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# CROSS NEUTRALIZATION OF SOME SNAKE VENOMS FROM AFRICA AND MIDDLE EAST BY VACSERA POLYVALENT SNAKE ANTISERA

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**Abstract**

An extensive study of neutralization of lethality of two species of elapid, seven species of genus Viper, and two species of *Macrovipera* by VACSERA polyvalent snake antisera**.**

The results showed that polyvalent snake venom antisera prepared by injecting horses with *Naja haje, Naja nigricolis,* and *Cerrastes cerrastes venom*) was highly effective in neutralizing the venoms specifically and neutralized Para-specifically others including *Vipera palastinae, Vipera xanthina, Vipera ammodytes, Echis coloratus, Echis carinatus, Cerastes vipera* and *pseudocerrastes-feildi* beside *Macrovipera* species including *Macrovipera lebetina obtuse, M. lebetina turanica.*

**Key words:** Egypt, VACSERA polyvalent snake, Africa, Middle East, Snake venom

## Introduction

Preparation of snake antivenom includes administration of the venom to a suitable animal, mainly horses and after an appropriate period collecting the specific antibodies from the serum of the inoculated animal (Joger *et al,* 2007). During such procedure the recipient animal may suffer different types of ill-health signs including, generalized asthenia, pallor, skin rashes, muscular pain, hemorrhages, cardiovascular, respiratory problems, nervous signs as paresis and paralysis, break down of tissues, and finally collapse and death, the severity and duration of the observed clinical signs depend on the nature, amount and site of the injected venoms (Rosenberg, 1990). Genus *Vipera* is widespread throughout Western and Central Asia. It is a genus in constant revision and recognizes some two dozen species and a number of subspecies (Stümpel and Joger, 2009; Thorpe *et al,* 2007). The genus *Macrovipera* extends from Eastern Europe to Western and Central Asia, as well as Mediterranean Africa (David and Ineich, 1999). Between 1999 and 2008, several genus-level name changes have occurred, most notably the transfer of some species of *Vipera* and *Macrovipera* of genera *Daboia* and *Montivipera* (Cox *et al,* 2022). Para-specificity (also known as cross-neutralization) refers to the capacity of antivenom to neutralize the venom of species not included in the immunization scheme of animals used for antivenom production at therapeutically useful doses, not more than those necessary for specific neutralization (Ursenbacher *et al,* 2008). It was studied among some genera, and sometimes extends beyond a genus (Ramos-Cerrillo *et al,* 2008). The therapeutic effectiveness of antivenom relied on the toxin-specific protective antibodies within, an immunological approach, such as ELISA or antivenomics, could be a way to evaluate the neutralizing potency against specific toxin activity (Calvete *et al,* 2014). Nevertheless, systematic information of the bona fide spectrum of Para-specific neutralization of lethality may be of use to treating clinicians in cases when the offending snake was not identified, or identified but not included in immunization protocol (Segura *et al,* 2010). The envenomation availability severity, and others, i.e. the expected safety of the antivenom and the danger of squeals even when symptomatic treatment would suffice to prevent death, must guide the choice to use antivenom in the absence of clinical validation of antivenom efficacy for particular species (WHO, 2010). In this investigation we generated polyvalent experimental equine antisera to study the Para-specific spectrum of

protection afforded by it against a collection of two Elapidae, seven *Vipera* and two *Macrovipera* venoms.

The study aimed to evaluate Para-specific neutralization, its extent and potency versus specific neutralization within and between each genus.

## Material **and Methods**

Venoms*:* All included *Naja haje, Naja nigricollis, Cerrastes cerrastes, Vipera palastinae, Vipera xanthina, Vipera ammodytes, Echis coloratus, Cerastes vipera, Pseudo-cerastes feildi, Macrovipera lebetina obtuse,* and *M. lebetina turanica* were prepared lyophilized and certified originally from Helwan Farm, Egyvac. All were dissolved in sterile normal saline solution as 1mg/1ml.

