

Treatment of snake bites by *Bothrops* species and *Lachesis muta* in Ecuador: laboratory screening of candidate antivenoms

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Abstract

Bothrops xanthogrammus/asper, *B. atrox* and *Lachesis muta* are probably responsible for most cases of severe envenoming in Ecuador. In recent years, the most widely used antivenom ('Myn' Ronti, imported from Mexico) has proved clinically ineffective. There is an urgent need to identify an effective alternative for clinical testing. Five antivenoms with activity against *Bothrops* venoms were compared using standard World Health Organization rodent and *in vitro* assays: (i) 'Myn', Ronti Mexico SA ('*B. atrox*', '*Crotalus terrificus*'), (ii) Instituto Butantan (*Bothrops* polyvalent, Brazil), (iii) Instituto Nacional de Higiene y Medicina Tropical (*Bothrops* polyvalent, Ecuador), (iv) Instituto Nacional de Salud (*B. asper*, *C. durissus* and *Lachesis muta*, Colombia), and (v) Laboratorios Probiol (*Bothrops*, *Lachesis* and *Crotalus*, Colombia). The venoms against which these antivenoms were tested were Ecuadorian *B. atrox*, *B. asper* and *B. xanthogrammus*. Brazilian antivenom proved to be the most effective, followed by the Ecuadorian and Colombian antivenoms. Mexican antivenom was completely ineffective in neutralizing the lethal effects of Ecuadorian *Bothrops* venoms. Monospecific Brazilian *L. muta* antivenom (Instituto Butantan) proved effective against Ecuadorian *L. muta* venom, but the Colombian polyspecific antivenoms did not. Clinical trials of Brazilian and Ecuadorian antivenoms are planned in the Amazon region of Ecuador in the near future.

Keywords: snake bite, antivenoms, *Bothrops* spp., *Lachesis muta*, Ecuador

Introduction

In Ecuador, snake bite reaches its highest incidence east of the Andes. In this Amazonian region (El Oriente) the principal species responsible for bites include *Bothrops atrox* (barba amarilla), *Bothriopsis bilineata smaragdina* (Amazonian tree viper), *Bothriopsis taeniata* (Castelnau's pit viper), and *Lachesis muta* (Bushmaster). Victims are mainly indigenous tribal groups such as the Huarani (Waorani) (LARRICK *et al.*, 1978) and Shuar and immigrant gold miners and farmers. West of the Andes, in the coastal region (La Costa), bites by *Porthidium nasutum* (hog-nosed pit viper) are common, but not severe, but in the north-west, *Bothrops xanthogrammus/asper* (equis or culebra X) (Fig. 1) is the major cause of severe

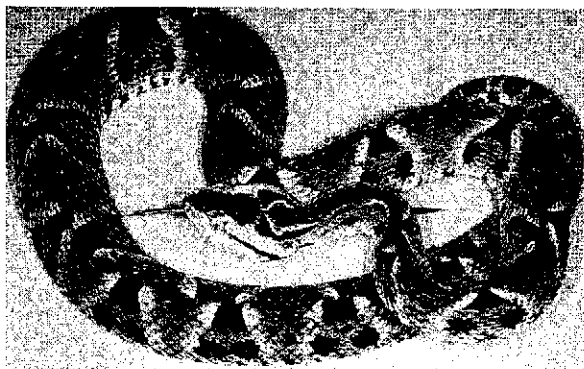


Fig. 1. *Bothrops xanthogrammus/asper*, the major cause of severe snake bite in the coastal region of Ecuador.

envenoming, although *L. muta* also causes some bites in the Montanas de Muisne and Rio Cayapas region. There are undoubtedly many deaths from snake bite in Ecuador, but mortality data for the country as a whole are still unavailable (SWAROOP & GRAB, 1954).

