Research Article

Venom immunization: IgG/IgE titers, safety, risk, and methods of the VIPRBITEM cohort

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Abstract

Background: This is the first study to examine a cohort that engages in the practice of immunization with snake venoms. In this practice, either fresh wet venom or venom reconstituted from freeze-dried form is used in vaccination protocols to produce hyper-immunity to venom. **Methods:** This is a retrospective community-initiated collaborative research (CICR) project that collated the records of venom immunization. Records of schedules, formulations, photographs, medical records, and diaries were collated from existing practitioners and evaluated by inspection and interviews. One accidental bite was observed over 3 days, with vital signs, and photographic records of swelling taken to verify reality of the bite. Over 74 snake-genera man-years, and 24 man-years of injection data from 8 participants, for 22 species of venomous snakes from Elapidae and Viperidae are represented. Six of those participants had detailed records of date, dose and effects. **Results:** IgG titers to 6 venoms for 4 cohort members tested of 8 included 2 with clear hyper-immune status. IgE titers were elevated for some. In 861 injections, records showed a rate of atopy/anaphylaxis of 4.3% , an infection rate of 0.58% and an abscess rate of 1.51% . Serious adverse reactions were rare and these appeared to be linked to overly aggressive immunization schedules and formulation accidents. We note that greater cross-immunity of IgE over IgG is suggested. Two basic protocols were followed, one was an approximate one month interval, the other was one or more injection(s) per week. In 176 envenomations, 175 were without antivenom treatment, two hospitalizations occurred, and one received full antivenom treatment. Dry bites were not included in our dataset. Envenomations showed a 1.14% rate of atopy/anaphylaxis, a 0.57% rate of infection and a 1.7% rate of abscess. **Conclusions:** Immunization of humans to snakebite is effective, and reasonably safe with care. Injection records suggest immune cross-reactivity between ophidians within the same family, and better cross-reactivity within the same genera. A cohort participant was pronounced dead based on EEG, and then recovered without treatment. A neurotoxin case with "brain death" EEG should stay on life support for 6 weeks to allow time for the immune system to clear venom.

Keywords: Venom immunization, Self-immunization, Envenomation, Bill Haast

1. INTRODUCTION

Of the races that inhabit the earth there is but one, the Psylli of Marmarica, who are unhurt by the fell bite of serpents. – Lucan [1].

1.1. Historical literature of venom immunization

There is a long history of humans using venoms to vaccinate against bites of venomous snakes. This recorded history of immunization against snake venoms goes back to Roman historians. In modern times, scientific research stopped in 1965 with Canan and Flowers while amateurs kept the practice going (Table 1). In this study, we present the first cohort of people vaccinating to snake venoms.

The colloquial term for this practice is self-immunization or SI. Herein, we will use the more precise terms of venom immunization or vaccination.

1.2. Community-Initiated Collaborative Research (CICR) venom cohort

The cohort was named Venom Immunization Protocol Research on Basic Immunology Therapeutic Experimental

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Table 1. History of venom immunization: Antiquity to modern times

Venom vaccination	Citations
Psylli tribe, Africa.	Dio, Circa 210 AD[2] Lucan, Circa 60 AD [1]
Colonel Serpa Pinto, Mozambique	Pinto, 1881 [3]
Galibi, Boni, Emerillon and Bosse, Africa. Curados de Culebras' use of rattlesnake fangs for injecting venom, Tuxpan.	Calmette, 1908 [4]
Wanyamwesi injection practice of *lukago*. Africa.	Carnochan and Adamson [5]
Indians and Egyptians using young snakes bite to begin, then older snakes to increase immunity.	Webb and Powell, 1946 [6]
Bill Haast's recovery from krait bite.	Haast and Winer, 1955 [7]
Charles Tanner, tiger snake immunization, test with 25 mg wet venom. Persistence for 3 months. Bite recovery 1960 uneventful.	Wiener, 1960 [8]
Herschel Flowers, 1 ml of his serum neutralized 30 mouse LD50's. Flowers serum could neutralize 100 mg of *Naja* (Indian cobra) venom.	Flowers, 1963[9] Canan and Flowers [10]
Bill Haast venom injections. Recovery from cobra and krait bites. Haast's serum is used to treat snakebites.	Kursh, 1965 [11]
Psylli of Africa, Marsi of Italy, Ophiogenes of Hellespont, Syrians ondf the Euphrates. Mexicans near Tampico, Mexico.	Klauber, 1979 [12]
Bill Haast venom injections from 1948 to 2011. Serum used to treat 21 snakebite victims.	Haast [13]

Bold fonts: Journal articles

LD50: Lethal dose 50%

Method (VIPRBITEM). There are parallels and differences between this study and community-based participatory research (CBPR) [14]. The primary difference of CICR from CBPR is that members of the cohort initiated the study, defined its protocols, decided independently to perform the procedures, and provided the data.

A consent form was created and signed by members of the cohort. Consent was based on the Harvard open IRB consent and guidelines [15]. IRB approval was obtained for the collection of blood samples for laboratory analysis and for collection of data, from the Institute for Regenerative and Cellular Medicine (IRCM) Santa Monica, California (IRB approval number: IRCM-2019-209) (See supplement for expanded discussion).

1.3. Reasons for immunization with venom

We asked members of the cohort why they wanted to go through the process of becoming immune to venom. The reasons they gave are presented in Table 2.

Table 2. The VIPRBITEM cohort rationales for pursuing hyperimmunity to venom

Rationale	Discussion
Survival when antivenom is unavailable	Hobbyists with exotic snakes are bitten with no antivenom available. Encounters may occur in the field without easy access to medical care [16]. In some locales, antivenom is hard to find.
Avoidance of financial ruin	Vial price is "$7900 and $39,652 per vial," average $10,000 [17]. Typical north American snake bite 10–30 vials averaging 12–18 vials [18]. Medical bankruptcy of underinsured is common [19].
Better protection and recovery from bites	Compartment syndrome is overdiagnosed and fasciotomy is overused, despite fasciotomy being strongly discouraged by venom treatment experts [20].
Avoidance of long-term sequelae	Joint fusion, arthritis, tendonitis, nerve pain, and malaise can persist for a lifetime. Long-term sequelae rate with antivenom may be 40% [21].
Hope of contributing to saving lives and preventing disfigurement	For some, this drives record keeping and is a motivator to be studied.

2. METHODS: VIPRBITEM COHORT

For those that wish to see more detailed information on the methods and data of the VIPRBITEM cohort, the supplement contains quite complete records of injections and effects leading up to demonstrated immunity by challenge.

2.1. Hyper-immunity vaccination series protocols

The VIPRBITEM cohort uses protocols for performing venom immunization based on experience handed down orally over roughly 70 years, with input from a few papers [22]. The principles are basically the same as insect venom desensitization [23]. No members of this cohort use filtration to remove bacteria nor have any attempted a toxoid, although both are discussed in Weiner's paper [8], which is known to the cohort.

Injections are done either intramuscularly or subcutaneously in the VIPRBITEM cohort. Weiner recorded 2 intradermal venom injections. Our recommendation is to use the subcutaneous route because it exposes muscles to less damage if a dose is miscalculated. Intradermal injections are excellent for immune system presentation but may leave a scar and maximize pain.

The most common injection locations are in the thigh and abdomen above the belt line. The forearm and upper arm are less often used. Areas near joints should be avoided, as should hands, feet, and fingers.

Antihistamines are used on an as-needed basis, and there is no reason not to take them pre- or post-injection. Most practitioners have an epinephrine injection kit available and are encouraged to use it, but so far no venom vaccinator we are aware of, in or out of this cohort, has used one when warranted. Most discuss what they do with their physician and get kidney and liver blood work done from time to time. The cohort was informed that the use of either antihistamine or epinephrine will not compromise immune response, which is a common question.

We recommend use of the curated dataset created for the companion meta-analysis article [24] to this one, and the worked examples in Table 1 of this article's supplement.

The hyper-immunization protocol consists of two phases, i.e., immunoglobulin (Ig)G generation and maintenance. There are two basic types of protocol: long-interval and short-interval ones.

2.1.1. Phase I: Generation of IgG

Doses in the VIPRBITEM cohort typically start at 0.01 mg or less of wet venom, which is prepared by serial dilutions, using drops. One drop of wet venom (approximately 1 mg) is mixed into 9 drops of diluent, then 1 drop of diluted venom is mixed into 9 drops of diluent. One more iteration gives roughly 0.001 mg/drop. This diluted mixture may then be diluted further, that is, another 1 drop into 9 drops of diluent. Diluent is boiled tapwater, distilled water, phosphate-buffered saline, or other fluid purchased at a pharmacy. A 10-unit volume of diluted mixture (more or less), where one unit is 0.1 mL, is drawn up into a syringe, which is typically an insulin injection syringe. On average, 0 units of 0.001 dilution is approximately 0.01 mg of wet venom, or else 0.001 mL if an additional dilution step is made. Note that while 1 standard drop is supposed to be 0.05 mL, drops of venom are typically considerably larger. Drop size can vary, depending on equipment, viscosity, and technique, so while workable, measurement has significant margin of error until the final dilution is drawn up into a syringe.

