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Clinical Trial of an F(ab')$_2$ Polyvalent Equine Antivenom for African Snake Bites in Benin

J.-P. Chippaux,* A. Massougbodji, R. P. Stock, A. Alagon, and the Investigators of African Antivipmyn® in Benin†

Unité de Recherche 010, Institut de Recherche pour le Développement, La Paz, Bolivia; Faculté des Sciences de la Santé de Cotonou, Cotonou, Benin; Instituto de Biotecnología, Universidad Nacional Autonónoma de México, Cuernavaca, Morelos, Mexico

Abstract. We report the results of a trial designed to measure the safety and efficacy of African Antivipmyn®, a new freeze-dried polyvalent equine F(ab')$_2$-based antivenom. We tested 289 envenomations. After treatment, 19% of treated patients had undesirable events, all benign. A possible adverse effect was attributed to this antivenom in 11% of the patients. Bleeding was observed in 48% of the patients; it stopped within 2 hours after treatment with antivenom in 60% of the patients. Blood incoagulability was observed in 80% of the patients. Restoration of coagulation was attained within 4 hours in 60% of the patients. Nine patients died; 6 arrived at the hospital in the final stage of complications and 5 arrived at the hospital more than 60 hours after the bite. The value of blood coagulation tests in diagnosis of envenomation and bleeding as an indicator of renewal of treatment are emphasized.

INTRODUCTION

Snake bites represent a public health problem particularly important in the savannah areas of sub-Saharan Africa.[1,2] In Benin, studies showed that annual incidence ranges between 200 and 650 snake bites per 100,000 people.[3] Annual mortality exceeds 10 deaths per 100,000 inhabitants. However, a large number of victims of snake bite resort to traditional medicine and, as a consequence, many envenomations are not treated by health services. Thus, notified annual morbidity is less than 85 envenomations per 100,000 persons.[4–6]

The effectiveness of antivenom therapy and its role in the treatment of the envenomation are well established and are not questioned, at least in Africa.[7–9] Two clinical trials conducted in northern Cameroon between 1993 and 1996 confirmed the good tolerance of purified F(ab')$_2$ administered either by infusion[10] or slow direct intravenous injection.[11] In addition, these trials resulted in establishing clinical (edema, bleeding) and biologic (whole blood coagulation test [WBCT] in a dry tube) criteria for antivenom therapy and its surveillance.[12,13] These surveys can be used as a basis for other clinical studies to validate new preparations of antivenins.

The limited availability of antivenoms has resulted in several manufacturers in developing countries to propose solutions for this problem.[7,8,14] An example is the Instituto Bioclon, a subsidiary of Laboratorios Silanes (Mexico City, Mexico), the largest producer of antivenoms in Latin America. An open, phase IV clinical trial using African Antivipmyn® (Laboratorios Silanes, Mexico City, Mexico) a freeze-dried antivenom composed of purified F(ab')$_2$ fragments of equine IgG, was performed under field conditions in Benin from July 2005 to July 2006. The main objective of this trial was to measure the early tolerance in terms of the incidence of undesirable events and severity of each adverse reaction observed.

Secondary objectives were to evaluate the efficacy this antivenom by comparing the incidence of the deaths with published data for the same zone (10–12.3%, which was rounded to 10%) and the incidence of patients cured without sequelae; identify symptoms facilitating the surveillance and the choice of dosage; and propose an algorithm for management of snake bites (signs of envenomation, doses, indicators of surveillance and criteria of cure).

MATERIALS AND METHODS

Antivenom. The lot of African Antivipmyn® that we used was composed of purified F(ab')$_2$ fragments of IgG from horses immunized with venoms of *Bitis gabonica*, *B. arietans*, *Echis ocellatus*, *E. leucogaster*, *E. pyramidum*, *Naje haje*, *N. melanoleuca*, *N. nigricollis*, *N. pallida*, and *Dendroaspis polylepis*. Each vial of antivenom contained 200 mg of protein and neutralized at least 250 50% lethal doses of each venom as determined by standard lethality tests.[15] The antivenom is in a freeze-dried form that is reconstituted in a 10-mL volume of an injectable saline solution.

Organization of the clinical trial. The protocol of clinical trial was reviewed and approved by the National Committee of Ethics of Benin in June 2005. Any patient who reported being bitten by a snake with obvious signs of envenomation (edema, bleeding, positive WBCT result, or neurologic disorder indicating elapid envenomation) and who signed an informed consent form was included in the study and treated in accordance with the protocol.

Patients who did not satisfy all entry criteria inclusion or refused to participate in the trial were treated in accordance with the recommendations and policies of the health services of Benin and were not included in the clinical trial. Patients who received antivenom other than African Antivipmyn® (polyvalent Africa; Serum Institute of India [Pune, India] or polyvalent antivenom; Bahrat [Mumbai, India] or FAV Afrigue; Sanofi Pasteur [Lyon, France]) were excluded from the study. Patients who left the hospital during treatment against doctors' advice were also excluded from the analysis. The trial was performed in 11 health centers of central and northern Benin (Figure 1), in a zone of Guinean-Sudanese savannah with extensive agricultural activity.

All patients included in the study received at least one dose of African Antivipmyn®. One dose was composed of two vials

* Address correspondence to J.-P. Chippaux, Unité de Recherche 010, Institut de Recherche pour le Développement, C.P. 9214 La Paz, Bolivia. E-mail: chippaux@ird.fr

† Investigators of African Antivipmyn® in Benin: E. Fassinou, A. Ndamadjo (Kandi); R. Soglo (Gogounou); B. E. Tamou (Bembéréké); A. B. Mama, A. Nguemezi (Nikki); A. Salako, J.-P. Hounyet, A. Koussémou (Boko); Y. A. Mongbo (Tchaourou); A. Santos, A. Batonon (Papané); L. Loukmann M. Tévoèdjrè (Ouéssé); A. Yaya (Savé); Y. Agbangla, S. Ahoui (Glazoué); C. Agossadou (Dassa).

