NEUTRALIZATION ON VENOMS FROM FIVE *BOTHROPS* SPECIES, PROVIDED BY TWO THERAPEUTIC ANTIVENOMS USED IN ARGENTINA.

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OBJECTIVES. To determine the specific and paraspecific neutralization provided by two antibothropic antivenoms produced by the Ministry of health in Argentina on the venoms of the five species of *Bothrops* of highest sanitary importance in the country.

FIGURE 1.

Figure 1.a

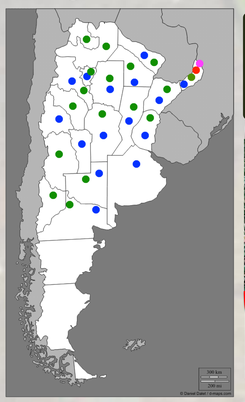
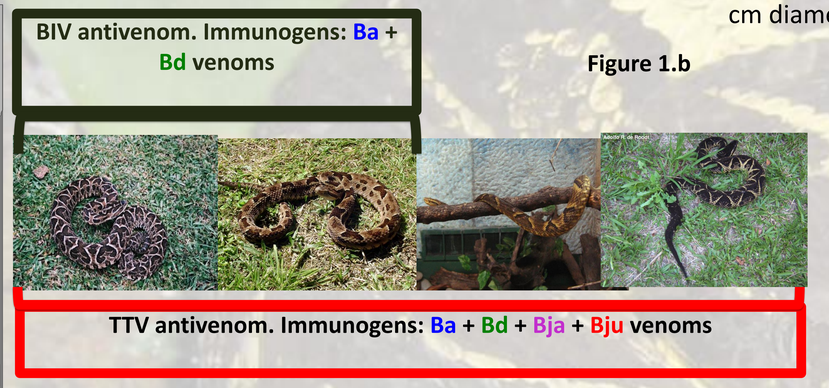


Figure 1.b



1.a Distribution of the species used for obtain the venomw to be used as immunogens. **1.b.** Venoms used as immunogens in the antivenom production. **Ba: *B. alternatus***; **Bd: *B. diporus*; Bja: *B. jararaca***; **Bju: *B. jararacussu*** venoms

CONCLUSIONS. In Argentina there exist at least 10 species of *Bothrops*, nevertheless only Ba and Bd are commonly found and widely distributed in the most crowded regions of the country, reason by which the BIV is the most distributed AV in Argentina. The production of TTV is not easy due the difficult obtaining of Bja and Bju venoms, present in low number in only one province (**See Figure 1**). By this reason, the search on immunogenic mixtures that could produce neutralizing antibodies with high paraspecificity, could facilitate the production and cover of AV. As a first step, we compare the specific and paraspecific neutralization of the BIV on different bothropic venoms and compare this with that provided by the specific TTV. Both antivenoms were equally efficient in the neutralization of the tested activities, independently of their immunogenic mixtures. In example, lethality (neutralization of lethality is the gold standard test to control antivenoms), coagulant, hemorrhagic and radial PLA_2 activities from Bja and Bju venoms were well neutralized by the BIV, which does not include them in their immunogenic mixture, while Bn venom was well neutralized by both AVs. The results showed that the cross neutralization on the tested activities from these venoms is high. Nevertheless, despite the good neutralization of PLA_2 and indirect hemolytic activity provided by both antivenoms, the neutralization of the indirect hemolytic activity of Bju venom seems to be higher using the specific TTV. In view of these results, the paraspecificity of BIV would be able to neutralize the non specific venoms (Bja and Bju), nevertheless, the apparently minor neutralization by BIV on indirect hemolytic activity must be deeper studied. Differences in neutralization, considering the volume of AV required to neutralize, were strongly related with the amount of specific $F(ab')_2$ fragments in the AVs.

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MATERIALS AND METHODS. We studied the neutralizing capacity of two anti-*Bothropic* AVs (antivenoms): 1) BIV, produced by immunizing horses with venoms of *Bothrops alternatus* [Ba] and *B. diporus* [Bd], and 2) TTV, produced by immunizing the both mentioned venoms plus *B. jararaca* [Bja] and *B. jararacussu* [Bju] venoms (**Figure 1**) on these same venoms and on *B. neuwiedii* venom (Bn). Their neutralizing capacity on lethality (mice, challenge $5LD_{50}$), hemorrhagic (rats, challenge 1.5 MHD, (minimal hemorrhagic dose*), coagulant (on plasma, challenge 1 MCD-P), proteolytic (gelatin, 1 hidrolytic dose*) and PLA_2 (egg yolk, 2 hydrolitic dose*) activities of Ba, Bd, Bn, Bja and Bju venoms, were studied (de Roodt et al., 1998, 2011; WHO 2016). In addition the toxicity of Bja and Bju venoms on myoblast C2C12 cells and their neutralization by AVs (8 ul of AVs versus 4 ug of venom) was assayed (Bustillo et al., 2017). (*)= dose of venom that causes 1 cm diameter of hydrolysis in plate or hemorrhage in skin.