Mixing venoms is common practice within species and across genera and even families. Given the extreme variability exhibited by venoms, this is probably a wise practice. In the VIPRBITEM cohort, we only show data from mixtures within genera.

2.1.2. Monthly inoculation: Long interval

Inoculation occurs at a rough 3–6-week interval, although it may have multi-month gaps. Doses are approximately doubled when increased during the first 4–12 months, but this slows down as doses get higher. Dose selection is based on effects of previous doses. It is common to do the same dose multiple times. This schedule corresponds to Carnochan's and Adamson description of *lukago* among the Wanyamwesi [5]. Note that re-exposure to antigen at shorter intervals can interfere with development of immunity [25].

2.1.3. Weekly inoculation: Short interval

Inoculation occurs 2 times per week a couple of days apart, similar to an allergy shot series. Doses should be maintained at the same level for 3–6 weeks just as with monthly inoculation. This has similarity to allergy abatement protocols, where injections are typically done twice per week. If an individual experiences atopic reactions that are worrisome, dose may be cut by a factor of 10 or more, and a switch from monthly to weekly inoculation may be made. Note that data records contain departures, for instance, daily injections at low dose for a month or two.

2.2. Phase II: Booster maintenance

Booster dose is chosen based on feel but typically does not exceed 60% of the lethal dose 50% (LD50) for the snake venom, although some are more than LD50. Some practitioners set their boosters at fairly low doses, on the order of one milligram. Some just continue their dilute inoculations (even on a weekly basis) at much lower doses. Others inject amounts that are quite significant, as seen in the graphs that follow. Low dose boosters have been injected as often as two or more times per week for life. High dose boosters are typically injected on an approximate 3-month basis. This low-dose frequent booster schedule is not optimal immunologically but it may have other interesting effects.

3. METHODS: INVESTIGATORS

3.1. Cohort composition and background

From the VIPRBITEM cohort of 10 people, there are data from a total of 9, all male, in our dataset (Figure 1), V0001-9. However, V0002 and V0009 did not have complete datasheets. The youngest age when immunizations started was 23, and the oldest member of the cohort was 52. The mean average age is 35. Defining an age for the experiment is complicated because it varies from a rough 20-year period to 7 years.

All participants in this cohort are self-selected participants who came from an online forum. Participation in the cohort was decided based on the primary investigator having discussions with participants, examining records, and judging the participants to be forthcoming on all aspects. Seven of the participants were located in North America, one was in

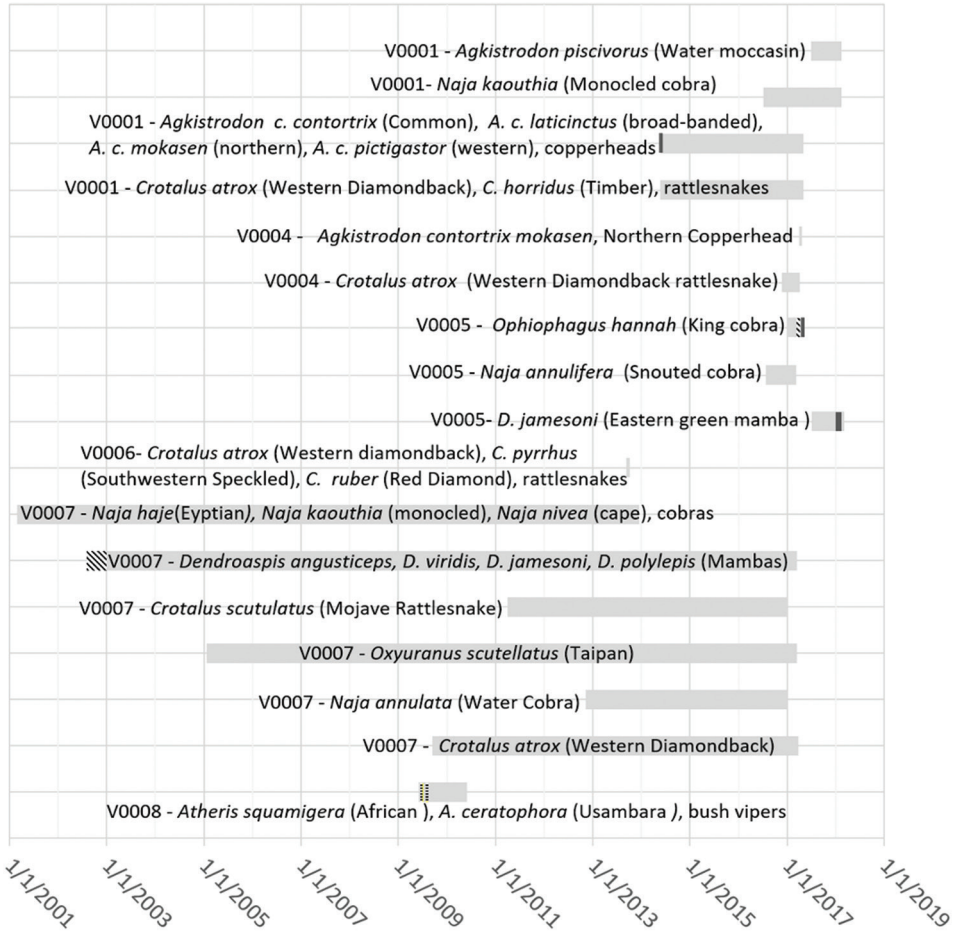

Figure 1. Cohort immunization periods. Diagonal stripes in immunization log-line show the period in our dataset when near anaphylaxis occurred, up to some degree of throat tightening (V0007–Mambas, V0005 King cobra). Light horizontal stripes indicate anaphylactic episodes up to the level of hives (V0008–bush vipers). Dark gray is indicative of experience of significant venom effect (V0001–Common copperhead, V0005–King cobra and Eastern green mamba).

Europe, one in Japan, and one in Southern Africa. Ethnicity was not recorded. All were self-reported in good health with no significant medical history that was not mentioned in the supplement. This is a CICR study, entirely voluntary, initiated by the cohort community.

The only female participant withdrew after being advised of teratogenic risk. One male participant died of unrelated causes during the study. The study used records kept by the participants over a period of two decades. Blood samples were only collected from participants located in the USA who volunteered to do so.

3.2. Data collection

Hyperimmunity data compiled by the VIPRBITEM cohort covering two decades were collected in our collaboration over a period of 8 years. Readers will see that these data have the ring of authenticity. Results reported contain the irregularity and idiopathic exceptions that tend to characterize real field data. In certain instances, photographic evidence and medical records were supplied on request.

The primary data collected were dose, date, pain, and swelling. In addition, notes on anaphylaxis, infection, and other health matters were collected. These immunizations represent over 68 snake-genera man-years and over 22 simple man-years of experience.

3.3. Snake venoms and dose toxicity in this hyper-immunity cohort

Venom values for minimum lethal dose (MLD) and LD50 wet and dry values are taken from the supplementary venom dataset provided using the algorithm of Hanley and Gross [24] to determine MLD within a set of studies, LD50, LD range, and LD 50% midpoint. Detailed discussion is provided in Supplement Section 1.

Elapids and viperids comprise all of the venoms in our dataset.

3.3.1. Elapidae

Elapid venoms included *Naja haje* (Egyptian cobra), *Naja kaouthia* (Monocled cobra), *Naja nivea* (Cape cobra), *N. haje* annulata (Ringed water cobra), *Naja annulifera* (Snouted cobra), *Ophiophagus hannah* (King cobra), *Dendroaspis angusticeps* (Eastern green mamba), *Dendroaspis viridis* (Western green mamba), *Dendroaspis jamesoni* (Jameson's mamba), *Dendroaspis polylepis* (Black mamba), and *Oxyuranus scutellatus* (Coastal taipan).

3.3.2. Viperidae

Viperid venoms included *Crotalus scutulatus* (Mojave rattlesnake), *Crotalus atrox* (Western diamondback rattlesnake), *Crotalus pyrrhus* (Southwestern speckled rattlesnake), *Crotalus ruber* (Red diamond rattlesnake), *Crotalus horridus* (Timber rattlesnake), *Agkistrodon contortrix* (Common copperhead), *Agkistrodon contortrix laticinctus* (Broad-banded copperhead), *Agkistrodon contortrix mokasen* (Osage copperhead), *Agkistrodon contortrix pictigastor* (Western copperhead), *Agkistrodon piscivorus* (Water moccasin), *Agkistrodon halys blomhoffii* (Mamushi), and *Atheris squamigera* (Green bush viper).

