Trans R Soc Trop Med Hyg
doi:10.1093/trstmh/tru038

ORIGINAL ARTICLE

Epidemiology of snakebite and use of antivenom in Argentina

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Received 26 December 2013; revised 10 February 2014; accepted 10 February 2014

Background: The incidence and case fatality rate of snakebite in Argentina are poorly known.

Methods: The authors used questionnaires provided with antivenoms by the primary manufacturer of antivenoms in Argentina.

Results: A total of 8083 completed questionnaires was collected between 1978 and 1998. The annual incidence of snakebite was 1.8 bites per 100 000 inhabitants, with a high geographical heterogeneity; in the northern provinces of the country, the incidence can exceed 150 snakebites per 100 000 people per year. *Bothrops* (pit viper) bites predominated, accounting for 96.6% (6720/6957) of envenomations; bites from *Crotalus* (rattlesnake) accounted for 2.8% (195/6957), and bites from *Micrurus* (coral snake) for 0.6% (42/6957). Most patients were young men, who were generally bitten during agricultural activities, i.e. while working in the fields. Most snakebites (78.9%, 5852/7419) were to the lower limb, including 58.3% (4322/7419) to the foot. The case fatality rate was <0.04% (3/8083). Most envenomations (90%, 7275/8083) were treated with specific antivenom during the first 4 h after the bite. The median dose of antivenom was two vials for viper bites (*Bothrops* and *Crotalus*) and three vials for *Micrurus* bites.

Conclusion: These preliminary results should enable manufacturers to increase the availability of appropriate antivenom and health authorities to improve the management of snakebites where they are most common.

Keywords: Antivenom, Argentina, *Bothrops*, *Crotalus*, *Micrurus*, Snakebite

Introduction

The incidence and severity of envenomation remains an underestimated public health problem in many countries. Current estimates are often based on partial health reports or medical publications representing only the local picture, and not necessarily the national and regional situation.[1,2] A better understanding of the epidemiology of snake, spider bites, and scorpion stings[3] would improve the management of these accidents and enable manufacturers of antivenom to adjust production and distribution to fit a country's needs, and eventually reduce costs. Health personnel would benefit from appropriate training and patients would profit from earlier treatment. Many countries have recently made notification of envenomation mandatory, in accordance with the WHO recommendation.[4] However, according to the respective Ministry of Health websites, results are as yet available for only a few countries, including Argentina, Bolivia, Brazil, Costa Rica, Peru and Uruguay.

In Argentina, there is an abundant snake fauna. Of the 136 species described, 18 can cause envenomation of varying severity. They belong to three genera: *Bothrops* (pit vipers; 10 species), *Crotalus* (rattlesnake; one species) and *Micrurus* (coral snakes; seven species).[5] Although snakebites are reported throughout the country, the geographical distribution is heterogeneous, being related mainly to climatic and topographic diversity and the ecological niche of each species,[6] and to human activities, including agriculture.[7] The *Bothrops* genus is almost ubiquitous in Argentina, while *Crotalus* and *Micrurus* are more restricted in their distribution (Figure 1).

When a snake is encountered, the probability and severity of a bite depends on the size of the specimen, its means of venom

