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VARIATION IN YIELD AND LETHALITY OF VENOMS FROM IRANIAN SNAKES

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M. Latifi. Variation in yield and lethality of venoms from Iranian snakes. Toxicon 22, 373-380, 1984. — The dangerous venomous terrestrial snakes of Iran belong to three groups: the Elapidae (cobras); the Viperinae (true vipers); the Crotalinae (pit vipers). Geographica dilstribution of each species was determined. Studies on the venoms extracted from the following Iranian snakes, Oxus cobra, Naja naja oxiana, Levantine viper (Afyi), Vipera lebetina, Carpet viper, Echis carinatus, Persian horned viper, Pseudocerastes persicus, Latifi viper, vipera latifii, Mountain viper, Vipera xanthina and Caucasus pit viper (Agkistrodon halys), indicated that the yield of venom varies in each species. Venoms were compared for their lethality (i.v. LD 50 in mice) and their rate of production. The antigenic components of the venoms were compared with their antisera by gel diffusion tests. To obtain the best results from antivenom treatment, the serum should be made against the venom of the local poplation of snakes or, at least, the commercial antivenom should be controlled for potency by testing with local reference venom.

Introduction

There are many reports indicating that the yield of venom and its potency depend on many factors, such as age, sex, stress, temperature, captive care feeding, etc. (Glenn et al., 1972). Studies on the extraction and production of venom have been reported by many authors (Bellumini, 1968; Deoras, 1966; Minton, 1975). As there was no information available on the yield and toxicity of Iranian snakes' venoms, the author began

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such a study in 1960. The venomous snakes in Iran can be classified in five groups: the Elapidae (cobras); the Viperinae (true vipers); the Crotalinae (pit vipers); the Colubridae (colubrids), such as Boiga trigonata, Malpolon monspessulanus, Malpolon moilensis, Telescopus fallax and Telescopus rhinopoma; the Hydrophidae (sea snakes), such as Enhydrina schistosa, Hydrophis cyanocinctus, Hydrophis spiralis, Hydrophis ornatus and pelamis platurus.

A large number of snakes were collected from various parts of Iran and extensive studies on the geographical distribution, toxicity, yield of venom and antivenom production were conducted, some of which have been reported previously (Latifi and Manhouri, 1966; Latifi and Farzanpay, 1973; Latifi, 1973, 1978). The results of further studies are presented in this communication.

Materials and Methods

Over 92,000 venomous snakes were collected from various parts of Iran studied for taxonomy; geographical distribution and venom production. The terrestrial venomous snakes of Iran are divided into two main groups: (a) the back-fanged snakes, Opisthoglypha; (b) the front-fanged snakes, Elapidae, Viperinae and Crotalinae. The schematic map (Fig. 1) shows the distribution of the front-fanged snakes of Iran. For venom collection the snakes were kept under conditions which ensured continuous venom producton. Healthy snakes were identified and milked. The techniqiue of milking varied slightly in solenoglypha and proteroglypha snakes (Latifi, 1978). The collected venoms were stored at - 20°C for a few hours and freeze dried. The yield of dry venom was determined for each snake. For further investigation, eight Iranian snakes (Oxus cobra, Naja naja oxiana, Levantine viper (Afyi), Vipera lebetina, Carpet viper, Echis carinatus, Persian horned viper, Pseudocerastes persicus, Latifi viper, Vipera latifii, Mountain viper, Vipera xanthina ssp. and Caucasus pit viper, Agkistrodon halys= intermedius) were selected. Ten snakes of equal size from each species of either sex of certain localities were milked and the venoms wre pooled. The yield of the venoms collected from right and left fangs and also pooled extractions of both fangs were recorded.

Venom lethality was determined-in-16-18g mice by i.v. in-

jection of various dilutions of venom solution in saline, and LD50 was estimated by the Spearman — Karber method (Finney, 1964). The variation in yield of venoms in different seasons for different species were compared by the Student's t-test (Bancroft, 1959). Six monospecific antivenoms against each venom were prepared by immunization of horses. The plasma was purified and concentrated by pepsin digestion and ammonium sulfate precipitation (Latifi, 1978). The potencies of antivenoms were expressed as mg of venom neutralized by one ml of serum (Latifi, 1978). The antigenic components of venoms (collected from pooled male and female snakes) were determined and compred with each other by the immunodiffusion technique (Latifi, 1972).