In addition to the aforementioned 23 species, the following 13 species have been reported in our cohort; however, data are not shown because the records for these are incomplete. These may have had some effect on ELISA results due to generation of broader cross-reactive immune response.

These venoms were *Bungarus caeruleus* (Common krait), *Crotalus tigris* (Tiger rattlesnake), *Crotalus viridis* (Western rattlesnake), *Crotalus vegrandis* (Uracoan rattlesnake), *Crotalus mitchellii* (Mitchell's rattlesnake), *Crotalus abyssus* (Grand Canyon rattlesnake), *Crotalus lutosus* (Great Basin rattlesnake), *Crotalus cerastes laterorepens* (Colorado Desert sidewinder), *Crotalus cerastes* (Mohave Desert Sidewinder), *Crotalus cerberus* (Arizona black rattlesnake), *Crotalus molossus* (Black-tailed rattlesnake), *Crotalus helleri* (Southern Pacific rattlesnake), and *Sistrurus miliarus* (Pygmy rattlesnake).

3.4. ELISA testing of cohort sera

ELISA plates (Millipore-Sigma) were made for *C. scutulatus*, *C. atrox*, *Bothrops atrox*, *Naja annulata*, *N. kaouthia*, and *D. polylepis* venoms (MToxins Venom Lab LLC, Oshkosh, WI; Reptile Rescue, Ranger, TX). Four cohort sera were tested on each venom plate together with normal control serum and Clodomiro Picado antivenom (AV) (Polivalente Anti-Botropico Anti-Crotalico Anti-Laquesico, Fundacion UCR Universidad Costa Rica). For IgG detection, Goat Anti-Horse IgG (ab102396, Abcam) was used for the AV wells and Goat Anti-Human IgG (AP112P, Sigma-Aldrich) for human sera. For IgE detection, Goat Anti-Human IgE (GTX77496, GeneTex) was used. Serial 4X dilutions of sera started at 100 with final dilution of 1.6384E6. AV dilution ended at 4.096E5. Plates were read on a microplate reader (MR9600T, Accuris) at 450 nm after HRP development. Signal was analyzed using Excel and a logistic regression (Equation 1). Titer positive/negative threshold (PNT) was selected conservatively based on the end of linear range. Intersection between fitted curve and PNT was calculated using equation 2.

Equations I and II: a = maximum, d = minimum, c = inflection, b = slope

$$y = \frac{a-d}{1+(x/c)^b} + d \quad (1)$$

$$x = c \cdot \left(\frac{a-d}{y-d} - 1 \right)^{\frac{1}{b}} \quad (2)$$

4. RESULTS

We expected to find more atopy/anaphylaxis, and a more consistent appearance when an individual showed symptoms. V0007 had one instance of hives after being bitten by a black mamba 2203 days (6 years, 11 days) after starting immunizations to mambas. V0007 and V0005 both experienced anaphylaxis symptoms at the beginning of an immunization series. For V0007, this occurred for injections from the *Dendroaspis* (mamba) species after previously being immunized to neurotoxic *Naja* (cobra) species. V0005 had an anaphylaxis episode with *O. hannah* (King cobra), prompting a vacation from this immunization series for 4 months. V0008 experienced severe itching, swelling, and hives for a few injections. Others experienced mild itching from time to time. We did not classify this latter as important enough to show in Figure 1. Epinephrine was not used in any case of anaphylaxis. This is probably, in significant part, due to the cost of replacing an autoinjector. It may be helpful to provide a cheaper vial and syringe or otherwise remove that cause of hesitation. Antihistamines were used in one case of anaphylaxis. Some cohort members prophylactically treated themselves with antihistamines. Prophylactic antihistamines should not interfere with the development of immunity.

One individual (V0009, data not shown), a Crocodile Dundee character who jumped on wild alligators and wrestled them, reported anaphylaxis symptoms, including some tightening of the throat with every bite. He drank alcoholic and caffeinated beverages to treat it and parked outside an ER when urged to go. He did not receive medical care in the period 2012–2016 for any bites but did have a 3-day intensive care unit (ICU) admission and a 3-day follow-up for an *A.*

contortrix bite in 1997. He also had a 5-day admission with an induced 3-day coma after a stunt with pouring a bucket of black widow spiders down his back (maximum of 20), probably *Latrodectus mactans*. Unlike all the others, he did not formally immunize except to help a friend for a few months. If he acquired hyper-immunity, it was through a series of accidents over years due to regular exposure in the field and stunts in bars. He intentionally maintained his probable hyper-immune status by accepting bites from smaller snakes in the North American viper family when he found them. Once, drunk in a bar, he had a copperhead bite him on the lip to show the woman carrying it that this was not a boa and he or she should not play with it. These data on V0009 were obtained by interview, photograph, and video. He drowned in a river trying to save a friend near where he had made a herpetological discovery [26].

4.1. ELISA IgG and IgE titers

It was practical only to obtain sera from 4 out of the 8 members of the cohort. The results in Tables 3 and 4 are presented as the multiple of the mean of all control sera on all plates. This was chosen because it is the most conservative method of presentation for this type of data. Raw ELISA values ranged up to the 1.6384 E6 final dilution.

V0001 was immunized to all the venoms except *N. annulata* and *D. polylepis*. At the time of this blood sample, (2019) V0001 had begun inoculations with *N. kaouthia* venom. V0002 was immunized to *C. atrox* and *B. atrox*. For V0001 and V0002, other ELISA values are cross-reactive. V0007 immunized to all venoms shown, among others. V0008 was immunized only to *A. squamigera*, *C. pyrrhus*, and *A. contortrix*, which were not tested. Thus, all of V0008's results were due to cross-reactivity.

Of the four sera tested, V0001 and V0007 displayed hyper-immune status by their tolerance to the venoms of species they were immunized to. V0001 did not attempt an *N. kaouthia* bite for another 9 months (≈12 more step cycles) and probably had much higher IgG titers at that time. It is not clear if V0002 and V0008's titers shown would be sufficient to protect against a significant envenomation.

4.2. Adverse reactions to injections

Swelling (not shown in Table 5, shown in Figure 2) ranged from quite minor to involvement of an entire limb. Some degree of swelling occurs in virtually every injection with significant amounts of venom.

4.2.1. Abscesses

Sterile abscesses (Table 5) tend to occur as cytotoxic venom dose increases, which is a reason to avoid intramuscular injections. Sterile abscesses are particularly associated with jumps in dose or accidental error in making up a dose. One member of the cohort, V0006, misunderstood how to perform injections and delivered 9–12 months of dose increases in one month causing severe abscess problems. While abscesses probably cannot be eliminated for cytotoxic venoms, they could be further minimized.

4.2.2. Infection

Serious infection (Table 5) is rare on a per injection basis and appears to be due to contamination of doses or carrying skin bacteria in with the needle. This should be preventable. However, without filtration, it is inevitable that venom doses carry mouth bacteria from snakes that can cause infections. Literature indicated that elapids [27] have a greater tendency to cause infections than viperids [28]. However, this may be an artifact of environment because cobra bites tend to be in tropical climates.

4.2.3. Allergic responses

Atopy (Table 5) is mostly just itching in this dataset. Two members of this cohort (V0005 and V0007) developed anaphylactic-type reactions that included throat and breathing problems, all going untreated. For both, this was restricted to one genera. We believe that this was due to an overly aggressive injection schedule in both cases. V0007 presumed a greater degree of IgG cross-reactivity from cobras to mambas than existed when choosing his attack dose. These data indicate that cross-reactivity to mamba venoms by the IgE raised to cobra venoms was higher than for IgG. Another episode consisting of itching and hives occurred after a dose

Table 3. IgG titer multiple of mean of control sera AV was made using Bothrops atrox venom. Only V0007 was immunized to all venoms shown

IgG	V0001	V0002	V0007	V0008	AV
Crotalus scutulatus	**587**	131	**662**	59	46
Crotalus atrox	**204**	**97**	**304**	31	88
Bothrops atrox	**338**	**171**	**252**	52	254
Naja annulata	24	7	**410**	2	<1
Naja kaouthia	**89**	31	**178**	4	254
Dendroaspis polylepis	15	10	**484**	4	8

AV: Antivenom; IgG: Immunoglobulin G; Immunized venoms are given in bold.

error. Insect venom has shown similar results for ultra-rush allergy series [23].