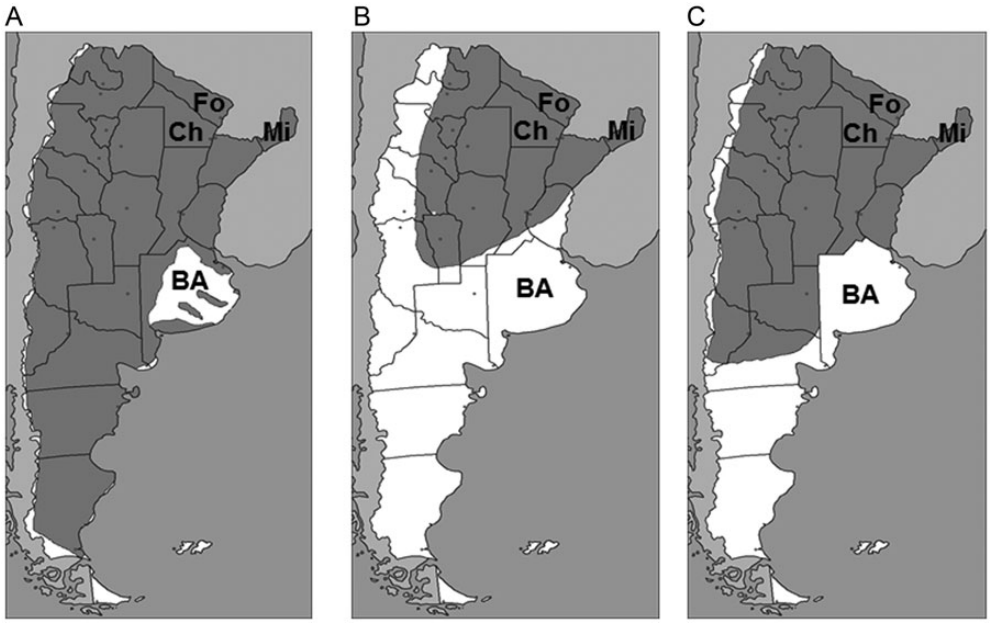

Figure 1. Distribution of venomous snakes in Argentina by genus. (A) The near-ubiquitous *Bothrops;* (B) *Crotalus;* (C) *Micrurus*; BA: Buenos Aires; Ch: Chaco; Fo: Formosa; Mi: Misiones.

delivery and the composition of the venom. Crotalinae (pit vipers, rattlesnakes) are solenoglyphous, with long fangs able to deeply inject large quantities of venom; Elapidae (coral snakes) are proteroglyphous, with shorter fangs but highly toxic venom.[8] *Bothrops* venom causes coagulation of the blood and induces necrosis, with functional sequelae; *Crotalus* venom is neurotoxic and myotoxic, leading to respiratory and kidney failure, and *Micrurus* venom is neurotoxic, causing respiratory paralysis.[9] All three genera can cause fatal accidents.

Antivenoms are manufactured by the Instituto Nacional de Producción de Biológicos (National Institute of Biological Production)-Administración Nacional de Laboratorios e Institutos de Salud (ANLIS) 'Dr Carlos G. Malbrán' (National Administration of Laboratories and Health Institutes), which is a public body. They are distributed throughout the country, even to Patagonian provinces in the far south where *B. ammodytoides*, a pit viper endemic to Argentina, occurs, except to the province of Buenos Aires; antivenoms are supplied to this region by a provincial manufacturer, the 'Tomás Perón' Biological Institute.[10] The antivenoms distributed by the Instituto Nacional de Producción de Biológicos are fragments $F(ab')_2$ in liquid form, obtained by salt precipitation, thermocoagulation and pepsin digestion of horse plasma. The antivenom against *Bothrops* is manufactured with the venom of *B. alternatus* and *B. diporus* (another one is produced for the province of Misiones with the addition of the venoms from *B. jararaca* and *B. jararacussu*). The antivenom against *Crotalus durissus terrificus* is monovalent and that against *Micrurus* is manufactured with a mixture of several Argentinian *Micrurus* venoms. In addition, a private laboratory (Biol) produces a lyophilized polyvalent antivenom against *Bothrops* and *Crotalus*, made from the venom of *B. alternatus*, *B. diporus* and *C. durissus terrificus*. Finally, the Central Laboratory of Public Health of the Province of Buenos Aires manufactures a bivalent anti-*Bothrops* antivenom (*B. alternatus* and *B. diporus*). The formulation is a liquid whole IgG precipitated by caprylic acid.

In Argentina, reported envenomations are the subject of a compulsory weekly notification.[11] However, the new system of data collection lacks information on the biting species, clinical symptoms, evolution of the envenomation (e.g. necrosis or sequelae and treatment). Accordingly, it was considered that it would be highly useful to conduct an epidemiological study, based on medical records returned from health centres during the period 1978 to 1998, to obtain information on which to base prevention strategies and, by evaluating the use of antivenoms, develop a rational programme of antivenom production.

