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Original Contribution

Antivenin remains effective against African Viperidae bites despite a delayed treatment

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Abstract

Background: Viperidae bites represent a public health issue in Africa and are responsible for a hemorrhagic syndrome with fatal outcome in the short term. A research on Medline database does not reveal any data definitively demonstrating the efficiency of antivenom in case of delayed administration. The aim of this study, based on a 12-year survey of viperine syndromes in republic of Djibouti, was to compare the normalization of the hemostasis disorders with an early administration of antivenin versus a delayed administration.

Methods: A retrospective study was conducted from October 1994 to May 2006 in the intensive care unit of the French military Hospital, in Djibouti. Seventy-three Viperidae-envenomed patients were included. Antivenin efficiency in correcting hemostatic disorders was analyzed in relation to time to treatment (before or after the 24th hour after the bite).

Results: Forty-two patients (58%) presented with bleeding. A consumptive coagulopathy was found in 68 patients (93%). Antivenin was observed to be effective in improving hemostasis, and the time to normalization of biologic parameters was similar, whether the treatment was started before or after the 24th hour after the bite.

Conclusion: Antivenin should ideally be administered as early as possible. However, in Africa, time to treatment generally exceeds 24 hours. The results of the present evidence-based study confirm an empirical concept: a delayed time to treatment should in no way counterindicate the use of antivenin immunotherapy, in the case of African Viperidae bites.

1. Background

Ophidian envenomations remain a public health issue in Africa, due not only to their frequency and seriousness but

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Fig. 1 *E pyramidum* (graph by P. Gillet, A. Dutheil, and S. Cocquet; Inf'Faune).

also to their difficult management. There may be 500 000 snakebite-related envenomations every year in Africa, with nearly 25 000 fatalities, but the numbers may be larger.

The association of clinical or biologic abnormalities of hemostasis and of a local syndrome (pain, edema, necrosis, or gangrene) represents the so-called viperine syndrome and is evocative in Africa of an envenomation by Viperidae [1].

Viperidae are responsible for more than 90% of the ophidian envenomations in tropical Africa. Two species dwell in Republic of Djibouti: *Echis pyramidum* (Fig. 1) and *Bitis arietans*. *Echis* sp. are the most significant snakes on the medical field, representing 10% of hospitalizations at some periods of the year, in some areas of Nigeria [1].

Although modern antivenoms have proved to be efficacious and safe, and despite the high lethality of viper bites in Africa when antivenom is not used, a research on Medline database does not reveal any data definitively demonstrating the efficiency of antivenom in case of delayed administration.

The aim of this study, based on a 12-year survey of viperine syndromes in Republic of Djibouti, was to compare the normalization of hemostasis with an early administration of antivenom (less than 24 hours), versus a delayed administration (more than 24 hours).

2. Methods

2.1. Population

A retrospective study was conducted in the French military hospital in Djibouti (Bouffard Hospital), which is a tertiary care center. The intensive care unit receives patients from all over the country. All consecutive patients presenting snakebites between October 1994 and May 2006 were studied. All data were collected in hospital's medical records by the same investigator. This medical doctor had a good knowledge of snake envenomations and applied a standardized data collection grid. However, this grid, useful for clinical symptoms, did not permit standardization of biologic parameters collection.

For each patient, the following data were collected: hemorrhagic signs, platelet count, prothrombin time (PT), activated cephalin time (ACT) ratio, and fibrinogen. All the laboratory tests were carried out at the hospital laboratory. Patients were considered as victims of a viperine syndrome, if they presented at least 1 of the following signs: bleeding or biologic coagulopathy defined as PT less than 45%, ACT greater than twice the normal values, platelets less than 80 Giga/L, or fibrinogen less than 1 g/L. Edema extension exceeding the elbow or the knee level or presence of vesicles or necrosis was also considered as a sign of viperine syndrome.