4.2.4. A brain death recovery

One instance of a 4-day hospitalization (Figure 3) was reported by V0007 in September of 2001, 194 days after beginning immunization. V0007 performed a demonstration bite from a monocled cobra (*N. kaouthia*) in his right bicep.

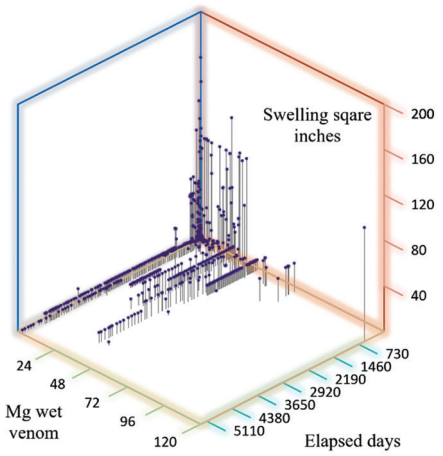

Figure 2. Swelling signaling inflammation. When estimating square inches of swelling, if, for example, swelling was from elbow to wrist in the whole limb, it would be the measurement of the distance from elbow to wrist times the approximate circumference of the forearm. If it was a patch on the arm, or leg, the measurement would be the dimensions of the swollen patch. Elapsed days start with the first immunization injection. Pain reports can remain very high even when swelling has dropped to a relatively minor level.

Table 4. IgE titer multiple of mean of control

IgE	V0001	V0002	V0007	V0008
Crotalus scutulatus	**9**	80	**8**	72
Crotalus atrox	**33**	**41**	**26**	1
Bothrops atrox	**63**	**42**	**8**	105
Naja annulata	4	14	**5**	142
Naja kaouthia	**2**	17	**32**	198
Dendroaspis polylepis	32	25	**77**	9

IgG: Immunoglobulin E; Immunized venoms are presented in bold.

This was not a problem. Over an hour later, (maximum of 3 h) he received an accidental bite from an Egyptian cobra on a finger in his left hand. 20 min later, he was unable to stand and went into a coma in ICU for 3 days. V0007's EEG went flat and he was declared brain dead. Last rites were performed on day 2, counseling was given to his wife about removing life support, with significant pressure. She refused, he regained consciousness on the 3rd day, and was ambulatory later that day. This is something important that attending physicians should be aware of. Given time (up to 6 weeks), a neurotoxin envenomation victim should recover if kept breathing, faster if provided with appropriate AV.

4.2.5. Fusion of a joint

Within the cohort, but outside the primary set of records, is a report of an accidental injection of a *C. viridis* venom dose into the distal joint of a finger, causing fusion of the joint. The injection occurred when the syringe was accidentally dropped by person A after removing the needle cover. Person B dove for the floor and made a successful catch, but the syringe penetrated his distal finger joint like a dart. After the catch, person A tripped over person B on the floor, and person A's fall pushed the plunger down, discharging the contents of the syringe (undiluted venom) into the joint.

Considerable time and labor can go into making up a syringe for an injection, so valuing the contents is understandable. However, if a syringe is dropped, we recommend to just let it go and start over. Fusion of a joint is virtually certain to occur from direct injection into synovium regardless of the level of circulating IgG. Treatment requires immediate surgery and lavage of the joint synovium with the use of AV.

4.3. Dose determination and dry bites

The exact dose for a live bite is difficult to determine with precision. Bite envenomation doses are presented here as nominal wet venom with error bars. Doses are estimated by combining range data from literature with venom milking data and size of the snake. There was no evidence that any of these bites were prepped by pre-milking the snakes. The

Table 5. Adverse reactions from injections

Injections	Abscess	Infection	Atopy/Anaphylaxis	Abscess (%)	Infection (%)	Atopy/Anaphylaxis (%)	*Total injections*
V0001	2	2	8	0.87[a]	0.87[a]	3.48[a]	*230*[a]
V0003	1	1	9	2.38[b]	2.38[b]	21.43[b]	*42*[b]
V0004	0	0	0	0.00[c]	0.00[c]	0.00[c]	*10*[c]
V0005	2	1	3	4.55[b]	2.27[b]	6.82[b]	*44*[b]
V0006	2	0	0	20.00[c]	0.00[c]	0.00[c]	*10*[c]
V0007	5	0	11	1.01[a]	0.00[a]	2.21[a]	*497*[a]
V0008	1	1	6	3.57[c]	3.57[c]	21.43[c]	*28*[c]
Total	*13*	*5*	*37*	1.51	0.58	4.30	*861*

Notes: [a]significant value greater than 100; [b]significant value greater than 30; [c]significant value lesser than 30

cohort reported that dry bites occurred multiple times that caused little pain and no swelling. Dry bite incidents were not reported for this study.

Figure 3's bite envenomation events for *Naja* spp. are shown as an example.

4.4. Adverse bite envenomation events in context of immunizations

V0001's first bite and infection (Table 6) was accidental and occurred at day 122 in his first immunization series. He sought medical attention at an ER immediately after seeking our advice. He was admitted for 2 days, given 12 vials of AV, and discharged. After discharge, he developed an antibiotic-resistant infection later, diagnosed as infection of *Proteus mirabilis* and *Enterococcus* spp. V0001 was readmitted for 21 days, receiving IV antibiotics, debridement, and distal finger joint amputation. V0001 is the only member of the cohort who had AV treatment for a bite. V0003's bite (Table 6) was untreated.

The allergic events that occurred after V0007's bites (Table 6) and also seen in Figure 4 consisted of severe hives, itching, and instances of throat swelling. With the exception of the throat involvement, symptoms appeared unexpectedly and did not recur.

4.5. Selected immunization series

Naive participants took a minimum of 90 days and a maximum of 18 months (Figure 5) to see doses of 10 mg or more with a minimum of inflammation. We take lack of inflammation as a signal of reasonably effective immunity. A number of venoms required considerably more time, on the order of 12–18 months, before sufficient immunity was seen. Exactly what drives this range is an open question that we will not speculate on.

Figure 4 shows high starting-dose immunizations conducted by an experienced member of the cohort (V0007) based on his assumption of cross-immunity. This participant had demonstrated effective hyperimmunity for other species within the elapids. For banded water cobra (*N. annulata*), wet venom dose started at 11.4 mg and progressed, within 60 days, to the chosen maintenance booster dose of 22.8 mg. Inflammation was a rough 3" × 3" region at this booster dose, which is considered tolerable. In contrast, the mamba (*Dendroaspis* spp.) species mixture resulted in anaphylactic reactions of varying intensities along with large inflamed regions. The participant did not suffer significant systemic effects, so cross-immunity was present and might have been mostly IgE based. What IgG cross-immunity existed was overwhelmed and the primacy of IgE is shown by the anaphylactic response. We make this diagnosis by

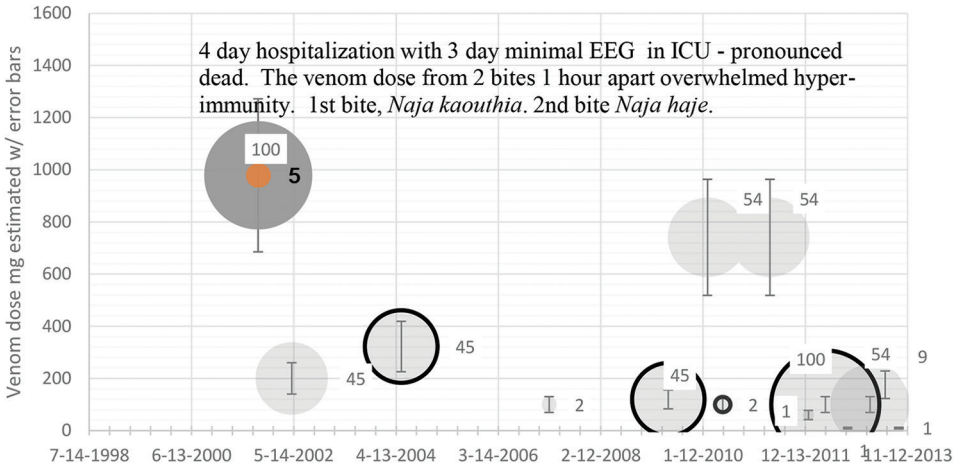

Figure 3. V0007 *Naja haje* (Egyptian cobra), *Naja kaouthia* (Monocled cobra), *Naja nivea* (Cape cobra) bites. Callout numbers are square inches of swelling. Black ring indicates abscess, dark shows necrosis. No medical care was sought for any bites except the first one. Total of 15 bites. The first bite bubble on the left is actually 2 bites. It had a 4-day hospitalization, and the participant was pronounced dead for 3 of them.

