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NEUTRALIZATION OF TWO NORTH AMERICAN CORAL SNAKE VENOMS WITH UNITED STATES AND MEXICAN ANTIVENOMS

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Abstract

Elapid snakes throughout the world are considered very lethal containing neurotoxic venoms that affect the nervous system. When humans are envenomated it is considered a serious medical emergency, and antivenom is the main form of treatment considered, in spite of the fact that some patients may only survive under intensive therapy treatment such as respiratory support. Coral snakes are part of the family Elapidae and envenomations by these snakes are very low (< 2% of total snakebites) in most countries from southeastern United States to Argentina. In the United States there are only two species of coral snakes of medical importance which belong to the *Micrurus* genera: *Micrurus fulvius fulvius* (Eastern coral snake) and *M. tener tener* (Texas coral snake). In 2006, Wyeth pharmaceutical notified customers that the production of the North American Coral Snake Antivenin (NACSA) in the U.S. was discontinued and adequate supplies were available to meet historical needs through the end of October 2008; and therefore, it is of utmost important to consider other antivenoms as alternatives for the treatment of coral snake envenoming. One logical alternative is the coral snake antivenom, Coralmyn, produced by the Mexican company, Bioclon. In order to compare neutralization between NACSA and Coralmyn antivenoms with the North American coral snake venoms, the venom lethal doses (LD_{50}) and antivenom effective doses (ED_{50}) were determined in 18–20 g, female, BALB/c mice. Additionally, venom comparisons were determined through a non reduced SDS-PAGE for *M. f. fulvius*, *M. t. tener* and the Mexican coral snake venom, *M. nigrocinctus nigrocinctus*. Coralmyn antivenom was able to effectively neutralize 3 LD_{50} doses of all venom from both *M. t. tener* and *M. f. fulvius*, while Wyeth antivenom only neutralized *M. f. fulvius* venom and was not effective in neutralizing 3 LD_{50} doses of *M. t. tener* venom. Coralmyn is effective in the neutralization of both clinically important coral snake venoms in the U.S.

Keywords

Coral snakes; *Micrurus fulvius fulvius*; *Micrurus tener tener*; venom, antivenom; neutralization, North American Coral Snake Antivenin; Coralmyn

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Introduction

The coral snakes are the only Elapids that exist in the Americas and are represented by three genera: *Leptomicrurus, Micruroides* and *Micrurus* (Sandner-Montilla, 1985; Pifano et al., 1986; de Brandao Prieto da Silva, 2001). In the United States there exist three coral snakes representing two genera, which are *Micruroides euryxanthus, Micrurus tener tener,* and *Micrurus fulvius fulvius*. The latter two are clinically more significant only because the former displays a shy, elusive behavior that rarely comes into contact with humans (Shaw, 1971). Although the venom of the *Micruroides* is less toxic than *Micrurus*, they all present neurotoxic symptoms (McColloguh and Gennaro, 1971; Wingert and Wainschel, 1975; Aird, 1991; Dalo, 1989; Parrish, 1967) . The *Micrurus* species can be found from south, eastern Florida westward to east and south Texas; *M. f. fulvius* are found east of the Mississippi river and *M. t. tener* are found west of the Mississippi river (Campbell and Lamar, 2004; Fig. 1). Envenomation by these snakes can cause death due to the alpha neurotoxins that cause muscle paralysis and respiratory arrest due to postsynaptic, nondepolarization blockage at the neuromuscular junction by binding competitively to the acetylcholine receptor (Lee, 1970 and 1972; Pettigrew and Glass, 1985; Vital-Brazil, 1987; Alape-Giron et al, 1996; Rosso et al, 1996; Francis et al., 1997). The current NACSA will no longer be available after October 2008; and therefore, it is important to determine the ability of other coral snake antivenoms to neutralize the toxic effects of the North American coral snake venoms. The Sonoran coral snake (*M. euryxanthus*) was not included in this study since it is a relatively harmless snake and no deaths have ever been attributed to its bite (McCollough and Gennaro, 1963; Parrish and Khan, 1967).