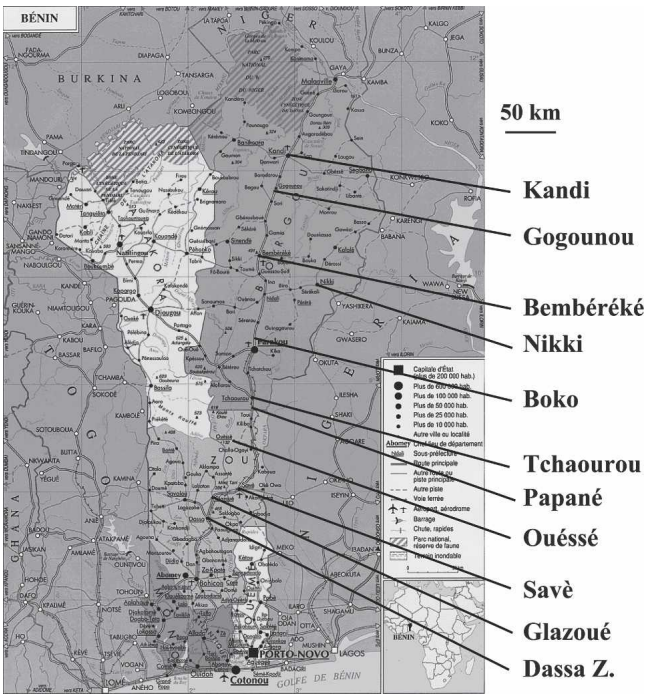

FIGURE 1. Map of Benin and location of trial centers.

of antivenom. However, for practical reasons the content of two vials was reconstituted in 10 mL of physiologic saline and injected slowly by direct intravenous route. The immediate and delayed tolerance was measured clinically.

Severity of envenomation and effectiveness of treatment were analyzed clinically and biologically according to the following criteria. Clinical scores, graduated according to severity, were used to measure the size of edema (0 = no edema, 1 = edema limited to only one articulation, 2 = edema reaching two articulations, 3 = edema of all members but excluding the root, 4 = edema including the root, and 5 = edema exceeding the root), necrosis (0 = no necrosis, 1 = cutaneous necrosis, 2 = muscular necrosis, and 3 = necrosis of tendon or bone), and bleedings (0 = no bleeding, 1 = persistence of bleedings at the bite site for more than one hour, 2 = bleeding of mucous membranes, 3 = bleeding of cutaneous lesions away from the bite site, and 4 = externalization of internal bleedings). Hematuria and proteinuria were measured using reactive strips. A coagulation test (WBCT) was carried out in a degreased dry tube and evaluated 30 minutes after sampling using 2 scores: time of coagulation (0 = < 30 minutes and 1 = 30 minutes) and quality of the clot (0 = normal clot, 1 = fragmented or fragile clot, 2 = absence of a clot).[12,13] A positive WBCT result was a coagulation time of more than 30 minutes and/or an abnormal clot, and a negative WBCT result was normal blood coagulation.

Additional treatment with antivenom was provided on the basis of clinical (persistence of edema or bleedings) or biologic (abnormal WBCT result) results. Patients were examined 1, 2, 4, 12, 24, 48, 72, and 96 hours and 1, 2, 12, 14, 24, 26, 48, 50, 72, and 74 hours after each treatment with antivenom.

Measurement of *E. ocellatus* venom by enzyme-linked immunosorbent assay (ELISA). A standard antigen-capture assay was developed using the antibodies in antivenom. Briefly, $F(ab')_2$ equine immunoglobulin fragments were purified by affinity chromatography using cyanogen bromide–activated Sepharose 4B (Sigma, St. Louis, MO) coupled by standard methods to *E. ocellatus* venom (Latoxan, Valence, France) at a concentration of 14 mg of venom per gram of dry resin. Bound antibodies were eluted using 100 mM acetic acid. Fractions were collected into 1 M Tris-HCl, pH 8 to neutralize the acid, dialyzed, quantitated by absorbance at 280 nm (1 optical density unit = 1.4 mg/mL), and stored at 4°C until use. For detection of venom after capture, a fraction of the immunopurified antibodies were biotinylated using biotin N-hydrosuccinimidyl ester (Pierce, Rockford, IL) at a biotin:antibody molar ratio of 20:1. Alkaline phosphatase–streptavidin conjugate (Zymed, South San Francisco, CA) and *p*-nitrophenyl phosphate were used as secondary reagents.

Plates were coated with a solution of antibody (5 μg/ml) in 100 mM sodium bicarbonate buffer, pH 9.6, overnight at 4°C and then blocked with Tris-buffered saline (TBS, 25 mM Tris-HCl, pH 8, 150 mM NaCl) supplemented with 0.5% (w/v) bovine serum albumin (BSA) and 0.2% (v/v) Tween 20 for two hours at room temperature. Samples were washed between incubations with TBS, 0.05% Tween 20. Plasma samples from patients (centers of Ouessé and Tchaourou) were collected during the study upon admission (H_0) and kept frozen until use. Before the assay, 600 μL of each plasma sample was delipidized with a one-third volume of chloroform (Merck, Rahway, NJ) by mixing for 3 minutes and centrifugation at 14,000 × *g* for 5 minutes. The aqueous fraction was stored frozen until use. Venom was measured in plasma samples at dilutions ranging from 1:2 to 1:20 (in TBS, 0.5% BSA, 0.05% Tween 20) a standard curve was generated using serially diluted (1:3) samples of *E. ocellatus* venom starting at a concentration of 0.33 μg/mL in at the corresponding percentage of pooled normal human serum treated in exactly the same way as the samples from the study. All samples were incubated for one hour at room temperature. After incubation, plates were washed and wells were incubated with biotinylated antibody at a concentration of 0.76 μg/mL (1:1,000 dilution) in the same solution used for sample dilution (100 μL/well), washed, incubated with streptavidin–alkaline phosphatase (1:2,000 dilution), and developed with *p*-nitrophenyl phosphate (Zymed). Absorbance was read at 405 nm at several time points. The values of the blank sample, which contained all reagents except venom, was subtracted and absorbance was plotted as a function of *E. ocellatus* venom concentration. Results are given as the mean ± SD of triplicate determinations.