Antivenom*:* polyvalent snake venom antisera from vacsera, Egypt which prepared by injection of horses by *Naja haje, Naja nigricolis,* and *Cerrastes cerrastes* venoms and after an appropriate period collecting the specific antibodies from the serum of the inoculated animal. Vacsera snake antivenom is a trivalent antiserum raised by immunizing three groups of horses by three previous mentioned venoms. Immunization scheme consisted of 12 doses starting with an initial dose of 500mg/horse of each venom mixture emulsified with complete Freund's adjuvant (CFA, Rockland, PA), followed by upgrading venom doses without adjuvant. All immunizations injections were subcutaneously and antibody titers were monitored regularly till time of plasma collection using the immunopheresis technique. Antiserum consisted of Equivolume pools of horses' sera.

Animals*:* For lethal potency and neutralization of lethality, 18-20g Swiss Albino male mice (VACSERA) were used, following the guide for care and use of laboratory animals (Conour *et al,* 2006).

Lethal potency*:* Different doses of each venom were injected IV in five mice using tthe conventional technique (WHO, 2010).

Mice deaths 48hrs post injection and lethal potency were calculated as ($LD_{50}$), venom dose in µg/mouse. The plot of mortality versus venom dose was analyzed by nonlinear regression (Casasola *et al*, 2008).

Neutralization of lethality: Different doses of antivenom were incubated with $LD_{50}$ of each venom species for 30 min at 37$^{O}$C. The samples were then injected intravenously in mice (n ¼ 5/dose). The dyed mice were recorded and the median effective doses ($ED_{50}$) were calculated as the antivenom dose protected 50% of mice. Antivenom potency was calculated using formula: Potency ¼ [(n-1)/ $ED_{50}$]? $LD_{50}$; where n-1 represented number of lethal doses of challenge minus one. $LD_{50}$ was subtracted from total challenge dose (n) was the dose caused 50% mice's death , i.e. calculation based on total challenge minus one was the actual venom quantity caused 100%mortality as neutralized by antivenom. As $ED_{50}$ was µg/µl (=mg/ml), or mg venom neutralized by 1 ml antivenom.

Statistical analysis*:* Data were presented as mean and standard deviation (SD) or with the 95% confidence intervals in parentheses. When necessary, Student's t test was used for comparisons. Data were analyzed by using combined Prism 4.0 software package (Barde and Barde, 2012).

## Results

Both *Cobra* and *Vipera* venoms were neutralized by polyvalent snake antisera ($LD_{50}$). The specific neutralization potency ranged from 80µl/ml *N. haje*, 35µg/ml *N. nigricollis* to 79.4µg/ml *Cerrastes cerrastes*, but Para-specific neutralization ranged from 7.32µg/ml *Vipera xanthine*, 10.64µg/ml *V. palastinae*, 21.25µg/ml, *Echis coloratus*, 25.5µg/ ml, *E. carinatus 28*µg/ml, *Pseudocerastes feildi* to 38.2µg/ml *Cerrastes vipera*. *Macrovipera* Para-specific neutralization was 18.4µg/ml in *M. obtusa*, and 18µg/ml for *M. turanica*.

*Cobra* venoms were the most potent venoms (2.1ug/mouce) for Egyptian cobra (*Naja haje)*, and spitting cobra (*N. nigricollis*) venom was (8.7ug/mouce). All vipers' venoms were significantly lethal than macrovipers. The most potent one was *V. ammodytes* (8.0µg/mouse) and the least one was *P. feil-*

*di* and *E. coloratus* (21.25 & 25µg/mouse respectively). In *Macrovipera*, the most lethal was *M. obtusa* (17.85µg) and the least one was *M. turanica* (20.4µg/mouse).

Details were given in tables (1 & 2) and figures (1 & 2).

Table 1: Median lethal dose of venom (ug/mouse)

| Venom | LD50 (-) |
|---|---|
| *Naja haje* | 2.1 |
| *Naja nigricollis* | 7.32 |
| *Cerastes cerastes* | 10.7 |
| *Vipera ammodytes ammodytes* | 8.0 |
| *Vipera xanthine* | 11.48 |
| *Cerrastes vipera* | 16 |
| *Vipera palastinae* | 19.1 |
| *Echis coloratus (Saw scaled viper)* | 25.5 |
| *Echis carinatus (Echis pyramidum)* | 28 |
| *Pseudo-cerastes feildi* | 21.25 |
| *Macrovipera lebatina obtuse* | 17.85 |
| *Macrovipera lebatina turanica* | 20.4 |

(-)confidence interval of <0.01 as just one intermediate survival value at very close doses (95%).