All patients admitted before August 2001 were treated by a 20-mL ampule of *Echis-Bitis-Naja* serum (Pasteur, Mérieux, Paris). From September 2001 onward, envenomed patients received one or two 10-mL ampules of the newly available FAV-Africa (Aventis-Pasteur), efficacious against *Echis*, *Bitis*, *Naja*, and *Dendroaspis* venoms. Depending on the clinical course, additional ampules were injected later in some patients. Time to treatment (hours) was defined as the elapsed time between hour of bite and hour of the first antivenom injection.

Table 1 Kaplan-Meier method

Time	No. patients presenting with an abnormal value	No. patients presenting with a normal value	Probability of normalization	Function of normalization
t_0	0	D_0	$Q_0 = 0$	$S_0 = 1$
t_1	V_1	D_1	$Q_1 = D_1/V_1$	$S_1 = S_0 p_1$
t_2	V_2	D_2	$Q_2 = D_2/V_2$	$S_2 = S_1 p_2$
…	…	…	…	…
t_i	V_i	D_i	$Q_i = D_i/V_i$	$S_i = S_{i-1} p_i$

V_i: number of patients presenting with an abnormal value of the parameter at the beginning of the time interval $t_i - t_{i-1}$.
D_i: number of patients presenting with a normal value of the parameter during the interval $t_i - t_{i-1}$.
q_i: probability of normalization of the parameter during the interval $t_i - t_{i-1}$: $q_i = D_i/V_i$.
S_i: function of normalization at the time t_i: $S_i = p_0 p_1 \ldots p_i = p_i S_{i-1}$.

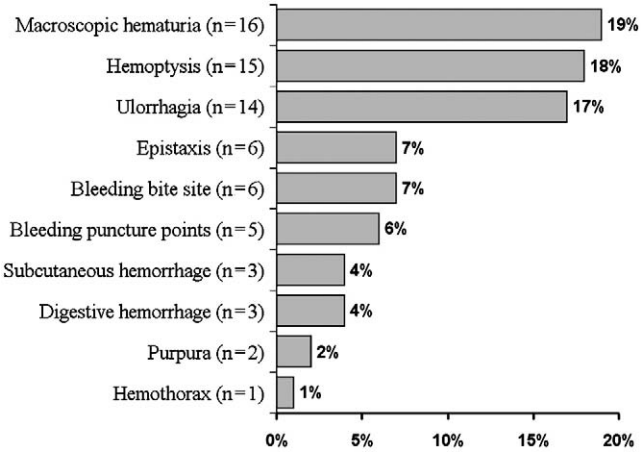

Fig. 2 Location of bleeding (n = 73).

Medical records data were collected anonymously. The study protocol was reviewed and approved by the French Military Health Service ethical commission.

2.2. Statistical analysis

Because collection of biologic parameters could not be standardized, curves of PT normalization and fibrinogen rate were constructed for envenomed patients according to the Kaplan-Meier method (Table 1). This method allowed calculating the probability of normalization each time a hemostatic disorder was recorded.

The analysis of the normalization function using the Kaplan-Meier method is represented by a graph with time on the abscissa and probability of normalization on the ordinate. This probability represents the population percentage whose parameter exceeds the threshold. The latter was arbitrarily fixed to define the disorder of hemostasis. The curve is in ascending stair steps. Each step represents the probability of normalization during the corresponding time interval. The step's upright represents the increased probability of the parameter's normalization noted at the *t* time. The log-rank test was used to compare curves of normalization ($\alpha = .05$), by taking account of patients who did not normalize.

3. Results

3.1. Studied population's characteristics

Eighty-four Djiboutian patients (64 men and 20 women) were admitted for snakebite between October 1994 and May 2006. Among these, 73 Viperidae-envenomed patients (87%) were included in the study, 64 (76%) among them having benefited from antivenom administration. Other patients were excluded because they were not considered as victims of a viperine syndrome. Two patients among the 73 envenomed patients were then excluded from the study of hemostasis evolution: 1 received heparin, and 1 had only been given subcutaneous antivenom in the proximity to the bite.

The average (SD) age was 27 (15) years (range: 1-60 years). Bites occurred in all ages but mostly occurred among males from 21 to 40 years. Sex ratio was 3:1 (55 men and 18 women). Children younger than 10 and those younger than 5 represented 16% and 5% of the sample, respectively.