Table 6. Bite envenomation adverse events

Injections	Abscess	Infection	Atopy/Anaphylaxis	Abscess (%)	Infection (%)	Atopy/Anaphylaxis (%)	Total bites
V0001	0	1	0	0.00[b]	11.11[b]	0.00[b]	*9*[b]
V0003	0	0	0	0.00[b]	0.00[b]	0.00[b]	*1*[b]
V0007	3	0	2	1.82[a]	0.00[a]	1.21[a]	*165*[a]
V0008	0	0	0	0.00[b]	0.00[b]	0.00[b]	*1*[b]
Total	*3*	*1*	*2*	1.70	0.57	1.14	*176*

Notes: [a]Significant value greater than 100; [b]significant value lesser than 30

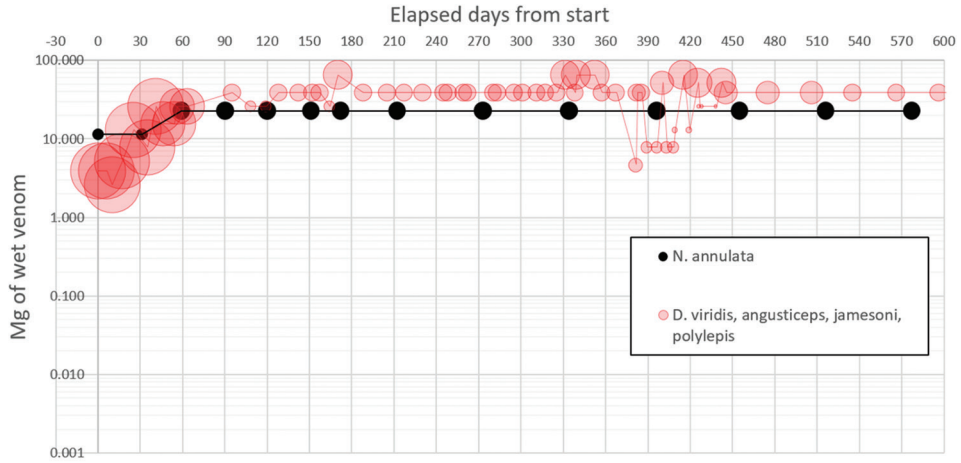

Figure 4. Cross-immunity immunizations. In this chart, mamba mix (*Dendroaspis spp*.) starts at 3.9 mg of undiluted mixed wet venom and Banded water cobra (*Naja annulata*) at 11.4 mg of undiluted wet venom. For a standard immunization series, such doses would not be seen for a minimum of 60 days and may take as long as 450 days to achieve. Bubble size shows swelling. We think that this schedule is overly aggressive.

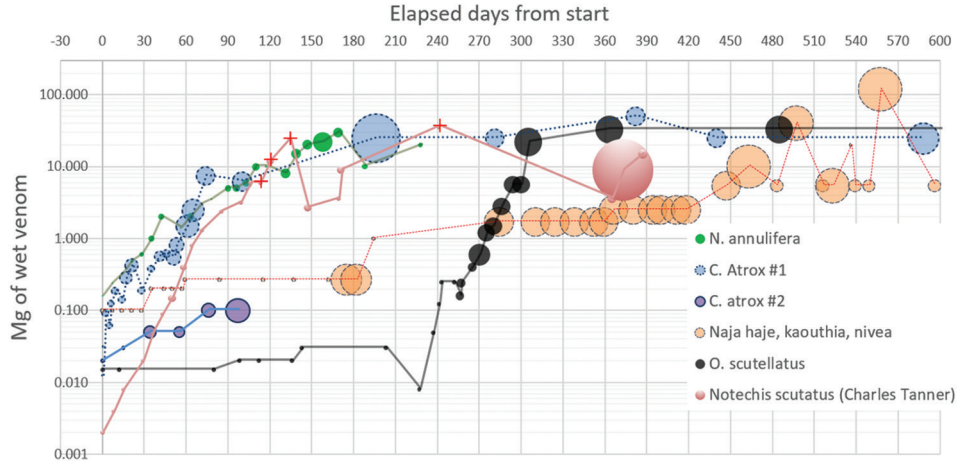

Figure 5. Naive participant series with no previous exposure to venom. Bubble size indicates square inches of swelling. Scaling callouts for swelling bubbles are available in the supplement detail. Charles Tanner's 1958-59 immunization is shown for comparison, with crosses (+) for the 4 toxoid injections. Tanner's immunizations matched that of the cohort, except that Tanner began at a lower initial dose.

examination of records. These anaphylactic reactions ceased abruptly around the 60 day mark, presumably because of development of sufficient IgG titers.

Figures 4 and 5, as well as Tables 3 and 4, provide a basis for the belief that a significant degree of cross-reactivity develops between snakes within the same family and greater cross-reactivity within the same genera. There is a great deal of conservation of sequence and structure in venom molecules, so cross-reactivity is to be expected. Mixtures of venoms are common to us for these vaccination series, and we believe that this is probably wise.

4.6. Pain of injections and bites by dose and elapsed days from starting injections

There was a good consistency in pain scale reports (Figure 6) across 6 different participants that provided pain data. One of the participants (V0007) captured a Northern paper wasp (*Polistes fuscatus*) and got it to sting several times. This was rated 2, which was consistent with the Starr scale [29]. The next day, a bite (5 bleeding fang holes) from a black mamba (*D. polylepis*) was rated 10 and declined to 8–9 (pain level) from h 1–3. In discussion with participants, the reason for the difference in subjective pain was ascribed to duration, intensity, and complexity. There are more tissues in a larger volume of the body that is affected by a snake bite than are affected by a typical hymenoptera sting.

Pain can be considerable in these inoculations, although some of the higher pain reports were associated with allergic reactions and infections. Once a booster dose is stabilized, the level of pain experienced from booster injections often remains high for years. Since pain has typically declined to a more manageable level within 2–3 h, there may be a place

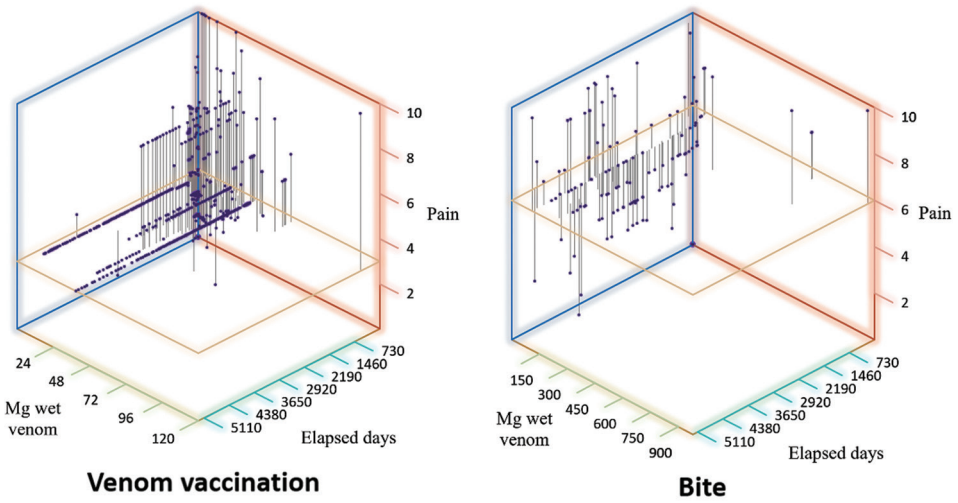

Figure 6. Pain. The z-axis of elapsed days sets day 0 as the first injection. Left: Venom vaccination injection pain. Horizontal cut plane from which drop lines originate is median pain level of 3. Right: Bite pain. Cut plane from which drop lines originate is mean average pain level for bites of all species (if no drop line is visible, the data point is on the plane.) As a reference, one member of the cohort rated a set of wasp stings as 2 on this scale.

for physicians to provide local anesthetic for mixing with injections or for using before injecting venom.

4.7. Swelling by venom dose and elapsed days from starting injections

As seen in Figure 2, swelling declined dramatically over the course of 2–3 years of immunization. In the earliest period, some instances involved most of an arm or leg, and this occurred with Charles Tanner as well. Major swelling events might sometimes be associated with anaphylactic reactions and infections.

