Papers

Crotaline snake bite in the Ecuadorian Amazon: randomised double blind comparative trial of three South American polyspecific antivenoms

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Abstract

Objective To compare the efficacy and safety of three polyspecific antivenoms for bites by pit vipers.

Design Randomised double blind comparative trial of three antivenoms.

Setting Shell, Pastaza, southeastern Ecuador.

Participants 210 patients with incoagulable blood were recruited from 221 consecutive patients admitted with snake bite between January 1997 and December 2001.

Intervention One of three antivenoms manufactured in Brazil, Colombia, and Ecuador, chosen for their preclinical potency against Ecuadorian venoms.

Main outcome measures Permanent restoration of blood coagulability after 6 and 24 hours.

Results The snakes responsible for the bites were identified in 187 cases: 109 patients (58%) were bitten by *Bothrops atrox*, 68 (36%) by *B bilineatus*, and 10 (5%) by *B taeniatus, B brazili*, or *Lachesis muta*. Eighty seven patients (41%) received Colombian antivenom, 82 (39%) received Brazilian antivenom, but only 41 (20%) received Ecuadorian antivenom because the supply was exhausted. Two patients died, and 10 developed local necrosis. All antivenoms achieved the primary end point of permanently restoring blood coagulability by 6 or 24 hours after the start of treatment in >40% of patients. Colombian antivenom, however, was the most effective after initial doses of 20 ml (two vials), <70 ml, and any initial dose at both 6 and 24 hours. An initial dose of 20 ml of Colombian antivenom permanently restored blood coagulability in 64% (46/72) of patients after 6 hours (P = 0.054 compared with the other two antivenoms) and an initial dose of <70 ml was effective at 6 hours (65%, P = 0.045) and 24 hours (99%, P = 0.06). Early anaphylactoid reactions were common (53%, 73%, and 19%, respectively, for Brazilian, Colombian, and Ecuadorian antivenoms, P < 0.0001) but only three reactions were severe and none was fatal.

Conclusions All three antivenoms can be recommended for the treatment of snakebites in this region, though the reactogenicity of Brazilian and Colombian antivenoms is a cause for concern.

Introduction

Each year in Ecuador, 1200-1400 cases of snake bite are reported in 19 of the 21 provinces. Reports from Santo Domingo de los Colorados, Guayaquil,[1] and Shell[2] confirm its importance. In "El Oriente," east of the Andes, the principal venomous species are *Bothrops atrox* (common lancehead) and *B bilineatus smaragdinus* (two striped forest pit viper, also known as *Bothriopsis bilineata smaragdina*). *B taeniatus* (speckled forest pit viper, also known as *Bothriopsis taeniata*), the less common *Bothrops* and *Bothrocophias* species and *Lachesis muta* (bushmaster) cause some bites.[3 4] The indigenous Amerindian peoples and people of mixed European ethnicity, whose occupations include farming and logging, are all at risk of snake bite. The principal clinical effects of envenoming by *B atrox* are life threatening bleeding and blood coagulation disorders, shock, and renal failure. Necrosis and bacterial infection at the site of the bite may cause permanent physical handicap. Envenoming by *B bilineatus* is usually less severe. Other species such as *B brazili* and *L muta*, although potentially as dangerous as *B atrox*, rarely bite people.[4]

In an earlier preclinical laboratory study, the neutralising potency of five antivenoms (Brazilian, Ecuadorian, Mexican, and two Colombian) was tested against the venoms of *B atrox*, *B asper*, and *B xanthogrammus*.[5 6] The Brazilian antivenom proved most effective, followed by the Ecuadorian and two Colombian antivenoms. The Mexican antivenom showed absolutely no neutralising activity against Ecuadorian *Bothrops* venoms, consistent with its poor clinical reputation in Ecuador.[5] We selected the three most effective antivenoms for a double blind randomised comparative clinical trial in Ecuador.

