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Review

Snake venoms: from research to treatment

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Abstract

The present work offers a brief overview on how the problem of snakebite has been confronted in Costa Rica, and especially, on how scientific and technological research has contributed to this goal. The origins of the antiophidic struggle in the country and the creation of the Instituto Clodomiro Picado, at the University of Costa Rica in 1970, are briefly summarized. The first stages of its work are described, as well as the evolution of different research areas on snakes, venoms and antivenoms; which have contributed during four decades to the existence of adequate and sufficient therapeutic resources to tackle snakebites in Costa Rica and in the Central American region.

Keywords: snake venoms, toxins, antivenoms, anti-ophidic sera, ophidism

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Estimates suggest that at least 400,000 people each year suffer from snake bite envenoming worldwide, mainly in tropical and subtropical countries situated in Africa, Asia and Latin America.[1-3] The actual number of cases may even be higher, since this disease ails of serious underrecording, because it affects mainly residents of zones with little access to health systems and thus, of entry into official statistics. A proportion of envenoming cases result in death. According to conservative estimates, these cases amount to at least 20,000 cases per year.[1] In addition to their life-threatening character, envenoming can cause damage to tissues, leaving permanent sequelae as serious as amputations and disability.[4, 5]

The world's situation with regard to this health problem is not flattering. Although prompt treatment with antivenom (anti-ophidic serum) has been known for over 120 years, there is a serious shortage of antidotes in many countries. The production of antivenoms is not commercially attractive for large pharmaceutical industries, whose priorities are focused on medicines with larger markets and on diseases affecting nations with high incomes. On the other hand, the efforts of public institutions that oversee health policy in countries located in the regions most affected by snakebite, have not always managed to solve the problem of production and supply of antivenoms.[6, 7] In addition, efficacy of antivenoms is geographically restricted, since their therapeutic coverage is limited to a group of species of venomous snakes whose toxins share immunological similarities. Therefore, an antivenom prepared against a snake speciesfrom a particular geographical area may have little or no neutralizing efficacy in another region, due to the antigenic variability of venoms from different species.

The scarcity of antivenoms in some regions of the world has been a reason for concern for decades. It led in 2009 to the classification of snakebite by the World Health Organization (WHO) in the category of neglected tropical diseases. Nevertheless, the political and economic support to resolve this problem has not improved significantly.

In Central America, snakebite accidents reach an annual incidence of nearly 4000 cases. Panama is the most affected country, with approximately 2000 envenomings; followed by Costa Rica, Nicaragua, Honduras and Guatemala, each with approximately 500 accidents per year. Figures in El Salvador and Belize amount to approximately 50 cases each.[8] Costa Rica was able to solve the problem of antivenoms supply four decades ago due to the creation of the Clodomiro Picado Institute, which has benefited not only Costa Rica's population, but also that of the Central American region and of some other Latin American countries. This article provides a brief description of how Costa Rica has faced the

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snakebite problem, and particularly, how scientific research and technology has contributed to this end.

Scientific legacy of Dr. Clodomiro Picado, a pioneer of the fight against snakebite in Costa Rica

Clodomiro Picado Twight (1887-1944) is widely known in Costa Rica as one of the most eminent scientists in national history. His life and work have been the subject of many analyses. These clearly draw upon his brilliance, wit, tenacity, and commitment to contribute to the solution of the population's problems through scientific research and its aplications.[9] The topic of snake venoms figured as a priority among the many different scientific topics that fascinated Dr. Picado in his laboratory, located in what today are the premises of the San Juan de Dios Hospital. His masterpiece from 1931, "Venomous snakes found in Costa Rica, their venoms, anti-ophidic serotherapy" is a fascinating compendium of observations and experiments that even to date amaze experts, despite the fact that they were made in very poor conditions. Dr. Picado laid the seed that would later germinate in the creation of a project to combat the scourge of snakebite in the country. Thus, it is not surprising that the son of Luis Bolaños, a close collaborator, was summoned in the mid-1960s to run a "Program of anti-ophidic sera", and then, to found and manage the Clodomiro Picado Institute.