Antivenom is expensive (average retail cost, US \$20 per ampoule) and scarce. The National Institute of Hy-

giene and Tropical Medicine in Guayaquil currently produces only about 800 ampoules per year raised against the venoms of '*B. asper*', '*B. xanthogrammus*' and *B. atrox*. The status of '*B. xanthogrammus*' and '*B. asper*' in Ecuador is controversial. Most older references cite both species as occurring west of the Andes. Recent research has revealed that only one large species of *Bothrops* occurs west of the Andes in Ecuador (CAMPBELL & LAMAR, 1992; SCHATTI & KRAMER, 1993). The oldest name for this form is *B. xanthogrammus* (COPE, 1868). SCHATTI & KRAMER (1993) regarded it as a subspecies of *B. atrox*, and restricted its range to western Ecuador and south-western Colombia. However, multivariate analysis of morphological characters by one of us (W. W.) suggested that '*B. xanthogrammus*' includes the Central American populations previously known as '*B. asper*' (see CAMPBELL & LAMAR, 1989), and indicated no conspecificity with *B. atrox*. Further evidence for the conspecificity of '*B. xanthogrammus*' and '*B. asper*' is the production of F2 progeny ('*B. xanthogrammus*' × '*B. asper*') by one of us (A.F.L.) and the observation that their venoms are identical by sodium dodecylsulphate-polyacrylamide gel electrophoresis (SDS-PAGE) (Figure). To supplement the small indigenous production of antivenom, the distribution of which is virtually confined to the coastal region and Loja, several different antivenoms have, at different times, been imported from Mexico, Brazil, Costa Rica, Colombia and the USA. For example, during one period of 19 months, 3000 ampoules of 4 different types of antivenom were imported at a total cost of US \$27 792. Many Ecuadorian doctors have come to doubt the clinical efficacy of some of these antivenoms, especially the one manufactured in Mexico which has been most widely distributed by the Ministry of Public Health in recent years. As a result, alternative methods of treatment, such as electric shock (GUDERIAN *et al.*, 1986) have gained popularity, despite their inherent dangers and the lack of evidence of their effectiveness.

The aim of the present study was, therefore, to assess, using rodent and *in vitro* assays approved by the World Health Organization (WHO, 1981; THEAKSTON & REID, 1983), a range of different antivenoms of possible use for treating cases of snake bite in Ecuador, 4 of which are

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¹The abbreviation *B.* refers to *Bothrops* throughout this paper.

currently in use in the country. It was hoped to identify promising candidate antivenoms for clinical testing in double-blind randomized trials in Ecuador.

Materials and Methods

Venoms

Pooled lyophilized venoms from 26 specimens of *B. atrox* (from Puyo and Tena), 12 specimens of '*B. xanthogrammus*' (from Bucay and Pallatanga), 23 specimens of '*B. asper*' (from South Esmeraldas, Costa) and a single specimen of *L. muta* (from Manabi) were kindly donated for the study by the National Institute of Hygiene and Tropical Medicine, Guayaquil. The samples of the 3 *Bothrops* venoms were aliquots of batches of pooled venom used in Ecuador for the preparation of the national antivenom (*siero antifidico*; THEAKSTON & WARRELL, 1991). Venoms were stored at 4°C in a dark bottle before use, and all solutions were prepared fresh for injection by dissolving them in physiological saline.

The three Ecuadorian *Bothrops* venoms were compared by subjecting them under non-reducing conditions to 15% SDS-PAGE (LAEMMLI, 1970).

Antivenoms

(i) *Suero antiolidico* (lot 17, expiry 5/95), produced by the Instituto Nacional de Higiene y Medicina Tropical 'Leopoldo Izquieta Perez', Guayaquil, Ecuador is raised against the venoms of Ecuadorian *B. atrox*, '*B. asper*' and '*B. xanthogrammus*'. According to the manufacturers, it provides cover against *Bothriopsis bilineata*, *B. taeniata* (formerly known as *B. castelnaudi*), *P. nasutum* and *Bothriechis schlegeli* (eyelash viper). It is a liquid ammonium sulphate-precipitated pepsin-digested preparation, 10 mL per ampoule.