The main lethal effect of the venoms of the crotaline (rattlesnake-like) pit vipers responsible for envenoming is intracranial or gastrointestinal haemorrhage resulting from vascular endothelial damage, platelet dysfunction, and consumption coagulopathy. These dangerous antihaemostatic disorders and their reversal by specific antivenoms are reflected by whole blood coagulability, which is easily assessed at the bedside with a simple but sensitive 20 minute whole blood clotting test.[7 8] We therefore used this test to measure the efficacy of the three antivenoms in restoring whole blood coagulability and observed early reactions to assess safety.

Methods

Participants

During the five year period January 1997-January 2002, patients of any age who presented with a history of snake bite at any time during the day or night to Hospital Vozandes del Oriente, Shell, Pastaza, Ecuador, were considered for the study, unless they had

received antivenom within the previous 6 hours. Patients were included if the result of the blood clotting test showed that their blood would not coagulate.[7] [8] Patients or their relatives gave informed consent for admission, investigation, and treatment. All patients were admitted to the hospital and kept under observation for at least 48 hours. History, physical examination, and other relevant details were recorded on standard proformas. All members of the medical team were involved in the study, providing 24 hour cover.

Antivenom treatment and randomisation

We randomised patients in blocks of six to treatment with one of three antivenoms[5] [9]: Suero Antiofidico (Instituto Nacional de Higiene y Medicina Tropical "Leopoldo Izquieta Perez" Guayaquil, Ecuador); Soro Antibotropico (Instituto Butantan, San Paulo, Brazil); and Antiveneno Polivalente (Instituto Nacional de Salud, Bogota, Colombia).

The expiry dates of all the antivenoms used was December 2000 but from January 2001 until the end of the study, the Bra-

Two striped forest pit viper (*Bothrops bilineatus smaragdinus*, also known as *Bothriopsis bilineata smaragdina*) from Napo Province, Ecuador, within the catchment area of this study. As many as 36% of our patients had been envenomed by this species
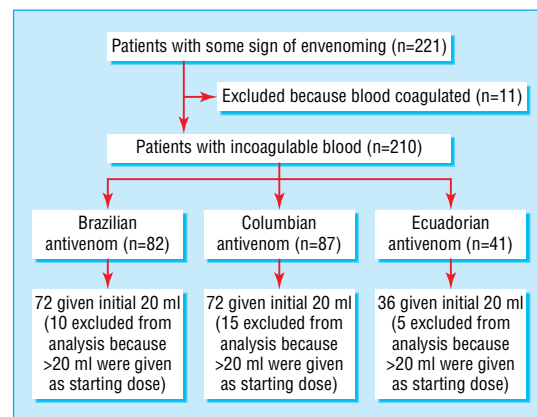

Fig 1 Flow of patient through study

zilian and Colombian antivenoms remained clear solutions and their in vivo neutralising potencies were undiminished.[10] By this time the Ecuadorian antivenom had already run out (see below).

We calculated that we would need to recruit 100 patients to each arm to detect an increase from 50% to 73% in the proportion of patients with clotting blood at 6 hours (80% power). After the 140th patient, the supply of Ecuadorian antivenom ran out, but strict randomisation was maintained to either Brazilian or Colombian antivenom. Allocation was determined by a table of random numbers. The antivenoms were packaged in numbered envelopes. The nurse on duty was responsible for drawing up the antivenoms, all indistinguishable colourless solutions, into an unmarked syringe so that the medical staff treating and assessing the patient and the patient were effectively blinded.

Most patients received an initial dose of 20 ml (two vials) of antivenom by intravenous injection over 10 minutes. A minority, considered on admission to be severely envenomed, were given higher initial doses of up to 70 ml (seven vials). Six hours after the start of antivenom treatment, we checked blood coagulability using the 20 minute clotting test. If the blood was still not coagulable, we administered a second dose of 20 ml of antivenom. Further doses were given at every six hours until blood coagulability was restored permanently.