Dr. Roger Bolaños and the creation of the Clodomiro Picado Institute (CPI)

Roger Bolaños Herrera (1931-2007) was familiar with the subject of venomous snakes from a very young age because his father worked with Clodomiro Picado. Dr. Bolaños studied microbiology at the University of Costa Rica and undertook further masters and doctoral studies in Brazil and the United States, respectively. He was called to lead an initiative to produce, for the first time in Costa Rica, the antidotes needed to provide treatment to victims of snakebite envenoming. The history of the creation of the Antiophidic Sera Program in 1966 and the subsequent founding of the Clodomiro Picado Institute in the University of Costa Rica on April 13, 1970, have been the subject of a recent compilation and analysis,[10] therefore, it will not be repeated in this article. However, some of the features with which Dr. Bolaños endowed the Institute, as its founding director, must be noted because they profoundly determined its historical development and lines of thought.

As a center for the production of antivenoms, the CPI is a very particular case because it was created within a public university, the University of Costa Rica (UCR). Due to the fact that it was embedded in a purely academic environment since its origin (in contrast to other health-related public institutions), and thanks to the vision and spirit instilled by Dr. Bolaños, the CPI was never envisaged as a mere immunotherapeutic product factory. It was conceived as an academic center that would combine production, intense scientific and technological research, teaching of undergraduate and graduate students and various social extension activities. All of these tasks would be closely related. In particular, the "production-research" pair, clearly mutually beneficial, became a key factor for the development, consolidation, competitiveness and growth of the CPI during its four decades of work on behalf of national and regional health. As a manager, but at the same time as an intellectual leader and researcher, Dr. Bolaños had the vision required to assign equal importance and interest to basic scientific research (for example, the study of the karyotypes of the snakes found in Costa Rica),[11] and technology research (for example, the development of an antivenom of Panamerican coverage against coral snakes).[12] This attitude is not common in the general Costa Rican culture (even among certain academic sectors), which tends to overvalue the immediate applications of research at the expense of work that seeks to answer fundamental scientific questions. The influence of the media, which encourages such culture, coupled with the discourse promoted by government institutions governing science-related policy, constitute elements which could undermine the philosophy of harmonious balance between science and technology promoted by Dr. Bolaños in the various research activities that were increasingly developed at the CPI.

From research to treatment: the early stages

The basic principles of antiophidic therapy, discovered in the late XIX century, have remained unchanged: the antivenoms are prepared from the plasma of animals immunized with one or more venoms, and which develop an adequate antibody response to achieve their neutralization.[13] Technologically, progress has being made in the purification of antibodies out of complete plasma, when compared to the rudimentary original methods. However, in essence, antivenoms are produced following the same principle and they become the physician's fundamental tool to treat patients who have been victims of an envenoming snakebite. Therefore, they are an essential element in the drug inventory of any health system.

During the early stages of research Dr. Bolaños and his small group of collaborators, which formed the initial staff of CPI, conducted research on several fundamental questions. This was necessary to structure the knowledge platform that would allow them to address the snakebite problem in a comprehensive manner in order to implement the production of the first indigenous antivenoms. On one hand, they investigated which are the species of venomous snakes found in Costa Rica, their geographical distribution,[14] the epidemiology of snakebite accidents and which were the most relevant species from the medical point of view.[15] On the other hand, snake collecting from different places in the country was initiated and a living collection in captivity was established. This allowed them to obtain and preserve the different venoms. These valuable biological materials allowed the performance of a series of very important studies that revealed the average yield per extraction in each species, its potential lethality in experimental models, or the theoretical quantities of antivenom needed to ensure therapeutic success in patients.[16] In addition, the first immunochemical analysis and biotests were made to determine the antigenic relationships between the venoms of different species, and their cross-neutralizations. Thus, an immunization formula was established to obtain a polyvalent antivenom, ie one antivenom capable of neutralizing the venoms of all snakes of the Viperidae family found in Costa Rica (Table 1).