Statistical analysis. Because of poor recruitment in Dassa Zoumé, data from this center were not included in some analyses (e.g., means and prevalences). Data were captured in Excel® (Microsoft, Redmond, WA) and analyzed with Epi Info version 6 (Center for Disease Control and Prevention, Atlanta, GAUSA), STATA version 8 (Stata Corp., College Station, TX), and other software (http://statpages.org/). Means were compared by analysis of variance or nonparametric tests (Kruskall Wallis or chi-square) for non-normal distributions or comparisons of distributions. We carried out bivariate analysis for all parameters of patients (age, sex, delay in treatment, doses, and clinical and biologic symptoms) and multivariate analysis using a generalized linear model to correlate the quantity of antivenom administered with the delay of treatment, inflammatory syndrome, hemorrhages, and WBCT results. The threshold of significance was P =

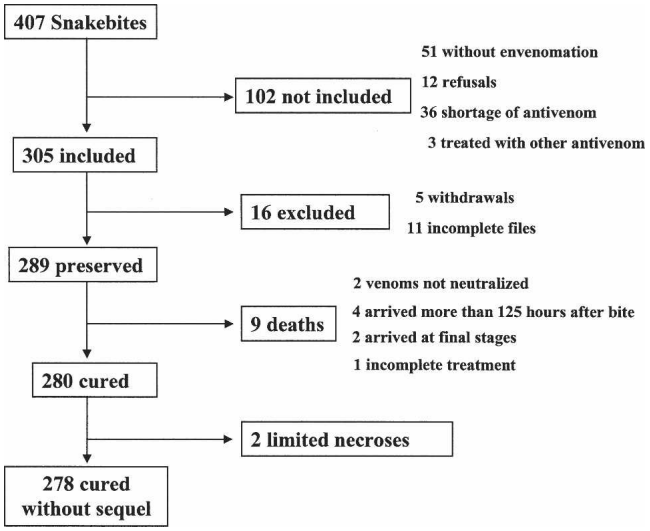

FIGURE 2. Structure of the trial.

0.05. Analysis of intention to treat included all patients who received at least one dose of two vials of antivenom at admission to a hospital.

RESULTS

We excluded 16 patients (5%) because of incomplete information or premature withdrawal from the study against or without the advice of the attending physician. To our knowledge, none of the patients excluded from the analysis died of envenomation or treatment (Figure 2). A total of 289 patients were included in the analysis.

The populations at different centers showed homogeneity for demographic status and circumstances of envenomation (Table 1). The average age of the patients ($F = 1.45$, degrees of freedom [df] $= 9$, $P = 0.17$) and sex distribution between children (< 15 years of age) and adults ($\chi^2 = 18.38$, df $= 27$, $P > 0.75$) at different centers were not significantly different. However, parameters of hospitalization differed significantly with regard to delay in time of consultation ($F = 3.97$, df $= 9$, $P < 10^{-4}$) and duration of hospitalization ($F = 10.32$, df $= 9$, $P < 10^{-4}$). Most (> 75%) bites occurred in fields or bush country during pastoral labor or hunting.

The first dose antivenom was given 36 ± 49 (mean ± SD) minutes (median = 30 minutes) after admission. Additional doses of antivenom were given on the basis of results of clinical examinations. A total of 551 doses of antivenom were given to the 289 patients. Patients received 1.9 ± 1.3 (mean ± SD) doses (3.8 ± 2.6 vials). We observed a significant difference between centers ($\chi^2 = 53.51$, df $= 9$, $P < 10^{-4}$, by Kruskal-Wallis test). Among various reasons for additional doses of antivenom, delay in treatment did have a significant effect ($F = 1.54$, df $= 4$, $P = 0.19$).

Clinical symptoms. Shock was observed in 3 (2%) of 183 patients in whom blood pressure was measured at the time of admission. The systolic blood pressure was < 60 mm of Hg in two patients and < 80 mm Hg in one patient. Blood pressure returned to normal one hour after treatment with antivenom. No other patients showed decreased blood pressure.

Anemia was observed in 51 (18.1%) of 282 patients. Anemia persisted for ≥12 hours in 10 (21.3%) patients with anemia. However, anemia disappeared within 20 hours after treatment with antivenom in 50% of the patients and 40 patients (78%) recovered.

Edema was present in 214 (86%) of 248 patients. Pain was reported in 260 patients (95%). Edema decreased linearly ($R = 0.96$, df $= 6$, $P < 0.005$) and disappeared after treatment within 10 days (half-life = 5 days).

Inflammatory syndrome (pain, edema, temperature ≥ 38°C, or proteinuria) (prevalence = 75%) decreased exponentially; data fitted a straight regression line ($R = 0.76$, df $= 7$, $P = 0.025$). This syndrome disappeared faster than edema. Edema persisted, in some cases with a high intensity, in some patients more than 5–8 days after the start of treatment. Inflammatory syndrome completely disappeared in 3–5 days in all patients. Two cases of limited necrosis (1%) were also observed.

