Effectiveness of Two Common Antivenoms for North, Central, and South American *Micrurus* Envenomations

Adolfo R. de Roodt, D.V.M., Ph.D.,[1,*] Jorge F. Paniagua-Solis, Pharm.D., Ph.D.,[2] Jorge A. Dolab, B.Sc.,[3] Judith Estévez-Ramiréz, M.Sc.,[4] Blanca Ramos-Cerrillo, B.Sc.,[5] Silvana Litwin, B.Sc.,[1] J. C. Dokmetjian, B.Sc.,[1] and Alejandro Alagón, M.D., Ph.D.[5]

[1]Área de Investigación y Desarrollo/Serpentario, Instituto Nacional de Producción de Biológicos—A.N.L.I.S. ''Dr. Carlos G. Malbrán'', Buenos Aires, Argentina
[2]Dirección de Investigación y Desarrollo, Laboratorios Silanes S.A. de C.V., Col. Del Valle, México DF, México
[3]Departamento de Vacunas y Sueros, Instituto Nacional de Producción de Biológicos—A.N.L.I.S. ''Dr. Carlos G. Malbrán'', Buenos Aires, Argentina
[4]Laboratorio Investigación y Desarrollo, Instituto Bioclón, Col. Toriello Guerra, México DF, México
[5]Departamento de Medicina Molecular y Bioprocesos, Instituto de Biotecnología de la UNAM, Cuernavaca, Morelos, México

ABSTRACT

Micrurus snakes (coral snakes) may produce severe envenomation that can lead to death by peripheral respiratory paralysis. Only few laboratories produce specific antivenoms, and despite the cross-reactivity found in some *Micrurus* species venoms, the treatment is not always effective. To test two therapeutic antivenoms against the venom of four species of *Micrurus* from Southern America, North of South America, Central America, and North America, the determination of the lethal potency of the venoms, the study of some biochemical and immunochemical characteristics, and the determination of the neutralizing activity of both antivenoms were studied. North American and South American antivenoms neutralized well venoms from *Micrurus* species of the corresponding hemisphere but displayed lower effectiveness against

Correspondence: Adolfo R. de Roodt, D.V.M., Ph.D., Área de Investigación y Desarrollo/Serpentario, Instituto Nacional de Producción de Biológicos—A.N.L.I.S. ''Dr. Carlos G. Malbrán'', Av. Vélez Sarsfield 563, CP 1281, Buenos Aires, Argentina; Fax: +54-11-4303-2492; E-mail: aderoodt@uolsinectis.com.

venoms of species from different hemispheres. It was concluded that the neutralization of *Micrurus* venoms by regional antivenoms could be useful to treat the envenomation by some *Micrurus* snakes but is necessary to evaluate carefully the antivenoms to be used with the venoms from the snakes of the region. Also considering the difficulties for coral snake antivenom production, the development of a polyvalent antivenom useful to treat the envenomation by coral snakes from different regions is necessary.

Key Words: *Micrurus*; Antivenoms; Venoms; Severe envenomation; Snakes; Therapeutics.

INTRODUCTION

Accidents by venomous snakes represent a health problem worldwide that varies in severity in different regions, ranging from a sanitary problem of scarce significance in the North of Europe to a very important sanitary problem in the whole of Africa (1).

In America the snakes responsible for the highest number of envenomations in humans belong to the Viperidae Family (pit vipers, lance-headed vipers). Information from Argentina, Brazil, Colombia, Costa Rica, and Mexico indicates that these snakes are responsible for over 95% of the accidents (2–8).

The remaining small percentage of accidents by venomous snakes is due to the American Elapids, represented by the Genus *Micrurus*, *Micruroides*, and *Leptomicrurus*. Most of the accidents are due to *Micrurus* species, owing mostly to their distribution range (9), from Patagonia (*M. pyrrhocryptus*) to the United States (*M. fulvius*). The American Elapids are named coral snakes because of their bright red color that contrasts with the black, white, or yellow bands along their body.

Coral snake venoms are extremely toxic and the bite of *Micrurus* constitutes a medical emergency because of high risk of death due to their high neurotoxicity. These venoms produce loss of muscle strength and, in general, death by respiratory paralysis of peripheral origin in animals and humans. The neurotoxicity of these venoms can be produced by a postsynaptic action (alpha neurotoxins, e.g., *M. frontalis*), block of the end-plate receptors (alpha neurotoxins), and inhibition of evoked acetylcholine release by the motor nerve endings (presynaptic-like action e.g., *M. corallinus*), or, venoms that block endplate receptors (alpha neurotoxins) and depolarize the muscle fiber membrane (cardiotoxins or myotoxic phospholipases A_2, i.e., *M. nigrocinctus* and *M. fulvius* (10). However, envenomation by these snakes is not frequent, and the accidents are uncommon for several reasons, among which we can summarize the following: Elapid venomous apparatus (proterogliphous) is not as efficient as the viper's (solenogliphous) in venom delivery; the small size of the snake's mouth and the inability to maintain a large aperture of the jaws hinders utility to bite a human; there is a need for some time to inject a high amount of venom by their unsealed venomous conducts; coral snakes are not aggressive, in fact they are rather shy snakes; and they live mostly underground. For these reasons, accidents by *Micrurus* are most common in snake handlers in serpentariums or in children in the forest, who take the snakes to play because of their nonaggressive character and very attractive colors. In the North of Argentina it is not unusual for children from the communities of the forest to play with coral snakes.