Bites mainly occurred at nightfall, between 7 and 9 PM: 15 cases (28% of 53 notified hours). The bitten area was the lower limb in 57% of cases and the upper limb in 41% of cases.

No death was recorded.

3.2. Hemorrhagic syndrome's characteristics

Forty-two patients (58%) presented bleeding. The most frequent hemorrhagic signs were macroscopic hematuria

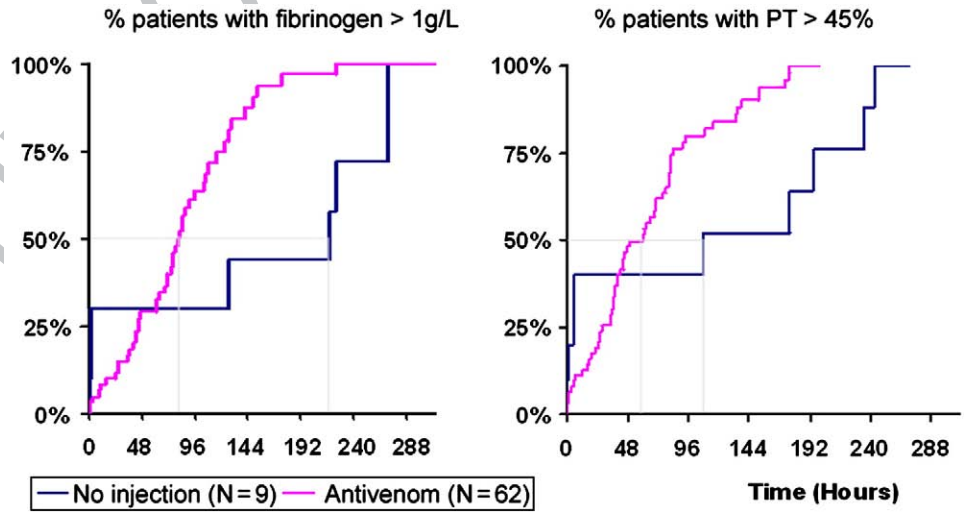

Fig. 3 Normalization of biologic parameters plotted against time, with or without antivenom (H0: hour of bite).

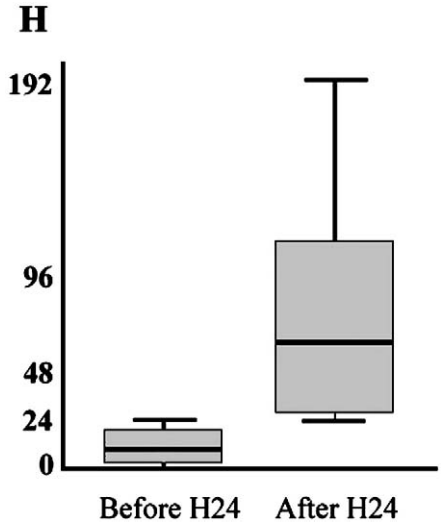

Fig. 4 Distribution of time to treatment.

(22%), hemoptysis (21%), and bleeding gums (19%) (Fig. 2). There was no significant link between bleeding and patient age, particularly for age less than 11 years versus age 11 years and greater (hazard ratio, 0.68; 95% confidence interval, 0.29-1.58). Parenthetically, age less than 11 years has been found to be of bad prognostic significance for snakebite severity [2].

Sixty-eight patients (93%) exhibited a coagulopathy, 26 of which did not present any bleeding (38%). At the first hemostasis measurement, PT of 57% of patients was nil, ACT of 56% was invaluable, and afibrinogenemia was found in 81%. Only 1 patient showed an afibrinogenemia without any other hemostasis disorders. A thrombocytopenia less than 150 000/mL was observed in 37 patients (51%), and the lowest recorded value was of 17/mL.

3.3. Course of hemostatic abnormalities: with and without antivenom

Among the 71 envenomed patients evaluated for hemostasis disorders, 62 (87%) patients benefited from an antivenom administration and 9 (13%) did not.

