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Venom yield and its relationship with body size and fang separation of pit vipers from Argentina

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ABSTRACT

The amount of venom that a snake can inject is related to its body size. The body size is related to head size and to the distance between fangs. To correlate snake body size, distance between fangs and distance between puncture wounds with the venom yield (and consequently with the venom dose potentially injected in a single snakebite), we studied these variables in two species of public health importance in South America, *Bothrops* (*Rhinocerophis*) *alternatus*, and *Crotalus durissus terrificus*. In all cases a positive correlation was observed between body length, fang separation distance, distance between puncture wounds and venom yield, with a regression coefficient over 0.5 for *Bothrops alternatus* and over 0.6 for *Crotalus durissus terrificus* in all cases, being the relation distance between punctures wounds and venom yield of 0.54 and 0.69 respectively. The difference between fang separation and puncture separation was never greater than 30%, with a mean difference around 13%. The strong relationships between body size, fang separation and venom yield may be useful for planning potential venom production in serpentariums. In addition, because puncture mark separation gives an approximate idea of the size of the snake, this provides a rough idea of the size of the snake that produced a bite and the potential amount of venom that could have been injected.

1. Introduction

The amount of venom that can be obtained by milking snakes is related to several factors. Among these, the size of the snake is one of the most important (de Roodt et al., 1998; Mirtschin et al., 2002; Hayes et al., 2002; Furtado et al., 2006; McCleary and Heard, 2010). It is known that the snake's length and body condition are related to venom production (Glenn et al., 1972; Fix, 1980; Tun and Cho, 1986; de Roodt 2002, Mackessy et al., 2003, 2006; McCue, 2006). In addition, the total body size and venom production are related to head size (Mirtschin et al., 2002) and distance between fangs (Sabbatini et al., 2004; de Roodt et al., 2012). These relationships illustrate the importance of the size of the snake in the amount of venom produced, and consequently the amount of venom that can be injected in a bite. Thus, the severity of envenomation can be related to the size of vipers (Ribeiro et al., 2001; Janes et al., 2010).

Nevertheless, the relationship of the snake size and the distance between fangs or punctures, with the potential amount of venom that can be obtained by milking or injection in a single snakebite, has not been systematically studied, at least for South American snakes. This absence of information is surprising, because these data could have practical value: first for venom production in a serpentarium, where venom yield predictions may help to determine the number of vipers acquired for high-quantity venom production. Second, in the field, separation between puncture marks could indicate the size of the snake that produced a bite and the puncture separation may be the only evidence physicians often have about the snake that delivered a bite. Considering that pit viper envenomation is a medical emergency requiring immediate attention and use of specific antivenom (Reid and Theakston, 1983; WHO, 2010), these data could be of particular value during the early phase of treatment, in the absence of reliable information regarding

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the size of the snake responsible for the bite. Although the quantity of venom injected in a bite is variable and not always predicted by the body size it is obvious that a newborn or young specimen possesses in its glands less venom relative to the content of an adult's. The fraction of stored venom that is injected in a single viper bite also varies, with a maximum around 30%–50% of the venom gland content (Tun and Cho, 1986; Kochva, 1987; Fernandes et al., 1993). Nevertheless, big snakes have more venom than smaller snakes (de Roodt et al., 1998; Mirtschin et al., 2002) and big snakes produce more severe envenomation than smaller snakes (Ribeiro et al., 2001; Janes et al., 2010).

For these reasons, we studied two species of vipers of great public health importance in South America: *Bothrops alternatus* ("yarará grande", "crucera", "urutú", "víbora de la cruz") and *Crotalus durissus terrificus* ("*mboi-chiní*", "víbora de cascabel" [rattlesnake]) (FUNASA, 1999; Carreira et al., 2006; Melgarejo, 2003; Ministerio de Salud, 2014). to relate the distance between fangs, the puncture distance and body size to venom yield.

2. Materials and methods

2.1. Study animals and housing

Data from 102 specimens of *Bothrops (B.) alternatus*, and 38 of *Crotalus (C.) durissus (d.) terrificus* were analyzed. Specimens were from different geographic regions of the country and were located in the Serpentarium of the National Institute for Production of Biologicals of the National Administration of Health Institutes and Centers of the Ministry of Health of Argentina (INPB-ANLIS "Dr. Carlos G. Malbrán"). All were healthy specimens maintained in plastic boxes, at 27 °C, with light-dark cycles of 12 h. Animals received drinkable water *ad libitum* and were fed with mice or rats (depending on size) every 15 days. Animal care was based on published guidelines for care of reptiles in academic institutions (Pough, 1991).