However, even if not frequent, accidents by *Micrurus* do happen and do constitute a medical emergency. As has been mentioned, venoms from *Micrurus* spp. are rich in α-neurotoxins that bind to alpha subunits of acetylcholine receptors at the myoneural plate, leading to muscular and respiratory paralysis and death (5,6,10,11). Although experimentally it has been documented that some *Micrurus* venoms may produce myotoxicity and local lesions (12–15), the paramount feature of the coral snake toxicity is the neurotoxicity, and all the cases are considered serious because of the high risk of death.

The only specific treatment for *Micrurus* envenomation is the application of the specific antivenom. Although *Micrurus* antivenoms are produced by several laboratories like Wyeth (United States), Bioclón (México), Clodomiro Picado Institute (Costa Rica), Butantan Insitute (Brazil), and the Instituto Nacional de Producción de Biológicos (Argentina) (3,5,6,16–18), they are not always available for treatment, even for use in the same country, and sometimes its production is discontinued. This lack of regular availability is due to various technical causes, among which are the difficulty in the obtention of coral snakes for venom extraction (in some regions of South America it is necessary to hunt the snakes in the jungle); the demands of maintaining *Micrurus* snakes in captivity in good state of health and feeding (19); and the small

amount of venom that can be extracted per snake (20). These facts make the production of *Micrurus* antivenom more difficult than the production of viper antivenom.

If α-neurotoxins are responsible for the toxicity of these venoms and all coral snakes have this type of toxins (11) and considering that cross-reactivity among several venoms of *Micrurus* has been reported (21–24), it may be hypothesized that the use of a given *Micrurus* antivenom could be used to treat envenomation caused by other species of *Micrurus*. However such statement seems not to be entirely true, since lack of neutralization of venoms from *Micrurus* by antivenom produced with venom from snakes of the same country has been also reported (25).

In spite of the above, and as a first step to the obtention of an effective polyvalent anti-*Micrurus* antivenom to be used in several regions of America, we studied the neutralizing capacity of two antivenoms of therapeutic use against the venoms of *Micrurus* snakes of different regions.

One of the antivenoms was from North America and another from South America. Both antivenoms were tested against venoms from *Micrurus* species from North America (*M. fulvius*), Central America (*M. nigrocinctus*), and South America (*M. surinamensis* and *M. pyrrhocryptus*) in order to evaluate their neutralizing capacity.

MATERIALS AND METHODS

Venoms

Venoms from healthy specimens of *M. fulvius* (La Florida), *M. nigrocinctus* (Costa Rica), and *M. surinamensis* (Leticia, Colombia) were provided from the Bank of Venoms of Bioclón Institute, México DF. Venom from *M. pyrrhocryptus* (Argentina) was provided from the Centro Zootoxicológico de Misiones, Oberá, Misiones, Argentina. All the venoms were obtained by manual extraction, and immediately frozen at −20°C and lyophilized. The venoms were aliquoted and stored at −20°C until use.

Determination of Lethal Potency

Mice (CF-1 strain, 18–22 g, 5 to 8 animals per dose level) were injected by i.p. route with different amounts of venom in 0.15 M NaCl. From the number of surviving animals 48 h after the injection, the LD_{50} was calculated by non-linear regression using the combined Prism and Stat-Mate softwares (GraphPad, Inc., San Diego, CA). It was defined as the amount of venom that produce the death of 50% of the challenged mice (26,27).

Electrophoretic Study

Samples prepared under non-reducing conditions were separated on a vertical slab of 12.5% acrylamide gel using the discontinuous buffer system described by Laemmli (28). For molecular weight estimation of venom proteins, a kit of molecular weight markers (BioRad Broad Range) was run in the same gel. Gels were stained with Coomasie Brilliant Blue R (Sigma).

Double Immunodiffusion

Double immunodiffusions were performed in Petri dishes (10 cm) containing 1% Agarose (Sigma) in PBS pH 7.4 as described by Siles Villarroel (29). Wells (0.3 cm) were punched and filled with 10 μl of the different *Micrurus* venoms (concentration 1 mg/ml). The venoms were confronted against serial dilutions of the different antivenoms. After 48 h, Petri dishes were washed with 0.15 M NaCl, dried at 37°C, and immunocomplexes were stained with Amido Black (Sigma).