Material and methods

For many years, standardised questionnaires were distributed along with antivenoms. Completed questionnaires returned to the Instituto Nacional de Microbiología 'Dr Carlos G. Malbrán' (since 1997, Instituto Nacional de Producción of Biológicos-ANLIS 'Dr Carlos G. Malbrán') indicated which stocks of antivenom needed to be replaced. The data covered all of Argentina, with the exception of the province of Buenos Aires; the latter was supplied with antivenoms produced locally and never input medical records to the national system. The questionnaires were reviewed by two of the authors (JAD and RF).

We calculated the incidence of snake bites in each province by sex, age, occupation, anatomic site of the bite, season, circumstances of the accident, geographic location of the bite, time between the bite and treatment, and antivenom administration. In most cases, the questionnaire stated what the patient was doing (work or leisure activity) at the time of the bite (e.g. farmers working in the fields, or children playing). The sex ratio was the male/female ratio. The age ratio was calculated by dividing the number of adults by the number of children. The different occupational categories were: housewife; farmer; migrant worker;

child <5 years old; child aged 5–11 years; and child aged 12–17 years. For the data analysis by age group, we considered 5-year age brackets (1–5, 6–10, 11–15, etc.), with individuals aged ≥16 years considered to be adult. Geographic locations of the bite were: at home; around home (outside the house, but in places near home where daily tasks are carried out); and rural areas (where individuals engaged in activities away from the home and its environs). However, the questionnaire lacks information regarding the time of bite.

Demographic data were based on Argentina's national census for 1991 – the median year of the study period[12] – to express the specific rates by sex and age.

The results were analysed by the χ^2 test, or Fisher's exact bilateral test when the expected value was less than five. The significance threshold of statistical tests was $p<0.05$, unless otherwise stated.

Because medical records reviewed did not always contain complete information, we specified for each variable the number from which the calculations were made. The data were processed anonymously for ethical reasons. Because of age of the data and the absence of personal or confidential information, the approval of an ethics committee was not required.

Results

The Institute 'Dr Carlos G. Malbrán' received, between 1 January 1978 and 31 December 1998, 8083 clinical records of snakebites treated with 'Dr Carlos G. Malbrán' antivenom.

Most envenomations occurred in the northern part of Argentina, particularly in the north-eastern provinces of Chaco, Misiones and Formosa (Figures 2 and 3).

In most questionnaires (6957/8083, 86.1%) the snake responsible for the bite was identified. The genus *Bothrops* represented 96.6% (6720/6957) of accidents, *Crotalus* 2.8% (195/6957), and *Micrurus* 0.6% (42/6957). *Bothrops* bites predominated in all provinces. *Crotalus* bites were more common in the province of Formosa. *Micrurus* bites were rare everywhere.

The sex ratio (SR) was based on 7979/8083 records (98.7%). Males were more often bitten than females, regardless of the snake concerned. The male:female ratio was 2.2:1, 3.3:1 and 2:1 for the genera *Bothrops*, *Crotalus* and *Micrurus* respectively. The SR was highly uneven in people aged 10–80 years, and least uneven in those aged 1–5 years. The highest age-specific incidence occurred in the age group 11–15 years (948/6811, SR=2), the next highest in those aged 16–20 years (743/6811, SR=2.1) and the next in 51–60 year-olds (643/6811, SR>3). The age-specific incidence of bites by each genus of snake differed significantly according to age (Table 1). *Crotalus* bites were equally distributed in all ages, while *Micrurus* and *Bothrops* bites predominated in the young (those under 25 years).

The site of the bite was noted in 7419/8083 patients (91.8%). The lower limb was the most often affected, but the site of the bite varied significantly according to age and sex (Table 2) and snake genus (Table 3). Adult males were more often bitten on the hand. The extremities (hands and feet) were more often affected by *Bothrops* than by the other two snake genera.

