Emergency Medicine Australasia (2004) **16**, 459–464

EMA

TOXICOLOGY

Cross reactivity between venomous, mildly venomous, and non-venomous snake venoms with the Commonwealth Serum Laboratories Venom Detection Kit

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Abstract

Objective: Studies have noted the relatively common occurrence of positive urine results with the Commonwealth Serum Laboratories Venom Detection Kit (VDK) when testing patients with suspected snakebite who are not envenomed. Possible explanations have been false positive test results or subclinical envenoming. We investigated a third possibility, that there is potential for the venom (or saliva) from mildly venomous and non-venomous snakes to give a positive reading with the VDK.

Methods: Venoms/saliva from three non-venomous and seven mildly venomous snake species were tested in the laboratory with the VDK, along with control venoms from four of the five major snake genera (Brown snake, Tiger snake, Death adder and Black snake).

Results: Two of the venom/saliva samples, from Gould's hooded snake (*Parasuta gouldii*), a mildly venomous snake, and the Black-headed python (*Aspidites melanocephalus*), a non-venomous snake, caused a positive test for the tiger snake genus. There was also cross-reactivity between black snake venoms and the tiger snake well of the VDK.

Conclusions: This study provides a further possible explanation for 'false positive' VDK results, that is venom/saliva presence or absorption from mildly or non-venomous snakes and cross reactivity with venomous snakes on VDK testing. It has implications for antivenom use should it ever be required for more severe envenoming syndromes from mildly or

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Conmpeting interstests: Professor George Jelinek is Chair of the Management Committee of the UWA EMERG Trust which funded the study, however is not a member of the Grants Review Board which allocates funding for individual projects; Dr Dania Lynch is a member of the UWA Animal Ethics Committee which approved the study.

moderately venomous snakes, and for further research. It reinforces the practice of only using VDK testing in patients who show definite evidence of envenoming.

Key words: *snake bites, snake envenomation, snake venoms, snakes, venoms.*

See also pp. 384–386

Introduction

The Commonwealth Serum Laboratories (CSL) Venom Detection Kit (VDK) is an ELISA test available commercially in Australia since 1979 for the identification of snake genus from wound and urine samples of envenomed patients. The rationale behind its development was to enable snake genus identification from the patient's skin or urine so as to enable monovalent antivenom to be given rather than polyvalent. Australian polyvalent antivenom contains antivenom to all five major snake genera. Each ampoule has a very much larger quantity of horse serum than monovalent (approximately 55 mL), thereby exposing the patient to greater risk of allergic reactions. Polyvalent antivenom is also considerably more expensive than monovalent and is often irrelevant in areas of Australia where limited genera of snakes are found. Several large studies have documented the value of the VDK in guiding monovalent antivenom therapy for envenomed patients.[1–4]

Concern has been raised about the test giving a positive result in the absence of clinical or laboratory evidence of envenoming. Clearly a positive skin swab result does not imply envenoming, but a positive urine VDK result may be interpreted as indicating envenoming. Blood testing for venom has long been regarded as unreliable and is not recommended. Positive urine VDK results in the absence of other evidence of envenoming have been reported by Sutherland in 1992 in 8 of 181 VDK results in a one year review of antivenom usage;[5] and by Mead and Jelinek in 1996 in 5 of 117 paediatric patients in Perth.[3] Additionally, Jelinek and colleagues in 1991 reported positive VDK results from the urine or blood of 9 of 193 suspected snakebite patients with no evidence of systemic envenoming from a general Perth population.[2]

To date, this has not been reported to result in administration of the incorrect monovalent antivenom, although in 2003 Barrett and Little reported two cases where envenoming was clinically clearly due to a Death adder, but the VDK reported a positive result for Brown snake from urine and the bite site.[6] This resulted in polyvalent antivenom being given rather than the appropriate monovalent antivenom, with the attendant increase in risk and cost.

The cause of positive urine VDK results in the absence of definite envenoming has not been established and has been presumed to represent either false positive results from the VDK or subclinical envenoming. A third explanation is that some of these cases represent bites and venom/saliva absorption from mildly venomous snakes (this group currently represented by approximately 80 known species), or non-venomous snakes, with cross-reactivity of the venom/saliva with that of the known dangerously venomous snakes. Cross-reactivity between the mildly venomous Bardick snake (*Notechis curtus*) of the tiger snake genus with Death adder on VDK testing was reported in a single case of mild envenoming by Marshall and Herrmann in 1984.[7] Otherwise, the cross-reactivity between venoms/saliva from mildly venomous and non-venomous snakes and the five genera of venomous Australian snakes used in the VDK has not previously been investigated.