Antivenoms

The antivenoms used were Suero Anti-*Micrurus*, from the Instituto Nacional de Producción de Biológicos—A.N.L.I.S. "Dr. Carlos G. Malbrán," Buenos Aires, Argentina (batch 111, expiration date June 20, 1999) with a protein content of 55 ± 2.5 mg/ml; and *Coralmyn*, from Instituto Bioclón, Mexico DF, Mexico (batch B-2D-06, expiration date October 16, 2004). The pharmaceutical presentation is lyophilized to be reconstituted in 5 ml of diluent, with a final protein content of 40 ± 1.5 mg/ml. Both antivenoms were $F(ab')_2$ fragments of equine immunoglobulins with similar degree of purity.

Neutralization Assay

This assay was performed as suggested by the World Health Organization (26,27). CF-1 mice (18–20 g) were injected i.p. with 3.0 LD_{50} of each venom preincubated for 30 min at 37°C with different doses of each antivenom (six animals per dose) in a final volume of 0.5 ml. After 48 h deaths were recorded and the data

analyzed by nonlinear regression using the software Prism (GraphPad Inc., CA). The neutralizing capacity was expressed in microliters as the effective dose 50% (ED_{50}), that is, the antivenom dose which protects half of the injected mice (26,27) or as the milligram of venom neutralized per vial of antivenom (30).

RESULTS

The electrophoretic profile showed differences between the venoms. The venom from *M. surinamensis* showed few proteins over 20 kDa with strong stained bands under 14 kDa whereas the venoms from *M. nigrocinctus* and *M. fulvius* showed strong stained bands around 6.4–50 kDa with differences in intensity and mobility between both venoms (Fig. 1).

Double immunodiffusion showed that *Coralmyn* strongly recognized the venom from *M. nigrocinctus* and *M. fulvius* and did not recognize the venom of *M. surinamensis* (Fig. 2) or *M. pyrrhocryptus* (data not shown) venoms. On the other hand, Anti-*Micrurus* antivenom recognized the venom of *M. surinamensis* and weakly the venoms of *M. nigrocinctus* and *M. fulvius* (Fig. 2).

The lethal doses found for the different venoms were 0.85 mg/kg (confidence interval, CI, 0.75 to 1.11 mg) for *M. nigrocinctus* venom, 0.48 mg/kg (CI 0.45 to

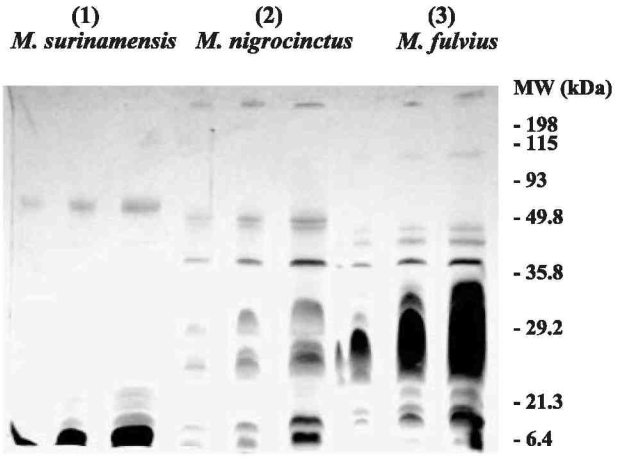

Figure 1. SDS-PAGE of venoms from different species of *Micrurus*. It was performed in a 12.5% Acrilamyde/Bisacrilamyde gel in not reducing conditions. (1) *M. surinamensis* venom (from left to right: 10, 20 and 30 μg); (2) *M. nigrocinctus* venom (20, 35 and 50 μg) and (3) *M. fulvius* (same as in *M. nigrocinctus*). The migration of the molecular weight markers is expressed in kDa in the right (Molecular weight markers BioRad Broad Range).