The location of the bite was recorded for two-thirds of cases (3509/8083) (Table 4). Most accidents occurred in rural areas away from home (2490/3509, 70.9%); the next most frequent (772/3509, 22%) were accidents at home, which occurred particularly during children's games.

The season when the bite occurred was specified for 99.4% of cases (8031/8083). Bites were more frequent in summer, between December and April (Figure 4), with a peak in February and March.

Whatever the genus of snake responsible, most envenomations (88.9%, 5394/6069) were treated within 4 h of the bite. With regard to snakebite treatment, the type of snake made no significant difference except for the dose of antivenom, which

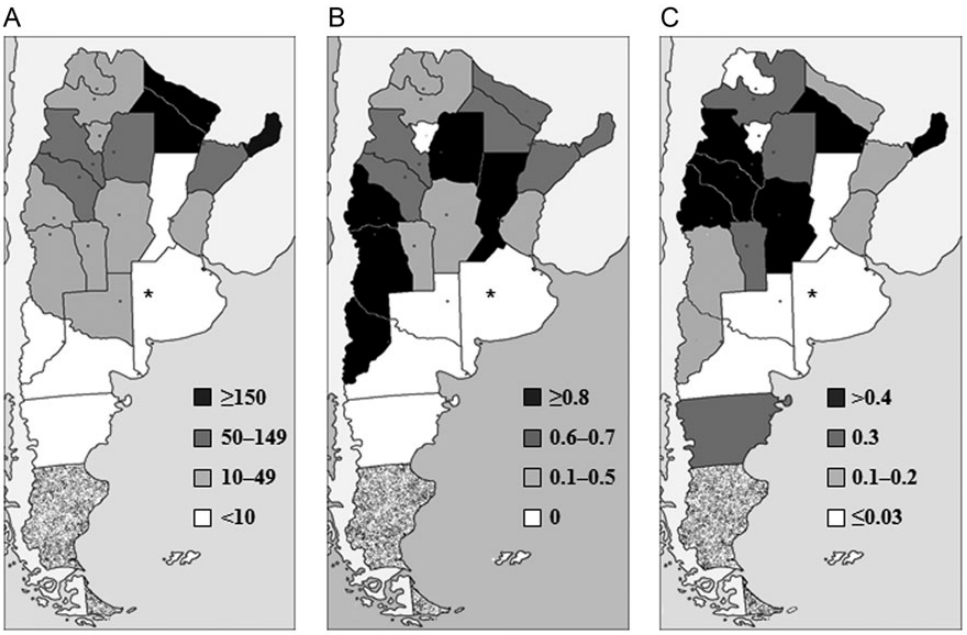

Figure 2. Incidence of snakebite by province in Argentina, 1978–1998, per 100 000 people, for (A) *Bothrops* (highest incidence); (B) *Crotalus*; (C) *Micrurus*.