Table 1: Venomous snakes from Costa Rica*

Viperidae family (vipers)	Elapidae family (coral snakes and sea snakes)
• *Bothrops asper* • *Crotalus simus (Crotalus durissus durissus)* • *Agkistrodon bilineatus* • *Lachesis stenophrys (Lachesis muta)* • *Lachesis melanocephala (Lachesis muta)* • *Porthidium ophryomegas (Bothrops ophryomegas)* • *Porthidium volcanicum* • *Porthidium nasutum (Bothrops nasutum)* • *Porthidium porrasi* • *Atropoides picadoi (Bothrops picadoi)* • *Atropoides mexicanus (Bothrops nummifer)* • *Cerrophidion godmani (Bothrops godmani)* • *Bothriechis nigroviridis (Bothrops nigroviridis)* • *Bothriechis schlegelii (Bothrops schlegelii)* • *Bothriechis lateralis (Bothrops lateralis)* • *Bothriechis supraciliaris*	• Micrurus nigrocinctus • Micrurus mosquitensis • Micrurus multifasciatus (Micrurus mipartitus) • Micrurus alleni • Micrurus clarcki • Pelamis platura **

* In brackets, synonyms with previous taxonomic designations

** Some taxonomic classifications include this marine species in the Hydrophiidae family

This formula, developed during the early research stages of the CPI with very limited tools remains valid, and includes the venoms of the species Bothrops asper (terciopelo), Crotalus simus (rattlesnake) and Lachesis stenophrys ("cascabel muda" or Central American Bushmaster). The Bothrops asper causes the greatest number of envenoming cases in Costa Rica.[15] At the same time, similar work was made to study the venoms of coral snakes belonging to the Elapidae family, which have very distinctive antigenic differences with respect to those of the Viperidae family. Using this, an immunization formula was found that uses a mixture of the venoms of Micrurus nigrocinctus and Micrurus mosquitensis. With it, an antivenom that covers all

Figure 1. Representative species of the two families of venomous snakes found in Costa Rica. A. Bothops asper and B. Nigrocintus Micrurus.

the species of coral snakes in the country is obtained (Table 1), except for one: the regal coral snake Micrurus mipartitus (multifasciatus).[17] No antivenom for this species is available. Fortunately, it is very rare in Costa Rica (this explains why it is difficult to access venom to produce the antidote against it). The same applies to the only species of sea snake in the country, Pelamis platura, that inhabits the Pacific's waters. The limiting factor for the development of antivenom against this species is the difficulty to obtain their venom.

Two types of antivenom, the polyvalent and the anticoral, simplify the treatment

In some regions of the world venomous snakes form complex antigenic groups, making clinical diagnosis and the choice of appropriate antivenom difficult. However, in Costa Rica, the two antivenoms produced using the formulas developed in the early research of the CPI simplify treatment. The physician does not need to know which particular species of snake caused the envenoming, only if it was caused by a viperid or an elapid, in order to apply either the polyvalent or the anticoral antivenom, respectively. The medical conditions caused by these two families of venomous snakes have their own characteristics and are distinguishable.

The viper venoms (such as "terciopelo", rattlesnake, bocaracá, parrotsnake, etc.) produce evident local manifestations, which can be highly visible, including: edema, pain, hemorrhage, dermonecrosis, myonecrosis, and blisters.[18,19] The intensity of these signs depends on the amount of venom injected by the bite, and to some extent, the aggressor species, as some venoms are more destructive than others. However, edema is an almost omnipresent sign in viper bites.[20] At the systemic level, the viper venoms induce other manifestations, such as coagulation abnormalities (prolongation of coagulation time, fibrinogen consumption), thrombocytopenia, other sites bleeding and, bruising. It may occur with significant hypotension and renal failure in the most severe cases.[21-23] Again, there may be exceptions to one or more of these manifestations, depending on the aggressor species. For example, some viper venoms lack procoagulant activity and do not significantly alter the coagulation parameters.[24] However, in general terms, the viper-induced clinical condition is recognized based on the above mentioned characteristics, without identifying the particular aggressor species, in order to provide specific treatment with polyvalent antivenom as soon as possible.