In the present study, the shape of curves was clearly in favor of a faster normalization with antivenom (Fig. 3), and log-rank tests were significant ($P = .002$ for fibrinogen and $P = .001$ for PT).

Half of treated patients had a normalized fibrinogen rate at the 80th hour after the bite versus at the 217th hour for 50% of untreated ones.

Half of treated patients had a normalized PT at the 50th hour after the bite versus the 107th hour for 50% of untreated ones.

3.4. Time to treatment 24 hours or less versus more than 24 hours

Approximately one third of patients (32%) were admitted before the 12th hour, 59% before the 24th hour, and 78% before the 48th hour. Average (SD) time to treatment was 37 (36) hours (range: 1-192 hours). Time to treatment was 24 hours or less in 39 patients and more than 24 hours in 23 patients. Median time was 10 hours for this first group and 57 hours for the second group (Fig. 4).

There was no significant relationship between the rate of normalization of fibrinogen and the time to antivenom administration ($P = .36$). The shape was very close for both curves (Fig. 5). A similar result was observed between the rate of normalization of PT and time to antivenom administration ($P = .93$).

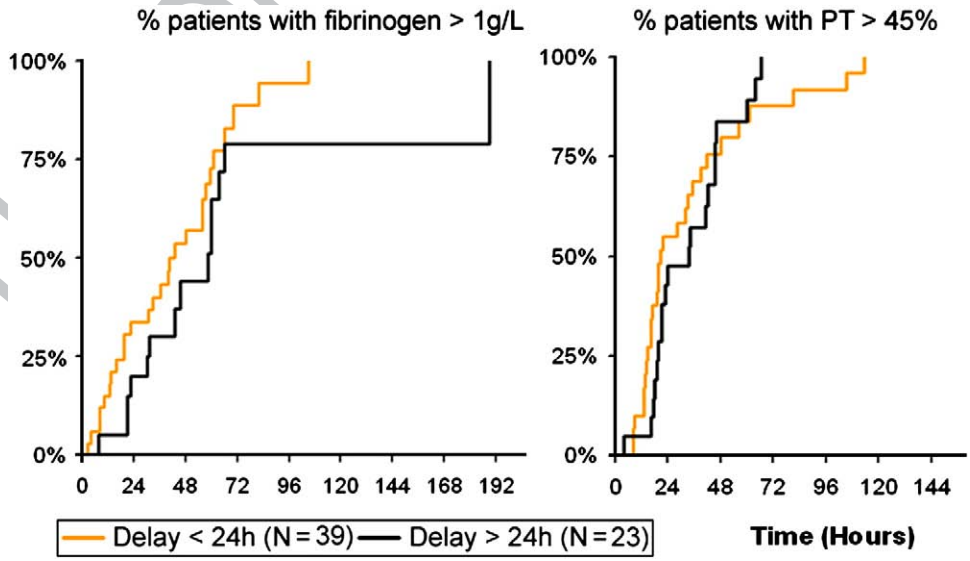

Fig. 5 Normalization of biologic parameters plotted against time and delay (H0: hour of antivenom injection).

4. Discussion

This retrospective study was exhaustive for snakebites treated in Djibouti Republic at Bouffard Hospital between 1994 and 2006. Others Djiboutian hospitals like civil national Peltier Hospital can rarely dispense antivenom because of cost. So, we may consider that although clearly underestimating the true incidence of the bites, our study gives an approximative estimation of incidence of snakebites treated with antivenom in this country.

This study confirms the previously demonstrated antivenom effectiveness in treating hemostatic disorders secondary to Viperidae bites. More importantly, the results also demonstrate that this immunotherapy remains effective whatever the snakebite-antivenom injection time.