Bleeding was observed in 138 patients (48%). However, in 28 patients (20% of those with hemorrhagic syndromes), bleeding appeared several hours after the first treatment with antivenom. Moreover, 40 patients (29% of those with hemorrhagic syndromes) had a relapse after the first remission. We observed a delay and/or a relapse of the hemorrhages in 63 patients (46% of patients with bleeding). Prevalence of hemorrhages differed significantly between centers ($\chi^2 = 47.86$, df $= 18$, $P < 10^{-3}$; Table 2). Arresting of bleeding occurred within 2 hours after the first treatment with antivenom in 60% of the patients and within 24 hours in 80% of

TABLE 1
Description of patients arriving at centers

Center	No.	Mean age (yr)	Men	Women	Boys	Girls	Deaths	Delay before treatment (hours)	Hospital stay (days)
Bembéréké	35	20	16	3	9	7	2	60	5
Boko	18	23	9	1	7	1	2	29	4
Dassa	1	29	1	0	0	0	0	–	–
Glazoué	35	24	14	10	6	5	0	19	2
Gogounou	22	23	9	5	6	2	0	20	2
Kandi	50	19	20	9	13	8	1	16	2
Nikki	16	17	5	4	4	3	0	13	3
Ouessé	36	21	18	5	9	4	1	8	4
Papané	26	23	16	4	3	3	1	30	7
Savé	29	28	12	6	8	3	1	19	2
Tchaourou	21	28	7	5	7	2	1	19	3
Total	289	22	127	52	72	38	9	24	3

TABLE 2
Prevalence of hemorrhage and whole blood clotting test (WBCT) results at different centers*

Center	Hemorrhage	Delay	Relapse	Delay and/or relapse	WBCT+	Delay	Relapse	Delay and/or relapse	No. of AA doses
Bembéréké	13	2	2	4	33	3	4	7	87
Boko	8	1	3	3	17	5	4	7	64
Dassa	0	0	0	0	1	1	0	1	1
Glazoué	10	0	2	2	21	3	1	3	44
Gogounou	15	4	4	8	13	2	0	2	32
Kandi	16	5	3	6	43	3	3	6	94
Nikki	13	2	4	5	14	5	0	5	36
Ouessé	27	3	15	18	32	5	3	8	66
Papané	12	3	1	4	22	8	1	8	47
Savé	16	7	3	9	6	0	0	0	34
Tchaourou	8	1	3	4	14	5	2	5	46
Total	138	28	40	63	216	40	18	52	551

* AA = African Antivipmyn.

the patients (Figure 3). The distribution of antivenom treatment according to prevalence of bleeding (Table 3) differed significantly ($\chi^2 = 66.71$, df = 8, $P < 10^{-3}$). The distribution of bleeding prevalence according to the delay of treatment was not significant ($\chi^2 = 13.72$, df = 8, $P > 0.05$).

The WBCT was conducted in 275 patients, including 259 at H_0. A total of 216 patients had a positive WBCT result (79% of the tests conducted) and, of these, 168 patients presented a positive WBCT (65% of the tests carried out) at hospital admission. We observed a delayed positive result in the WBCT in 40 patients (19% of those with a positive WBCT result) and a relapse in 18 (8% of those with a positive WBCT result) patients. Overall, a delay in a WBCT-positive result or a relapse in a patient with a positive WBCT result were observed in 52 patients (25% of those who had a positive result in the WBCT). The prevalence of abnormal WBCT results differed significantly according to the center ($\chi^2 = 85.65$, df = 18, $P < 10^{-4}$; Table 2). The difference in the distribution of antivenom according to a delay in a positive WBCT result, a relapse, or an anomaly in a WBCT result (Table 3) was significantly different ($\chi^2 = 42.07$, df = 8, $P < 10^{-3}$). We observed a transitory decrease in the number of positive results in the WBCT after each treatment with antivenom (Figure 4). Distribution of abnormal WBCT results according to the delay of treatment was not significant ($\chi^2 = 9.83$, df = 8, $P > 0.25$).

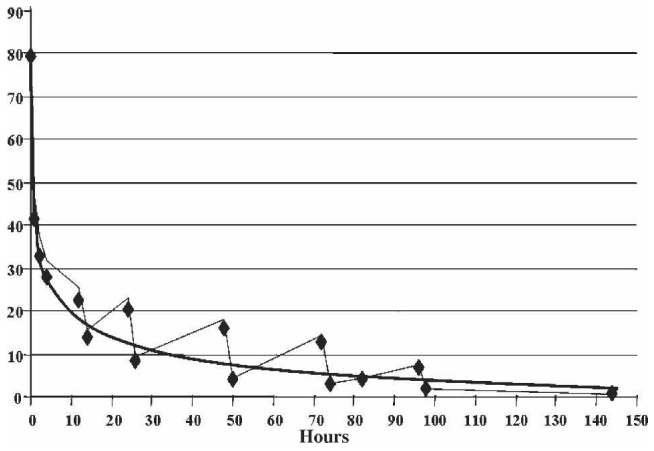

FIGURE 3. Post-therapeutic evolution of bleeding. Values along the y-axis are % of persons with bleeding.

Nine deaths (3.1%) were observed (Table 4). The snake was not identified in any of the deaths. However, the description of the snake by the victim or his family and clinical and biologic symptoms allow some speculation on the identity of the snake. Most were probability *E. ocellatus*, and in one in Tchaourou, possibly *Atractaspis*.

The deaths were divided into three groups. The first group included 6 patients who were admitted with serious complications; five had a delay in consultation and treatment greater than 60 hours, and one who was admitted 12 hours after the bite, showed signs of cerebral hemorrhage and died 3 hours later. The second group included one girl who died because of a shortage of antivenom supply at that particular center. The third group included two patients who were admitted less than four hours after being bitten but died inexplicably; this group included the patient who was probably bitten by *Atractaspis*, whose venom is not neutralized by the antivenom tested.

Several potential risk factors and some indicators of severity were evaluated. These included delay of treatment, hematologic disorders, and number of doses given. Six (4%) of 138 patients who had bleeding died compared with 3 (2%) of 151 patients without bleeding, but the difference was not significant ($\chi^2 = 1.33$, df = 1, $P = 0.25$). Seven (3%) of 216 patients who had a positive WBCT result died compared with 2 (3%) of 73 patients without blood coagulation disorders ($\chi^2 = 0.05$, df = 1, $P > 0.75$). The case fatality rate according to doses of antivenom was not significant between the two groups ($\chi^2 = 3.31$, df = 4, $P = 0.05$). Mortality was also not significantly different ($\chi^2 = 0.83$, df = 1, $P > 0.25$) between patients who received 1 or 2 doses (8 deaths in 220 subjects, 4%) and those who received 3 or more doses (1 death in 69 patients, 1%). Conversely, mortality according to delay of treatment was significant ($\chi^2 = 13.09$, df = 4, $P < 0.025$).