Since coral snake antivenom became available in 1967 (Kitchens and Van Mierop, 1987), no deaths have been reported in the U.S.; however, before coral snake antivenom, the estimated case fatality rate was 10% (Parrish and Khan, 1967; Norris and Dart, 1989; German et al., 2005), and the cause of death was respiratory or cardiovascular failure. The best and most acceptable form of treatment after coral snake envenoming is with the use of antivenom. This study compared the efficacy of the Mexican antivenom, Coralmyn®, and U.S. antivenom, Wyeth's North American Coral Snake Antivenin to neutralize the toxicity effects of *M. t. tener* (Texas coral snake) and *M. f. fulvius* (Eastern coral snake) venom. Ultimately, the purpose of this study is to determine if Coralmyn antivenom is as effective in neutralizing the U.S. coral snake venoms. It is crucial to find a safe and effective antivenom that will replace the current U.S. one, which will no longer be available after October 2008 (Peterson, 2006).

Methods

2.1. Venoms

Two pools of venom from *M. f. fulvius* (Eastern coral snake) were purchased from Biotoxins Incorporated in St. Cloud, FL (Pool I-Lot#: MF/05A; Pool II-Lot#: MF/038.). Each pool contained venom from approximately 60 specimens found in south central Florida. Venom from *M. t. tener* was extracted from coral snakes found in the Houston area (Pool I) and South Texas area (Pool II) at the Natural Toxins Research Center (NTRC) on the average of every two weeks. Each pool contained venom from approximately 10 specimens. The snakes were allowed to bite into a para-film over a 15 mL test tube. The venoms were centrifuged for 5 min at 23°C at 12,800 × g to remove cellular debris. The venom supernatant was then transferred to vials with the proper labels and stored at −90°C until lyophilized.

2.2. Antivenoms

Coralmyn® is a polyclonal antivenom $(Fab)_2$ fragment with an equine origin, and produced by Instituto Bioclon in Mexico using venom from *Micrurus nigrocinctus nigrocinctus* (Black banded coral snake). The North American Coral Snake Antivenin is a polyclonal IgG with an equine origin, and produced by Wyeth® located in the United States using venom from *Micrurus fulvius fulvius* (Eastern coral snake).

2.3. Lethal Dose (LD_{50})

Five groups of eight mice for each venom were housed in cages and observed throughout the quarantine period and experiments. The endpoint of lethality of the mice was determined after 48 hr. The LD_{50} of the venoms listed in Tables 1 were determined in BALB/c mice. Venoms were dissolved in 0.85% saline at the highest test dose per mouse. Serial dilutions of 1.5 using saline were made to obtain four additional concentrations. All solutions during the experiment were stored at 0° C and warmed to 37°C just before being injected into mice. The lethal toxicity was determined by injecting 0.2 mL of venom (containing dosages ranging between 2.5 to 13.5 μg/mouse for *M. f. fulvius* venoms and dosages ranging from 4.5 to 22.5 μg/mouse for *M. t. tener* venoms) into a tail vein of 18–20 g female BALB/c mice. The injections were administered using a 1-mL syringe fitted with a 30-gauge, 0.5-inch needle. Saline controls were used. The LD_{50} was calculated by the Spearman-Karber method for each pool of venom (n=3 ±SD).

2.4. Antivenom efficacy dose (ED_{50})

Five groups of eight mice were challenged with a mixture of antivenom containing 3 LD_{50} of venom. Five doses of antivenom were used. Coralmyn and NACSA vials were reconstituted with 5 or 10 mL, respectively with WFI (water for injection) and all subsequent dilutions were made with sterile 0.85% saline. Stock venom solutions were freshly prepared at 0°C before being used. For each group of mice, 3 venom LD_{50} were mixed with antivenom and incubated at 37°C for 30 min. Each mouse was injected with 0.2 mL (NACSA) or 0.5 mL (Coralmyn) of venom/antivenom freshly mixed and into the tail vein of mice. The mice were observed for 48 hr and the percent survival and ED_{50} was calculated by the Spearman-Karber method for both antivenoms (n=3 ±SD).

2.5. Non-reduced SDS PAGE of Micrurus venoms

A total of 6 μg of each venom (*M. t. tener* pools I –II, *M. f. fulvius* pools I – II and *M. n. nigrocinctus*) were run non-reduced on a 10–20%Tris-Glycine SDS PAGE (Invitrogen™). A XCell *Sure*Lock™ system with Tris-Glycine SDS running buffer (10X) diluted to 1X at a voltage of 130 for 90 min using a Bio-Rad PowerPac™ Basic was used. SeeBlue Plus2 markers ranging from 3–250 kDa were used as controls.