(ii) *Suero antifidico polivalente* 'Myn' (lot R9H01, expiry 16/8/94) produced by Ronti Mexico SA and distributed by Grupo Pharma S.A. de C.V., Zapata Laboratories, Mexico City, Mexico. According to the manufacturer it is raised against the venoms of Mexican '*B. atrox*' and '*Crotalus terrificus*' (tropical rattlesnake), which do not occur in Mexico (see Discussion). The antivenom is produced as a lyophilized 10 mL salt-precipitated pepsin-digested preparation. The manufacturers claim that it neutralizes the venoms of all Mexican, Central and South American *Bothrops* and *Crotalus* species (THEAKSTON & WARRELL, 1991).

(iii) *Soro antibiotopico* (batch 9305088, expiry 5/96) produced by the Instituto Butantan, São Paulo, Brazil, is a polyspecific *Bothrops* antivenom raised against a mixture of venoms of Brazilian species (*B. jararaca* 50%, *B. jararacussu* 8.25%, *B. alternatus* 8.25%, *B. moojeni* 8.25%, *B. neuwiedi* 8.25%, *B. cotiara* 8.25%, *B. pradoi* 8.25%; THEAKSTON & WARRELL, 1991), of which one, *B. moojeni*, is similar to *B. atrox* (see WUSTER *et al.*, in press). It is a liquid ammonium sulphate-precipitated, pepsin-digested preparation, 10 mL per ampoule.

(iv) *Antiveneno polivalente* (lot 070992, expiry 2/96), referred to as Colombian (INS) in this paper, produced by the Instituto Nacional de Salud, Bogota, Colombia, is raised against Colombian *B. asper*, *C. durissus* and *L.*

muta venoms. It is a liquid ammonium sulphate-precipitated preparation, 10 mL per ampoule (THEAKSTON & WARRELL, 1991).

(v) *Sueros antifidicos liofilizados* (lot 12928, expiry 5/97), referred to as Colombian (LP) in this paper, produced by Laboratorios Probiol, Bogota, Colombia, is raised against the venoms of Colombian species of *Bothrops*, *Crotalus* and *Lachesis*. It is a lyophilized salt-precipitated pepsin-digested preparation, 10 mL per ampoule after reconstitution.

(vi) *Soro antilaquetico* (batch 9207170, manufactured 2/7/92, expiry 15/7/93) produced by the Instituto Butantan, São Paulo, Brazil, is raised against Brazilian *L. muta* venom. It is a liquid preparation (10 mL) made by ammonium sulphate precipitation and pepsin digestion (THEAKSTON & WARRELL, 1991). The labelling on the ampoule and information on batch number, expiry and manufacture dates on the outer packaging was hand-written. The following information was printed in Portuguese on the outer packaging; '1 mL neutralizes 3.5 mg of *L. muta* venom for treatment of accidents caused by snakes of the genus *Lachesis*'.

Determination of the protein content of antivenoms

The protein content (mg/mL) of each antivenom was determined by measuring the absorbance at 280 nm (E280 1.45).

Tests on antivenoms in vivo and in vitro

Tests for estimation of whole venom toxicity (LD₅₀) and the neutralizing effects of each antivenom in mice (ED₅₀), the neutralization of venom haemorrhagic, necrotizing, procoagulant and *in vivo* defibrinogenating activities were performed exactly as described by THEAKSTON & REID (1983) and LAING *et al.* (1992). The results quoted are the means of 2 assays for all except the LD₅₀ and ED₅₀ estimations, for which 95% confidence limits were estimated using probit analysis (FINNEY, 1971).

Results

All 3 Ecuadorian *Bothrops* venoms produced similar

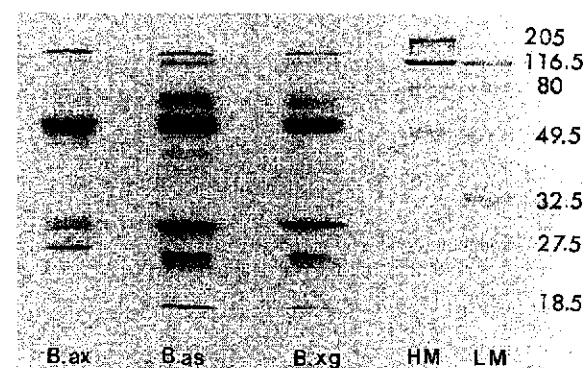


Fig. 2. Examination by SDS-PAGE of Ecuadorian *Bothrops* venoms: B.ax, *Bothrops atrox*; B.as, '*B. asper*'; B.xg, '*B. xanthogrammus*'. HM, high molecular weight markers; LM, low molecular weight markers. Molecular masses are given in kDa.