RESULTS. In all the cases BIV and TTV neutralized all the activities of the venoms. Volumetrically the neutralizing doses (mg of V [venom] neutralized by ml of AV [antivenom]), TTV showed more potency. However, when the neutralizing potency was expressed as the mg of AV, the doses turn very close. **See Table 1**. Proteolysis in gelatin was inhibited and the hemorrhage was well neutralized in all the cases, 0.4 ug of each antivenom neutralized 100% the hemorrhage of 1,5 MHD of venom. The heterologous neutralization of Bja and Bju venoms on myotoxicity on C2C12 cells was similar, since 8 ul of each antivenoms neutralized 100% the toxicity on these muscle cells. **See Figures 2 and 3.** PLA_2 activity (radial hydrolysis of phospholipids and indirect hemolysis) and plasma coagulation were neutralized in all the cases and all the doses got close when were considered as the amount of AV protein required for neutralizations. **See Tables 2 and 3.**

TABLE 1. NEUTRALIZATION OF THE LETHALITY

Venoms	Antivenoms			
	ED_{50} BIV		ED_{50} TTV	
	µl	mg	µl	mg
B. alternatus	112 (104–120)	2.02 (1.87–2.16)	50 (48–53)	2.15 (2.06–2.28)
B. diporus	104 (94–115)	1.87 (1.69–2.07)	33 (30–36)	1.42 (1.2–1.55)
B. neuwiedi	150 (111–162)	2.7 (2.00–2.92)	43 (39–48)	1.85 (1.68–2.06)
B. jararaca	86 (82–89)	1.55 (1.48–1.60)	33 (32–34)	1.42 (1.38–1.46)
B. jararacussu	210 (188–233)	3.78 (3.38–4.19)	82 (80–85)	3.53 (3.44–3.66)

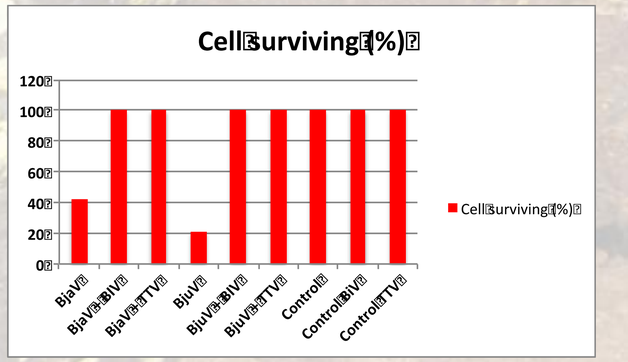
TABLE 2. NEUTRALIZATION OF COAGULANT ACTIVITY

Venoms	Antivenoms			
	BIV		TTV	
	µl	mg	µl	mg
B. alternatus	50	0.90	25	1.08
B. diporus	50	0.90	50	2.15
B. neuwiedi	100	1.80	50	2.15
B. jararaca	25	0.45	25	1.08
B. jararacussu	50	0.90	25	1.08

TABLE 3. NEUTRALIZATION OF PLA_2 ACTIVITY

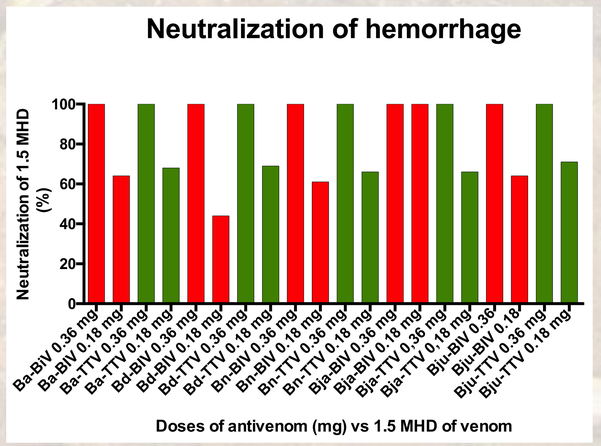
Venoms	Antivenoms			
	BIV		TTV	
	µl	mg	µl	mg
B. alternatus	5.9 (3.7–9.5)	106.2 (66.6–171)	5.4 (4.6–6.3)	232.2 (197.8–270.9)
B. diporus	6.6 (6.1–7.3)	118.8 (109.8–131.4)	2,8 (2.3–3.5)	120.4 (98.9–150,5)
B. neuwiedi	5.7 (4.3–7.6)	102.6 (77.4–136.8)	1,5 (1.2–1.7)	64.5 (51.6–73.1)
B. jararaca	4.4 (2.6–7.4)	79.2 (46.8–1332.2)	1,2 (1.0–1.4)	51.6 (43.0–60.2)
B. jararacussu	15.9 (12.9–19.7)	286.2 (232.2–354.6)	4,5 (4.9–6.8)	294.4 (210.7–292.4)

FIGURE 2.



Neutralization of the toxic effect on muscle cells (C2C12 cells). Cells were challenged with 4ug of venom incubated with 8 ul of each antivenom.

FIGURE 3.



Neutralization of the hemorrhagic activity caused by 1.5 MHD of toxic effect on muscle cells (C2C12 cells). Cells were challenged with 4ug of venom incubated with 8 ul of each antivenom.