Results

Venom lethality and antivenom efficacy

The LD_{50} of two North American coral snakes venoms and ED_{50} of two commercial antivenoms against the North American coral snakes were determined by intravenous route (Table 1 and Table 2). *Micrurus fulvius fulvius* venom was 3.4 times more toxic than *M. t. tener* venom 0.23 and 0.8 mg/kg, respectively (Table 1). Coralmyn antivenom was able to neutralize both North American coral snake venoms effectively with a mean ED_{50} of 507.5 and 319 LD_{50}/5 mL vial for *M. f. fulvius* and *M. t. tener*, respectively (Table 2). The NACSA was effective in neutralizing *M. f. fulvius* venom with a mean ED_{50} of 449 LD_{50}/10 mL vial, but was not effective in neutralizing at least 44 LD_{50} of *M. t. tener* venom.

Non-reduced SDS PAGE of Micrurus venoms

Micrurus tener tener showed approximately 19 bands, *M. f. fulvius* showed 15 bands, and *M. n. nigrocinctus* showed approximately 17 bands (Fig. 2). All protein bands ranged between 150 to 5 kDa. In general, these three *Micrurus* venoms share some common and some different characteristics.

Discussion

Parrish and Khan (1967) reported that bites of the Eastern coral snakes are believed to be more severe than that of the Texas coral snakes, and the lethality doses in our study confirms their findings since the LD_{50} for *M. f. fulvius* and *M. t. tener* averaged at 0.279 and 0.779 mg/kg, respectively (Table 1). The majority of the symptoms of coral snake envenomation in the United States have been reported as a result of Eastern coral snakes (McCollough and Gennaro, 1963; Kitchens and Van Mierop, 1987; German et al., 2005). Many of these symptoms include local swelling, respiratory arrest, seizures, paresthesias, vomiting, nausea, dizziness, lethargy, ptosis, among others (Ramsey and Klickstein, 1962; Mosely, 1966; McCollough and Gennaro, 1963; Pifano et al., 1986). Morgan et al. (2007) reported that these symptoms maybe isolated to mainly the Eastern coral snake with the exception of paresthesias. These same investigators reported that 37 patients bitten by the Texas coral snake did not develop neurologic symptoms, and many had more local pain and swelling than that reported for the Eastern coral snake envenomations (Morgan et al, 2007). Nonetheless, Eastern coral snake envenomations will still remain an issue in treatment when the NACSA is no longer available. Eastern coral snake venom does produce neurological symptoms that warrant treatment with antivenom.

The manufacturers of the NACSA state that their antivenom, one vial equaling 10 mL, is able to neutralize 250 LD_{50}, which is equivalent to 2 mg of *M. f. fulvius* venom. On the other hand, the manufacturers of Coralymn state that their antivenom, one vial equaling 5 mL, is able to neutralize 450 LD_{50}, which is 5 mg of *M. n. nigrocinctus* venom. In this study using BALB/c mice, NACSA was able to neutralize an average of 449 LD_{50} of *M. f. fulvius* venom per vial and less than 44 LD_{50} for *M. t. tener* venom. Coralmyn antivenom was able to neutralize an average of 507.5 LD_{50} of *M. f. fulvius* venom and 319 LD_{50} of *M. t. tener* venom (Table 2).

In this study, the two North American coral snake venoms showed differences in lethal potency. *Micrurus fulvius fulvius* had an LD_{50} of 0.268 to 0.291 mg/kg, which is similar to the LD_{50} of *M. fulvius* venom in other studies (Arce et al., 2003; de Roodt et al., 2004). On the other hand, *M. t. tener* had an LD_{50} of 0.736 to 0.822 mg/kg, which is similar to the LD_{50} of the Mexican coral snake venom, *M. n. nigrocinctus* (de Roodt et al., 2004).