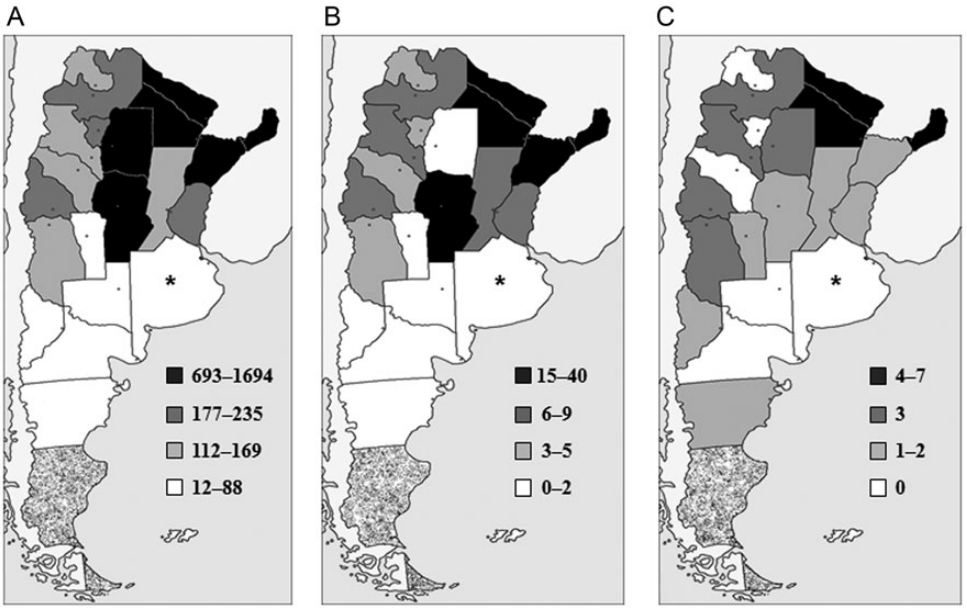

Figure 3. Number of snakebites by province in Argentina, 1978–1998, per 100 000 people, from (A) *Bothrops*; (B) *Crotalus*; (C) Micrurus.

Table 1. Notifications of snakebite (1978–1998) in Argentina, by age and snake genus

Age (years)	*Bothrops*	*Crotalus*	*Micrurus*	Total
1–5	502	15	5	522
6–10	730	8	6	744
11–15	935	9	4	948
16–20	729	11	3	743
21–25	565	13	2	580
26–30	481	15	0	496
31–35	420	14	3	437
36–40	453	9	0	462
41–45	402	11	3	416
46–50	317	7	2	326
51–55	333	13	1	347
56–60	284	12	0	296
61–65	198	8	1	207
66–70	97	6	1	104
71–75	94	4	0	98
76–80	38	7	0	45
≥81	35	5	0	40
Total	6613	167	31	6811

differed depending on the genus (Table 5). Of the patients who received *Bothrops* antivenom, most (60%, 4017/6681) were given up to two vials, 32% (2140/6681) received three or four vials and 8% (524/6681) five vials or more. Most bites from *Crotalus* and *Micrurus* (respectively 91% [179/197] and 95% [35/37]) were treated with up to five vials of antivenom. The median (and 25% and 75% quartiles) of the number of antivenom vials administered after bites from *Bothrops*, *Crotalus* and *Micrurus* were, respectively, two (1,3), two (1,4) and three (2,3). Antivenom doses were significantly different between the genera, showing an increasing trend with *Crotalus* and *Micrurus* in relation to *Bothrops*.

Three deaths were reported (3/8083, 0.04%), all occurring as a result of a *Bothrops* bite.

Discussion

The survey covered a 20-year period, and reported an annual average of 404 snakebites treated by an antivenom produced by the Institute 'Dr Carlos G. Malbrán'. The annual incidence of snakebites in Argentina was shown by the survey to be nearly two bites per 100 000 inhabitants; a high geographical heterogeneity was marked by the predominance of envenomations in the north of the country (Figures 2 and 3). The annual variation of incidence was not specified because the questionnaires were not provided annually, but only when new antivenoms were ordered, which did not corresponded to the dates of snakebites.

The analysis of these relatively old data (1978–1998) may be a useful basis for future studies on trends and the impact of interventions. However, several epidemiological studies in Europe[13,14] and Africa[15] have shown the national incidence of snakebites to be quite robust and consistent over time. In contrast, mortality can significantly decrease in response to improved health management, particularly accessibility of health centres, development of more effective antivenoms, better training of medical personnel to treat envenomation and, possibly, earlier hospital attendance by the patients.