We sought to test the hypothesis that venoms or saliva from some non-venomous or mildly venomous snakes could produce positive results for venomous snakes with the VDK. We aimed to do this by testing the available venoms of three non-venomous and seven mildly venomous snake species with the CSL VDK, along with a control group of eight venomous Australian snake species, to determine if any of the mildly venomous and non-venomous snakes tested positive for any of the five medically significant genera of Australian snakes, namely Brown, Tiger, Death adder, Black and Taipan.

Methods

Snake husbandry

The snakes used for this study were kept by one author (BB, a trained herpetologist) or were caught

and re-released during visits to rural regions of Western Australia. BB was responsible for performing the 'bite/milking' procedure in which a latex membrane was stretched over a sterile collection container to mimic the human skin surface, allowing for the snake to bite and release venom. The term 'venom' is used throughout this paper, although for non-venomous snakes the 'venom' is saliva. Unless otherwise stipulated, VDK testing was performed on the same day as venom recovery in order to simulate the time frame occurring in a human situation, that is, patients presenting to a medical centre for definitive care not long after suspected envenoming has taken place.

Venom Detection Kit testing

After allowing both mildly venomous and venomous snakes to bite individual latex membranes, the venom was allowed to air dry for a few hours. The membranes were then swabbed in identical fashion to that used clinically to test wound swabs according to the CSL VDK instructions. With the non-venomous species, a swab of the soft mouth tissue both before and immediately after feeding was found to be adequate to detect venom. VDK testing was performed in all cases by one individual (NM), a trained laboratory technician experienced in VDK testing, in accordance with manufacturer's instructions. When a positive result was obtained on VDK testing, the venom was tested a second time with another VDK to ensure repeatability.

Deviation from the Venom Detection Kit protocol

After consultation with CSL, an additional dilution step was included in the standard protocol to avoid overwhelming the assay system with neat venom. The swab stick was first placed in 1.5 mL of 0.85% saline solution to moisten it and then swabbed over the latex membrane to collect venom. It was returned to the saline solution and gently mixed. A sample of this solution was further diluted (1 : 36) in the diluent supplied in the VDK.

Species of snake

The following venomous, mildly venomous and non-venomous snakes were used in the study:

- Common (and quick to bite) small mildly venomous snakes:
  Black-backed snake (*Parasuta nigriceps*); Reticulated whip snake (*Demansia p. reticulata*); Rosen's snake (*Suta fasciata*); Gould's Hooded snake (*Parasuta gouldii*); Little spotted snake (*Suta punctata*); Monk snake (*Parasuta monachus*); Moon snake (*Furina ornata*).
- Non-venomous snakes:
  Black-headed python (*Aspidites melanocephalus*); Carpet python (*Morelia spilota imbicata*); and Stimson python (*Antaresia stimsoni stimsoni*).
- The control group of venomous snakes:
  Western Tiger (*Notechis scutatus occidentalis*); Dugite (*Pseudonaja affinis affinis*); Ringed brown snake (*Pseudonaja modesta*); Mulga snake (*Pseudechis australis*); Spotted Mulga (*Pseudechis butleri*); Death adder (*Acanthophis pyrrhus*); Southern Death adder (*Acanthophis antarcticus*); and Pilbara Death adder (*Acanthophis wellsi*). No taipan venom was available for testing.

Some snakes of the same species but from different geographical locations were tested for possible variation.

Results

Table 1 summarizes the findings. As expected, the venomous snakes tested strongly positive for the appropriate venoms. Of note, a mildly venomous snake, Gould's hooded snake (*Parasuta gouldii*) and a non-venomous snake, the Black-headed python (*Aspidites melanocephalus*) each caused a positive test for the tiger snake genus. There was also cross-reactivity between black snake venoms and the tiger snake well of the VDK, with both black and tiger snake wells changing colour simultaneously. Both positive (well 7) and negative (well 6) test controls showed colour development as expected. There was some variability in the extent of colour change for the different species of Death adder.

Initial testing of dugite venom revealed a weak but distinct result for well 2 (Brown snake) despite the positive test control well indicating a strong colour change after 10 min (results not shown). The test was repeated, but an additional dilution step was incorporated as outlined in the methodology, and the VDK returned a strong positive test result for well 2 (Brown snake). Therefore, testing of all snake venoms in this study included an additional dilution step. Without dilution, all five wells in the kit can change colour, or the test can be overwhelmed as above and a false negative possibly recorded.[8]

Table 1. A comparison of Venom Detection Kit (VDK) results of venom/saliva taken from venomous, mildly and non-venomous Australian snake species