Both antivenoms showed different neutralizing efficacy against both species of coral snakes (Table 1 and Table 2). The NACSA was effective against *M. f. fulvius* venom (ED_{50}=10 mL of antivenom neutralizing a mean of 449 LD_{50} of venom); however the antivenom was not effective in neutralizing 3 LD_{50} doses of *M. t. tener* venom. At 10 mL, the NACSA was still not capable of neutralizing 44 LD_{50} of venom (Table 2). On a milligram basis, the NACSA neutralized *M. f. fulvius* effectively (10 mL neutralizing a mean of 2.4 mg and less than 0.76 mg for *M. t. tener* venom). Coralmyn antivenom was effective in neutralizing both *M. f. fulvius* and *M. t. tener* venoms. Neutralization was more effective against *M. t. tener* venom on a milligram basis (ED_{50}= 10 mL of antivenom neutralizing a little over 10 mg of venom), while neutralization of *M. f. fulvius* venom was only half as effective (ED_{50}=10 mL of antivenom neutralizing an average of 5.5 mg of venom) (Table 1). However, on an LD_{50} basis, Coralmyn was more effective in neutralizing *M. f. fulvius* venom (10 mL neutralizing a mean of 507.5 LD_{50} and 319 LD_{50} for *M. t. tener* venom) (Table 2). As in previous studies

(Consroe et al., 1995;Arce et al., 2003;Sánchez et al., 2003), the more toxic venoms on an LD_{50} basis are easier to neutralize because less venom is required to administer an LD_{50}; and thus a more logical way to test antivenom efficacy is first protect the mouse with antivenom and then determine the LD_{50} as previously suggested (Sánchez et al., 2003). The ability of Coralmyn to neutralize the Texas coral snake venom more effectively than the NACSA could be due to the fact that the Texas coral snake and the Mexican black-banded coral snake are more geographically related (Campbell and Lamar, 2004). According to the SDS-PAGE, *M. t. tener* and *M. n. nigrocinctus* venom shared more common bands than *M. n. nigrocinctus* and *M. f. fulvius* venom (Fig. 2). The similarity of *M. n. nigrocinctus* and *M. t. tener* venom profiles is consistent with the ability of Coralymn to neutralize these venoms effectively.

Until recently, most of the published article pertaining to coral snake neutralization in the USA has been limited to that of the *M. f. fulvius* (Cohen et al., 1968 and 1971; Amuy et al., 1997; de Brandão da Silva et al., 2001; Wisniewski et al., 2003; de Roodt et al., 2004). This present study reveals a lower effectiveness of the NACSA to neutralize the venom of the Texas coral snake, and recent assessments of Texas coral snake bites have questioned the necessity of antivenom administration when bitten by these Texas snakes (Standford et al., 2005; Borys et al., 2005; Morgan et al., 2007). Further studies are needed to completely rule out the need for antivenom in the cases of Texas coral snake bites. Nonetheless, our study shows that the Mexican antivenom, Coralmyn, is effective in neutralizing the Texas coral snake venom in a mouse model; and thus, could be an alternative in treating patients with both Eastern and Texas coral snake envenomations. Unlike the NACSA, Coralmyn is an $F(ab')_2$ antibody and it less likely to cause adverse reactions such as anaphylaxis in patients (Sutherland, 1977; Dart and McNally, 2001); furthermore, (Fab')2 antibodies are distributed faster into the tissue of Coralmyn (Chippaux and Goyffon, 1998) and the reconstitution is immediate as opposed to 45 min for NACSA (Table 3) (Kanavage et al., 2006).

The goal of this study was to determine if Coralmyn antivenom was capable in neutralizing the North American coral snake venoms so that the U.S. may have an effective alternative in treating these envenomations when the NACSA will no longer be available. The results not only show that Coralmyn is effective in neutralizing both coral snake venoms tested, but it is more effective in neutralizing *M. t. tener* venom, than the NACSA. Furthermore, Coralmyn is able to neutralize perhaps the most clinically important coral snake venom, *M. f. fulvius*, with a mean of 507.5 LD_{50} per vial versus 449 LD_{50} per vial of the North American approved product.

Acknowledgments

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Figure 1.
Geographical distribution of *Micrurus fulvius fulvius* and *M. tener tener*.