The frequency of identified snakes and the distribution of species responsible for the bites were similar to those observed in other South American countries.[16–20] The abundance of *Bothrops*, their aggressiveness and probably superior adaptation to the human environment may explain the higher incidence of bites by species of this genus in periurban and rural areas.[21–23]

Table 2. Notifications of snakebite (1978–1998) in Argentina, by bite site and age

Bite site	No. of patients	Gender		Sex ratio	Child (C)	Adult[a] (A)	Age ratio
		Female (F)	Male (M)	M/F	(n)	(n)	A/C
Foot	4322	1398	2924	2.1	665	3101	4.7
Leg	1530	525	1005	1.9	298	1030	3.5
Hand	1352	320	1032	3.2	176	1027	5.8
Arm	141	35	106	3.0	24	97	4.0
Head	37	7	30	4.3	17	17	1.0
Thorax	37	7	30	4.3	18	18	1.0
Total	7419	2292	5127	2.2	1198	5290	4.4

[a] Individual aged ≥16 years.

Table 3. Notifications of envenomation (1978–1998) in Argentina by bite site and snake genus

Bite site	*Bothrops*	*Crotalus*	*Micrurus*	Total
Foot	3119 (61.2)	97 (54.4)	16 (44.4)	3232 (57.2)
Leg	1102 (20.3)	52 (28.1)	11 (30.5)	1165 (20.6)
Hand	1038 (19.1)	27 (14.6)	7 (19.4)	1072 (19.0)
Arm	125 (2.3)	15 (8.1)	1 (2.8)	141 (2.5)
Head	18 (0.3)	2 (1.1)	1 (2.8)	21 (0.4)
Thorax	18 (0.3)	2 (1.1)	0 (0)	20 (0.3)
Total	5421 (100)	185 (100)	36 (100)	5651 (100)

Values are n (%).

Crotalus' rattle emits a sound that may warn any potential victim,[24] and in Argentina *Micrurus* snakes are semi-nocturnal and burrowing, and therefore unlikely to be in contact with humans.[25] The scarcity and characteristics of *Micrurus* bites in Argentina have been described elsewhere.[26] Accidents occur when the snake is discovered in its resting place, which may explain why it is mostly children who are bitten by species of this genus;[26] unlike a knowledgeable adult, they may be more likely to be attracted to a brightly coloured, less intimidating snake.

The seasonal distribution of the bites followed the pattern of agricultural activities. However, the distribution of hazard rates showed differences that might contribute to the development of strategies for surveillance and prevention.

The characteristics of the victims provide further more robust evidence, with a predominance among them of young males undertaking agricultural activities. Most bites were to lower limbs, emphasising the risk of accidents while walking or during non-mechanised farming activities. Therefore, labour regulations should encourage the wearing of shoes and other appropriate protective clothing.[27] Community participation in the preparation of prevention information will help ensure that recommendations made are culturally acceptable and appropriate to the climatic conditions.

Most envenomations were managed within a reasonable time (less than 4 h), which could explain the effectiveness of the treatment and low mortality. The therapeutic dose of antivenom is controversial. Its calculation is based on the amount of venom inoculated by the snake, which is difficult to determine. Although the capacity of the venom glands can be evaluated by venom extraction,[28] the yield of venom inoculated during a defensive bite may be only 10–50% of that capacity.[8,29–31] More precise assessments have been attempted by determining the venom concentration in patients bitten by a snake.[32]

In Argentina, the therapeutic dose is based on empirical data and studies performed in Brazil,[33] which should be confirmed by local clinical studies. The neutralising capacity of *Bothrops* antivenom used in Argentina during the study period (1993–1998) was 47±5 mg/vial for the venom of *B. alternatus* and 40±5 mg/vial for that of *B. diporus*. The average neutralising capacity of *C. durissus terrificus* antivenom was 20±9 mg/vial, and that of *Micrurus* antivenom was 7.5±2.5 mg/vial (all neutralising capacity data from Dolab, unpublished data). The increasing doses of antivenom required according to the genus of snake reflects the decreasing neutralising capacity of antivenoms against *Bothrops*, *Crotalus* and *Micrurus* respectively.

The use of antivenom and the dose administered seems to be determined more by availability than by the neutralising capacity of antivenoms or the clinical symptoms or therapeutic needs of the patient. The production of viper antivenoms (*Bothrops* and *Crotalus*) poses no difficulty, and these agents are readily available. However, the production of *Micrurus* antivenom is more challenging because these snakes are difficult to keep and yield only small amounts of venom. Accordingly, the poor availability of *Micrurus* antivenom limits its use, regardless of actual needs.