Common name	Species name	Geographical locality	Test results				
			Well 1	Well 2	Well 3	Well 4	Well 5
Venomous snakes							
Tiger							
Western Tiger	*Notechis scutatus occidentalis*	Bibra Lakes — metro WA	+ + +	–	–	–	–
Western Tiger	*Notechis scutatus occidentalis*	Lort River — rural WA(A)	+ + +	–	–	–	–
Brown							
Dugite	*Pseudonaja affinis affinis*	Stoneville — metro WA	–	+ + +	–	–	–
Ringed brown	*Pseudonaja modesta*	Paynes Find — rural WA	–	+ + +	–	–	–
Black							
Mulga	*Pseudechis australis*	Port Hedland — rural WA(B)	+ +	–	+ + +	–	–
Spotted Mulga	*Pseudechis butleri*	Yalgoo — rural WA	+ +	–	+ +	–	–
Death adder							
Death adder	*Acanthophis pyrrhus*	Port Hedland — rural WA	–	–	–	+ +	–
Southern Death adder	*Acanthophis antarcticus*	Bickley — metro WA	–	–	–	+ + +	–
Pilbara Death adder	*Acanthophis wellsi*	Munjina — rural WA(C)	–	–	–	+ +	–
Mildly venomous snakes							
Black-backed	*Parasuta nigriceps*	Mundrabilla — rural WA(D)	–	–	–	–	–
Reticulated whip	*Demansia p. reticulata*	Port Hedland — rural WA(E)	–	–	–	–	–
Rosen's snake	*Suta fasciata*	Port Hedland — rural WA(F)	–	–	–	–	–
Rosen's snake	*Suta fasciata*	Tom Price — rural WA(G)	–	–	–	–	–
Gould's Hooded	*Parasuta gouldii*	Balladonia — rural WA(H)	+ +	–	–	–	–
Little spotted snake	*Suta punctata*	Port Hedland — rural WA(I)	–	–	–	–	–
Monk snake	*Parasuta monachus*	Menzies — rural WA(J)	–	–	–	–	–
Moon snake	*Furina ornata*	Newman — rural WA(K)	–	–	–	–	–
Non- venomous snakes							
Black-headed python	*Aspidites melanocephalus*	South of Hedland — rural WA	+ +	–	–	–	–
Carpet python	*Morelia spilota imbricata*	Mt Helena — rural WA	–	–	–	–	–
Stimson's python	*Antaresia s. stimsoni*	Karratha — rural WA	–	–	–	–	–

Geographical locality: For those snakes captured from rural sites of Western Australia (WA) for re-release, venom was collected and stored at 4°C for the following number of days prior to testing: (A) 33; (B) 29; (C) 25; (D) 28; (E) 23; (F) 23; (G) 35; (H) 38; (I) 23; (J) 27; and (K) 32. Test Results: Well 1: Tiger; Well 2: Brown; Well 3: Black; Well 4: Death Adder; Well 5: Taipan. + + positive; + + + strongly positive.

Discussion

Although a simple question, the possibility that some apparent false positives from VDK samples may arise from bites by non-venomous and mildly venomous snakes has not been tested. This study revealed cross reactivity between a non-venomous (Black-headed python) and mildly venomous (Gould's hooded) snake and the tiger snake genus.

As previously noted, Marshall and Herrmann reported a case of envenoming by the mildly venomous Bardick snake (*Notechis curtus*), a snake of the tiger snake genus not known to cause serious illness.[7] It tested positive with the VDK to death adder. Pearn and colleagues showed that the Red-bellied black snake (*Pseudechis porphyriacus*), a species of Black snake not yet known to cause fatal envenoming, caused a moderately severe envenoming in five cases, with local inflammation and necrosis as well as systemic features of nausea and vomiting, and chest pain.[9] Sutherland and Tibballs indicate that this snake is capable of causing the death of a child but probably not an adult.[8] Despite this snake being of the black snake genus, VDK testing is positive for tiger snake

venom, and tiger snake antivenom is recommended in cases where envenoming requires treatment, although black snake antivenom can be used.

Similarly, there is cross-reactivity between a number of other black snakes and tiger snake venom on VDK testing, and tiger snake antivenom is recommended for many of them, including the Blue-bellied black snake (*Pseudechis guttatus*) and Collett's snake (*Pseudechis colletti*).[8] This is in accord with our finding of cross-reactivity between both the Mulga snake (*Pseudechis australis*) and Spotted Mulga snake (*Pseudechis butleri*) and the tiger snake well of the VDK. Indeed it was only after unsuccessful use of tiger snake antivenom in a fatal case of Mulga snake envenoming in 1969 that CSL changed their recommendation to the use of Papuan black snake antivenom, then raised a specific antivenom to this snake, now black snake antivenom.[8]

White reported mild envenoming by the Five-ringed brown snake (*Pseudonaja modesta*).[10] It tested positive to Brown snake with the VDK as expected, although unlike other Brown snakes it has no procoagulant activity. Brown snake antivenom would be suitable if required for more severe envenoming. Isbister and Currie reported mild envenoming by the Northern small-eyed snake (*Rhinoplocephalus pallidiceps*) and the Black whip snake (*Demansia atra*).[4] No VDK testing was performed, and no antivenom was required in these cases. Sutherland and Tibballs noted a case of a 10-month-old infant envenomed by Stephen's banded snake (*Hoplocephalus stephensi*), where a VDK result was positive for tiger and black snake.[8] Others have reported only tiger snake venom detection in envenomings by this snake.[8] Our own laboratory has noted several instances of simultaneous colour change in the brown and death adder wells of the VDK, however, they report the finding as positive for Brown snake if they note coexistent coagulopathy (pers. comm. Nick Michalopoulos).