In contrast, envenoming by coral snakes does not induce significant local manifestations, except for a feeling of numbness or mild pain. It is characterized by symptoms of peripheral neurotoxicity, together with a progressive neuromuscular blockade, that leads to a characteristic myasthenic syndrome or flaccid paralysis.[15,25] Bipalpebral ptosis is a sign of an advanced stage of envenoming and there is a significant risk of respiratory failure. The appropriate antivenom (anticoral) should be given promptly to prevent, to the extent possible, the occupation of the acetylcholine receptors at the neuromuscular junction by the potent neurotoxins of these venoms, among other pharmacological targets.

In recent years, the wide availability of both types of antivenoms in Costa Rica and the cumulative experience of hospitals that provide attention to the largest number of snakebite cases have benefited the treatment protocols through an increasing uniformity or standardization[26] (Table 2). Intradermal tests that attempt to predict a possible allergic reaction to equine proteins (the species in which antivenoms are produced in Costa Rica and in most production centers) have been abandoned internationally due to their lack of sufficient predictive value.[27] However, once envenoming in the patient, either by a vipid or an elapid, has been established, the urgent administration of the respective treatment is performed with all necessary care, in order to address potential immediate adverse reactions. These may relate, albeit rarely, to a true anaphylactic reaction (hypersensitivity type I, mediated by the pre-existence in the patient of IgE antibodies against equine proteins), or, more commonly, to an anaphylactoid type reaction, which does not involve IgE, but the activation of the complement system and the direct release by the antivenom of several inflammatory mediators.[27] Some clinical studies that have evaluated the antivenoms produced in the CPI describe a frequency of immediate adverse reactions close to 15-25%, which is acceptable for therapeutic products of this nature.[28-30]

Unlike the early adverse reactions to anti-ophidic serum therapy, delayed reactions are more common. These are mediated by a Type III mechanism of hypersensitivity (serum sickness), caused by the abundant formation of immune complexes between equine proteins and their respective antibodies, that peaks between the first and second week after treatment. The frequency of these reactions is more difficult to establish, as in most cases they occur when the patients have abandoned treatment centers. This condition is generally very benign and self- limiting and responds well to treatment with antihistamines and steroids.[27]

The antivenoms continue to evolve through research

Polyvalent and anticoral antivenoms,[31] prepared in the CPI since its origins and to date, have been improved over the years through the hard research work of academic and technical staff. One of the major changes was the substitution of the ammonium sulphate precipitation technique for immunoglobulins initially used, with the use of caprylic acid (octanoic acid) for the removal of albumin and other plasma proteins. This method, adapted and optimized for equine immunoglobulins,[32] allows to obtain an antivenom with a higher purity and with a physicochemical profile above that of ammonium sulphate. Besides, it provides a higher final performance and lower processing time. The current antivenoms compare favorably with the ones produced decades ago, and continue to experience a series of gradual improvements, which are carefully assessed, in areas such as: potency, safety, stability, lyophilization, content of protein aggregates, and others. Apart from the technological improvements introduced in the processing of hyperimmune equine plasma; changes in immunization procedures have also been achieved[33], which have resulted in a better overall condition of the animals, as well as in the introduction of various techniques in accordance with modern practices in manufacturing and quality control of the final product.

Table 2: Hospital treatment of snakebite envenoming (adapted from Ref.[26])

1. Diagnosis and assessment

Determine if there is envenoming and if it corresponds to a snake from the Viperidae (vipers) or Elapidae (coral) family based on signs and symptoms (see Section 5 of the text). In the former case polyvalent antivenom is administered, and in the latter, the anticoral one.

2. Preparation

- Provide immediate intravenous access through two IV lines (one for the antivenom and another for other drugs and fluids)
- Dilute 100 mL of the appropriate antivenom (10 vials of 10 mL) in 500 mL of 0.9% Sodium Chloride Solution (200 mL in the case of children).