We analyzed factors that could influence the decision to provide new treatment (Table 5). The generalized linear model showed that severity of the inflammatory syndrome, bleeding, delay of bleeding after the first dose of antivenom, or relapse after a transitory period of cure were significantly associated with treatment with 1 dose (2 vials). Inflammatory syndrome and bleeding were significantly associated with treatment with 2 or 3 doses (4–6 vials). Treatment with 4–9 doses was significantly associated with the inflammatory syndrome. The prevalence of any positive WBCT results was not significant.

TABLE 3
Distribution of prevalence of hemorrhage and WBCT+ according to African Antivipmyn® doses administered*

AA doses	Bleeding	Delay	Relapse	Delay and/or relapse	WBCT+	Delay	Relapse	Delay and/or relapse	No. of patients	Total no. of AA doses
1	46	9	4	11	97	22	3	24	152	152
2	38	7	17	23	55	6	7	10	68	68
3	24	4	8	12	33	9	2	9	37	37
4	18	4	6	9	19	2	5	7	20	20
≥5	12	4	5	8	12	1	1	2	12	12

* For definition of abbreviations, see Table 2.

Adverse effects. All unexpected reactions occurring at times specified in the protocol (0, 1, 2, 4, 12, 14, 24, 26, 48, 50, 72, 74, 96, and 98 hours) until discharge and three weeks after the first treatment with antivenom. Unexpected events were observed in 39 (13%) patients after the first treatment with antivenom and in 55 (19%) patients after all treatments. Symptoms observed were always benign. The nature and frequency of symptoms is shown in Figure 5.

Late tolerance was investigated three weeks after the first administration of antivenom and was measured in 77 patients (27%). Two (3%) had symptoms (persistent cough and low blood pressure) but these could not be attributed to antivenom because of their nature. Thus, there was no serum sickness in the 77 patients examined 3 weeks after the first treatment with antivenom.

Venom detection. *Echis ocellatus* venom was detected in 9 (82%) of 11 patients (Figure 6). Venom could not be detected in the blood of any of these patients after treatment with antivenom (limit of detection of the assay was approximately 1.3 ng/mL).

DISCUSSION

Although antivenoms had existed since the end of the 19th century, clinical trials to evaluate them constitute a recent practice first reported some 20 years ago, at least in Africa.[10,11,16,17] Clinical trials generally have two objectives: assessment of efficacy and assessment tolerance (safety). The former assessment implies a comparison either with a placebo, which in our study would not have been ethical, or with a gold standard antivenom. Efficacy can be determined only if there are several products so that one can determine the most efficacious one or demonstrate their equivalence. In addition to the difficulty in choosing the gold standard product, it should be stressed that a statistical comparison that considers neutralizing properties measured by experiments in animals, which are similar for most current antivenoms, requires a considerable number of patients to observe a statistically significant difference.

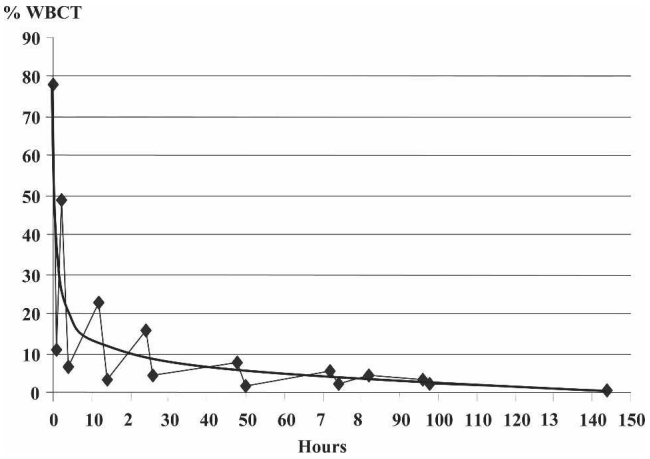

FIGURE 4. Post-therapeutic improvement in whole blood clotting test (WBCT) results. Values along the y-axis are % of persons with a positive WBCT result.

Evaluation of safety is absolutely necessary because unpurified or poorly purified antivenoms frequently lead to severe side effects.[9] However, in Africa and from a public health perspective, the problem to address is not that of comparing several products but rather the lack of even one antivenom that satisfies specific criteria.[7,8,14,18,19] These criteria include neutralization of venoms of relevant African snakes, which can be measured in potency tests in animals[15]; clinical safety, which requires laboratory quality control during manufacture and clinical confirmation; stability, which can be obtained by freeze-drying the product to tolerate tropical temperatures and avoid the requirement of a cold chain; and accessibility, both financial (reasonable cost) and commercial (availability in remote health centers). At the time of this trial, there was no antivenom available or in preparation that met all of these criteria. Thus, the main goal of the clinical trial was evaluation of tolerance. We also studied clinical effectiveness by comparing our data with previous studies in the study area.

We chose Benin for reasons of safety, speed, and economy. First, health infrastructure and personnel were a guarantee of correct management of snake bites. Second, the high incidence of snake bites in this area of Benin, which was investigated for more than a decade, would provide a sufficient recruitment within a reasonable time. Lastly, health centers were selected relatively close to each other to simplify surveillance and to allow comparability of results.

Patient populations attending the centers were homogeneous for sex, age, and circumstances of envenomation. A prolonged delay of consultation and treatment in all health centers partly confirmed this homogeneity. The significant difference in time of arrival between the centers may be explained, at least in part, by their medical expertise and type of equipment. However, delay in consultation did not affect the prevalence of bleeding or positive WBCT results or the number of administered doses. Duration of hospitalization might be understood in terms of severity of envenomation and decisions of medical teams.