Table 2: Lethality by VACSERA neutralization of polyvalent antivenom.

| Snake Venom | *$ED_{50}$ = µg/ml |
|---|---|
| *Naja haje* | 80 |
| *Naja nigricollis* | 35.0 |
| *Cerrastes cerrastes* | 79.4 |
| *Vipera ammodytes ammodytes* | 15.3 |
| *Vipera xanthine* | 7.32 |
| *Cerrastes vipera* | 38.2 |
| *Vipera palastinae* | 10.64 |
| *Echis coloratus* | 21.25 |
| *Echis carinatus(pyramidum)* | 20.4 |
| *Pseudo-cerastes feildi* | 25.5 |
| *Macrovipera lebatina obtuse* | 18.4 |
| *Macrovipera lebatina turanica* | 18 |

## Discussion

Snakebites are a common problem in Medical and Veterinary Medicine. Vipers are member of the family Viperidae, a group of snakes found worldwide (Peterson, 2007).

Snake antivenoms are the specific treatment for snakebites envenomation (Elfiky *et al,* 2023). Anti-venoms can prevent or reverse snakebites effect and minimize mortality and morbidity as toxicity differs among species. A list of snakebite envenoming was given (Williams *et al.* (2019)

There is an urgent need to have safe, effective and affordable antivenoms, particularly for developing countries, and to improve regulatory control over the manufacture, import, and sale of antivenoms (WHO, 2010). "Specific" antivenom means that it was developed specifically to neutralize the venom of the snake that bit the patient, and also neutralized the venoms of related species or Para-specific neutralizati-on (Fathi *et al,* 2022). VACSERA polyvale- nt antiserum was specifically neutralized by Egyptian cobra, Spitting cobra, and *C. cera-stes* venom. Ad hoc it was neutralized Para-specific by *Vipera* venoms including *V. ammodytes, V. xanthinae, C. vipera, E. coloratus, E. carinatus, P. feildi,* and *Macrovipera* venoms as *M. l. obtuse,* and *M. l. turanica*. But, the Elapidae venoms were significantly more lethal than that of *Vipera*, or *Macrovip era*.

In the present study, Elapidae venoms the $LD_{50}$ of *Naja haje* venom were 2.1µg/mouse (0.105mg/kg) by I.V. injection. This r nearly agreed with Shaban and Hafez (2003), they found that $LD_{50}$ of *N. haje* venom was 2.1 µg/mouse by IV. But, $LD_{50}$ of *N. nigricollis* was 7.2µg/mouse (0.36mg/kg). Also, this agreed with Abd El-Aziz *et al.* (2019), they found that $LD_{50}$ of *N. nigricollis* was 0.34

mg/kg and 5.5µg/mouse respectively in spite of difference in injection roots. The *Vipera* venoms were significantly more lethal than *Macrovipera* ones as $LD_{50}$ of *C. cerrastes* venom was 10.7µg/mice (0.535mg/kg). This nearly agreed with Seddik *et al.* (2002), who reported 9µg/mouse. $LD_{50}$ of *V. ammodytes* venom was 8.25µg/mouse (0.412mg/kg), which agreed with Garcia-Arredondo *et al.* (2019), who reported a dose was 8.4µg and 8.07µg/mouse respectively. *V. xanhina* venom $LD_{50}$ was 11.65µg/mouse (0.582mg/kg). This agreed with Archundia *et al.* (2011), they reported .2µg/mouse. $LD_{50}$ of *C. vipera* venom was 19.2µg/ mouse (0.9mg/kg). This nearly agreed with Saber e*t al.* (2019), who reported 18.3µg/mouse (0.915mg/kg). $LD_{50}$ of *V. palastinae* venom was 19µg/mouse (0.95mg/kg), but was 8.4µg/mouse (Arhundia *et al,* 2011). The differences may be due to geographical distribution. $LD_{50}$ of *E. coloratus* venom was 25µg/mouse (1.25mg/kg). This nearly agreed with *S*eddik *et al.* (2002), who reported 20µg/mouse in Sudan species. $LD_{50}$ of *E. carinatus* was 28µg/ mouse (1.25mg/kg). This more or less agreed with Abd El-Aziz *et al.* (2019), who reported 1.744mg/kg, but it was 30µg/mouse for Sdan species, and 25µg/mouse for Saudi ones (Seddik *et al,* 2002).