A hemorrhagic syndrome is responsible for more than half of the morbidity and the mortality due to snakebites in the world [3] and should always benefit from antivenom administration [2]. In Africa, a hemorrhagic syndrome must initially evoke an *Echis* bite, but it may sometimes be the sign of an envenomation by other Viperidae or exceptionally by opisthoglyphous Colubridae such as *Dispholidus typus*. In the present study, the clinical and biologic presentation (significant edema, bleeding, and initial afibrinogenemia, absence of large necrosis or neurologic signs, and sometimes identification or description of the snake) led us to regard *E pyramidum* as the main culprit for envenomations in Djibouti, excepting some cases possibly due to *B arietans*.

Occurrence of clinical signs is frequently delayed compared with earlier changes in laboratory studies. Warrell et al [4] observed in Nigeria that coagulopathy appears 75 minutes to 27 hours after Viperidae bites. The hemorrhagic syndrome can include persistent bleeding at the bite site, puncture points, or mucous membranes (bleeding gums, epistaxis, hematuria, digestive hemorrhage, and hemoptysis). Hemorrhagic shock or subarachnoid hemorrhage is a usual cause of death. Cases of abundant intracavitary bleeding (hemoperitoneum, hemothorax) have been described, but these are atypical.

Multiplicity of biologic signs and normalization times show the complexity of venom composition and the variability of inoculated quantity. There is often discordance between laboratory and clinical signs [5]. In a study carried out in Cameroun, 30% of snakebites involved hemostasis disorders without clinical signs [7].

E pyramidum and *B arietans* venoms contain many complex proteins, especially enzymes, which interfere with all steps of hemostasis:

(1) Hemorrhagins that induce disorders of capillary permeability
(2) Phospholipases A2, serine proteases, and metalloproteinases, L-amino-acido-oxydases, phosphoesterases, disintegrins, and C-type lectins that may either activate or inhibit platelets. Platelets inhibition reduces their efficiency, whereas pathologic activation reduces the number of circulating platelets).
(3) Proteins interfering with coagulation: either procoagulant proteases (prothrombin activators and factor X activator) or anticoagulant proteases (factor IX/X inhibitors, protein C activator, phospholipases A2) [6]. In the case of African Viperidae, these proteases never promote thrombotic syndrome in vivo. When coagulation processes are activated, they persist until exhaustion of 1 or more factors (consumption phenomenon) and frequently lead to a hemorrhagic syndrome, mostly due to afibrinogenemia.
(4) Lastly, some fibrinolytic enzymes activate fibrinolysis and have plasmin-like properties. Fibrinogen may be reduced, as well as fibrin.

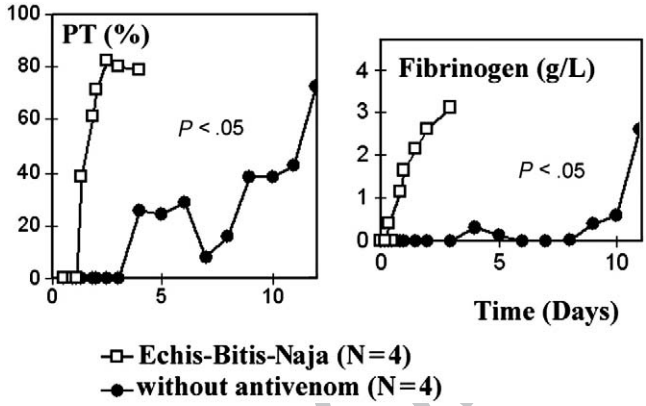

Fig. 6 Normalization of PT and fibrinogen in a previous pilot study with (n = 4) or without (n = 4) *Echis-Bitis-Naja* antivenom ($P < .05$, *U* test [9]).

The bite is definitely not responsible for thrombin overproduction, but for heparin-insensitive meizothrombin production and primitive fibrinogenolysis. Moreover, platelet count, antithrombin III, factor XIII, and D-dimers may be normal [7]. These are no biologic features of a true disseminated intravascular coagulation, which is defined by mandatory biologic criteria. "Venom induced consumptive coagulopathy" would be a more appropriate descriptive term.