General treatment

All medical staff responsible for administering antivenom were familiar with the signs of early anaphylactoid reactions and watched the patients carefully for such signs. These were treated with subcutaneous adrenaline (epinephrine) and intravenous diphenhydramine and hydrocortisone, as was the current practice in Ecuador at the time of the study. Pain was treated with oral paracetamol, intravenous pethidine, or tramadol. Patients received fresh compatible HIV negative blood if their packed cell volume fell below 20%, and routine tetanus prophylaxis was given. Local necrosis was treated by immediate surgical debridement, gentamicin, and chloramphenicol.

Snakes responsible for the bites

Dead snakes brought to the hospital were labelled and preserved in 10% formalin or 70% ethanol. They were formally identified at the Fundacion Herpetologica Gustavo Orcés in Quito.

Laboratory investigations

Venous blood was sampled on admission, at 6, 12, 18, and 24 hours later, and then daily until patients were discharged from hospital. Blood coagulability was determined by the 20 minute clotting test. We obtained background levels of absorbance in the enzyme immunoassay from blood samples taken from 100 rela-

Table 1 Comparison of groups of patients bitten by snakes in Ecuador, at randomisation before treatment with antivenom*

	Colombian (n=87)	Brazilian (n=82)	Ecuadorian (n=41)
Mean (SD) age (years)	28.3 (18.4)	27.3 (17.1)	23.3 (15.6)
Age range (years)	2-80	4-62	4-54
Male/female	39/48	42/40	25/16
Mean (SD) time between bite and antivenom (hours)	6.8 (9.1)	8.3 (14.6)	8.4 (10.7)
Range of time before antivenom (hours)	0.2-51	0.2-102	0.3-40
Site of bite:			
Foot/leg	47	34	20
Hand/arm	32	40	18
Other	6	6	2
Snake species identified	74	76	37
Snake brought with patient:			
B atrox	5	7	1
B bilineatus	7	4	1
B taeniatus	1	0	1
Unidentified	0	1	1
Snake identified by venom EIA:			
B atrox	45	44	20
B bilineatus	25	27	16
B taeniatus	3	2	1
B brazili	0	1	0
Lachesis muta	1	2	0

EIA=enzyme immunoassay.
*Numbers do not always sum to total because of missing data.

tives or friends of patients who had not been bitten in the past and were considered representative of the normal population within the study area.[11] We froze residual plasma/serum from the blood clotting test for detection of specific venom antigen by enzyme immunoassay using the technique described by Ariaratnam et al[12] but with an additional step before the wells were coated with antiserum. Four millilitres of each antiserum was passed over a CN-Br activated Sepharose 4B affinity column (Sigma, Poole, Dorset) to which was bound 5 mg of each venom to retain as much venom specific antibody as possible. Enzyme immunoassays were developed for five different venoms (*B atrox*, *B bilineatus smaragdinus*, *B taeniatus*, *B brazili*, and *L muta*). This assay had been validated in a separate study by testing admission samples from 57 patients selected because all had brought in the dead snake (personal communication). Therapeutic antivenom concentrations were measured by enzyme immunoassay in 103 patients.[11]

Statistical analysis

We constructed a database using Microsoft Access and compared the efficacy of antivenoms and reaction rates with 2×3 tables. The relation between the dose of antivenom required and the initial serum concentration of venom antigen was determined with the Kruskal-Wallis test.

Results

Randomisation and clinical features

We recruited 210 patients with incoagulable blood (fig 1). Eighty seven patients (41.4%) received Colombian INS antivenom, 82

Table 2 Clinical features of envenoming in 210 patients bitten by snakes. All had incoagulable blood before randomisation to antivenom therapy*