Sutherland and Tibballs note that a positive result from well 1 (Tiger snake) indicates that this is the appropriate antivenom to administer if required, and not necessarily the genus of the biting snake.[8] For instance, a positive result from the tiger snake well in the VDK can result from Red-bellied black snake as discussed previously, but also from a Copperhead (*Austrelaps* sp.) or Rough-scaled snake (*Tropidechus carinatus*). Sutherland and Tibballs however, state that a positive result from any of the other four wells, that is Brown, Black, Death adder and Taipan identifies the genus as well as the antivenom. This is not always true, given the cross-reactivity between the Bardick snake (tiger snake genus) and death adder well on the VDK reported by Marshall and Herrmann in 1984.[7] Additionally, Williams and White in 1990 reported that the venom of two specimens of the Yellow-faced whip snake (*Demansia psammophis*) from the same geographical region were neutralized by brown snake antivenom, yet one had a positive reaction with Brown snake on VDK, and the other Tiger snake.[11]

However, Sutherland and Tibballs assert in their chapter on 'snakes of uncertain or lesser medical importance' that in the unlikely event that antivenom might be required for one of these snakes not generally considered dangerous then *possibly* tiger snake antivenom may prove beneficial.[8] They note that little research has been done on this topic. Our research has shown that tiger snake cross-reactivity may also potentially result from a bite by a non-venomous (Black-headed python) and mildly venomous (Gould's hooded) snake.

Further research may assist in identifying other non-venomous snakes which may show cross-reactivity with venomous snakes on VDK testing, thereby creating potential confusion about antivenom treatment. Such research may also be useful in situations where snakes previously thought of as non-venomous actually produce a syndrome of envenoming. The particular cross-reactivities they show may provide a clue as to appropriate antivenom treatment in cases where envenoming progresses and is felt to require treatment.

False positive VDK results are not unusual.[2–5] While it is likely that the majority are truly false positive results, and some may be the result of subclinical envenoming, we have shown that there is also the possibility that some may be due to bites by non-venomous or mildly venomous snakes. Non-venomous snakes can leave a diagnostic semicircular row of fang marks, however, many bites by Australian snakes leave no clearly visible mark, so this may not assist in differentiation of non-venomous from venomous bites.

The VDK cross reactivity we have found between non-venomous, mildly venomous and venomous snakes has other clinical implications. Mead and Jelinek first highlighted the importance of not using the VDK as a tool to confirm envenoming from snakebite.[3] Tibballs had previously used VDK detection of snake venom in the urine as proof of envenoming[12] and this view is commonly held amongst clinicians. Currently evolving

emergency medicine practice in suspected snakebite is to test swabs from the skin and urine but only if the patient shows clinical signs of envenoming, thereby preventing false positive VDK results from influencing treatment decisions. Isbister and Currie have trialed such a policy, collecting swabs from all patients with suspected snakebite, but only VDK testing those who develop evidence of envenoming.[4] They reported no cases where nonenvenomed patients were treated with antivenom, a situation previously reported commonly in a number of large suspected snakebite series.[2,3]

In contrast, a publication from the Australian Venom Research Unit, a recognized authority in toxinology, has recommended VDK testing on all patients with suspected snakebite[13] and this is also recommended on their website (http://www.pharmacology.unimelb.edu.au/avruweb/doctors.htm). This practice is also recommended by Sutherland and Tibballs.[8] This may cause confusion in situations where there is some suggestion of envenoming clinically, but not enough to warrant antivenom, but the VDK is positive, either due to subclinical envenoming, a false positive result or because of cross reactivity after a non-venomous or mildly venomous snakebite. Particularly in inexperienced hands, the positive VDK result may result in needless administration of antivenom, with its attendant risks and cost.

Conclusions

Mildly venomous and non-venomous Australian snake venoms may cross react with venoms from venomous snakes on VDK testing. This finding reinforces the currently evolving clinical practice of only VDK testing patients with definite evidence of envenoming.

Acknowledgements

The authors acknowledge financial support from the Emergency Medicine Education and Research Grants (EMERG) Trust of the University of Western Australia. Ethics approval to conduct this study was gained from the University of Western Australia Animal Ethics Committee (approval no. 240/97).

Accepted 16 July 2004

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