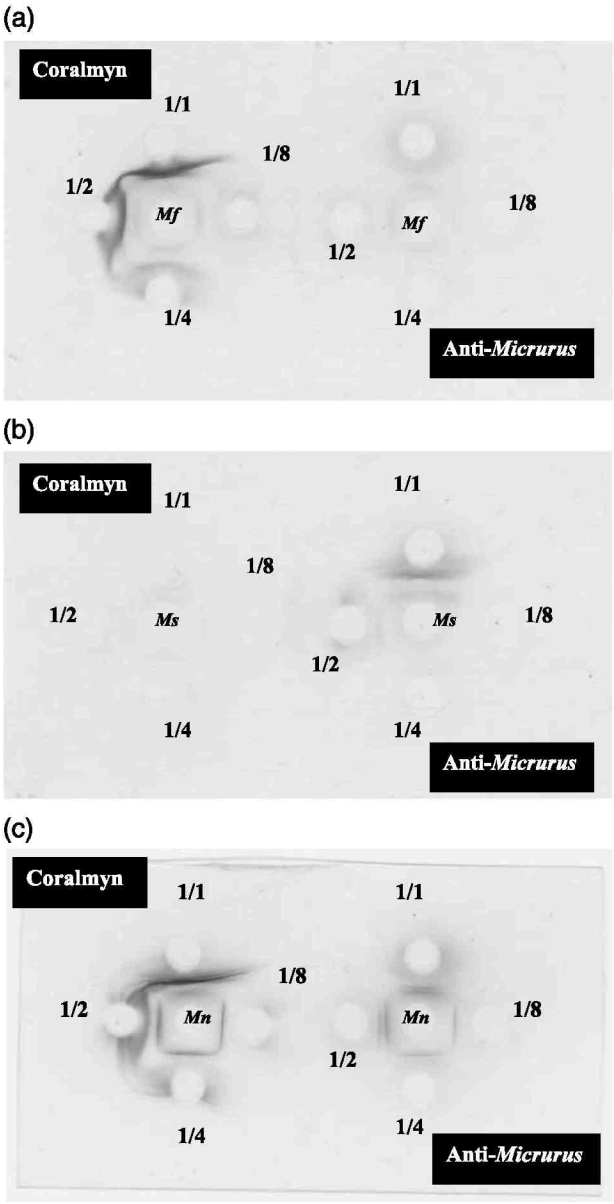

Figure 2. Double immunoprecipitation (Ouchterlony method) in 1% agarose of the venoms of *M. fulvius*, *M. nigrocinctus* and *M. surinamensis* against Coralmyn (left side of the gels) and Anti-*Micrurus* (right side of the gels) antivenoms. The central wells were filled with 10 μl of a solution of 1 mg/ml of *M. fulvius* (***Mf***, a), *M. nigrocinctus* (***Mn***, b), or *M. surinamensis* (***Ms***, c) venoms. The peripheral wells were filled with 10 μl of dilutions of the antivenoms (1/1, $^1/_2$, $^1/_4$ and 1/8 in NaCl 0.15 M). After 48 h the gels were dried and stained with Amido Schwarz.

Table 1. Neutralizing capacity of the antivenoms on the lethal potency of the different *Micrurus* venoms.

Antivenoms	*Coralmyn*		Anti-*Micrurus*	
Venoms	ED_{50}	Potency per vial (5 ml)	ED_{50}	Potency per vial (5 ml)
M. fulvius	39 µl (25–58)	2.46 mg	385 µl (360–412)	0.25 mg
M. nigrocinctus	63 µl (50–78)	2.69 mg	123 µl (114–131)	1.4 mg
M. surinamensis	> 500 µl	< 0.15 mg	36 µl (29–44)	2.11 mg
M. pyrrhocryptus	> 500 µl	< 0.52 mg	88 µl (82–95)	2.95 mg

The ED_{50} (Effective Dose 50%) indicate the amount of antivenom required to protect 50% of mice challenged with 3.0 i.p. doses of each venom and it is expressed in microliters. The 95% intervals of confidence are indicated into brackets. The potency per vial expresses the amount of venom (in milligrams) neutralized by one vial of antivenom.

0.50) for *M. fulvius* venom, 0.38 mg/kg (CI 0.25 to 0.95) for *M. surinamensis* and 1.3 mg/kg (CI 0.6 to 1.9) for *M. pyrrhocryptus* venom.

The antivenoms showed different neutralizing capacity against the different venoms (Table 1). *Coralmyn* was effective against venoms from *M. fulvius* and *M. nigrocinctus* (ED_{50}s of 39 µl and 63 µl, respectively) but did not neutralize the venom from the South American *Micrurus* since the ED_{50}s were over 500 µl of antivenom. On the other hand, Anti-*Micrurus* antivenom was effective in the neutralization of *M. surinamensis* and *M. pyrrhocryptus* venom (ED_{50} of 36 µl and 88 µl, respectively), but it showed lower neutralizing capacity against *M. fulvius* venom (385 µl) and *M. nigrocinctus* venom (123 µl), being necessary to duplicate the dose to neutralize *M. nigrocinctus* venom or to use tenfold the dose to neutralize *M. fulvius* venom when compared with the ED_{50} determined for these venoms with *Coralmyn* (Table 1).

The calculated neutralizing potency per vial of antivenom (30) for the Anti-*Micrurus* were 2.95 mg for *M. pyrrhocryptus*, 2.11 mg for *M. surinamensis*, 0.25 mg for *M. fulvius*, and 1.4 mg for *M. nigrocinctus* venoms. For *Coralmyn* the potency per vial was 2.46 mg for *M. fulvius* venom and 2.69 mg for *M. nigrocinctus* venom.

DISCUSSION

The venoms showed differences in lethal potency and in electrophoretic pattern. A differential immunochemical reactivity of the venoms with the different antivenoms was also observed, which was consistent with the data obtained from the experiments of seroneutralization of lethality.

The lethal potencies of the venoms found in this study are close to those reported for other *Micrurus* species (31), and the electrophoretic profiles of these venoms in general have the major characteristics described for *Micrurus* venoms (31–34) with most of the Coomassie-stained material under 20 kDa.

The immunochemical assay showed that the antivenoms show greater reactivity toward venoms from geographically related snakes. The neutralization assays showed similar results. *Coralmyn*, an antivenom widely used in North America and Central America, showed a good neutralization capacity against the venom of *M. nigrocinctus* (the venom used as immunogen) from Costa Rica and *M. fulvius* from La Florida (United States) but was not effective against venom of *M. surinamensis* (Colombia) or *M. pyrrhocryptus* (Argentina). On the other hand, Anti-*Micrurus*, an antivenom used in Argentina, was effective against the venoms of *M. surinamensis* and *M. pyrrhocryptus* (the latter used as immunogen), but it confers very low protection against venom from *M. nigrocinctus* and *M. fulvius*.