Table 1. Median lethal intravenous doses of Ecuadorian *Bothrops* venoms and median intravenous effective doses of antivenoms in mice

Venom LD ₅₀ ^a Ecuadorian	Median lethal dose (μg/18–20 g mouse)		Antivenom ED ₅₀ ^b		
	Mexican	Ecuadorian	Brazilian	Colombian (INS)	Colombian (LP)
' <i>B. asper</i> ' ^c 22.7 (9.9–40.6)	NE	21.3 (12.7–29.2)	8.1 (6.5–10.1)	33.1 (23.7–41.7)	27.3 (18.9–35.5)
' <i>B. xanthogrammus</i> ' ^c 23.2 (9.1–35.5)	NE	19.4 (9.7–27.4)	6.2 (4.4–8.3)	41.5 (36.4–50.8)	34.1 (26.4–40.9)
<i>B. atrox</i> 43.9 (38.5–48.4)	NE	70.0 (52.6–87.4)	36.6 (30.5–40.9)	138.2 (114.3–229.1)	84.1 (57.9–124.9)

^aMedian lethal dose in μg/18–20 g mouse (95% confidence intervals in parentheses).

^bMedian effective dose in μL/18–20 g mouse; NE=not effective at the maximum level of the assay system (200 μL of antivenom injected) (95% confidence intervals in parentheses).

^cProbably conspecific (see text).

Table 2. Median lethal intravenous dose of Ecuadorian *Lachesis muta* venom and median intravenous effective doses of one Brazilian monospecific and two Colombian polyspecific antivenoms in mice

Venom LD ₅₀ ^a	Brazilian (Butantan)	Antivenom ED ₅₀ ^b Colombian (INS)	Colombian (LP)
40.5 (-75.5-72.7)	27.6 (16.1-43.8)	NE	77.9 (63.4-105.3)

^aMedian lethal dose in µg/18-20 g mouse (95% confidence intervals in parentheses).

^bMedian effective dose in µL/18-20 g mouse; NE=not effective at the maximum level of the assay system (200 µL of antivenom injected) (95% confidence intervals in parentheses).

Table 3. Results of tests *in vivo* and *in vitro* on three Ecuadorian *Bothrops* and *Lachesis muta* venoms^a

Species	MHD (µg/rat)	MND (µg/rat)	MDD (µg/ mouse)	MCD-P (µg/mL)	MCD-F (µg/mL)
' <i>B. asper</i> ' ^b	30.0	175.0	5.0	2.60	38.00
' <i>B. xanthogrammus</i> ' ^b	37.0	120.0	7.0	0.31	48.00
<i>B. atrox</i>	43.0	175.0	2.0	3.00	42.00
<i>L. muta</i>	24.0	97.0	5.0	22.00	17.20

^aMHD=minimum haemorrhagic dose, MND=minimum necrotizing dose, MDD=minimum defibrinogenating dose, MCD=minimum coagulant dose (P=plasma, F=fibrinogen).

^bProbably conspecific (see text).

protein patterns when examined by SDS-PAGE. There were slight differences in the protein bands between *B. atrox* and '*B. asper*' venoms. However, there was no difference between those of '*B. asper*' and '*B. xanthogrammus*' (Fig. 2), supporting our impression that these are conspecific (SCHÄTTI & KRAMER, 1993).

The LD₅₀ values of the 3 Ecuadorian *Bothrops* venoms are shown in Table 1, and that of Ecuadorian *L. muta* venom in Table 2. Results of the other tests *in vivo* and *in vitro* on all 4 Ecuadorian venoms are given in Table 3.