Results and Discussion

Considering the distribution of venomous snakes in Iran (Fig. 1), an effort was made to collect the snakes from various localities of Iran for venom production. The maximum, minimum and the mean ($\pm$ S.D.) of venom extracted per snake at differ-

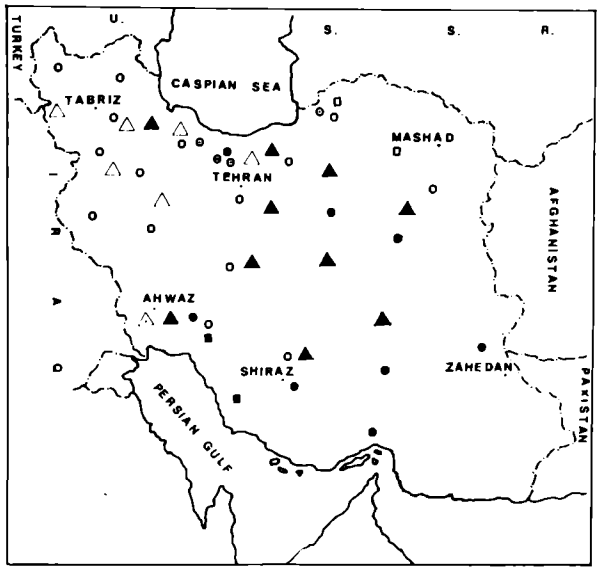

FIG. 1. DISTRIBUTION OF VENOMOUS SNAKES IN IRAN.
□ Oxus cobra (*Naja naja oxiana*), □ Desert black snake (*Walterinnesia aegyptia*), ▲ Persian horned viper (*Pseudocerastes persicus*), △ Egyptian sand viper (*Cerastes cerastes*), ● Carpet viper (*Echis carinatus*), ⊛ Latifi viper (*Vipera latifii*), O Levantine viper or Afyi (*Vipera lebetina*), ⊖ Orsini's viper (*Vipera ursinii*), △ Mountain viper (*Vipera xanthina* ssp.), ⊙ Caucasus pit viper (*Agkistrodon halys*).

ent seasons of the year are indicated in Table 1. The snakes were usually milked only once, except certain groups which were kept for a second and third extraction. The low volume of venom in freshly captured snakes was a poor indicator of the snake's ability to produce venom, since two facts were unknown: (1) how much venom was lost during the capture period; (2)

TABLE 1. VARIATION IN YIELD AND PRODUCTION OF VENOMS

Venom	Numbers of snakes milked	Yield of venom per snake (mg)		
		Maximum	Minimum	Mean ± S.D.
Naja naja oxiana				
Spring	3642	113	19	66 ± 27 (43)
Summer	2054	159	37	101 ± 36 (19)
Autumn	275	208	72	124 ± 39 (13)*
Winter	120	135	81	111 ± 25 (5)*
Total	6091			87 ± 25
Echis carinatus				
Spring	12474	33	6	16 ± 3 (41)
Summer	2112	40	9	18 ± 3 (14)
Autumn	2520	18	9	12 ± 2 (14)
Winter	1121	22	8	15 ± 2 (6)
Total	18227			15 ± 2
Pseudocerastes persicus				
Spring	2399	69	21	42 ± 4 (22)
Summer	273	100	18	49 ± 6 (9)
Autumn	2362	80	17	41 ± 4 (8)
Winter	898	61	26	45 ± 3 (13)
Total	5932			44 ± 4
Vipera latifii				
Spring	1400	11	2	6 ± 2 (25)
Summer	6630	10	2	6 ± 1 (9)
Autumn	673	9	3	6 ± 2 (5)
Winter	38	2?	2?	2 (1)
Total	8741			6 ± 2
Vipera xanthina ssp.				
Spring	3037	13	8	10 ± 1 (6)
Summer	1255	18	8	12 ± 2 (5)
Autumn	154	7?	7?	7 (1)
Winter				
Total	4446			10 ± 2
Vipera ursini				
Spring	65	2	1	2 ± 1 (2)
Summer	2473	4	1	2 ± 1 (15)
Autumn	215	3	2	3 ± 1 (2)
Winter				
Total	2753			2 ± 1
Vipera lebetina				
Spring	8581	79	16	47 ± 4 (55)
Summer	654	91	35	69 ± 7 (13)*
Autumn	332	71	22	38 ± 4 (6)
Winter	159	43	28	34 ± 3 (3)
Total	9726			49 ± 16
Agkistrodon halys				
Spring	2094	5	1	3 ± 1 (7)
Summer	13294	7	1	4 ± 1 (38)*
Autumn	184	4	3	4 ± 1 (2)
Winter	58	3	2	3 ± 1 (2)
Total	15630			4 ± 1