3. Infusion

- Ensure availability of epinephrine and equipment for basic cardiorespiratory assistance to manage a possible adverse reaction. Hypersensitivity skin test lacks reliability.
- Start IV infusion with slow drip (1-2 mL every 3-5 min), ensuring the patient's direct medical observation. If no adverse reaction occurs in the first 5-15 min, increase the flow so that all the solution is infused in 1 hour.
- In the event of an adverse reaction (urticaria, hypotension, headache, nausea, bronchospasm, chills), discontinue infusion and assess the subcutaneous administration of 1:1000 epinephrine, as well as IV administration of antihistamines and corticosteroids.
- Once the reaction is controlled, in about 15-20 min, restart the infusion of antivenom.

4. Complementary treatment

IV fluids, prophylaxis with tetanus toxoid and antibiotics, diuretics for the management of renal function.

One of the current challenges for the improvement of antivenoms, as described below, is to achieve higher antibody response to snakebite toxins of major clinical relevance but with low immunogenicity. The identification of venom components that are poorly recognized by antivenoms has advanced significantly thanks to the introduction of an analytical strategy known as antivenomics,[34,35] based on modern proteomics techniques recently introduced by the CPI.

Research areas on snakebite

Throughout its four decades of existence, the CPI has developed a range of research areas around its central objective: to contribute to the solution of the snakebite problem in Costa Rica and in the region. This variety of activities cover mainly the following topics:

(a) *Epidemiology of the snakebite accident:* Compilations and statistical analyzes are performed on the number, characteristics and consequences of snakebite envenoming recorded in the country.[36-42] Recently, the epidemiology of other envenomings has also been studied, such as those by African bees.[43] A novel aspect in the field of snakebite epidemiology is the application of georeferencing techniques to construct risk maps and seek the strengthening of the health facilities with higher incidence, as well as the distribution and rational use of the valuable therapeutic resources embodied in antivenoms. This type of analysis, already undertaken by countries such as Nicaragua and Argentina, [44,45] is underway in Costa Rica.

(b) *Herpetological studies and of natural history of snakes:* Analysis are carried out about the national herpetofauna, its distribution, ecology, reproductive biology, ontogenetic shape changes, taxonomy, improved survival in captivity, venom production, and others.[46-60] These biological studies are being extended to the main scorpion species found in Costa Rica, aiming at the eventual development of an antivenom.

(c) *Studies on clinical and immune response in equines immunized for the production of antivenom:* Progress has been achieved on the optimization of the process of equine immunization with snake venom, which has led to a reduction in the dose (with the consequent decrease in the lesions induced by the venoms) and a rational design of bleeding schemes to obtain the hyperimmune plasma as the raw material of the process of antivenom development. These studies have been based on clinical, haematological, serological and blood chemistry parameters of equines.[33,61-64] At the same time, research is conducted on the effect of new immunological adjuvants,[65] of options such as immunization with DNA encoding for toxins[66] and of venom combinations on the equine antibody response.[67] They are aimed at obtaining higher levels of antibodies and a more complete coverage of the various antigens (toxins) in the venoms.

(d) *Studies on plasma processing techniques and improvement of the final characteristics of antivenoms:* Research is being conducted on new techniques of immunoglobulin purification at an industrial scale and on

alternative ways to process and stabilize these proteins, either in a solution or lyophilised.[68-71] Also, basic studies are conducted in this area aimed at understanding the mechanisms leading to the development of early adverse reactions in a percentage of patients treated with antivenoms.[72-75]

(e) *Studies on quality control and preclinical assessment of the neutralizing capacity of antivenoms:* Controlling an immunobiological product for therapeutic use, such as antivenoms, is complex. Thus, it requires careful standardization, as well as a preclinical evaluation on animal models, using techniques that reliably predict subsequent clinical performance. Within this area of research, [76-81] work has been done on the comparative properties of antivenoms prepared as whole IgG (undigested) or their proteolytic fragments $F(ab')^2$ and Fab, on animal models.[82] The antivenoms are produced in the CPI under the first modality (IgG), since research to date does not show a superiority in the use of fragments $F(ab')^2$ or Fab.[82]

(f) *Clinical research studies on antivenoms:* During the last two decades some clinical trials have been developed to assess performance and other characteristics of the antivenoms produced in the CPI in patients who have suffered from snakebite envenoming. Such studies have been conducted mainly in collaboration with clinical experts in Colombia and Nigeria.[28-30, 83,84] Although to date clinical trials with antivenoms have not been performed in Costa Rica (partly due to the unclear regulatory situation that prevails), it would be ideal for addressing important questions in the field of serotherapy.

