça da Silva I, Sampaio VS, et al. (2015) Snakebites and scorpion stings in the Brazilian Amazon: identifying research priorities for a largely neglected problem. Plos Neglected Trop Dis 9: e0003701.

33. Santos MC (2003) Serpentes peçonhentas e ofidismo no Amazonas. In: Cardoso JLC, França FOS, Wen FH, Malaque CMSA, Haddad-Junior V (Org.). Animais peçonhentos no Brasil. Biologia, Clínica e Terapêutica dos Acidentes. 1ed Savier Ed. FAPESP: 115–125.

34. Feitosa RFG, Melo IMLA, Monteiro HSA (1997) Epidemiologia dos acidentes por serpentes peçonhentas no Estado do Ceará—Brasil. Rev Soc Bras Med Trop 30: 295–301. PMID: 9265225

35. Lima JS, Martelli Júnior H, Martelli DRB, Silva MS, Carvalho SFG, Canela JR, et al. (2009) Perfil dos acidentes ofídicos no norte do Estado de Minas Gerais, Brasil. Rev Soc Bras Med Trop 42: 561–564. PMID: 19967240

36. Sharma SK, Koirala S, Dahal G, Sah C (2004) Clinico-epidemiological features of snakebite: a study from Eastern Nepal. Trop Doct 34: 20–22. PMID: 14959965

37. Mohapatra B, Warrell DA, Suraweera W, Bhatia P, Dhingra N, Jotkar RM, et al. (2011) Snakebite mortality in India: a nationally representative mortality survey. PLoS Negl Trop Dis 5: e1018. doi: 10.1371/journal.pntd.0001018 PMID: 21532748

38. Harrison R, Hargreaves A, Ward A, Laloo DG (2008) Snake envenoming: a disease of poverty. PLoS Negl Trop Dis 3: e569. doi: 10.1371/journal.pntd.0000569 PMID: 20027216

39. David S, Matathia S, Christopher S (2012) Mortality predictors of snake bite envenomation in southern India: a ten-year retrospective audit of 533 patients. J Med Toxicol 8: 118–123. doi: 10.1007/s13181-011-0204-0 PMID: 22234395

40. Sharma SK, Chappuis F, Jha N, Bovier PA, Loutan L, Koirala S (2004) Impact of snake bites and determinants of fatal outcomes in southeastern Nepal. Am J Trop Med Hyg 71: 234–238. PMID: 15306717

41. Sharma SK, Bovier P, Jha N, Alirol E, Loutan L, Chappuis F (2013) Effectiveness of rapid transport of victims and community health education on snake bite fatalities in rural Nepal. Am J Trop Med Hyg 89: 145–150. doi: 10.4269/ajtmh.12-0750 PMID: 23568287

42. World Health Organization (2015) Neglected tropical diseases. Available: http://www.who.int/neglected_diseases/diseases/snakebites/en/. Accessed 16 March 2015.

43. Ozay G, Bosnak M, Ece A, Davutoglu M, Dikici B, Gurkan F, et al. (2005) Clinical characteristics of children with snakebite poisoning and management of complications in the pediatric intensive care unit. Pediatr Int 47: 669–675. PMID: 16354222
44. Ennik F (1980) Deaths from bites and stings of venomous animals. West J Med 133: 463–468. PMID: 7467305

45. Ribeiro LA, Albuquerque MJ, Pires-de-Campos VAF, Katz G, Takaoka NY, Lebrão ML, et al. (1998) Óbitos por serpentes peçonhentas no Estado de São Paulo: avaliação de 43 casos, 1988/93. Rev Assoc Med Bras 44: 312–318. PMID: 9852651

46. Pinho FMO, Zanetta DMT, Burdmann EA (2005) Acute renal failure after Crotalus durissus snakebite: a prospective survey on 100 patients. Kidney Int 67: 659–667. PMID: 15673314

47. Amaral CFS, Rezende NA, Silva OA, Ribeiro MMF, Magalhães RA, Reis RJ, et al. (1986) Insuficiência renal aguda secundária a acidentes ofídico botrópico e crotálico. Análise de 63 casos. Rev Inst Med Trop Sao Paulo 28: 220–227. PMID: 3563305

48. Otero R, Gutiérrez J, Beatriz-Mesa M, Duque E, Rodríguez O, Arango JL, et al. (2002) Complications of Bothrops, Porthidium, and Bothriechis snakebites in Colombia. A clinical and epidemiological study of 39 cases attended in a university hospital. Toxicon 40: 1107–1114. PMID: 12165312

49. Pinho FMO, Yu L, Burdmann EA (2008) Snakebite-induced acute kidney injury in Latin America. Semin Nephrol 28: 354–362. doi: 10.1016/j.semnephrol.2008.04.004 PMID: 18620958

50. Albuquerque PLMM, Jacinto CN, Silva-Junior GB, Lima JB, Veras MDSB, Daher EF (2013) Acute kidney injury caused by Crotalus and Bothrops snake venom: a review of epidemiology, clinical manifestations and treatment. Rev Inst Med Trop Sao Paulo 55: 295–301. doi: 10.1590/S0036-46652013000500001 PMID: 24037282

51. Ribeiro LA, Gadia R, Jorge MT (2008) Comparação entre a epidemiologia do acidente e a clínica do envenenamento por serpentes do gênero Bothrops, em adultos idosos e nãodes. Rev Soc Bras Med Trop 41: 46–49. PMID: 18368270

52. Albuquerque PLMM, Silva GB, Jacinto CN, Lima JB, Limia CB, Amaral YS, et al. (2014) Acute kidney injury after snakebite accident treated in a Brazilian tertiary care centre. Nephrology (Carlton) 19: 764–770.

53. Mosquera A, Idrovo LA, Tafur A, Del Brutto OH (2003) Stroke following Bothrops spp. snakebite. Neurology 60: 1577–1580. PMID: 12771244

54. Santos-Soares PC, Bacellar A, Povoas HP, Brito AF, Santana DLP (2007) Stroke and snakebite: case report. Arq Neuropsiquiatr 65: 341–344. PMID: 17607441

55. Machado AS, Barbosa FB, Mello GS, Pardal PPO (2010) Acidente vascular cerebral hemorrágico associado à acidente ofídico por serpente do gênero Bothrops: relato de caso. Rev Soc Bras Med Trop 43: 602–604. PMID: 21085881

56. Bard R, Lima JCR de, Sá-Neto RP, Oliveira SG, Santos MC (1994) Ineficácia do antiveneno botrópico na neutralização da atividade coagulante do veneno de Lachesis muta muta: relato de caso e comprovação experimental. Rev Inst Med Trop Sao Paulo 36.