In the case of viper envenomation, one might consider the use of a bivalent (*Bothrops* and *Crotalus*) antivenom. However, the benefit is limited because in Argentina the symptoms of *Bothrops* and *Crotalus* bites easily distinguish them. In addition, the low incidence of *Crotalus* bites compared to those by *Bothrops* does not justify production of large amounts of anti-*Crotalus* antivenom, even in

Table 4. Notifications of snakebite (1978–1998) in Argentina, by bite location and snake genus

Bite location	*Bothrops*	*Crotalus*	*Micrurus*	Undetermined	Total
At home	748 (27.7)	15 (15.4)	4 (33.3)	5 (0.7)	772 (22.0)
Around home	239 (8.8)	6 (6.2)	0 (0)	2 (0.2)	247 (7.1)
Fields	1717 (63.5)	76 (78.3)	8 (66.7)	689 (99.1)	2490 (70.9)
Total	2704 (100)	97 (100)	12 (100)	696 (100)	3509 (100)

Values are n (%).

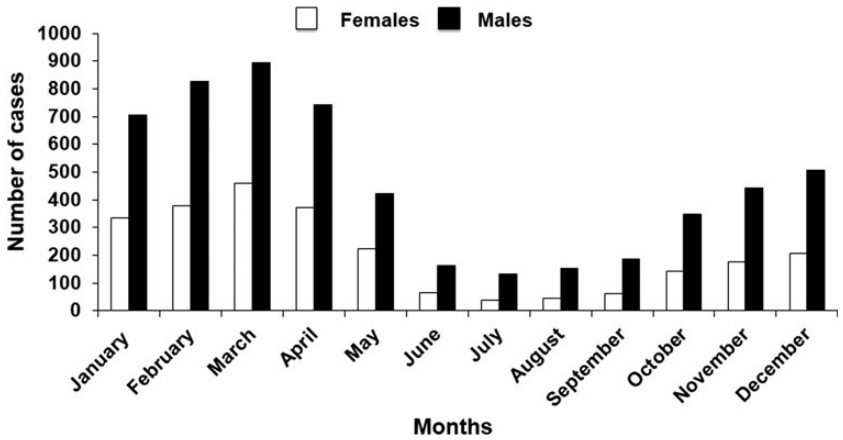

Figure 4. Notifications of envenomation in Argentina, 1978–1998, by season (note the summer distribution of cases) and by gender of patients (n=8031).

association with a bothropic antivenom. Accordingly, Argentina's Ministry of Health recommends use of the simpler and cheaper to manufacture monovalent antivenom.[9] Nevertheless, although long considered less effective, polyvalent antivenoms are now widely used because they do not require the identification of the snake responsible for the bite and they seem to have a better neutralising capacity; the latter is linked to high paraspecificities that potentiate the immune response of animals used for their manufacture.[34,35] Regarding *Micrurus* envenomations, the incidence is even lower, which does not justify a high rate of production. In addition, the production of antivenoms against elapids is more difficult than the manufacture of viper antivenoms.

Of the three recorded deaths in our survey, all were caused by *Bothrops* bites. In two cases (a 35-year-old woman bitten in Chaco province and a 53-year-old man bitten in Formosa province), the antivenom was administered late (after >24 h). A 12-year-old boy died after receiving the wrong antivenom; he was given a crotalic antivenom designed for *C. durrissus terrificus* instead of antivenom against *Bothrops*.

The Directorate of Statistics and Health Information (DEIS) of Argentina's Ministry of Health reported a median of five deaths annually (range two to seven) for the period 1994–1998, which included those individuals who did not receive antivenom (Roodt et al., unpublished data). Even including the latter, taking into account the geographical location and climatic characteristics, rates of morbidity and mortality from snake envenomation are relatively low in Argentina.[36,37] However, in some provinces snake bite remains a major public health problem with significant morbidity, especially in regard to the socioeconomically vulnerable population with limited access to health services.