(g) *Studies on the composition of venoms:* The complex protein formation in venoms of snakes found in Costa Rica has been studied permanently since 1960 and in a more rapid and accurate manner since the introduction of improved techniques for chromatographic separation and protein biochemistry. The introduction of new proteomics technologies, based on the development of mass spectrometry, has significantly accelerated progress in this area of research. Thanks to the support of CONARE and the UCR, as of 2010, the CPI inaugurated a modern laboratory for proteomic analysis, with which it is now possible to know in detail the composition of venoms,[34] as well as that of other biological samples of interest for the country's scientific community. A very useful derivation of proteomic analysis of venoms (venomics) has been the development of a strategy, called "antivenomics", used to determine the recognition of each of the venom's components by the antibodies present in the antivenoms.[34.35]

(h) *Isolation, characterization and study of the mechanism of action of snakebite toxins:* In order to better understand snake venoms and their pathological actions in the body, the different toxins are isolated and studied in a variety of experimental models that have allowed a deeper understanding of their mechanisms of action and of the physiopathology of snakebite envenoming.[85,91]

(i) *Experimental pathology of snakebite envenoming:* The pathogenesis of the toxic effects in experimental animal models is researched by using complete venoms and their isolated components and employing techniques of histology, immunohistochemistry, immunofluorescence and inflammatory exudates proteomics.[19, 92.93]

(j) *Search for, characterization and assessment of inhibitors with therapeutic potential against snake venom:* The possibility of obtaining purified toxins from the venoms has allowed work on a research area whose objective is to find alternative substances to antibodies, whether of a natural or synthetic origin, which are also capable of inhibiting their toxic effects.[94-98] These studies have provided some promising compounds as candidates for an eventual therapeutic application, which could complement the use of antivenoms. However, this goal depends on the possibility of performing controlled clinical trials, once the preclinical research stage has been overcome. As mentioned (section f), greater support would be required for this purpose.

(k) *Development of new types of antivenoms:* Although still in an early stage, the ICP is undertaking research on the potential development of equine antivenoms against bees and scorpions. In a first stage, research has been conducted on the characteristics of bee envenoming in an experimental model[99] and on the immune response to their major toxins. Also, the effects of venoms from scorpions found in Costa Rica and their biochemical proteomic composition has been researched. Moreover, the experience in the development of snakebite antivenoms for the region has allowed the development and production at the CPI of two new antivenoms for relevant snakes in Nigeria[100] and Papua New Guinea[101]; therefore contributing to alleviate this health problem in these regions of high snakebite envenoming incidence.

From research to treatment: a look at the future

When viewed in the context of their historical evolution, the scientific and technological activities carried out in the CPI show positive indexes, both in the generation of knowledge (scientific publications) and in their production aspect (number of antivenom vials), as shown in Fig.2. Looking towards the future, in the short and medium term, and based on indigenous and foreign research, the question to be asked is what improvements and changes could be expected in connection with antivenoms, the key therapeutic element to address the problem of snakebite. Although it is difficult to predict on issues involving scientific and technological aspects, given the sudden and radical changes that may occur due to a fundamental discovery or a high impact innovation, some suppositions may be outlined. The use of therapeutic biotechnology products, such as recombinant human antibodies, or their fragments, such as scFv or others produced in various prokaryotic or eukaryotic expression systems, will probably not constitute a viable option as antivenoms in the short term. The reasons for this are related, on the one hand, to the high production costs for the production of elements of this nature that fulfill the requirements of an injectable product at an industrial scale, and on the other, to the complex composition of the venoms, which would require the development of a "cocktail" of recombinant antibodies or fragments. However, the possibility that antidotes of this nature can be developed to combat some particular types