Many studies demonstrate the clinical efficiency of antivenom [8]. In a prospective pilot study at the same hospital, we previously showed that antivenom therapy with Fab'_2 fragments (*Echis-Bitis-Naja*) was efficacious and safe for *Echis* bites in Djibouti (Fig. 6). Bleeding generally stopped within 12 to 30 hours, and a single injection was often enough. Antivenom restored PT within 13 hours, fibrinogen within 19 hours [9].

The present retrospective study did not permit a plotting of evolution curves of biologic parameters because of dissimilar timing in biologic measurements in successive patients. The fact that data collection could be not standardized, concerning the biologic parameters constitutes a potential statistical bias, but normalization times constitute an interesting approximation: it allows the prediction at

any (*t*) time of the percentage of patients who present a normalization of the studied parameter.

For 50% of patients, fibrinogen and PT normalization had occurred by the 80th and the 50th hours after the bite, respectively (Fig. 3). Without antivenom administration, time for correction of biologic disorders in 50% of patients was nearly 3 times longer (fibrinogen) or twice longer (PT). Strictly speaking, the log-rank test is appropriate only if the group curves do not cross. However, crossing observed in our data is probably an artifact due to the low number of patients in the untreated group (n = 9).

Like in our previous prospective study, it can be noted that the hemostatic parameters spontaneously normalized on the 10th day, and the present results seem consistent with those of the pilot study if we consider the present longer time to management (37 ± 36 hours).

During the present study, 2 antivenoms were successively used: *Echis-Bitis-Naja* for the first group of the patients, then FAV-Afrique. Previous studies [5] demonstrated the same efficacy for these 2 preparations of immunoglobulin fragments F(ab′)2 (as long as *Dendroaspis* is not involved, of course). An initial dose of a 20-mL ampule was systematically used for *Echis-Bitis-Naja*, whereas FAV-Afrique was begun at a 1 or 2 ampules according to patients weight. However, it seems that there is a weak relationship between antivenom dose and recovery time [8]. We did not notice any significant difference between the 2 antivenoms nor between an initial dose of 1 or 2 FAV-Afrique ampules. Although *E pyramidum* venom is neither used for manufacturing of *Echis-Bitis-Naja* nor FAV-Afrique, correction of bleeding disorders and absence of deaths among these patients testify to the cross-reactivity of those 2 antivenoms towards Djiboutian *E pyramidum* venom.

Concerning evolution according to time to treatment, it is known that most of snakebites-related fatality and morbidity are related to delays in time to management. Warrell et al [4], like Pugh and Theakston [10], noted that a short time to treatment lead to a better outcome. Another study found that lethality was more frequent with a late medical management of *Echis ocellatus* bites [5]. The incidence of complications is directly proportional to the duration of venom's presence in blood before neutralization by antivenom [11]. Renal abnormalities significantly correlate with late onset of treatment, and early antivenom administration prevents renal damage [12]. Thus, early institution of antivenom therapy is beneficial in preventing complications however severe the envenomation.

However, as is frequent in Africa, time to management in Bouffard Hospital intensive care unit frequently exceeds 24 hours. Several reasons may explain this delay. First, recruitment in the department serves the all country and distance clearly delay management. Second, more than half of the patients in West Africa first consult a tradipratician. Lastly, because of antivenom costs, snakebite handling is subjected to an agreement between Djiboutian Hospital and Bouffard Hospital: Djiboutian physicians refer snakebite victims to Bouffard Hospital intensive care unit, a situation that also extends the delay between the bite and immunotherapy.

Our data show that it is possible to treat successfully patients arriving several days after the bite and save their lives. The nonsignificant log-rank test concerning time to treatment could be the consequence of a lack of power. However, the log-rank test was significant concerning antivenom administration, despite the low number of patients in untreated group (n = 9 versus n = 62 for treated group). Concerning time to treatment, group numbers were more comparable (n = 39 for time <24 hours and n = 23 for time >24 hours). Therefore, although a nonsignificant test does not formally demonstrate an absence of difference of normalization time according to time to treatment, our data suggest that any pertinent difference would have been detected.

Chippaux et al [13] did not find significant link between the incidence of hemostatic disorders and the delay of consultation. The fact that antivenom remains effective despite several days time is known to physicians who usually deal with patients bitten by Viperidae but has not been demonstrated before.