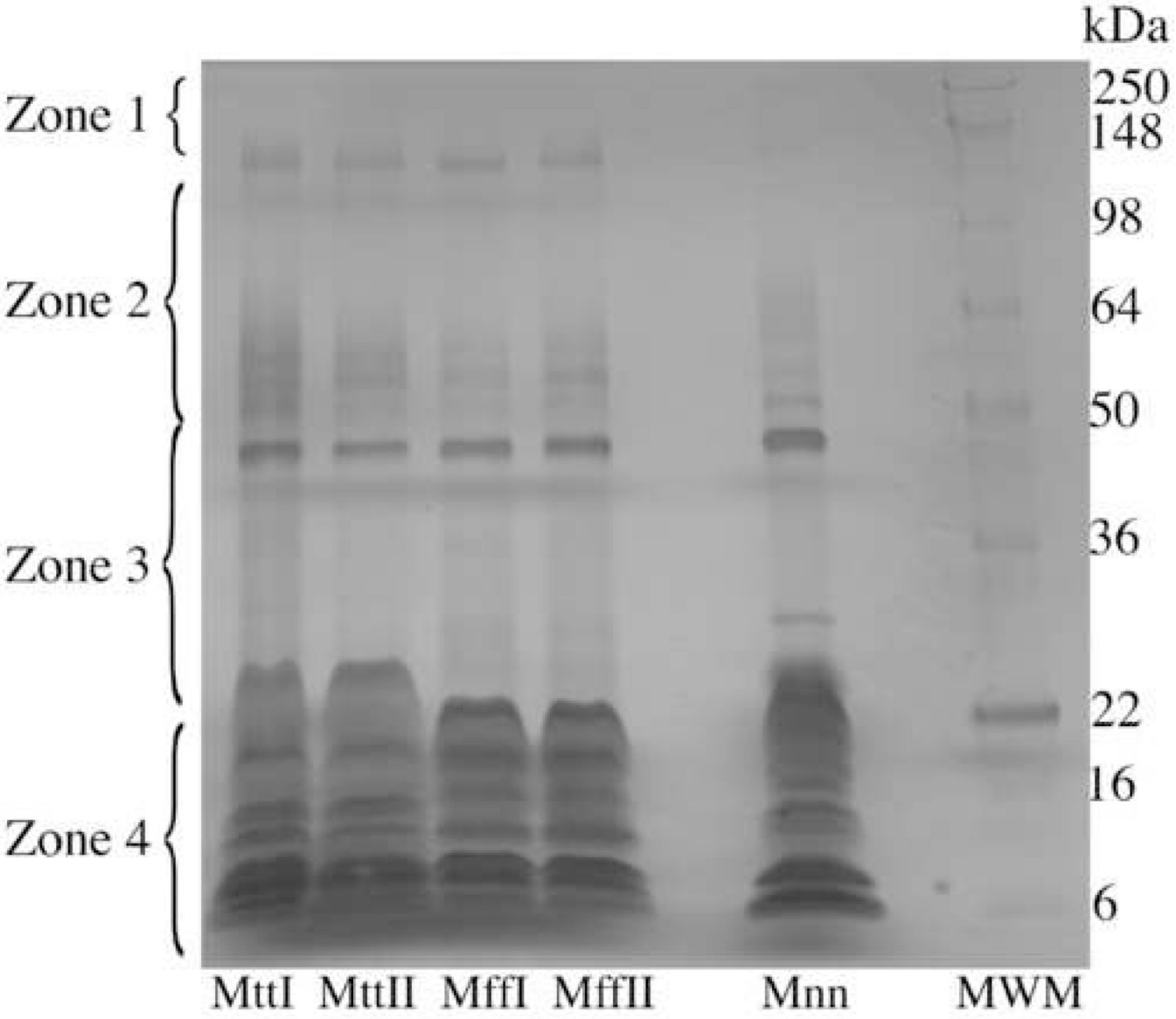

Figure 2.

Non-reduced SDS PAGE of *Micrurus* venoms. A 10–20% Tris-Glycine gel was used to run 6 μg of *M. t. tener* venoms pools I and II, *M. f. fulvius* venoms pools I and II and *M. n. nigrocinctus* venom. An XCell *Sure*Lock™ electrophoresis system with Tris-Glycine SDS running buffer was used. The gel was run at 130V, 90 min on a Bio-Rad PowerPac™ Basic. The markers used were SeeBlue Plus2 (Invitrogen). Mtt: *M. t. tener*, Mff: *M. f. fulvius*, and Mnn: *M. n. nigrocinctus*.