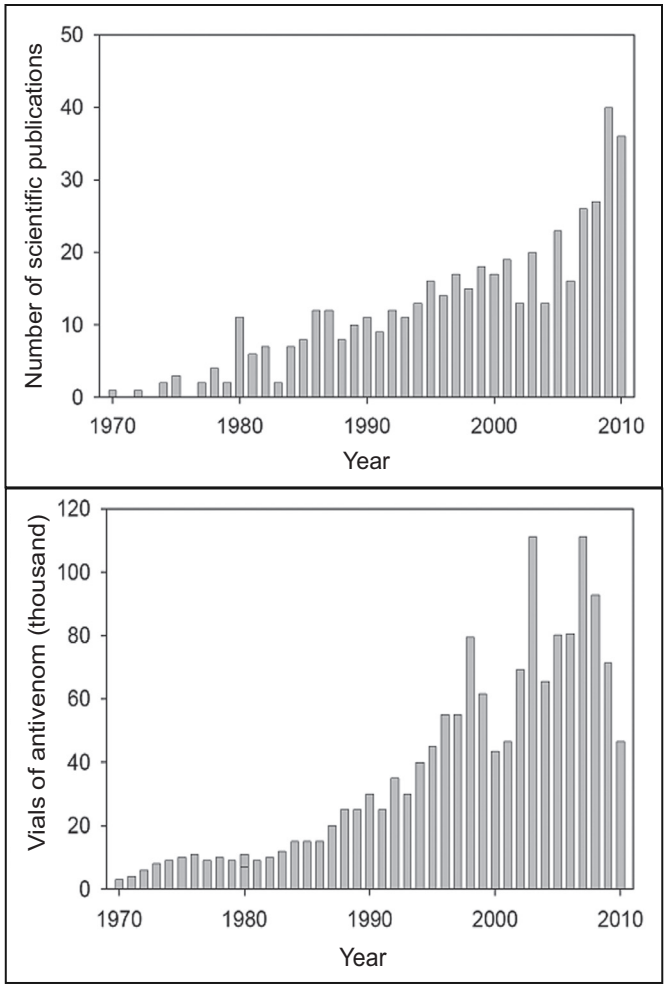

Figure 2: Scientific publications and antivenom production at the ICP (1970-2010).

of envenoming should not be ruled out, such as those caused by some snakes of the Elapidae family. This is the case of species were it is possible to show that the toxicity of the venom is attributable to one or two of the main components. A good candidate for this might be the case of the redtail coral snake Micrurus mipartitus, for which there is no antivenom available in Costa Rica. The recent proteomic analysis of its venom has revealed the presence of a prevalent neurotoxin,[100] which could be a suitable target for the development of a neutralizing monoclonal antibody, and its eventual mass production for therapeutic purposes.

It is likely that improvements and changes in antivenoms resulting from research will be introduced in a gradual rather than radical way. Some of the issues for which an evolution in the short term can be expected are those related to improvements in the safety and security of antivenoms, due to the development of increasingly efficient methods of processing, purification and final stabilization of immunoglobulins. At the same time, the therapeutic efficacy of the antivenoms may be increased through immunization strategies that can overcome the limitations in the immunogenicity of some snakebite toxins, which play a key role from the clinical standpoint. Also, it is to be expected that the detailed analyses of the venomic composition of snakes found in various geographical origins and the development of new antigenic formulas on such rationales lead to a significant expansion of the antivenoms' coverage areas. This could contribute to improve the availability of treatment in countries or regions where the shortage of antivenoms has dramatic consequences for the population, which suffers high mortality and morbidity indexes. Finally, an introduction to clinical practice of some non-immunologic inhibitors that block specific toxins is possible. However, this requires a greater interest from the medical community towards undertaking controlled clinical studies, despite not having the sponsorship and support of large international pharmaceutical companies.

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