It has been demonstrated in the clinical setting that immunoglobulins lose their effectiveness when injected more than 4 hours after bites of the Australian elapid, *Oxyuranus scutellatus*. In this last case, treatment relies on symptomatic measures such as artificial ventilation, which should be pursued until the natural elimination of toxins [14]. The present absence of deleterious impact of time to management on hemostasis normalization may be explained by the enzymatic nature of proteins contained in Viperidae venoms. Contrary to Elapidae venoms toxins [15], enzymes do not remain fixed to receptors but persist in tissues and blood stream.

In conclusion, although antivenom should ideally be administered as early as possible, it preserves a constant efficacy on African Viperidae venoms–related hemostasis disorders, whatever the time to treatment. In the event of the late management of Viperidae bites, frequently occurring in Africa, a long time to treatment should in no way contraindicate the use of antivenomous immunotherapy.

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References

[1] Pugh RNH, Theakston RD. Incidence and mortality of snake bite in savanna Nigeria. Lancet 1980;ii:1181-3.
[2] Manent P, Mouchon D, Nicolas P. Envenomation by *Echis carinatus* in Africa: clinical study and evolution. Indications for antivenoms. Med Trop 1992;52:415-21.
[3] White J. Snake venoms and coagulopathy. Toxicon 2005;45:951-67.

[4] Warrell DA, Davidson NMD, Greenwood BM, Ormerod LD, Pope HM, Watkins BJ, et al. Poisoning by bites of the saw-scaled or carpet viper (*Echis carinatus*) in Nigeria. Q J Med 1977;46:33-62.

[5] Einterz E, Bates M. Snakebite in northern Cameroon: 134 victims of bites by the saw-scaled or carpet viper, *Echis ocellatus*. Trans R Soc Trop Med Hyg 2003;97:693-6.

[6] Mion G, Olive F, Hernandez E, Martin YN, Vieillefosse AS, Goyffon M. Action of venoms on blood coagulation: diagnosis of hemorrhagic syndromes. Bull Soc Pathol Exot 2002;95:132-8 Review. French.

[7] Singletary EM, Rochman AS, Bodmer JC, Holstege CP. Envenomations. Med Clin North Am 2005;89:1195-224.

[8] Chippaux JP, Lang J, Amadi-Eddine S, Fagot P, Rage V, Peyrieux JC, et al. Clinical safety of a polyvalent $F(ab')^2$ equine antivenom in 223 African snake envenomations: a field trial in Cameroon. Trans R Soc Trop Med Hyg 1998;92:657-62.

[9] Mion G, Rüttimann M, Saïssy JM, Olive F. Efficacy of antivenom therapy (*Bitis-Echis-Naja*) in the treatment of bites by the carpet viper (*Echis carinatus*). Intensive Care Med 1997;23(Suppl 1): S179.

[10] Pugh RNH, Theakston RDG. A clinical study of viper bite poisoning. Ann Trop Med Parasitol 1987;81:135-49.

[11] Narvencar K. Correlation between timing of ASV administration and complications in snake bites. J Assoc Physician India 2006;54:717-9.

[12] Vijeth SR, Dutta TK, Shahapurkar J. Correlation of renal status with haematological profile in viperine bite. Am J Trop Med Hyg 1997;56: 168-70.

[13] Chippaux JP, Amadi-Eddine S, Fagot P. Diagnosis and monitoring of hemorrhage due to viper envenomation in the African savanna. Bull Soc Pathol Exot 1999;92:109-13.

[14] Trevett AJ, Lalloo D, Nwokolo NC, Naraqi S, Kevau IH, Theakston RD, et al. The efficacy of antivenom in the treatment of bites by the Papuan taipan (*Oxyuranus scutellatus canni*). Trans R Soc Trop Med Hyg 1995;89:322-5.

[15] Larréché S, Mion G, Daban JL, Goyffon M. King cobra envenoming. Anaesthesia 2007;62:1076-7.