With the exception of *M. corallinus* venom, that possesses presynaptic neurotoxins in addition to the alpha neurotoxins (10,35,36), the principal toxic components in *Micrurus* venoms are the latter (10,24, 37–40). For this reason, in Brazil an antivenom raised against venom of *M. frontalis* and *M. corallinus* is used (6,23,41). However, *M. corallinus* venom seems to be effectively neutralized by antivenoms raised against other venoms (22).

An important cross-reactivity among *Micrurus* venoms has been well described (22–24,43–46). Bolaños (22) proposed four antigenically related groups: 1) *M. fulvius*, *M. nigrocinctus*, and *M. carinicauda*, 2) *M. corallinus*, *M. frontalis*, and *M. spixii*, 3) *M. halleni* and *M. mipartitus* and 4) *M. surinamensis* venom as a separate group. In addition, Alapé-Girón (42) described, by means of monoclonal antibodies, three antigenically related groups: 1) *M. nigrocinctus*, *M. fulvius*, *M. dumerilii*, and *M. albicinctus*, 2) *M. frontalis* and *M. brasiliensis*, 3) *M. alleni* and *M. spixii* that present features of groups 1) and 2) but with particular characteristics. Venoms from *M. surinamensis*, *M. corallinus*,

M. ibicoca, *M. hempritchi*, *M. lemniscatus*, and *M. mipartitus* have characteristics that preclude their inclusion in any of the groups.

Owing to the fact that these venoms have important cross-reactivity, a PanAmerican anti-*Micrurus* antivenom raised with the venoms from *M. frontalis*, *M. nigrocinctus*, and *M. fulvius* was prepared in the past (22). This antivenom was effective for neutralization of venoms from several *Micrurus* species but was not useful for the treatment of *M. surinamensis* envenomation.

Several studies made in Brazil with venom from various species of *Micrurus* showed that they display significant cross-reactivity (23,24) but that the best neutralization is conferred by antivenoms generated against homologous venoms (23). In Brazil, as has been mentioned, an antivenom raised against venom of *M. frontalis* and *M. corallinus* is used. However, this antivenom seems to have a low neutralizing ability against the venom of *M. altirrostris* (25), which is surprising considering that this species was considered years ago a subspecies of *M. frontalis*. In a past study, the antivenom used in Argentina displayed a good neutralizing capacity against 5.0 LD_{50} of the venom from *M. pyrrhocryptus* (ED_{50} around 80 µl) but had a lower neutralizing capacity on the venom of *M. corallinus* and *M. balyocoriphus* (formerly, *M. mesopotamicus*) since doses of 400 µl could not protect 50% of mice challenged with *M. corallinus* venom and the ED_{50} for *M. balyocoriphus* was around 350 µl (de Roodt, unpublished results). This is also surprising, considering that both snakes were considered few years ago to be subspecies of *M. frontalis* (5).

In this study it is shown that both antivenoms could neutralize venom of *Micrurus* species not used as immunogen. For example, *Coralmyn* and Anti-*Micrurus* antivenoms neutralized the heterologous *M. fulvius* and *M. surinamensis* venoms, respectively, with near half of the dose required to neutralize the homologous venom (see Table 1). However, neutralization of the venoms from the other hemisphere was very low. Two recent papers also found good neutralization of *M. fulvius* venom with antivenoms obtained by immunization of horses with the venom of *M. nigrocinctus* (45,46).

Unfortunately the scarcity of *Micrurus* venoms make study on the neutralizing potency of their specific and non-specific antivenoms difficult. However, special attention must be paid to the improvement of the available antivenoms to treat the *Micrurus* envenoming in several regions, as has been shown in this study and by the Brazilian experience (25).

Taking into account the technical difficulties involved in the production of antivenom to treat coral snake bites, and until a good polyvalent antivenom is available in the regions where it could be useful, special attention must be paid to the evaluation of the antivenoms used for treatment of a particular coral snake bite. In addition, complementary measures for treatment, such as the use of inhibitors of acetylcholinesterases and atropin, or the use of artificial respiration, have to be systematically evaluated (6,10,11,47–50).

At present, we are working on the development of a polyvalent experimental anti-*Micrurus* antivenom to be used therapeutically in the neutralization of venoms from *Micrurus* species of North, Central, and South America (51).