5. DISCUSSION

The practice of immunization with snake venom has been considered dangerous and unwise. It is opposed by many and has been declared by some to be impossible. The opposition does not make sense even before this cohort study because there were previous papers that presented venom immunization as successful [7-9,30]. The VIPRBITEM protocols confirmed Weiner's results on Charles Tanner, as we show in Figure 5. At the end of this section, we treat the major objections within the fields of herpetology and venom studies stemming from an old criticism by Rosenfeld [31]. Our data clarified that the practice of venom immunization is highly effective and safe when practiced correctly. We saw indications that immunity carried over within genera and even across genera within families. In our cohort, there were no cases of liver or kidney damage from venom immunization, which was probably because IgG titers to the venom components rose together with the dose increasing. Thus, the net systemic venom dose for the hyper-immunity remained minimal.

5.1. IgE has higher cross-immunity than IgG

IgE appeared to display greater cross-immunity between genera than IgG, and IgE response might last longer than IgG. So far, this has not been a problem except for overly enthusiastic immunization schedules for a new and distantly-related species.

5.2. Key relevant characteristics of venom

Venoms are pH-neutral or close to being pH-neutral, which means that pain is the result of venom components. Fresh, wet snake venom is approximately 60–83% water [32] with most venoms falling in the range of 70–80%. We used 75% as the nominal value in reconstitution and calculations when converting dry to wet form [33].

Wet venoms are extremely stable and have been stored for up to 80 years at room temperature in simple stoppered bottles, retaining their activity, even when some oxidation took place [34]. Desiccated neurotoxic venoms lost no activity in 8–9 years when stored in stoppered bottles in the dark, but hemorrhagic venoms lost roughly half in the same time period and roughly half again after 13 years. Desiccated venoms lost more than half of their toxicity in a year if the bottles were opened frequently [35]. This indicates that oxygen is the primary agent that depletes toxicity of desiccated venom and probably also of wet venom. However, injections with oxidized, deactivated, venom should be a kind of toxoid.

Study of field conditions also showed stability without refrigeration under adverse conditions [36]. Venoms contain antibacterial components to both Gram-negative and Gram-positive microorganisms [37].

Freeze-drying of venom has a significant effect on bacterial load, with greater killing of Gram-negative bacteria than their Gram-positive counterparts [38]. Freeze-drying without vacuum or storage in nitrogen exerts greater effect than using lyophilizing equipment. The freeze-drying procedure used by this cohort was to place wet venom in a vial with a cracked open top in an approximately −20°C freezer inside a jar packed with desiccant. While this *per se* does not suffice to sterilize stored venom, and sterilization is not practical, it can be a helpful option. Venom components cause necrosis, neurotoxicity, myotoxicity, cardiotoxicity, hemorrhage, and thrombosis. PLA_2, serine proteases, and Zn^{2+}-metalloproteases are the primary toxins of interest [39]. These can create an environment friendly to bacteria after injection.

Most practitioners keep supplies refrigerated or freeze dried. Concerns with whole venom instability over the time periods that most practitioners are likely to use them are minimal.

5.3. Kinetics of hyperimmunity

The kinetics of venom hyperimmunity should be the stoichiometry of the venom dose versus antibody present, both IgG and IgE [40]. Roughly half of IgG circulates in blood/lymph, the rest in interstitial spaces with interstitium circulation playing a major role [41]. In addition to elimination through kidneys, any cell that absorbs a venom molecule bound to antibody bearing an IgG Fc chain will bind TRIM21 and be ubiquitinylated for destruction by the proteasome and further presented to the immune system [42,43]. TRIM21 is a mechanism that is not available to most AVs due to lack of human Fc chain. We saw no evidence of antibody binding resulting in longer-term persistence of venom within our cohort.

Low molecular weight toxins, such as neurotoxins, diffuse rapidly, and high molecular weight toxins, such as cyto/myotoxins, spread slowly from the injection site [38]. Hence, neurotoxins and most hemotoxins will disseminate in the body and maximize their exposure to circulating antibodies. Conversely, to neutralize myotoxic/cytotoxic components, circulating antibodies have to be brought to the site.

For smaller venom molecules that permeate out into the body from the envenomation, this can be modeled simply against the total antibody to that antigen. For larger molecules, it needs to be modeled through transport of blood-bearing antibodies to the bite site. In practice, this can be a concern with bites to fingers.

5.4. Limits to hyper-immunity, polyclonality of antibodies, and overconfidence

As shown by the participant who required ICU care for 3 days with a flat EEG before regaining consciousness, there were limits to hyper-immunity. Our interpretation of this incident is that this person's IgG was highly polyclonal because this person could accept 2 milking-style bites in quick succession without serious harm. It only required one antibody attaching to a protein (or virus) for the cell that encounters it to route that protein to the proteasome through the TRIM21 system. After a period of hours had passed from the first bite, most neurotoxic molecules of this first bite had soaked up at least double the number of antibodies required. This dramatically depleted the IgG antibodies available for the next bite. In the early phases of immunization, antibody levels are considerably lower than they can be later. We caution against overconfidence, particularly during the early stages.

5.5. Stoichiometric computation of hyperimmunity limits

The limits to hyperimmunity in humans have not yet been determined experimentally. It is also unknown what the practical limits of antibody generation are in humans, and these limits probably vary significantly. Here, we present guideline estimates.

The current limit understanding is presented here through an example based on a large specimen of Gaboon viper, *Bitis gabonica*. This kind of bite is probably beyond the ability of immunization to fully neutralize, except possibly for a human of approximately 440 lbs (200 kg).

A single milking of a Gaboon viper yielded as much as 2 g of dried venom [44]. Venom is 70–80% water. That implies 6–8 mL of wet venom. Average yield is 200–1000 mg of dry venom.

We will assume 1000 mg of dry venom, which corresponds to an approximate 4 mL bite from a large snake, assuming 75% water in the venom. We will use an average venom molecular weight of 13,000 g (13 kDa).

1 g of venom ÷ 13,000 g/mole ≈ 7.69E-5 moles of venom (76.9 micromoles)

7.69E-5 · 6.0221409E23 ≈ 4.63E19 molecules of venom.

A 70 kg adult human male has about 5 L of blood, with roughly 3 L of plasma/lymph, mostly in interstitial tissues. The plasma/lymph has a higher ratio of antibodies because it is just serum. Plasma makes up approximately 55% of blood volume so the 3 L of plasma/lymph in the body should have an equivalent load of IgG given by:

3 L ÷ 0.55 ≈ 5.45. This gives us a total of roughly 10.45 L (104.5 dL) of blood equivalent antibody-bearing fluid, in a 70 kg adult human male.

Normal total IgG levels average about 1000 mg per dL, with a normal adult range from 639 to 1349 mg of IgG per dL [45]. This allows for the calculation of a range.

In animals used for the production of antivenin, the best estimate of IgG fraction specific to venom is 1–2% [46]. We corresponded on the ELISA results from V0007 [47] who has hyper-immunized himself to several snakes, and if those were correct, V0007 may have roughly doubled the normal level. But even with that, V0007 should not have more than about 15% of that possible doubled IgG devoted to one snake species, in the most generous estimate. Cross-reactive antibodies are possible, and there is definitely evidence of that, but we will ignore it for this example.

Table 7 indicates a range from about 9–42 micromoles within normal human range, and in Table 8, maximum range may be as high as roughly 210–630 micromoles of specific IgG to a venom. The values in Table 8 are probably overestimates, but we cannot rule them out at this time. A fairly large Gaboon viper would inject approximately 77 micromoles of toxins. A very large one might inject 150 micromoles. It is evident that, within the normal range, such a snake should overwhelm antibody-based immunity, although it will be helpful. However, the problem is more complicated than that.

Working through the problem of calculating the limits to hyperimmunity is not as simple as stoichiometric balancing as if the human body was a beaker of venom and antibody. Antibody is dispersed throughout the bloodstream and interstitial plasma/lymph. The interstitial plasma turns over about once every 24 h normally. To better understand, this would require a good simulation model and comparison with experimental subjects.

For hyperimmunity to function, there must be perfusion of antibody to the venom molecules, to allow for binding. Binding is followed by ingestion by a cell so that the protein toxin is destroyed or eliminated by the kidneys.

Different venom components will have different requirements to neutralize them. The easiest are the neurotoxins because they must perfuse through the body to act, which puts them into contact with antibody. Second to these are the hemotoxins which have overwhelming effect at the site of the bite and then travel through the blood stream, counteracting clotting mechanisms. Perhaps, the most problematic for hyperimmunity are the cytotoxins which move slowly in the body and stay concentrated, causing tissue damage.