Means ± S.D. based on dry weight of venom are presented. Number of experiments (venoms obtained from different batches and each batch contained the venom of several snakes) are shown in parenthesis.

*The values are significantly different when compared to the spring season (Student's *t*-test, $P < 0.05$).

when did the snake last use its venom for feeding. Another factor influencing the volume obtained from a snake in the wild is the season of the year when the snake is captured. All the snakes were kept under the same conditions for four weeks before extractions were performd.

The yield of venom and its lethality varies not only accorring to the age, size, forcefeeding, fasted condition, period of milking, stress, photoperiods, temperature, captive care, etc. (Glenn et al., 1972), but also depends on the season and geographical distribution. Variation of the lethality of venom in different species, was noticed, as were slight differences between venom obtained from the right and left fangs of a single snake (Table 2). The yield of venom obtained from Naja naja oxiana was highest in autumn and winter, while the venoms of V. lebetina and Agkistrodon haly = intermedius were highest in summer. The yield of venoms of pseudocerastes persicus, V. latifii, V. xanthina ssp. and Echis carinatus showed no significant variation in different seasons. There was no significant difference in

Venoms	LD_{50} (i.v.) (μg/mouse)		LD_{50} male snake (μg/mouse)		LD_{50} female snake (μg/mouse)	
	Male	Female	Left fang	Right fang	Left fang	Right fang
Naja naja oxiana	12.8 (10.8 - 15.4)	6.8 (5.5 - 8.3)	15.9 (13.3 - 19.0)	15.2 (11.8 - 16.5)	5.2 (4.2 - 6.4)	6.3 (5.2 - 7.7)
Echis carinatus	6.6 (4.6 - 9.2)	3.0 (2.4 - 3.8)	19.1 (14.8 - 24.5)	20.0 (12.0 - 32.0)	3.7 (2.7 - 4.9)	3.9 (2.5 - 4.3)
Pseudocerastes persicus	18.1 (14.7 - 22.5)	15.1 (13.3 - 19.0)	11.5 (9.4 - 14.1)	14.2 (11.9 - 17.0)	17.7 (14.9 - 21.2)	15.0 (12.5 - 18.1)
Vipera lebetina	15.1 (11.8 - 19.4)	14.2 (11.5 - 17.5)	10.0 (8.3 - 12.1)	13.5 (10.3 - 17.9)	13.6 (11.3 - 16.4)	11.5 (8.8 - 14.8)
Vipera latifii	5.6 (4.7 - 6.7)	5.8 (3.2 - 10.5)	5.1 (4.1 - 6.4)	5.0 (4.1 - 6.1)	6.4 (5.2 - 7.9)	6.4 (5.2 - 7.9)
Vipera xanthina ssp.	6.1 (4.9 - 7.4)	5.7 (4.6 - 7.1)	6.9 (5.7 - 8.3)	7.3 (6.1 - 8.7)	7.3 (6.1 - 8.7)	6.9 (5.7 - 8.3)
Agkistrodon halys	10.8 (9.2 - 12.5)	7.6 (6.3 - 9.0)	14.3 (11.4 - 17.9)	12.1 (9.5 - 15.3)	6.4 (5.2 - 7.8)	7.3 (5.9 - 9.0)

The venoms were obtained from a total 10 snakes of equal size of each adult species. The LD_{50} for each venom was calculated by the Spearman - Karber method (FINNEY, 1964). The range of all values is given in parentheses.