	Colombian (n=87)	Brazilian (n=82)	Ecuadorian (n=41)
Local:			
Pain	76	79	37
Swelling	70	75	33
Local lymphadenopathy	27	26	10
Bleeding from bite site	44	38	26
Erythema	44	28	15
Blistering	25	7	6
Ecchymosis	27	26	13
Systemic:			
Nausea	38	33	16
Vomiting	23	15	6
Fever (>37.5°C)	22	19	8
Syncope	10	13	7
Gingival bleeding	44	35	13
Blood in sputum/saliva/vomit	37	34	9
Epistaxis	12	15	4
Gross haematuria	16	17	10
Bleeding skin/mucosa	7	9	4
Blood in stool (melaena)	7	9	4

*Numbers do not always sum to total because of missing data.
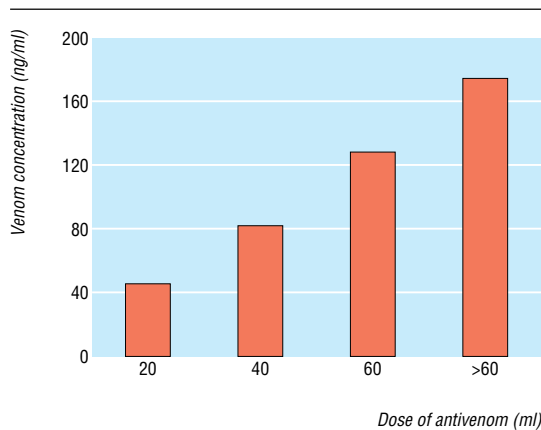
Fig 2 Relation between median serum concentrations of venom antigen on admission in patients requiring treatment with 20 ml (two vials), 40 ml (four vials), 60 ml (six vials), and >60 ml of antivenom

(39%) received Brazilian antivenom, but only 41 (19.5%) received Ecuadorian antivenom because the supply ran out after the 140th patient (table 1). The groups of patients receiving the three different antivenoms were similar in all respects on admission to hospital and before they received treatment (tables 1 and 2). Table 2 shows the clinical features of recruited patients.

Snakes responsible for bites
Twenty nine patients (14%) brought in the snake responsible for the bite: 13 (45%) were identified as *B atrox*, 12 (41%) as *B bilineatus smaragdinus*, 2 (7%) as *B taeniatus*, and 2 (7%) remained unidentified (table 1). Specimens of *B atrox* ranged from 20-90 cm (mean 43 cm, SD 22 cm) and *B bilineatus* 15-50 cm (34 cm, SD 14 cm) and the two *B taeniatus* specimens measured 150 cm and 180 cm. With the help of herpetologists and the results of enzyme immunoassay we identified the snake responsible for envenoming in 187 (89%) cases: 109 (58%) were bitten by *B atrox*, 68 (36%) by *B bilineatus*, 6 (3%) by *B taeniatus*, 1 (1%) by *B brazili*, and 3 (2%) by *L muta*. The distribution of bites by the different species was similar among the three groups (table 1).

Venom antigen detection
We assessed the serum concentrations of venom antigen from admission to discharge in 148 patients out of the 180 who received an initial dose of 20 ml of antivenom (samples from 32 patients were lost during transport from Shell to Quito). Concentrations on admission correlated significantly with the total volume of antivenom required to restore blood coagulabil-

ity permanently (P < 0.0001). In the small group of patients who required more than 60 ml of antivenom, median venom antigen concentrations were about fourfold to fivefold higher than those in patients who required only 20 ml of antivenom (fig 2). The time to venom clearance was also longer in patients who required higher doses of antivenom (fig 3).

Efficacy of antivenom treatment
Two patients died. A 4 year old boy treated with Brazilian antivenom and a 38 year old woman treated with Colombian antivenom died 11 hours and 3 days, respectively, after being bitten by *B atrox*. Both developed acute pulmonary oedema from which they could not be resuscitated. Ten patients (5% in each treatment group) developed local necrosis. All other patients were well on discharge from hospital.