5.6. Concerns regarding autoimmunity induction were not seen in or outside this cohort

We tried to address the issue of possible generation of auto-immunity from venom immunization by examining this cohort and searching for reports of long-term health issues in those outside the cohort. While the sample was small, no suggestions of auto-immunity being an issue have shown up. Practitioners arrived at their second half-century in excellent health. Several long-term practitioners outside the dataset, on the order of 100 man-years, showed no evidence of auto-immunity, and we speculate that this practice might be protective. Bill Haast, with 64 man-years of practice, was lively and active until near his death at the age of 100, without noticeable arthritis or anything else that was auto-immunity related. Granted, Mr. Haast injected lower doses than most in this cohort, and he suffered from serious damage to his hands. In animal studies of auto-immunity, Freund's complete adjuvant is typically used with conjugated self-proteins which venom injections do not have.

5.7. Cautionary notes regarding public claims of venom immunization

We established that not all who claim to be immunizing with venoms actually are including a prominent proponent. Careful evaluation and patience may result in admission that either immunization did not occur at all or it was greatly exaggerated after an original intent to do so. The pain induced by these injections, even from a miniscule dose, is no laughing matter. People vary greatly in their pain threshold and their ability to handle pain. When ELISA results do not match claims, or if

Table 7. Range of IgG and moles of IgG in a 70 kg human. 1 mole of IgG is about 150 kg

Range of IgG	Moles of IgG range	Moles of specific IgG
104.5 dL·639 mg/dL=66,775 mg	66,775 mg÷150,000 g=4.45E-4 mole	4.45E-4 mole·0.02=8.9E-6 mole
104.5 dL·1000 mg/dL=104,500 mg	104,500 mg÷150,000 g=6.97E-4 mole	6.97E-4 mole·0.02=1.4E-5 mole
104.5 dL·1349 mg/dL=140,970 mg	140,970 mg÷150,000 g=9.40E-4 mole	9.40E-4 mole·0.02=1.9E-5 mole

Ig: Immunoglobulin

Table 8. Maximum moles of specific IgG in a 70 kg human

Multiples of midrange	Moles of IgG	Maximum specific IgG
Assuming 15% of×1 IgG midrange	6.97E-04	6.97E-4 mole·0.15=2.1E-4 mole
Assuming 2% of×2 IgG midrange	1.39E-03	1.39E-3 mole·0.02=2.8E-5 mole
Assuming 15% of×2 IgG midrange	1.39E-03	1.39E-3 mole·0.15=6.3E-4 mole

Ig: Immunoglobulin

there is a veering response to requests for corroborating data, or data record sheets look "too good," scientists need to be careful about accepting claims at face value. A sympathetic approach is needed to this kind of investigation on the human side that many laboratory scientists are unfamiliar with but should be a quite familiar territory to healthcare workers and psychologists. A person may find themselves painted into a corner by their initial promises.

5.8. Risks of injecting with snake venoms

5.8.1. Teratogenic effects of venom injections

Per Langley, "*the fetus can be seriously harmed by systemic envenomation that does not cause local effects in the mother"* [48]. With no experimental data that clarify what the systemic dose range might be for the venom component toxins and variants that could harm a fetus, great caution should be exercised for injection of venom during pregnancy. It is likely that teratogenic effects are greater at the earlier time of pregnancy.

IgG crosses the placenta because of the neonatal Fc receptor (FcRn), and, to a small extent, by perfusion. The molecular weight of IgG is in the range of 146–150 kDa, whereas the great majority of venom peptides are in the range of 5–80 kDa. Relatively small peptide molecules, such as most venom components, should perfuse more easily across the placenta than immunoglobulins do. Density of FcRn in the placenta peaks at 37 weeks (the end of the third trimester) [49]. For most of the pregnancy, if any venom molecules cross the placental barrier, the fetus should not be expected to have meaningful maternal antibody present for protection. Within our dataset, injected doses sometimes had enough systemic effect to be noticed, indicating that immunization is not likely to be able to neutralize all teratogens before their crossing of the placenta.

The practice of venom immunization is rare among women. The women who considered joining the VIPRBITEM cohort decided to withdraw and ceased injections after being informed of potential risks. We recommend that if a woman immunizes to venom, that she performs a pregnancy test prior to all injections and cease injections if she plans on pregnancy or is pregnant.

5.8.2. Atopy/anaphylaxis

It is significant that animal study indicated that IgE response in mouse was essential to the protection against mortality from venom [40]. If IgE was absent, the animals did not survive as well. This suggests that, in the development of hyperimmunity to snake venoms in humans, IgE could be significantly involved.

In our dataset, near-anaphylaxis symptoms were relatively rare and the serious symptoms occurred in response to overly aggressive dosing or accidental overdosing, particularly early in the immunization schedule. Anaphylaxis also appears to be related to cross-immunity when IgE binds better than IgG from a different species.

5.8.3. Infection from venom immunization injections

There are two significant incidents of infection, for V0003 and V0008, in the cohort. In V0003's case, it was due to contamination of the dilution buffer. In V0008's probable infection, there was drainage and local inflammation but no fever was recorded and recovery occurred without resorting to antibiotics. We classed this as an infection because the dose was virtually the same as doses that occurred before and after it that had no similar effect. The inflammation proceeded, up the arm, over days with the appearance of cellulitis, the infection followed the location of a tendon, and V0008 self-assessed it as locally infected. In discussion with V0008, the most likely explanation was the lack of swabbing of the injection site.

It is possible that V0007 suffered an infection subsequent to a miscalculation/overdose with cytotoxic venom. However, this abscess did not include fever or malaise signaling infection, and the scenario fits the sterile abscess occurrence in animals. It was drained and dressed at home and healed well without antibiotics. V0007 did not self-assess it as an infection.

Others logged self-assessed minor infections that did not require antibiotics or medical care and these are logged as infections in this cohort. They were treated at home without antibiotics. Outside the cohort, we saw documentation of an anonymous report posted publicly, in which rapid onset cellulitis occurred following an injection. This was probably due to contamination of the dilution buffer or other mishandling.

Our cohort dataset, and what published literature reported on immunization with venom, tells us that infection is a low risk. With proper care of venom and dilution buffer solutions, the risk should be very low, even without treatment with antibacterial agents, such as thimerosal. However, the bacteria are present in unfiltered venom, and anyone doing this should be vigilant to avoid contamination or conditions where diluted venom has opportunity to grow bacteria that will be present.

5.8.4. Infection from bites in the context of hyperimmunity

One cohort participant developed a serious infection that required a 21-day hospital stay after an initial 2-day course of treatment. This individual was in the earliest stage of immunization and immediately sought medical care and received AV. After discharge, the fingertip became infected. Bacteria cultured were *P. mirabilis*, along with *Enterococcus*

species and an unidentified lactose fermenter Gram-negative rod. These are common in wound infections found in hospitals as well as soil and the mouths of snakes. This infection after a *C. atrox* bite to a finger may be due to an attempt to self-debride a necrotic fingertip and it is also possible that the infection could have been nosocomial, environmental, or delivered in the bite of the snake.

Along the lines of this *C. atrox* bite, the literature had a case of Herschel Flowers who had an infection in a finger after a *Naja naja* bite that caused significant necrosis. Also found in the drainage in Herschel's case was *Proteus spp*. In this case, his finger necrosis had been closed before the development of this infection, which suggests that the bacteria came with the puncture. Flowers had immunized against *Naja naja* venom and had high titers. He previously recovered from a bite on the wrist without complications.

A possible complication of the Flowers' case was a significant degree of compartment syndrome restricting blood flow to the finger and hence preventing sufficient circulation of neutralizing antibody and neutrophils to the site. This would mean that local venom effects could be similar to the effect on a non-immunized person, despite protection against systemic effects. With dead cell materials, poor circulation, and few leukocytes circulating to the area, a small inoculum of mouth bacteria from a snake could freely multiply.

Studies of oral bacteria of snakes showed a wide variety of opportunistic bacteria common in human wounds. *Proteus*, *Pseudomonas*, *Staphylococcus,* Enterobacter*,* Enterococcus*,* Klebsiella, Morganella, Aeromonas, Citrobacter, *Salmonella*, *Clostridium*, *Bacteriodes*, and Corynebacterium were all found [50-53]. One study identified 92 bacterial species by culture.

We think that infection risk after snakebite is probably lower in hyper-immune people. However, bites to extremities can still result in necrosis if circulation is compromised, due to insufficient IgG to the site. The outcome stoichiometrically depends on the dynamic balance between IgG and venom molecules.

5.8.5. Abscess and necrosis

Sterile abscess formation from inoculation with venom is a known problem in animals used for the production of AV [54]. The data collected from our cohort confirmed that probable sterile abscesses could occur with an adequately high dose of cytotoxic venom.