The protein contents of the antivenoms were as follows: Ecuadorian *Bothrops* polyspecific, 47 mg/mL; Mexican polyspecific, 8.9 mg/mL; Brazilian *Bothrops* polyspecific, 44 mg/mL; Colombian (INS) polyspecific, 61 mg/mL; Colombian (LP) polyspecific, 52.5 mg/mL; Brazilian *Lachesis* monospecific, 81 mg/mL. All antivenoms possessing activity against *Bothrops* venoms therefore had a similar protein content (44-61 mg/mL) except for the Mexican antivenom which contained 5-7 times less protein than the others.

The neutralization assays *in vivo* (ED₅₀) showed that the Brazilian *Bothrops* antivenom was approximately 3

times more effective (v/v and w/w) than the Ecuadorian antivenom in combating lethal effects of Ecuadorian '*B. asper*' and '*B. xanthogrammus*' venoms and twice as effective against Ecuadorian *B. atrox* venom (Table 1). Both Colombian antivenoms, raised against Colombian '*B. asper*', *C. durissus* and *L. muta* venoms, were between 2 and 4 times less active (v/v) (3-5 times less active w/w) than the Brazilian antivenom against Ecuadorian '*B. asper*' and *B. atrox* venoms; they were also 6 to 7 times less active (v/v) (6-8 times less active w/w) against the particular batch of '*B. xanthogrammus*' venom used (Tables 1 and 4). The Mexican antivenom was completely ineffective (Table 1) up to the limit of the assay system (200 µL of antivenom injected).

The Brazilian and Colombian (LP) antivenoms were equally effective (v/v, w/w) in neutralizing the haemorrhagic activity of the 3 venoms, followed by the Ecuadorian antivenom (Table 4). The Colombian (INS) antivenom was moderately effective, and the Mexican anti-venom virtually ineffective up to the limit of the test (Table 4).

The Mexican, Colombian (INS) and Brazilian antivenoms were slightly more effective (v/v) than the Colombian (LP) and Ecuadorian antivenoms in neutralizing the necrotizing activity of the 3 venoms (Table 4). On a w/w basis, the Mexican antivenom appeared to be much more effective, but this was due to non-inclusion of a dilution factor in the estimation.

Total venom procoagulant activity (MCD-P) of the venoms was most effectively neutralized (v/v) by the Ecuadorian antivenom, followed by the Brazilian and Colombian (INS) products (Table 4). However, while the Colombian (LP) antivenom possessed moderate activity against the thrombin-like enzyme (TLE) of Ecuadorian *B. atrox* venom, it contained virtually no anti-TLE activity against Ecuadorian '*B. asper*' or '*B. xanthogrammus*' venoms (Table 4). The Mexican antivenom was surpris-

Table 4. Neutralizing activities of Brazilian, Ecuadorian, two Colombian and Mexican *Bothrops* antivenoms against three Ecuadorian *Bothrops* venoms

Antivenom	Venom	ED ₅₀	MHD	Neutralizing activity ^a		MCD-P	MCD-F
				MND	MDD		
Brazilian	<i>B. atrox</i>	35.56	10.00	40.00	5.00	0.25	0.60
	' <i>B. asper</i> '	8.11	5.00	20.00	5.00	0.20	0.80
	' <i>B. xanthogrammus</i> '	6.16	5.00	20.00	10.00	0.50	0.80
Ecuadorian	<i>B. atrox</i>	70.00	40.00	50.00	6.00	0.05	0.40
	' <i>B. asper</i> '	21.29	10.00	30.00	5.00	0.05	0.20
	' <i>B. xanthogrammus</i> '	19.43	10.00	20.00	3.00	0.09	0.40
Colombian (INS) ^b	<i>B. atrox</i>	138.20	20.00	30.00	5.00	0.50	1.50
	' <i>B. asper</i> '	33.12	20.00	25.00	5.00	0.30	1.50
	' <i>B. xanthogrammus</i> '	41.48	20.00	20.00	5.00	0.30	1.50
Colombian (LP) ^c	<i>B. atrox</i>	84.12	10.00	25.00	5.00	0.25	0.75
	' <i>B. asper</i> '	27.34	5.00	50.00	5.00	0.40	80.00
	' <i>B. xanthogrammus</i> '	34.15	5.00	25.00	5.00	0.05	90.00
Mexican	<i>B. atrox</i>	NE	>200.00	15.00	10.00	5.00	0.50
	' <i>B. asper</i> '	NE	200.00	20.00	25.00	5.00	0.50
	' <i>B. xanthogrammus</i> '	NE	>200.00	25.00	35.00	2.00	1.00

^aResults are the means of 2 assays, in µL; NE=not effective at the maximum level of the assay system. ED₅₀=median effective dose; other abbreviations as in Table 3.