Table 3 gives details of the comparative efficacy of the antivenoms. One hundred and eighty patients were treated with an initial dose of 20 ml (two vials). In those who received this dose of Colombian antivenom, blood coagulability was restored in 64% (46/72) at 6 hours compared with 49% (35/72) and 42% (15/36) of those who received Brazilian and Ecuadorian antivenoms, respectively (P = 0.054). Permanent restoration of coagulability was recorded within 24 hours after starting treatment in almost all patients given 20 ml of Colombian antivenom (64/65; 99%), in 60/67 (90%) treated with the Brazilian antivenom, and in 32/35 (91%) treated with the Ecuadorian antivenom. Colombian antivenom also proved superior when the initial dose was < 70 ml and after any initial dose of antivenom (table 3). Eighty one patients (45%) required more than one dose of antivenom, including 11 patients whose blood became incoagulable again after a normal result from the whole blood clotting test at 6 hours. There was no difference in median total doses of the three antivenoms.

Therapeutic concentrations of antivenom
Of the 103 patients in whom we measured serum concentrations of therapeutic antivenom, 63 had received an initial dose of 20 ml with or without subsequent doses. Antivenom was detectable in the serum when serum venom antigenaemia had become undetectable and for at least 48 hours, even in those who had received only a single dose of antivenom (fig 4).

Antivenom reactions
Early reactions to antivenom were common, including rash, vomiting, abdominal pain, fevers and chills, pruritis, and, more seriously, dyspnoea and hypotension. In those receiving an initial antivenom dose of 20 ml, reaction rates were 19% (7/37) in those receiving Ecuadorian antivenom, 73% (56/71) for Colombian,

Table 3 Permanent restoration of blood coagulability after various initial doses of antivenom at 6 and 24 hours after start of treatment

Initial dose	At 6 hours		At 24 hours	
	No (%) of patients	P value*	No (%) of patients	P value*
20 ml				
Brazilian	35/72 (47)	0.054	60/67 (90)	0.10
Colombian	46/72 (64)		64/65 (99)	
Ecuadorian	15/36 (42)		32/35 (91)	
<70 ml				
Brazilian	37/74 (50)	0.045	60/68 (88)	0.06
Colombian	48/74 (65)		66/67 (99)	
Ecuadorian	15/36 (42)		32/35 (91)	
Any initial dose				
Brazilian	41/81 (51)	0.012	66/75 (88)	0.16
Colombian	59/87 (68)		77/80 (96)	
Ecuadorian	17/40 (43)		36/39 (92)	

*For comparison of Colombian antivenom with others.
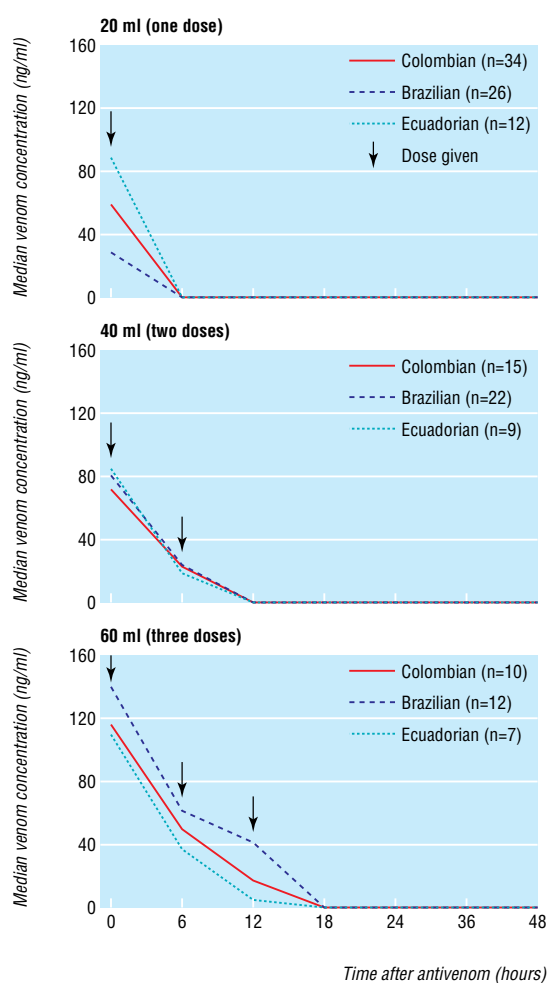
Fig 3 Clearance of venom antigen from 148 patients treated with one dose (venom antigen completely cleared by all three antivenoms within 6 hours of start of antivenom); two doses (venom antigen completely cleared from circulation within 12 hours after start of antivenom in patients whose coagulopathy did not respond to initial 20 ml dose); and three doses (venom antigen finally completely cleared from circulation within 18 hours after start of antivenom in patients whose coagulopathy did not respond to first two 20 ml doses)