TABLE 3. VARIATION IN YIELD OF VENOMS FROM LEFT AND RIGHT FANGS OF MALE AND FEMALE SNAKES

Venoms	Average venom per snake (mg)*		Venom of male snake (mg)*		Venom of female snake (mg)*	
	Male	Female	Left fang	Right fang	Left fang	Right fang
Naja naja oxiana	99	66	57	51	29	31
Echis carinatus	53	35	32	35	19	19
Vipera lebetina	48	48	24	23	30	18
Pseudocerastes persicus	68	77	27	26	26	35
Vipera latifii	16	11	8	7	4	5
Vipera xanthina ssp.	11	11	9	8	5	5
Agkistrodon halys	9	5	3	3	4	4

*Figures indicating the mean yield of dry venom in mg/snake were obtained from a total 10 snakes of equal size of each adult species.

yield of venom in males versus females, although in some species the average yield of venom from male snakes appeared more than from female snakes (Table 3). No variation was observed in venom pH in different seasons in E. carinatus (5.7), V. ursini (5.7), A. halys (6.2), while in Naja oxiana, V. latifii, (5.9 $\pm$0.2), V. xanthina ssp. (5.5$\pm$0.5), V. lebetina (5.8$\pm$0.2)

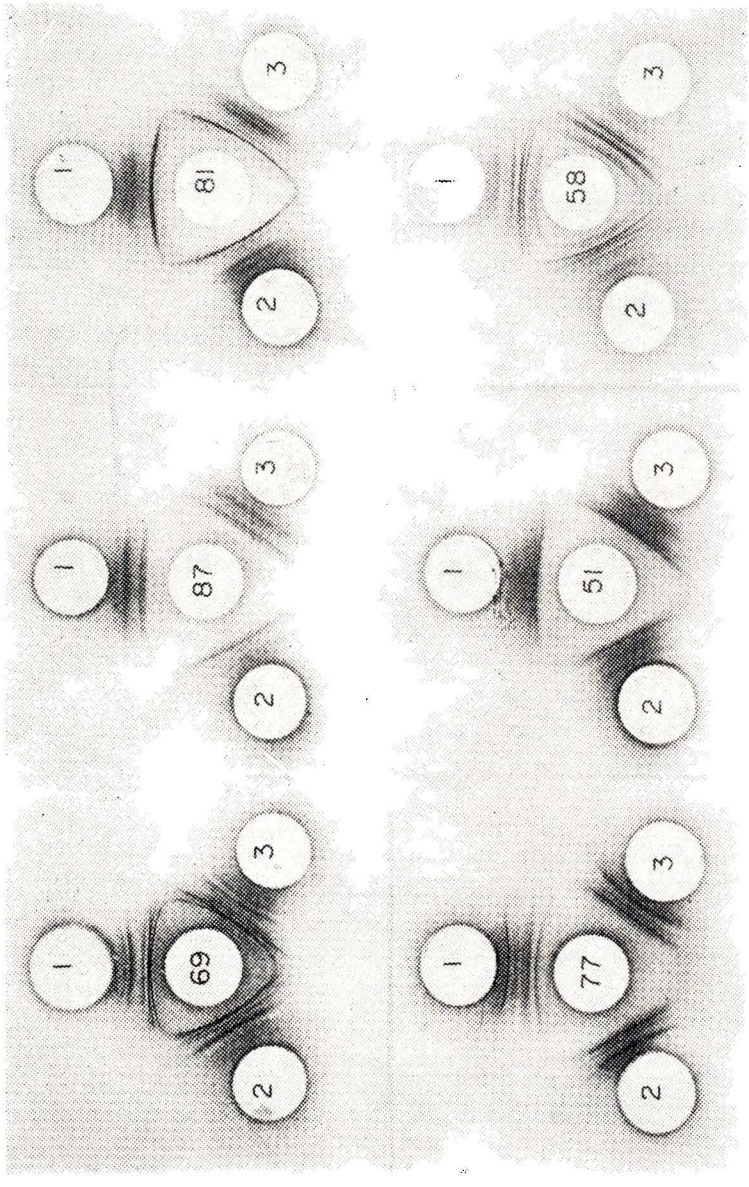

Fig. 2. Comparative precipitation bands formed in agar by venoms against Different antivenoms. Immunodiffusion tests of Naja antivenom (69), Echis antivenom (87), Lebeting antivenom (81), Pseudocerastes antivenom (77), Latifii antivenom (51) and Agki-strodon antivenom (58) against pooled (1), male (2) and female (3) venoms collected from related snakes.

TABLE 4. VARIATION IN POTENCIES OF VENOM AND ANTIVENOM

Venoms	LD_{50} (i.v.) (μg/mouse)	Antivenom neutralized (mg/ml)	
		Monospecific	Polyspecific
Naja naja oxiana	8.3 ± 0.9(10)	1.0 ± 0.4(8)	0.3 ± 0.1(20)
Echis carinatus	5.0 ± 1.1(10)	1.8 ± 0.1(5)	2.0 ± 0.5(20)
Pseudocerastes persicus	16.2 ± 2.7(8)	1.4 ± 0.3(6)	1.1 ± 0.1(20)
Vipera lebetina	6.4 ± 1.3(10)	2.2 ± 0.4(3)	1.3 ± 0.3(20)
Vipera latifii	5.5 ± 0.8(6)	1.2 ± 0.3(5)	0.9 ± 0.4(20)
Vipera xanthina ssp.	7.1 ± 1.0(3)		0.8 ± 0.2(20)
Agkistrodon halys	13.7 ± 0.7(5)	0.9 ± 0.3(5)	0.6 ± 0.2(20)

Potencies were determined i.v in mice (16 – 18 g) and the LD_{50} was estimated by the Spearman – Karber method (FINNEY, 1964). Mean ± S.D. are given. Number of experiments (venoms and antivenoms of different batches) indicated in parentheses.

and P. persicus (5.5 ± 0.1) pH varied slightly.