^bManufactured by Instituto Nacional de Salud, Bogota, Colombia.

^cManufactured by Laboratorios Probiol, Bogota, Colombia.

ingly effective in neutralizing the thrombin-like enzyme(s) (MCD-F) in all 3 *Bothrops* venoms (v/v), but not the overall venom procoagulant activity as assessed by the MCD-P (Table 4). Since *Bothrops* venoms contain other components active on clotting factors (e.g., prothrombin and factor X activators), it is possible that these were not neutralized by the Mexican antivenom. However, this result may also reflect the extreme dilution of this antivenom compared with the others.

Butantan monospecific anti-*Lachesis* antivenom and the Colombian (LP) antivenoms were both effective, the former being approximately 3 times more active (v/v) (twice as active w/w) against the lethal effects of Ecuadorian *L. muta* venom (Table 2). The polyspecific Colombian (INS) antivenom was ineffective up to the maximum antivenom dose used in the assay system (Table 2), and was only weakly effective in neutralizing the other venom activities. Overall, the Brazilian antivenom was slightly more effective than the Colombian (LP) antivenom in neutralizing venom haemorrhagic and procoagulant activities and much more effective in neutralizing venom necrotizing, TLE and *in vivo* defibrinogenating activities (Table 5).

Table 5. Neutralizing activities of Brazilian monospecific and two Colombian polyspecific antivenoms against Ecuadorian *Lachesis muta* venom^a

Antivenom	ED ₅₀	MHD	MND	MDD	MCD-P	MCD-F
Brazilian	27.60	20.00	75.00	10.00	4.00	0.50
Colombian (INS)	NE	100.00	150.00	>100.00	70.00	20.00
Colombian (LP)	77.86	25.00	150.00	100.00	5.00	3.00

^aResults are the means of 2 assays, in μ L; NE=not effective at the maximum level of the assay system; other abbreviations as in Tables 3 and 4.

Discussion

Clinicians treating the large numbers of snake bite victims in Ecuador are currently denied adequate supplies of an effective antivenom. This laboratory study has confirmed clinical doubts about the efficacy of imported Mexican antivenom in the treatment of envenoming by Ecuadorian crotaline snakes. The main reason for this low efficacy is probably its lack of specificity. The distributors (Grupo Pharma, personal communication, 1989) claim the '*B. atrox*' and '*C. terrificus*' venoms of Mexican origin were used for preparing the antivenom. However, these species do not occur in Mexico (CAMPBELL & LAMAR, 1989), and the venoms are probably those of Mexican *B. xanthogrammus/asper* and *C. durissus*. The *Bothrops* species is geographically remote from Ecuador and so its venom may be antigenically different, while neutralization of *Crotalus* venom is irrelevant for Ecuador where no *Crotalus* species occurs. Another problem with the Mexican antivenom is its low protein concentration, 5-7-fold less than that of the other *Bothrops* antivenoms tested. As the protein contents of all the *Bothrops* antivenoms, with the notable exception of the Mexican product, were within a similar range, their potencies can be compared by volume rather than by protein content, which is more convenient for clinicians treating patients in the field.

Brazilian antivenom was far superior to the Colombian (INS) and Mexican antivenoms in combating lethal effects of the 3 Ecuadorian *Bothrops* venoms, and should therefore be tested clinically in Ecuador as soon as possible. The Brazilian and Colombian (LP) antivenoms, followed by the Ecuadorian antivenom, were most effective in neutralizing venom haemorrhagic activity. It is this activity which is generally considered to be the most important lethal agent in humans (REID, 1968).