The main limitation of this study is that it considered only envenomations treated with 'Dr. Carlos G. Malbrán' antivenoms. Untreated cases or those treated with other antivenoms were ignored, particularly in the province of Buenos Aires; the latter accounted for 13 million inhabitants (nearly 40% of the national population) during the study period. However, the Buenos Aires region is highly urbanised (almost 90%, during the study period), so the incidence of snakebite is low, not exceeding 5% of total snakebites in Argentina (de Roodt et al., 2010).[36] In addition, our findings confirm those of other recent independent studies reporting less than 1000 bites per year during the study period and a low mortality between 2007 and 2011.[36,37] The Instituto Nacional de Producción of Biológicos-ANLIS 'Dr Carlos G. Malbrán' is the main source of antivenoms across the country, except in the province of Buenos Aires, which allows us to consider that, even if the data are not exhaustive, they represent the overall situation in Argentina. Our study undoubtedly covered most of snakebites that occurred in Argentina during the period 1978–1998. Some patients in the studied provinces might not have presented for treatment, or might have received antivenom from different sources, but they most probably represented a low proportion of cases.

Table 5. Doses of antivenom administered for snakebite (1978–1998) in Argentina by antivenom type

No. of vials	*Bothrops* antivenom	*Crotalus* antivenom	*Micrurus* antivenom	Total
1	2184	64	8	2256
2	1833	42	9	1884
3	1541	37	15	1593
4	599	16	2	617
≥5	524	38	3	565
Total	6681	197	37	6915

Conclusion

This study showed that, in Argentina, snakes of the genus *Bothrops* are responsible for more than 95% of snakebites; bites by *Crotalus* represented nearly 3% of the total and those by *Micrurus* less than 1%. The greatest risk was concentrated in areas of subtropical forest and in the northern part of the country, especially between December and April. Young adults, mainly male rural workers, are mostly bitten by snakes belonging to the genera *Bothrops* and *Crotalus*, while children under 12 years of age are more vulnerable to the much less frequent *Micrurus*.

At any age, the parts of the body most affected are the feet and hands, with a higher proportion of bites to the head, chest and legs in children than in adults.

Most bites (90%), irrespective of the genus of the snake, were treated within 4 h, with satisfactory results. Notification of bites or stings from venomous animals has been compulsory in Argentina since 2007,[11] and computerisation of the centrally held records allows quick access to information, although information now retrieved is less diverse than that presented here. In addition, free treatment and distribution of antivenoms by the health authorities covers the entire country. However, an effort should be made to improve the generic diagnosis of the snake responsible when bites occur, to allow the use of appropriate antivenom. At the same time, health authorities should reconsider the manufacture of bivalent antivenom (i.e. *Bothrops*+*Crotalus* antivenom) in addition to the monovalent antivenoms.

This study will help guide the appropriate allocation of resources and implementation of snakebite prevention strategies and also the definition of priority criteria for epidemiological research, including its location, taking into account the known range of snakebites in the country.

Authors' contributions: JAD, ARdR and RF conceived the study; JAD and ARdR designed the study protocol; RF and ARdR collected the data; JAD, ARdR, EHdT, SIG, RF and ODS carried out the epidemiological and clinical analysis; JPC participated in the analysis and interpretation of results. JAD, ARdR and JPC drafted the manuscript. EHdT, SIG and ODS critically revised the manuscript for intellectual content. All authors read and approved the final manuscript. ARdR and JPC are guarantors of the paper.

Acknowledgements: Engineer Raúl Funes died before the preparation of the manuscript. He instigated the project and was active in collecting and analysing the data. Without him, this project would not exist and we dedicate this work to him.

Funding: The study was funded by Instituto Nacional de Producción de Biológicos-ANLIS 'Dr Carlos G. Malbrán', Ministry of Health, Buenos Aires, Argentina.

Competing interests: None declared.

Ethical approval: Not required.

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