Table 1

LD_{50} of North American Coral Snake Venoms and ED_{50} of Wyeth and Bioclon Antivenom

Species	Pool	LD_{50} (*i.v.*) (mg/kg ± SD)[c]	Mean LD_{50} for species	NACSA ED_{50} (mL/mg±SD)[d]	NACSA Mean ED_{50} (mL/mg)	NACSA ED_{50} (mL/mg±SD)[e]	Coralmyn ED_{50} (mL/mg ± SD)[d]	Coralmyn ED_{50} (mL/mg ± SD)[e]	Coralmyn Mean ED_{50} (mL/mg)
M. f. f.	I[a]	0.268±0.029	0.279	10/2.17± 0.4	10/2.39	1/0.217 ± 0.04	10/4.86 ± 0.7	1/0.486 ± 0.071	10/5.5
M. f. f.	II[a]	0.291±0.055		10/2.61± 0.3	<10/0.76	1/0.261 ± 0.03	10/6.11 ± 1.2	1/0.611 ± 0.124	10/11.17
M. t. t.	I[b]	0.822±0.118	0.779	<10/±0.76		< 1/ 0.076	10/10.11 ± 1.7	1/1.011 ± 0.172	
M. t. t.	II[b]	0.736±0.133		<10/ 0.76		< 1/ 0.076	10/12.23 ± 3.9	1/1.223 ± 0.395	

[a] Pooled venom obtained from Biotoxins Inc., St. Cloud, FL.

[b] Pooled venom obtained from the NTRC, Kingsville, TX.

[c] The LD_{50} is the concentration of venom (mg/kg body weight) required to kill 50% of the BALB/c mice injected iv with 0.2 or 0.5 mL of the various snake venoms. LD_{50} was calculated using the Spearman-Karber method.

[d] Expressed as mL of antivenom/mg of venom neutralized; ED_{50} values were determined against 3 LD_{50} of venoms. n=3

[e] Expressed as the amount of protein (mg) neutralized by 1 mL of antivenom.

Table 2

LD_{50} per mouse and ED_{50} per vials

Species	Pool	LD_{50} mg/mouse	NACSA ED_{50} (mL vial/LD50)[a]	NACSA Mean ED_{50} (mL vial/LD_{50})	NACSA ED_{50} (mL/LD_{50})[b]	Coralmyn ED_{50} (mL vial/LD_{50})[c]	Coralmyn Mean ED_{50} (mL vial/LD_{50})	Coralmyn ED_{50} (mL /LD_{50})[b]
M. f. f.	I	0.00509	10/426	10/449	1/42.6	5/477	5/507.5	1/95.4
M. f. f.	II	0.00552	10/472		1/47.2	5/538		1/107.6
M. t. t.	I	0.01561	10/< 44	<10/44	1/<0.44	5/323	5/319	1/64.6
M. t. t.	II	0.01398	10/< 44		1/<0.44	5/315		1/63

[a] Wyeth's insert states that one 10 mL vial neutralizes 250 LD_{50} of *M. fulvius* venom.

[b] Expressed as the number of LD50 neutralized by 1 mL of antivenom.

[c] Bioclon's insert states that one 5 mL vial neutralizes 450 LD_{50} of *Micrurus* ssp venom.

Table 3

Comparison of NACSA and Coralymn

Characteristics	NACSA	Coralmyn
Produced	United States of America	Mexico
Origin	Equine	Equine
Antibody Type	IgG	(Fab')2
Molecular Weight	110 kDa	50 kDa
Reconstitution (min)	45[a]	Immediate[b]
Distribution (h)	>3[c]	3 [c]
Elimination (h)	>100 [c]	60 [c]
Tissue Affinity	High [c]	Moderate-High [c]
Elimination Route	Immune Tissue [c]	Immune Tissue [c]
Binds Complement	Yes [c]	No [c]
ED_{50} [d]	1 vial (10 mL) neutralizes 250 LD_{50}	1 vial (5 mL) neutralizes 450 LD_{50}

[a] Kanavage et al., 2006

[b] Current study

[c] Chippaux and Goyffon, 1998

[d] Manufacture's antivenom insert information