The results of the gel diffusion tests indicated that there were only slight antigenic differences between the pooled venom and those of male and female Pseudocerastes persicus snakes. No antigenic differences were, however, observed between venoms collected from males and females of other species (Fig. 2).

The LD50 values from first milking of Naja naja oxiana, Echis carinatus and Pseudocerastes persicus were 13 μg 8.1 μg and 14.4 μg, respectively, while in the second milking of the same species the values were 10μg, 3.9 μg and 12μg, respectively. Although certain similarities were seen among the venoms of Iranian vipers, the degree of resemblance observed was insufficient to choose a representative venom to replace other venoms in the production of antivenom intended for the treatment of snakebite.

It was not possible to collect sufficient Walterinneasia aegyptia and Cerastes cerastes snakes to allow the incidence of these two species in Iran to be assessed. Vipera latifii was found in an isolated area of Lar (near Tehran), while Vipera xanthina ssp. was distributed in many areas. There are still many areas of Iran in which snakes have not yet been collected.

Since 1960 the production of antivenom has greatly increased in order to satisfy the needs of Iran and neighbouring countries, whereas the number of snakes supplied to the institute has decreased. Effective antivenom production should be based upon the use of the pooled venom of each separate species. It is advisable to use venom from pools representing numerous milkings from the same species caught in different localities.

With reference to the variation in potencies of antivenoms

(Table 4), it seems that in most cases the polyspecific antivenom is suitable for the treatment of bites of all the Iranian snakes. However in Naja naja oxiana bites the monospecific antivenom is preferable. Best results are obtained with the use of antivenoms prepared from venoms of snakes of the local population or at least the commercial antivenom should be controlled for potency by testing with local reference or approved venom (Warell et al., 1974). Comparative output per snake (Table 1) gives an indication to the clinician as to how much venom to expect in the most severe cases of envenomation.

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