We could not detect any difference in the ability of the three antivenoms to eliminate or reduce local effects of envenoming. Only 10 patients (5%) developed local necrosis, the main cause of persistent morbidity in those who survive severe envenoming. The pathophysiology of local envenoming may involve direct and indirect inflammatory mechanisms that are independent of neutralisation of venom toxins mediated through antivenom.[19–22]

The high incidence of symptoms of early anaphylactoid reactions (including rash, vomiting, abdominal pains, fevers, chills, pruritis, dypnoea, and hypotension) in our patients was reminiscent of results of a study in Brazil in which reaction rates as high as 84% were recorded.[13] In other studies, early reaction rates ranged from 3% to 54%.[23–25] Among 178 patients who could be closely monitored, 100 (56%) suffered early reactions. The antivenoms differed significantly in their rates of reaction (Ecuadorian 19%, Brazilian 53%, Colombian 73%, P<0.0001). The higher reactogenicity of Colombian antivenom may reflect its higher protein content (61 mg/ml) compared with the two other antivenoms (Ecuadorian 47 mg/ml, Brazilian 44 mg/ml)[5] and
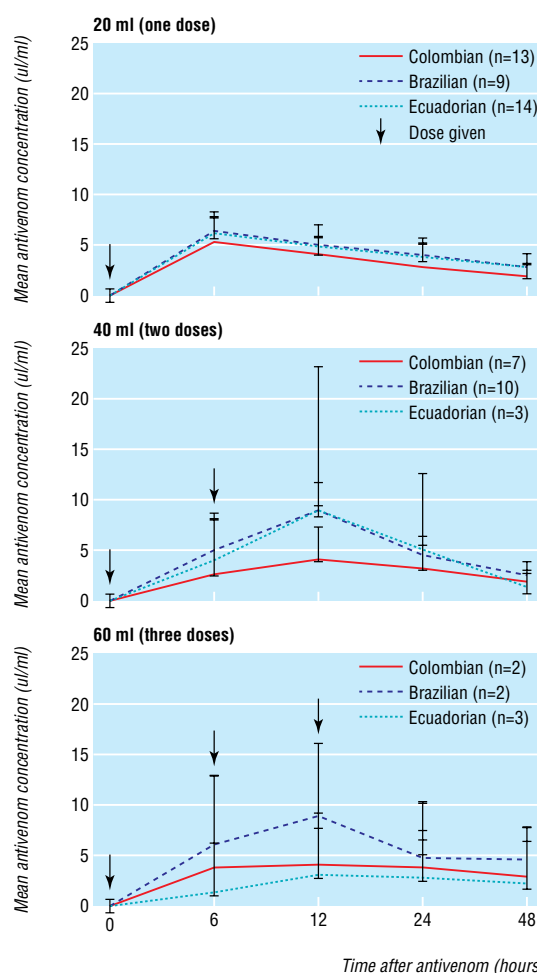
Fig 4 Therapeutic antivenom concentrations in 63 patients according to dose of antivenom on admission: one dose (antivenom clears venom from the circulation within 6 hours after start of antivenom; antivenom concentrations are maximum at 6 hours and remain high for more than two days); two doses (antivenom clears venom within 12 hours from start of first dose of antivenom; antivenom concentrations are maximum at 12 hours); and three doses (antivenom clears venom within 18 hours from start of first dose of antivenom; antivenom concentrations are maximum at 12 hours). Results are means (2 SE) showing overlapping error bars at each time point

and 53% (37/70) for Brazilian antivenom. These rates were significantly different from each other (P<0.0001). Two patients who developed hypotension had been treated with Colombian antivenom, one with Brazilian antivenom, and one with Ecuadorian antivenom.