REFERENCES

1. Chippaux JP. Venins de Serpent et Envenimations. IDR Éditions. Paris: Institute de Recherche pour le Développement, 2002.
2. Fan HW, Cardoso JL. Clinical toxicology of snake bites in South America. In: Meier J., White J., eds. Handbook of Clinical Toxicology of Animal Venoms and Poisons. Boca Raton: CRC Press, 1995:667–688.
3. Gold BS, Dart RC, Barish RA. Bites of venomous snakes. N Engl J Med 2002; 347(5):347–356.
4. Segre L, Dolab JA, Funes RF, de Titto E, Salomón OD, de Roodt AR, Haas AI, García SI. Accidentes humanos por ofidios en Argentina. Revista Brasil de Toxicol 2000; 13(suppl 1):41.
5. Ministerio de Bienestar Social, Secretaría de Estado de Salud Pública, Subsecretaría de Medicina Sanitaria. Dirección Nacional de Promoción y Protección de la Salud. Guía de prevención y tratamiento de las mordeduras por serpientes venenosas. Buenos Aires, 1980.
6. Ministerio de Saúde. Fundacáo nacional de saúde. In: Manual de Diagnóstico e Tratamento de Acidentes por Animais Peconhentos. Brasilia: Fundacáo Nacional de Saude, 1999.
7. Gutiérrez JM. Comprendiendo los venenos de serpientes: 50 años de investigaciones en América Latina. Rev Biol Trop 2002; 50(2):377–394.
8. Russell FE, Walter FG, Bey TA, Fernández MC. Snakes and snakebite in Central America. Toxicon 1997; 35(10):1469–1522.
9. Campbell JA, Lamar W. The venomous reptiles of Latin America. Comstock, Ithaca: NY University Press, 1989.
10. Vital Brazil O. Pharmacology of coral snake venoms. Mem Inst Butantan 1990; 52(suppl):32.
11. Vital Brazil O. Coral snake venoms: mode of action and pathophysiology of experimental

envenomation. Rev Inst Med Trop Sao Paulo 1987; 29(3):119–126.
12. Barros ACS, Fernández DP, De Lima Ferreira LC, Dos Santos MC. Local effects induced by venoms from five species of Genus *Micrurus* sp. (Coral snakes). Toxicon 1994; 32(4):445–452.
13. de Roodt AR, Gimeno E, Litwin S, Dokmetjian JCH, Estévez J, Dolab JA, Paniagua JF. *Micrurus phyrrocryptus* (''mboi-chumbé-guazú'') venom produces myotoxicity in rats and mice. Abstracts of Papers. 6th Asia-Pacific Congress on Animal, Plant and Microbial Toxins and 11th Annual Scientific Meeting of the Australasian College of Tropical Medicine, Cairns, Australia, July 8–12. International Society on Toxinology, 2002:115.
14. Gutiérrez JM, Chaves F, Rojas E, Bolaños R. Local effects induced by *Micrurus nigrocinctus* venom in white mice. Toxicon 1980; 18(5–6): 633–639.
15. Gutiérrez JM, Lomonte B, Portilla E, Cerdas L, Rojas E. Local effects induced by coral snake venom: evidence of myonecrosis after experimental inoculations of venom from five species. Toxicon 1983; 21(6):77–83.
16. Russell FE. Snake venom immunology: historical and practical considerations. J Toxicol Toxin Rev 1988; 7(1):1–82.
17. Rawat S, Laing G, Smith DC, Theakston D, Landon J. A new antivenom to treat Eastern coral snake (*Micrurus fulvius fulvius*) envenoming. Toxicon 1994; 32(2):185–190.
18. Theakston RDG, Warrell DA. Antivenoms: a list of hyperimmune sera currently available for the treatment of envenoming by bites and stings. Toxicon 1991; 29:1419–1470.
19. Saez L, Litwin S, Haurigot L, Gould E, Galarce PP, Vidal J, de Roodt AR. Observaciones acerca de la Variación de peso en elápidos y colúbridos en cautiverio sometidos a Alimentación forzada. Abstracts of VI. Reunión de Comunicaciones Herpetológicas, Santa Fe, Argentina, 1998; Asociación Herpetológica Argentina, S.M. del Tucumán, 1998:30.
20. de Roodt AR, Dolab JA, Galarce PP, Litwin S, Dokmetjian C, Segre L, Vidal JC. A study on the venom yield of snake species from Argentina. Toxicon 1998; 36(12):1949–1957.
21. Alape-Giron A, Lomonte B, Gustafsson B, Da Silva NJ, Thelestam M. Electrophoretic and immunochemical studies on *Micrurus* snake venoms. Toxicon 1994; 32(6):713–723.
22. Bolaños R, Cerdas L, Avalos JW. Venom of coral snakes (*Micrurus* spp.): report on a multivalent antivenin for the Americas. Bull PanAm Health Organ 1978; 12(1):23–27.
23. Higashi HG, Guidolin R, Caricati CP, Fernandes I, Marcelino JR, Morais JF, Yamagushi IK, Stephano MA, Dias-da-Silva W, Takehara HA. Antigenic cross-reactivity among components of Brazilian Elapidae snake venoms. Braz J Med Res 1995; 28(7):767–771.
24. Prieto da Silva AR, Yamagushi IK, Morais JF, Higashi HG, Raw I, Ho PL, Oliveira JS. Cross reactivity of different specific *Micrurus* antivenom sera with homologous and heterologous snake venoms. Toxicon 2001; 39(7):949–953.
25. Moraes FV, Sousa-e-Silva MC, Bárbaro KC, Leitao MA, Furtado MF. Biological and immunochemical characterization of *Micrurus altirrostris* venom and serum neutralization of its toxic activities. Toxicon 2003; 41(1):71–79.
26. Theakston RDG, Reid HA. Development of simple standard assay procedures for the characterization of snake venoms. Bull W H O 1983; 61:949–956.
27. World Health Organization. Progress in the Characterization of Venoms and Standarization of Antivenoms. WHO, Geneva: Offset Publication, 1981.
28. Laemmli UK. Cleavage of structural during the assembly of the head bacteriophage T4. Nature 1970; 227:680–685.
29. Siles Villarroel MS, Furlanetto RS, Rolim Rosa R, Zelante F, Navas J. Contribuçao ao estudo imunoquímico de venenos botrópicos III. Análise dos componentes antigénicos comuns atraves da dupla difusao em gel de agar. Mem Inst Butantan 1976/1977; 40/41:241–250.
30. Ministerio de Saúde, Secretaría de Vigilancia Sanitaria. Normas Técnicas de Fabricacáo e Controle de Qualidade dos Soros Antiofídicos, Antitóxicos e Antirrábico Aprobada pela vigilancia sanitaria. Brasil: Ministerio de Saúde, 1996.
31. de Roodt AR. Estudio Inmunobilógico del Veneno de las Serpientes de Importancia Sanitaria de la Argentina. Ph. D. Thesis. Facultad de Farmacia y Bioquímica de la Universidad de Buenos Aires, 2002.
32. Furtado MF, Rocha MT, Travaglia-Cardoso SR. Quality control in snake venom. Abstract of the VII. Simpósio da Sociedade Brasileira de Toxinología, Pirenópolis, Goiás, Brazil, September 16–20, 2002; Sociedade Brasileira de Toxinología, 2002:96.
33. Moura Da Silva AM. Caracterizacao de venenos