All *Bothrops* antivenoms tested were moderately effective in neutralizing venom necrotizing activity. The Mexican antivenom was surprisingly effective in this respect, considering its overall lack of efficacy. However, neutralizing of venom necrotizing activity in this laboratory model may not have much clinical significance as it

seems necessary to administer the antivenom very soon after the bite which, in practice, rarely happens in human cases of envenoming (WARRELL, 1992; CARDOSO *et al.*, 1993). Antivenom has in the past been shown to be relatively ineffective in neutralizing necrotizing activity in humans (REID, 1964; WARRELL *et al.*, 1976). Well-controlled studies have also shown that tissue damage is initiated almost immediately after experimental envenoming in animals (IDDON *et al.*, 1987), and it is therefore likely that development of such local effects cannot be prevented unless antivenom treatment is administered very soon after the bite.

Venom procoagulant activity was most effectively neutralized by the Ecuadorian antivenom; this result was predictable as the antivenom is prepared against the venoms of Ecuadorian *Bothrops* species, and may be directly related to the amount of procoagulant antigens present in the venom pool. It is surprising that this antivenom did not prove to be the most effective of the 5 antivenoms tested in all the assays. In view of its proven lack of efficacy in experimental assays and its apparent clinical inefficacy in patients, it is also surprising that the Mexican antivenom was fairly effective against the

TLE(s) present in all the Ecuadorian *Bothrops* venoms. These results indicate that the Mexican antivenom contains anti-TLE of similar neutralizing potency to the Ecuadorian and Brazilian antivenoms. The fact that it did not effectively neutralize the plasma coagulant activity (Table 4) implies that it did not contain activity against procoagulant venom enzymes. This also explains its low efficacy against defibrinogenation *in vivo*. Another reason for the lack of efficacy is the extreme dilution of this antivenom but, without specificity against the procoagulant enzymes of Ecuadorian *Bothrops* venoms, no amount of immunoglobulin would be effective for neutralization. One surprising observation, which is difficult to explain, is the minimal anti-TLE activity of the Colombian (LP) antivenom against Ecuadorian '*B. xanthogrammus*' and '*B. asper*' venoms (Table 4).

The Brazilian monospecific *Lachesis* antivenom and the Colombian (LP) polyspecific antivenoms satisfactorily neutralized the lethal effect of the venom of Ecuadorian *L. muta*, whereas the Colombian (INS) polyspecific antivenom, which should have activity against the venom of this species, was completely ineffective against the lethal activity (Table 2) and relatively ineffective in neutralizing venom procoagulant, defibrinogenating, haemorrhagic and necrotizing activities (Table 5). This was probably due to a combination of a higher level of anti-*Lachesis* venom activity in the Brazilian monospecific antivenom and more dilution of this activity in the Colombian (INS) polyspecific antivenom. Although the antivenom of choice here is the Brazilian monospecific antivenom, the Colombian (LP) antivenom would also be worth serious consideration on the basis of these observations. The latter also has the advantage that it is almost as effective as the Ecuadorian *Bothrops* antivenom against the venoms of Ecuadorian *Bothrops* species.

The murine ED₅₀ assays indicated the relative efficacy of the antivenoms in mice. These results cannot necessarily be extrapolated to human snake bite victims. For example, KEEGAN *et al.* (1964, 1965) showed that an antivenom against the venom of the Malayan pit viper (*Calloselasma rhodostoma*) was effective in mice but not in

humans. Similarly, a clinical trial, which compared the efficacy of Pasteur and Behringwerke antivenoms in saw-scaled viper (*Echis ocellatus*) envenoming in Nigeria, showed that the former was the more effective in humans, while laboratory toxicity (ED₅₀) studies found the latter to be more effective in mice (WARRELL *et al.*, 1980). However, the use of an extended panel of tests *in vitro* and in rodents has more recently shown closer correlation with clinical observations (WARRELL *et al.*, 1986; LAING *et al.*, 1992; CARDOSO *et al.*, 1993).

The results obtained in this study suggest that the Brazilian, Ecuadorian and Colombian (LP) antivenoms may be effective. The next step will be to carry out a randomized double-blind comparative clinical trial of these antivenoms in Ecuador. There is no ground for predicting any clinical efficacy for the Mexican antivenom at doses which would be safe or affordable in Ecuador.

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