Severity of envenomation differed significantly at the centers. This finding could be explained by the significantly different delays from center to center, although its impact

TABLE 4
Description and probable cause of fatalities*

Center	Sex	Age (years)	Delay (hours)	AA doses	Life time (hours)	Restoration of hemostasis	Possible or probable (bold) causes
Bembéréké	F	13	60	2	160	Récidive J_2	Severe anemia
Bembéréké	M	35	122	1	122	No	Hemorrhages
Boko	M	10	60	6	190	Récidive J_2	Severe anemia
Boko	M	40	12	2	15	No	**Cerebral hemorrhage**
Kandi	F	65	72	1	73	–	**Respiratory paralysis**
Ouessé	M	35	1	1	2	No	?
Papané	M	27	120	2	124	No	Hemorrhages
Savé	F	13	9	1	12	No	**Inadequate dose**
Tchaourou	M	13	4	1	8	–	*Atractaspis*

* AA = African Antivipmyn.

seemed limited. Three possible explanations can account for these differences: 1) differences in the bite circumstances; however, this explanation was not confirmed by demographic data; 2) variation in the fauna of the different localities; and 3) patients could have undergone a process of selection for any number of reasons, without significantly modifying the distribution of age and sex.

The difference of fauna likely affected the results because landscapes and climates are not identical in the area of the trial. Although *E. ocellatus* is responsible for most snake bites in western Africa,[20–22] its density varies in a north-south gradient in this region. It might have been more judicious to choose health centers distributed along an east-west transect. However, in this case monitoring would have been more complicated due to lower accessibility to centers because main roads run north to south in this region. We hoped to limit this factor by using centers close to each other (less than 400 km between the two centers the furthest apart).

Selection of patients also could affect the results and several factors may have played a role. Some centers were remote and poorly equipped health facilities that receive patients from a limited area, whereas others were reference hospitals that treat more severe cases coming from remote health centers. Moreover, some were public centers and others were private centers. This finding implies that policies of cost recovery and management of patients were different, which could influence treatment. Understanding differences in the delay in consultation might be important because reference hospitals would receive patients who have gone through other health centers and/or arrive from remote locations without suitable means of transportation. This also might explain, for example, the significantly different mean dosages administered in different centers (Figure 7), although guidelines for treatment were theoretically identical in all centers.

Shock was rare (2% of all cases), and anemia (18%) was not a specific sign because of other causes of anemia in Africa, notably malaria. The inflammatory syndrome, particularly edema, showed a high prevalence at all centers. Its usefulness in diagnosis is important because of its frequency. Nevertheless, it was not useful in surveillance of clinical evolution during treatment because of slow improvement even after successful treatment.

Bleeding (48%) was frequent but irregular (frequent delays or relapses) in half of the patients with clinical hemorrhagic syndrome. At admission, hemorrhage, especially systemic, indicated severe envenomation. Delay and relapse of bleedings after treatment indicated an insufficient number of doses and resulted in additional treatment with antivenom.

Prevalence of abnormal WBCT results (75–80%) was similar to the prevalence of inflammatory syndrome (75–85%). Abnormal WBCT results were a useful indicator of hemorrhagic syndrome in a latent state. Its diagnostic value and use as a criterion of severity must be considered.[12,13] However, its role in the surveillance of treatment was limited by its instability and a normalization that was slower than that of bleeding. The kinetics of recovery of coagulation factors (such as fibrinogen and platelets) in coagulopathies observed in dangerously severe hemorrhagic syndromes are poorly understood in terms of envenomation by *E. ocellatus*. Thus, it is theoretically possible that levels of fibrinogen required for normalization of a biologic test result such as the WBCT and the restoration of coagulability in the patient may differ. This might in turn explain differences we observed between the arrest of bleeding and continued abnormal WBCT results.

TABLE 5
Influence of factors on treatment renewal

Factors	Odds ratio	95% CI	*P*
Inflammatory syndrome	2.63	1.93–3.58	10^{-4}
Bleeding	2.51	1.89–3.33	10^{-3}
Delay or relapse of bleeding	2.09	1.63–2.68	10^{-4}
Delay or relapse of WBCT	1.24	0.97–1.59	0.09
WBCT	1.15	0.90–1.48	0.26

* CI = confidence interval; WBCT = whole blood clotting test.

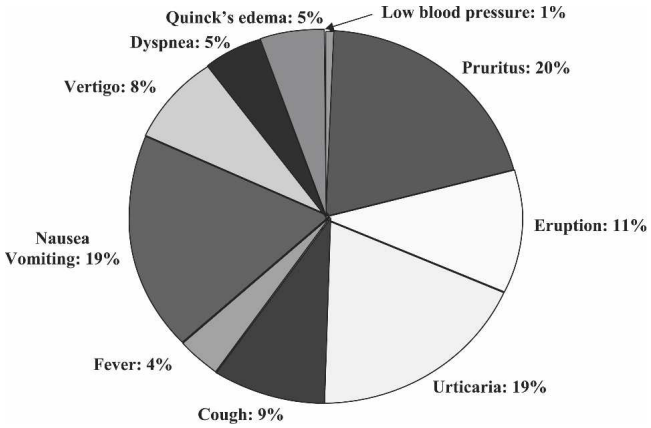

FIGURE 5. Prevalence of symptoms observed after administration of antivenom.