de serpentes por eletroforese em poliacrilamida. Mem Inst Butantan, Bol Biotecnol 1992; 3: 3–7.
34. Soares AM, Anzaloni-Pedrosa LH, Fontes RMM, Da Silva RJ, Giglio JR. Polyacrilamyde gel elctrophoresis as a tool for the taxonomic identification of snakes from the Elapidae and Viperidae families. J Venom Anim Toxins 1998; 4(2):137–142.
35. Cruz Hofling MA, Rodriguez-Simioni L, Vital Brazil O. Ultrastuctural changes in neuromuscular junctions of mouse diaphragm caused by the venom of the coral snake *Micrurus corallinus*. Mem Inst Butantan 1983/84; 47/48:95–105.
36. Vital Brazil O, Dias Fontana M. Acoes prejuncioais de peconha de cobra coral *Micrurus corallinus* na juncao neuromuscular. Mem Inst Butantan 1983/84; 47/48:13–26.
37. Alape-Giron A, Stiles B, Schmidt J, Giron-Cortés M, Thelestam M, Jornvall H, Bergman T. Characterization of multiple nicotinic acetylcholine receptor-binding proteins and phospholipases A2 from the venom of the coral snake *Micrurus nigrocinctus*. FEBS Lett 1996; 380(1–2):29–32.
38. Rosso JP, Vargas-Rosso O, Gutiérrez JM, Rochat H, Bougis PE. Characterization of alpha-neurotoxin and phospholipase A2 activities from Micrurus venoms. Determination of the amino acid sequence and receptor-binding ability of the major alpha-neurotoxin from *Micrurus nigrocinctus nigrocinctus*. Eur J Biochem 1996; 238(1): 231–239.
39. Francis BR, da Silva Junior NJ, Seebart C, Casaise Silva LL, Schmidt JJ, Kaiser II. Toxins isolated from the venom of the Brazilian coral snake (*Micrurus frontalis frontalis*) include hemorrhagic type phospholipases A2 and postsynaptic neurotoxins. Toxicon 1997; 35(8):1193–1203.
40. Vital Brazil O, Dias Fontana M, Pellegrini Filho A. Physiopathologie et therapeutique de l'envenomation experiméntalle causée par le venin de *Micrurus frontalis*. Mem Inst Butantan 1976/77; 40/41:221–240.
41. Mourao MM, María WS, Velarde DT. Antigen components for coral antivenom preparation. Abstracts of the VII. Simpósio da Sociedade Brasileira de Toxinología, Pirenópolis, Goiás, Brazil, September 16 to 20, 2002; Sociedade Brasilerira de Toxinología, 2002:257.
42. Alape-Giron A, Gustafsson B, Lomonte B, Thelestam M, Gutiérrez JM. Immunochemical characterization of *Micrurus nigrocinctus nigrocinctus* venom with monoclonal and polyclonal antibodies. Toxicon 1994; 32(6):695–712.
43. Barrio A, Miranda ME. Estudio comparativo morfológico e inmunológico entre las diferentes entidades del gnero *Micrurus* Wagler (Ophidia Elapidae) de la Argentina. Mem. Inst. Butantan 1966; 33(3):869–880.
44. Cohen P, Seligman EB Jr. Immunologic studies of coral snake venom. Mem Inst Butantan 1966; 33(1):339–347.
45. Wisiniewski MS, Hill RE, Havey JM, Bogdan GM, Dart RC. Australian tiger snake (*Notechis scutatus*) and Mexican coral Snake (*Micrurus* species) antivenoms prevent death from United States coral snake (*Micrurus fulvius fulvius*) venom in a mouse model. J Toxicol, Clin Toxicol 2003; 41:1–70.
46. Arce V, Rojas E, Ownby CL, Rojas G, Gutiérrez. Preclinical assessment of the ability of polyvalent (Crotalinae) and anticoral (Elapidae) antivenoms produced in Costa Rica to neutralize the venoms of North American snakes. Toxicon 2003; 41(7): 851–860.
47. Bucaretchi F. Elapidic envenomation: clinical features. Mem Inst Butantan 1990; 52(suppl):33–34.
48. Flanchsenberger W, Mirtschin P. Anticholinesterases as antidotes to envenomation of rats by the death adder (*Acantophis anctarticus*). Toxicon 1994; 32(1):35–39.
49. Reid HA, Theakston RDG. The management of snake bite. Bull W H O 1983; 61(6):885–895.
50. Trevett AJ, Lallo DG, Nwokolo N, Kebau IH, Warrell DA. Analysis of referal letters to asses the mannagement of poisonous snake bite in rural Papua New Guinea. Trans Royal Soc Trop Med Hyg 1994; 88:572–574.
51. de Roodt AR, Accatoli CT, Gelmi LV, Dokmetjian JCh, Litwin S, Estevez J, Paniagua J. Faboterápico polivalente Aniti-Micurus. Comunicación preliminar. Abstracts from the VI. Reunión de Expertos en Envenenamiento por animales Ponzonosos, Cuernavaca, Morales, México, March 13–15, 2003; México, D.F.: Institute of Biotechnology of the UNAM, Cuernavaca/Silanes Laboratories, 2003:73.