Discussion

In this randomised double blind comparative trial of three polyspecific antivenoms in 210 patients with venom induced coagulopathy, the resolution of coagulopathy at 6 and 24 hours after the initial dose were the primary end points. We chose the 6 hour time interval because of the frequently reported observation that, in envenomed patients, blood coagulability is usually restored within 3-6 hours of the administration of a dose of antivenom sufficient to neutralise circulating procoagulant toxins.[13–18] If an initial two vials (20 ml) of antivenom failed to correct the coagulopathy within 6 hours, a further two vials were given. Judged by this criterion, Colombian antivenom proved the most effective.

the fact that it is a whole IgG ammonium sulphate precipitated preparation, whereas the other two antivenoms are pepsin digested F(ab´)$_2$ fragments.[5] Severe reactions were rare and were promptly reversed by use of adrenaline, antihistamines, and corticosteroids.

Enzyme immunoassay was useful for identifying the snake responsible for envenoming. Concentrations of venom antigen on admission correlated with the severity of envenoming, as in studies of envenoming by *Vipera aspis*,[26] *B lanceolatus*,[27] and *Daboia siamensis*.[28] Patients whose venom antigenaemia was relatively low required treatment with only two vials (20 ml) of antivenom, whereas those with higher levels needed repeated doses of antivenom. Venom clearance by antivenom occurred more rapidly in patients with lower venom antigenaemias on admission, as in patients bitten by *B jararaca*.[29]

B atrox and *B bilineatus smaragdinus* are responsible for most snake bites in the Pastaza region. *B atrox* is notorious as the leading cause of severe snake bites wherever it occurs in South America.[4][16] In contrast, *B bilineatus*, a distinctive bright green arboreal snake, is less well known as a snake of real medical importance.[30] In this study 36% of our patients were bitten by this species and 6% by *B taeniatus*, *B brazili*, and *L muta*. Other crotalines including *B pulcher* (formerly *B albocarinatus* and also known as *Bothriopsis pulchra*), *Bothrocophias* (formerly *Bothrops* and *Porthidium*) *hyoprora*, and *Bothrocophias microphthalmus* are known to inhabit this area of unusual diversity of venomous snakes.[4]

Conclusions

All three of the tested antivenoms were clinically adequate in eastern Ecuador. Colombian antivenom, however, was the most effective judged by speed and efficiency in permanently correcting venom induced coagulopathy. The Ecuadorian antivenom was significantly less likely than either of the other two antivenoms to cause early anaphylactoid reactions. We recommended that the Ecuadorian Ministry of Health should increase production of this antivenom or, failing that, import either Colombian or Brazilian antivenoms to improve the treatment of snake bite envenoming in the Ecuadorian Amazon region.

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What is already known on this topic

Most studies on effectiveness of antivenom in snake bite have been carried out by estimating neutralising potency in experimental animals

Few well designed clinical trials have been carried out on the treatment of human snake bite in the rural tropics

What this study adds

A simple, accurate, and robust whole blood clotting test can be used to assess the ability of three antivenoms (Brazilian, Colombian, and Ecuadorian) to eliminate venom induced coagulopathy permanently

Enzyme immunoassay enabled the accurate identification of the snake species responsible for envenoming

Although the Colombian antivenom proved the most effective, all three antivenoms proved suitable for treating snakebite victims in this region

The Brazilian and Colombian antivenoms caused a high incidence of anaphylactoid reactions. This should prompt their manufacturers to improve production procedures

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bmj.com 2004;329:1129

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