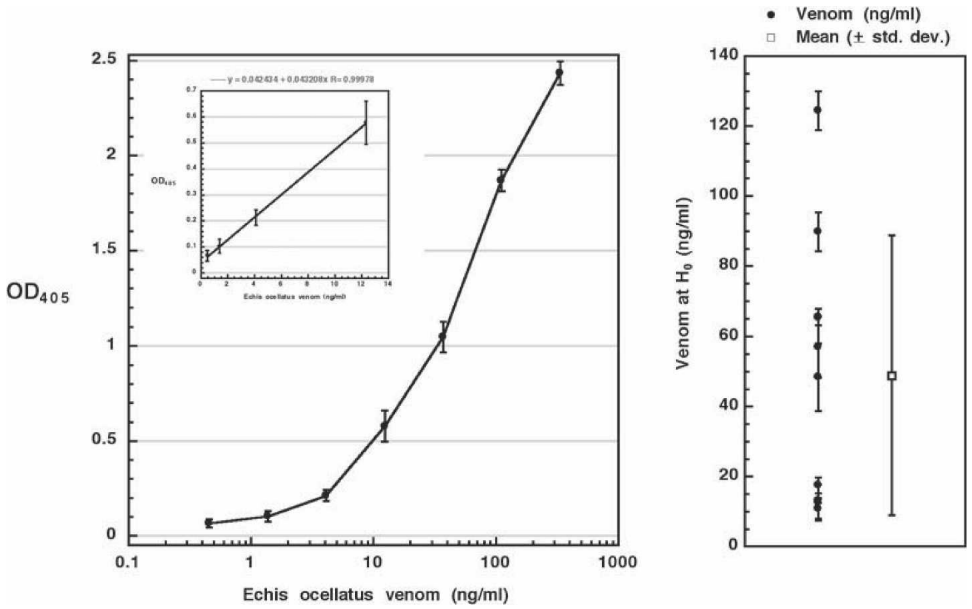

FIGURE 6. Measurement of *Echis ocellatus* venom by enzyme-linked immunosorbent assay. **Left**, Calibration curve of *E. ocellatus* venom with a linear interval used for calculations (**inset**). **Right**, venom levels in nine patients and mean venom levels at admission. OD = optical density; Std. dev. = standard deviation. Error bars show the SD.

Furthermore, recovery of coagulation factors may depend on parameters of envenomation other than blood venom levels at admission or during treatment (such as delay of treatment, and/or other concomitant conditions such as anemia caused by malaria and/or hepatic compromise). More studies must be done to test these *a priori* hypotheses.

Comparison of the case-fatality rate in this trial with those observed in other studies was not our principal objective, mainly because of limited significance of comparisons in a non-controlled and non-randomized study. Nevertheless, an analysis of deaths and their incidence can be performed.

Five of nine deaths occurred after a delay of consultation and treatment greater than 60 hours. Six of the patients who died were admitted to a hospital with severe complications or in a final stage of envenomation. This phenomenon is frequent and explains the high hospital mortality observed in Africa.[3] It is also likely that many patients die before reaching a hospital. Surprisingly, a significant distribution of fatalities regarding delay of consultation and treatment was not observed when prevalence of bleeding or positive WBCT results were considered, even though these indicators are directly involved in causes of death. This suggests that these symptoms, which are observed early in the clinical course, have a time-delayed action that leads to an effect on early treatment.

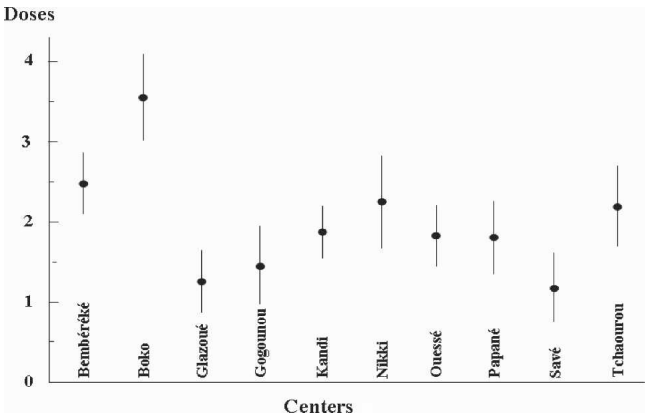

FIGURE 7. Mean doses of antivenom used in different centers. Error bars show standard deviations.

Comparison of case-fatality rates between patients included in our study (9 [3%] of 289 patients died) and patients not included in the trial for various reasons (7 [10%] of 67 envenomed patients died) was significant ($\chi^2 = 6.81$, df = 1, $P < 0.01$). However, the difference might be caused by factors other than treatment with antivenom. Case-fatality rate in the literature were generally higher ($\chi^2 = 226.01$, df = 2, $P < 10^{-3}$). Mortality varied between 2.6% for all bites including dry bites (without envenomation)[1] and 23% in reference hospitals where more severe envenomations were treated.[5] Our results were similar to the case-fatality rate observed after treatment with Ipser Afrique® antivenom (Institut Pasteur, Paris, France) (2%) under similar conditions and in a comparable geographic area.[10]

Additional treatment with antivenom was required mainly because of severity of inflammatory syndrome and bleeding. Although the inflammatory syndrome can be impressive, it does not have a direct influence on a fatal outcome. Conversely, bleeding has a direct impact on lethal risk. The generalized linear model showed a significant odds ratio only for 1–3 doses of antivenom (2–6 vials). This finding suggests that treatment with more than three doses of antivenom was not necessary and that some patients received an overdose. However, average applied doses varied widely according to center. Four explanations could explain this finding. 1) Severity of envenomation differed significantly according to center and required appropriate dosages. 2) Availability of antivenom at no cost could result in additional doses either as a precaution or to reassure the patient without considering other criteria. 3) Management of patients differed from center to center.

4) Therapeutic policies used before the trial could have influenced the decision to provide additional treatment, with some centers being accustomed to provide additional treatment with antivenom in cases of severe envenomation (e.g., Bembéréké), and others avoiding overuse for economic reasons (e.g., Savè). Thus, assessment of efficacy of the antivenom as a function of doses is speculative, and circumstances described constitute an important potential bias that limits evaluation of a minimal therapeutic dose.

Adverse reactions to the antivenom can be analyzed at two levels (circumstances of symptom onset and etiologic findings) that include several criteria (Table 6). All patients generally received tetanus sera simultaneously with antivenom. Although the amount of protein injected in tetanus serum was less than that in antivenom, tetanus serum could interfere with the antivenom. Thus, in addition to adverse events caused by antivenom, we measured all adverse all reactions observed during surveillance, even in patients who did not receive additional doses of antivenom (Table 7). Distribution of symptoms and adverse reactions, excluding those observed at H_0, where all patients received one dose of antivenom, was similar ($\chi^2 = 6.11$, df = 7, $P > 0.5$). On the basis of criteria in Table 6, the prevalence of adverse events caused by antivenom was 11%. Prevalence of symptoms in patients who did not receive additional doses of antivenom, but were examined at corresponding stages of monitoring, was 7%. This finding suggests that some symptoms could result from disorders related to envenomation, rather than the antivenom.