In the present study, $LD_{50}$ of *P. fieldi* venom was 21.25µg/mouse (1.06mg/kg), but it was 6.0µg/mouse by Seddik *et al.* (2002). The $LD_{50}$ of *M. lebatina* venom was 18µg/mouse (1.25mg/kg) for *M. obtusa*, and 20.0 µg/mouse (1.02mg/kg) for *M. turanica*. This agreed with Warrell (2010), who reported that *M. l. obtusa* was 12-18µg/mouse, and Garcia-Arredondo *et al.* (2019), who reported 16.32µg/mouse for *M. obtusa* and 18.36 µg/mouse for *M. turanica*. As the venoms responsible for lethality were antigenically conserved and spread among species/subspecies Garrigues *et al.* (2005), VACSERA snake antiserum was specifically neutralized *Naja haje,* and *N. nigricollis* venom ranged were from 80 to 35.0µl/ml, and *C. cerrastes* venom by 79.4µl/ml, but Para-specifically neutralized other *Viper* venoms ranged from 7.32 to 38.2µl/ml. The lowest Para-specific neutralization potency for *V. xanthina* was (7.32µl/ml). This could reflect the antigenic difference between the specific venoms used in immunization, as the differences were in limited significance.

In the present study, $ED_{50}$ was expressed as µl venom neutralized by 1ml of polyvalent antivenom with 95% confidence intervals. Also, the present Elapidae venoms were neutralized specifically 80µl/ml for *N. haje*. This agreed with Seddik *et al.* (2002), who reported 80µl/ml, also neutralized specifically 35.0µl/ml of *N. nigricollis* venom was 30-µl/ml. But, in Viperidae venoms neutralized specifically *C. cerastes* by 79.4µl/mouse. This agreed with Seddik *et al.* (2002), who found 80µl/mouse. But, 1 ml VACSERA snake antisera neutralized Para-specifically other vipers as *C. viper* by 38.2µl/ml, which nearly agreed with Seddik *et al.* (2002), who found 25 µl/ml. *V. ammodytes* was neutralized by 15.3µl/ml by VACSERA snake antisera, but it was 11.28µl/ml for Inoserp Europe antivenom (Alejandro *et al,* 2019). Also, *V. xanthina* was neutralized Para-specifically by 40µl/ml, while it was 16.13µl/ml for Inoserp Europe antivenom.

In the present study, Ad-hoc VACSERA snake antisera neutralized Para-specifically *V. palastinae* by 10.64µl/ml, *E. coloratus* by 21.25µl/ml and *E. carinatus* by 20.5µl/ml, but it was 20µl/ml and 17.5µl/ml for Sudan & Saudi species respectively (Seddik *et al,* 2002). Also, VACSERA snake antisera neutralized Para-specifically *E. carinatus* by 20.5µl/ml, and *P. feildi* by 25.5µl/ml, but it was 15µl/ml & 20µl/ml respectively (Seddik *et al,* 2002). Also, *Macrovipera* VACSERA snake antisera neutralized Para-specifically *M. l. obtusa* by 20µl/ml and *M. l. turanica* by 22µl/ml, but it was 3.5µl/ml for *lebatina* without subspecies (Seddik *et al,* 2002).

## Conclusion

The preclinical neutralization outcome results showed that VACSERA snake antivenom effectively neutralized the lethality of

the venoms analyzed proving its specificity and Para-specificity.

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Explanation of figures

Fig. 1: Median venom lethal dose (ug/mouse).

Fig. 2: VACSERA neutralization lethality by polyvalent snake venom