Marcel Dekker, Inc. **ARTICLE/CHAPTER INFO SHEET**

Product information:	D/Journal:	CLT
	D/Volume:	42
	D/Issue:	0
	C/ISSN/ISBN: Print	0731-3810
	C/ISSN/ISBN: Web	1097-9875
Article information:	D/Article ID number:	120030943
	C/Article title:	Effectiveness of Two Common Antivenoms for North, Central, and South American <emphasis type="italic">Micrurus</emphasis> Envenomations
	D/Unarticle or Article type:	
	C;A/Key words:	<emphasis type="italic">Micrurus</emphasis>, Antivenoms, Venoms, Severe envenomation, Snakes, Therapeutics
	D/Copyright holder:	MDI
AUTHOR INFORMATION:	C/Author number:	1
	C/Author first name:	Adolfo
	C;A/Author middle initial:	R.
	C:A/Author last name:	de Roodt
	A/Suffix to last name:	
	C;A/Degrees:	D.V.M., Ph.D.
	C;A/Author e-mail address:	aderoodt@uolsinectis.com
	C;A/Author fax:	+ 54-11-4303-2492
	C;A/Author phone:	+ 54-11-4303-2492
Affiliation(s) at time of authorship	C;A/Title	
	C;A/Department	Área de Investigación y Desarrollo/Serpentario
	C;A/Institution	Instituto Nacional de Producción de Biológicos—A.N.L.I.S. “Dr. Carlos G. Malbrán”, Av. Vélez Sarsfield 563
	C;A/Street Address	Buenos Aires
	C;A/City	MISSING DATA S/B QUERIED TO THE CONTACT AUTHOR
	C;A/State/Province	CP 1281
	C;A/Postal code	Argentina
	C;A/Country	
Current affiliation(s): Repeat if same as affiliation at time of authorship	C;A/Title	
	C;A/Department	Área de Investigación y Desarrollo/Serpentario
	C;A/Institution	Instituto Nacional de Producción de Biológicos—A.N.L.I.S. “Dr. Carlos G. Malbrán”, Av. Vélez Sarsfield 563
	C;A/Street address	Buenos Aires
	C;A/City	
	C;A/State/Province	CP 1281
	C;A/Postal code	Argentina
	C;A/Country	
Article data:	D/Submission date:	
	D/Reviewed date:	
	D/Revision date:	
	D/Accepted date:	
	D/Pub date print:	
	D/Pub date web:	

Author/Editor Query Form

Journal: CLT
Article No.: 120030943

Queries and/or remarks

AQ/EQ No.	Details
1	Please provide full name of J.C. Dokmetjian
2	In view of change in article title, running head was changed . Pls. check if appropriate.
3	Please check if citations is still correct in light inserted new reference.
4	Please check if citations is still correct in light inserted new reference.
5	Please supply first name initial for Gutierrez of new Ref. 46