Bites after hyper-immunity has developed can still cause necrosis. Bill Haast is considered a pioneer of American venom immunization. He was bitten on the hands multiple times by cobras, and these bite envenomations left him with quite damaged fingers, despite surviving without AV treatment [55]. Thus, systemic effects may be avoided, while the effect on the fingers may be problematic for bites. We can say, on the basis of the VIPRBITEM cohort, that effects in fingers appeared highly dependent on the degree of immunity. The VIPRBITEM member with the highest antibody titers (0007) has experienced little or no necrosis from cytotoxic bites to fingers.

Bill Haast practiced fairly low-dose inoculations compared to the majority of data in this cohort. Based on our dataset, a person with longer history of immunizations and higher doses will do better compared to those that have less. Moreover, maintenance of circulation by movement and massage is important for the hyper-immune, which is the opposite of the standard practice with people who are not hyper-immune to venom.

5.9. Gastão Rosenfeld's rejection of immunization to snake venoms

Rosenfeld stated a view about venom immunization that is widely held in herpetology and the venom studies field [31]:

> *"Active immunization of man was tried with success (Haast and Winer, 1955; Wiener, 1960; Flowers, 1963), but an a priori affirmation can be expressed that it is not satisfactory due to several factors. Instituto Butantan employees working at the serpentarium have been bitten repeatedly and showed no immunity to subsequent bites. Horses immunized for antivenin production have a shorter life-span than normal. Necropsy [animals] showed tissue degeneration of organs, such as the turbid swelling of the liver (Vaz and Araujo, 1948) probably as a result of the repeated venom injections needed to maintain high antibody count.*
>
> *The injection of small and repeated doses of venom induces a good level of antibody formation. Soon after stopping, antibody titer decreases and comes almost to zero. When a new immunization series is started in these animals, the high antibody titer is reached sooner than the first time, but this state will take days or weeks." - Gastão Rosenfeld 1971*

Rosenfeld's accomplishments were seminal, and he laid the groundwork for the development of angiotensin-converting enzyme-inhibiting drugs. He deserves the respect he commands in the fields of herpetology and venom studies. However, in light of more current understanding, we can address the objections he raised to venom immunization in humans.

As above mentioned, Rosenfeld stated that immunization in humans was, indeed, successful. Charles Tanner was immunized to *Notechis scutatus* (Tiger snake), and Charles tested with 25 mg of wet venom [8]. Charles Tanner showed

that his immunity persisted for 3 months, and his *N. scutatus* bite recovery without AV was uneventful [8]. Just 1 mL of serum from Flowers neutralized 30 mouse LD50's, and Flowers' serum should have been able to easily neutralize 100 mg of *Naja naja* (Indian cobra) venom [9]. Bill Haast survived a *Bungarus candidus* (Blue krait) bite without AV [7]. A small *Bungarus multicinctus* (Multi-banded krait) is what killed Joseph Slowinski from a bite that did not appear to break the skin [16].

Taking Rosenfeld's first point, envenomated employees would be treated, which blunts B-cell response, and even without treatment, stimulations at long intervals will not produce hyper-immunity, which is not a normal state for human immune systems. Both points were made by Flowers in the second paragraph of his paper [9]. That said, based on snakebite symptomatic response, one member of the VIPRBITEM cohort did appear to exhibit hyper-immune status primarily from receiving regular snakebites. Unfortunately, this person drowned accidentally a week before serum could be acquired.

To Rosenfeld's second point about life span of horses used for AV production, the dose escalation schedule used in AV production is far more aggressive than anything used on humans. Based on this study, it would take 12–18 months for an animal to comfortably develop hyperimmune status for snake venoms. However, there may be quirks in different animal species that change the schedule and upper limit somewhat. Members of this human cohort increased doses slowly, except for one who increased rapidly because of error and had to stop. As Rosenfeld mentioned above, liver and kidney damage in animals occurs from overly rapid dose escalation. In the VIPRBITEM cohort, blood work showed no indication of harm to the liver or kidneys. If antibody levels rise so as to neutralize venom effectively, harm to liver and kidneys is not expected.

Further addressing Rosenfeld's second point about life span of animals, which implies cutting life span of humans, he could not have known in 1972 that Bill Haast would live to be 100 (22 years longer than Gastão). Bill continued his weekly practice of injections until shortly before his death in 2011, spanning 64 years. We do not see any indications within the cohort that venom vaccination injections cause harm that could shorten life span. The first author is very sensitive to this because he works on increasing health span and potential life span in humans.

In the VIPRBITEM cohort, the longest interval that anyone has gone between a booster and getting bitten without needing treatment by AV was nearly a year. This individual has a two-decade history of injections and bites and extraordinary antibody titers to venoms. Our data indicated that boosters every 3 months once, hyperimmunity has been established are probably adequate, and there may be room to increase the interval. We do not know, at this time, what the optimal booster interval is, nor how human hyper-immunity behaves over time with precision. This warrants more studies. However, it is quite clear, from our ELISA results, that hyper-immune status does not rapidly return to zero as Rosenfeld described. Without numbers attached to what Rosenfeld thought a small venom dose was, and without defining what a "good level of antibody" means, we can only presume that these doses were very small, as was the antibody response, and that Rosenfeld's experiments bore little resemblance to the schedule presented in Weiner's paper on Charles Tanner [8].

6. CONCLUSION

The venom vaccination procedure appears to produce good to excellent protection against the effects of venomous snake bites. Systemic effects are largely avoided as long as dose does not overwhelm immunity. We reported one case of short-term systemic effect in the supplement. Local effects of venom are greatly reduced, and swelling as well as pain occur, however, most effects will clear up in 48–72 h. Highly cytotoxic venoms such as *C. atrox* (Western diamondback rattlesnake) can have a fairly long tail of mild malaise that could last for 2–5 weeks.

Good cross-immunity was seen in our dataset for snakes within the same genera. Across genera, within the same family, our dataset suggests that significant cross-immunity will exist, skewed toward IgE. The consequence of cross-immunity is the possibility of strong allergic responses.

A corollary to sections 5.4–5.5 on limits to hyperimmunity is that the lower the LD and likely bite LD multiple are, the lower the antibody titers can be for protection. Some of these snakes, such as the krait family, one of which killed Joseph Slowinski [16], have extremely low LD. This, combined with the complete lack of pain, suggests that herpetologists may want to consider immunization for a specific subset of venoms that are most likely to be lethal in the field, due to their extreme toxicity, low bite dose, and lack of pain.

Krait venom has been little used by immunizers to venom and was only mixed with other venoms (which provides pain sensation) when highly diluted. Venom doses are made by the cohort participants themselves, and venom immunizers know that sometimes dose errors are made. To do this with krait venom would be so easy, and the result is so likely to be fatal that this group is reluctant to do so. In addition, near total lack of sensation from a bite, and the necessity to milk the krait at home when not immunized, adds to this concern.

However, this does not mean that a properly managed program by academic or industrial laboratories could not produce an immunization series and boosters that would be

nearly painless and highly effective. We would call this one of the "low-hanging fruit" for venom hyper-immunity.

We caution physicians faced with neurotoxic "brain death" from venom proteins that these patients should recover if given enough time or enough AV. Keeping them alive on life-support may not yield results for 3–6 weeks, but the patient should recover. This is true for pre-synaptic and post-synaptic venom neurotoxins because the immune system and cellular processes will destroy protein-based toxins. This is different from molecules that the body cannot destroy that has low off-rates. In this latter case, in the brain, if the molecule comes off the binding site, it will usually bind right back. Moreover, if the molecule kills the cell, it will find another binding site on another cell.

A question that people considering this practice have to ask themselves is whether they are willing to accept the level of pain and inflammation that most venoms produce. As currently practiced, venom immunization is not for the faint of heart. If the pain and inflammation of a wasp sting or a fire-ant bite is intolerable, the current form of venom immunization is likely to be more challenging. It may be feasible for a physician to provide lidocaine, bupivacaine, or some other local anesthetics to mix with venom or inject before inoculation. However, there will still be significant discomfort when it wears off. One venom immunizer outside the cohort uses lidocaine with his injections.

There is room for improvement in the protocols provided here, and there are some risks. It is hoped that we have provided sufficient information to allow physicians to supervise those choosing to immunize themselves. The supplement contains more detailed graphs for each member of the cohort.

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CONFLICT OF INTEREST

All authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Conceptualization: Brian P. Hanley
Formal analysis: All authors
Investigation: Brian P. Hanley
Methodology: All authors
Writing – original draft: Brian Hanley
Writing – review & editing: All authors

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

IRB approval was obtained for the collection of blood samples for laboratory analysis and for collection of data, from the IRCM Santa Monica, California (IRB approval number: IRCM-2019-209) [15].

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA

Data used in this work is available from the corresponding author upon reasonable request.

FURTHER DISCLOSURE

First part of this study can be accessed at https://doi.org/10.14440/jbm.2024.0037.

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