This inference is supported by the finding that 34 patients who had suggestive symptoms at H_0 did not have any symptoms after additional treatment with antivenom (between 2 and 5 treatments each). Adverse reactions, however, reappeared after subsequent treatments with antivenom in 6 of the 45 patients with unexpected events who received at least 2 doses of antivenom. This finding suggests that 13% had actual adverse reactions (2% of the treated patients). Thus, adverse reactions caused by antivenom ranged from 2% to 11% of the treated patients with a corrected prevalence of 4%. Prevalences of adverse reactions between antivenom (19%) and FAV Afrique® (Aventis Pasteur, Paris, France) (17%) were not significantly different ($\chi^2 = 0.34$, df = 1, $P > 0.5$).

TABLE 6
Criteria of imputability of African Antivipmyn®*

Circumstances of onset
Absence before the start of treatment.
Appearance in the hour following injection.
Disappearance upon discontinuation of treatment (not used in this study).
No re-occurrence after renewal of treatment.
Etiologic arguments
Very suggestive symptoms: abrupt decrease in arterial pressure or shock in the minutes that follow injection of African Antivipmyn®, pruritus, cutaneous eruption, or glottal edema.
Suggestive symptoms that could nonetheless be related to the envenomation itself: digestive trouble (nausea, vomiting), respiratory problems (laryngeal irritation, cough, dyspnea), inflammatory sydrome (fever), neurovegetative manifestations (vertigo).
Absence of other reasonable etiology.

* Absolute criteria are in bold.

TABLE 7
Prevalence of adverse reactions

Time of treatment	Examination of non-treated patients	Symptoms	AA* doses	Adverse reactions	
				Possible	Probable
H_0	2	2 (–)	287	39 (14%)	–
H_1	78	11 (14%)	69	4 (6%)	–
H_2	67	5 (7%)	74	5 (7%)	–
H_4	102	6 (6%)	21	4 (19%)	13%
H_{12}	72	3 (4%)	33	3 (9%)	5%
H_{24}	60	3 (5%)	28	4 (14%)	9%
H_{48}	54	3 (6%)	14	0 (–)	–
H_{72}	21	2 (10%)	15	3 (20%)	10%
H_{96}	11	1 (9%)	8	0 (–)	–
Total	467	34 (7%)	549	62 (11%)	4%

* AA = African Antivipmyn.

Although the emphasis of the study was clinical, *E. ocellatus* venom levels in some patients for which samples were available were measured by an ELISA developed for this purpose. The measurement of *E. ocellatus* venom levels was done in 11 patients admitted into the study, of which 9 (82%) were positive upon admission. Venom levels varied widely (mean ± SD = 48.8 ± 39.9 ng/mL, range = 12.6–124.4 ng/mL, n = 9), which was expected given the age range of the patients (mean ± SD = 20.4 ± 18.3 years, range = 4–60 years), time of arrival, and the fact that *E. ocellatus* is a small snake (average length = 30 cm) that generally produces small amounts of venom compared with larger species. Venom levels are consistent with those reported in another study [17] and because most severe envenomations reported in the western African savannah are caused by this species.

Disappearance of venom after treatment in venom-positive patients in our small sample may also contribute to the assessment of efficacy, albeit in a limited manner, because clinical manifestations occur at different times independent of measured venom levels. Furthermore, the delay between the time of bite and treatment, which is often long in the African countryside, could be expected to make inferences regarding venom dosage and clinical severity of envenomation rather unfeasible. For example, it is conceivable that long periods (up to several days) may manifest themselves in relatively low levels of venom in blood but a very complicated clinical picture of envenomation. We are currently conducting studies to explore if significant relationships may be found between clinical picture upon arrival and its evolution during antivenom treatment and venom levels in patients. Our specific aim is to assess factors that may determine recovery of critical functions known to be disrupted during envenomation by *E. ocellatus* (such as blood coagulability) that can be clinically measured by bleeding and biologically measured by the WBCT.

This clinical trial, which was conducted in Benin in conditions of current medical practice, showed satisfactory tolerance of the new antivenom. Prevalence of adverse reactions was 11% and may be as low as 4% if symptoms related to envenomation are considered. Moreover, severe adverse effects were not observed, even in patients given high doses of antivenom.

Inflammatory symptoms were frequently observed and did not show any particular characteristics when compared with published data. The variable role of edema was confirmed. Its presence indicates envenomation and a need for treatment

with antivenom. However, edema is not useful in monitoring treatment or assessing cure. However, it could result in unnecessary additional treatment with antivenom.

Hemorrhagic symptoms, either bleeding or abnormal WBCT results were the main signs of envenomation in most patients. The sensitivity of the WBCT showed that this test was useful in diagnosis of envenomation. However, bleeding was more specific and a better indicator in prognosis. Thus, bleeding is the best indicator of evolution of recovery from snake bite during treatment with antivenom. Stoppage of bleeding with antivenom was observed in most patients 1–2 hours after treatment with 2 vials of antivenom. In some severe cases of bleeding, 2–3 doses (4–6 vials) were required. Persistence or relapse of bleeding after administration of 2–4 vials of antivenom was a useful indicator of additional treatment with antivenom. Treatment with more than three doses (six vials) may be excessive and unnecessary. A new study is in progress to specify minimal doses and to define a protocol of administration of this new antivenom.

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Authors' addresses: J.-P. Chippaux, Unité de Recherche 010, Institut de Recherche pour le Développement, La Paz, Bolivia. A. Massougbodji, Faculté des Sciences de la Santé de Cotonou, Cotonou, Benin. R. P. Stock and A. Alagon, Instituto de Biotecnología, Universidad Nacional Autonónoma de México, Cuernavaca, Morelos, Mexico.

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