2.2. Venom extraction

Venom was manually extracted, immediately vacuum dried and stored at −20 °C, from animals with 15 days' fast. When analyzing venom yield, we only included samples for which no loss of venom occurring during milking. A subgroup of 10 *B. alternatus* was milked selectively by individual fangs, to determine any differences in venom yielded by right vs. left venom glands.

2.3. Variables measured

All measurements were recorded and analyzed descriptively (mean, median, quartiles, standard deviation and 95% confidence intervals). In addition the correlation between the variables was studied by linear regression. The measures considered were: ***Body weight*:** snakes over 250 g were weighed in a mechanical scale with sensitivity of 1 g. Small snakes (under 250 g) were weighed in an electronic balance with a sensitivity of 0.1 g. The body weight was expressed in g. ***Body length*:** a metric rule from 0 to 150 cm was affixed to a solid surface where the snakes were extended and measured. When the length was greater than 1.5 m, a portable ruler was used for the extension. The body length was expressed in cm. ***Body condition*:** this was defined as the ratio body weight/body length and expressed as g/cm. ***Venom yield*:** dried venoms were weighed using an analytical scale with a sensitivity of 0.1 mg. Venom yield was expressed in mg. ***Distance between fangs* (DBF):** this measure was obtained using a digital caliper (sensitivity 0.01 mm). Fangs were extended with a rounded metallic bar in order to take the measure in the maximal angle of rotation and to avoid injury to the snake's oral mucosa. Although the DBF was taken in the base, midpoint and tips of the fangs, measures used for calculations were those of the midpoint of the fang, which did not show significant differences ($p > 0.52$; $t < 0.65$) from the distances between the tips of the fangs (data not shown). Measurement at the midpoint is safer and faster for the operator. The DBF was expressed in cm. ***Distance Between Punctures* (DBP):** this was determined by measuring the marks between punctures. Snakes (20 of each species) were stimulated with a plastic cylinder (around 15 cm diameter) covered with a plastic soft envelope (Parafilm NR). Immediately after each bite, the separation between the marks of the fangs was identified and measured with a caliper *in situ*. The DBP of each snake was estimated as the mean of the several bites (3–6) and it was expressed in cm. Only snakes that readily completed at least three measurable bites within a period of 5 min were included in the study. Relationships between DBF and DBP were analyzed using linear regression, and the mean and maximum differences (in percentage) between DBF and DBP were calculated for each species.

2.4. Data analysis

Descriptive results were expressed as mean ± standard deviation or mean and 95% confidence interval when appropriate. To evaluate the normality of the distribution of data, the Kolmogorov–Smirnov test was used. Relationships between the different variables (venom yield, body length, DBF, DBP) were studied by simple and multiple linear regression. To assess the fit of the studied variables to a simple linear regression model, data were analyzed by a polynomial regression fit through the evaluation of the significance of the coefficients of each model (linear, quadratic and cubic).For the multiple regression model, a stepwise approach was used to decide the order of predictors in the model. To compare the fit to the models obtained by simple and multiple linear regression analysis, the Akaike Information Criterion (AIC) was used for the most relevant variables.

To correlate the venom yield with DBF and DBP (Fig. 3a and b) and with body length (Fig. 3c and d), data were grouped into intervals and the venom yields included in each interval were plotted. Interval grouping shows how the DBF or DBP or the body length can help the interpretation of the venom that can be yielded from snakes with different size or that caused different DBP. Although these data can be obtained from the regression lines, results presented in groups of intervals shows the venom yield that can be expected from snakes of determinate size. Because only 20 snakes per species had been used in the DBP determination, the theoretical DBP was estimated. Mean theoretical DBP (tDBP-m) and maximum theoretical DBP (tDBP-mx) were obtained by adding the measured DBF to its product with the calculated mean and maximum percent differences for the species. These values were compared with other variables.

To evaluate the predictive value regarding the venom yield, in addition, we applied the linear relationship $y = m.x + b$, where y = dependent variable (the venom yield) and x = independent variable (body length or DBF), m = the slope and b = the Y intercept. We studied the relationship of body length with venom yield in the vipers up to 100, 120 and 140 cm and between the venom yield and the SBF up to 1.5, 2.0, 2.5 and 3.0 cm.

Prism 4.0 (Graph Pad, CA, USA) software was used for statistical analyses.

3. Results

- Characteristics of the snakes studied are summarized in Table 1.