USE OF HEN EGG DERIVED IMMUNOGLOBULIN AGAINST SCOLOPENDRA (*Scolopendra gigantea*) VENOM

Uso de Inmunoglobulina Derivada de Huevo de Gallina contra el Veneno de Escolopendra (*Scolopendra gigantea*)

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RESUMEN

Las picaduras de escolopendra (*Scolopendra gigantea*) en seres humanos y animales domésticos representan un accidente agudo y muy doloroso. La necrosis y otros daños ocasionados por este veneno pueden ser prevenidos, si se inyecta un antiveneno. Este estudio propone producir anticuerpos policlonales en gallinas hiper-inmunizadas contra el veneno de escolopendra (*Scolopendra gigantea* Linneaus 1758). Un grupo de gallinas fue inyectado subcutánea e intramuscularmente con diluciones de venenos, de acuerdo con tres rutinas diferentes. Los huevos fueron recogidos diariamente y los anticuerpos en la yema fueron purificados con un método modificado de polietilen-glicol y cloroformo. Los niveles de anticuerpos en yema fueron calculados con prueba de precipitación de gel de agar y pruebas de protección (ED_{50}). Los huevos cosechados 15 días post-inyección tenían los títulos más altos de anticuerpos. Después de seis meses, los anticuerpos liofilizados y guardados a 5°C mantenían su actividad. Ratones envenenados, inyectados posteriormente con anticuerpos purificados, tuvieron un 100% de supervivencia al compararse con los controles. La limpieza, la eficacia, y la sencillez de producir los antivenenos en gallina, y la incapacidad de estos anticuerpos (IgY) para fijarse al complemento humano, formulan una alternativa interesante a otros antivenenos producidos en mamíferos. Este estudio puntualiza que los anticuerpos de huevo de gallina pueden ser provechosos como un instrumento terapéutico para tratar escolopendrismo en seres humanos y animales domésticos. Además, abre un campo terapéutico para la fabricación de otros antivenenos contra el espectro amplio de las toxinas y como probable herramienta de diagnóstico.

Palabras clave: Inmunoglobulina Y, antiveneno de escolopendra, escolopendrismo, *Scolopendra gigantea.*

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ABSTRACT

A scolopendra sting in humans and domestic animals is an acute and highly painful accident. The present study was an attempt to raise specific hyper-immune polyclonal antibodies against scolopendra centipede (*Scolopendra gigantea* Linneaus 1758) venom. A group of hens were injected with venoms subcutaneously and intramuscularly according to three different routines. This protocol was found to be effective for hyperimmunization. Eggs were gathered daily and antibodies were purified from yolk with a polyethylene-glycol and chloroform modified method. Titers of antibodies in yolk were estimated with an agar gel precipitation test, and a serum protection (ED_{50}) test. Eggs harvested at 15 days post-injection had maximum antibody titers. After six months, antibodies lyophilized and stored at 5°C still maintained their activity. Envenomed mice were injected with purified antibodies, which induced 100% recovery as compared to those not treated with the antibodies. The cleanliness, effectiveness, and simplicity of producing antibodies against scolopendra venom in avian egg yolk, and their incapability to attach human complement, formulates an interesting option to equine and other mammalian antivenoms. This study infers that avian egg yolk antibodies may be useful as a therapeutic tool in treating scolopendrism in humans and domestic animals. It also opens a field for the production of other antivenoms against the wide spectrum of toxins as well as the use of these antibodies as a diagnostic tool.

Key words: Immunoglobulin Y, scolopendra antivenom, scolopendrism, *Scolopendra gigantea*.

INTRODUCTION

Scolopendrism is the envenomation by species of *Scolopendra* [12]. The venom is toxic to humans (the venom contains serotonin, acetylcholine, histamine, lipids, polysac-

charides, enzymes such as proteases, hyaluronidases and esterases [1,12] and causes a local painful burn (similar to a hornet-sting), severe edema, chills, fever (up to 39°), weakness, erythema, local lymphangitis, dizziness, necrosis at the site of the sting, myonecrosis and acute renal damage [1,17]. At experimental levels the *Scolopendra* venom causes, according to the dose, death in mice (*Mus musculus*) within a few minutes [31]. Accidents are not rare in human adults and severe envenoming followed by death is an exception. Nevertheless, some fatal cases have been reported [20]. When the scolopendra is irritated, it inoculates its venom through claws or fangs; each gland drains its toxic contents through a small aperture near the tip of the fang, and drops into the wounds causing the referred symptoms.

Passive immunization with antibodies derived mainly from horse's (*Equus caballus*) blood is widely used to treat snake (*Viperidae*), scorpion (*Buthidae*), and spiders (*Arachnoidea*) envenomations [17]. However, there is no specific treatment offered for scolopendra envenomation and the production of antivenom is necessary for its treatment.

The current study was intended to produce and purify hen (*Gallus domesticus*) specific hyper-immune polyclonal antibodies against scolopendra venom. Evaluation of antibodies was carried out through agar gel precipitation and serum protection (ED_{50}) tests, in order to evaluate the antivenom as an efficient therapeutic agent.

MATERIALS AND METHODS

Animals

Scolopendra. A group (twelve specimens) of *Scolopendra gigantea*, coming from Zulia and Falcon States, Venezuela, were maintained in the Immunochemistry Section of the Institute of Tropical Medicine at the Universidad Central de Venezuela. The animals, once identified, were placed in boxes and fed monthly with arthropods or neonates mice.

Mice. Male mice (INH strain) weighing 18-22 g from the Instituto Nacional de Higiene "Rafael Rangel", Caracas, Venezuela were used. The colony of mice was kept in boxes in a room maintained at 23°C on a 12/12-hr light/dark cycle as outlined in the "Guide of Principles of Laboratory Animal Care" [3].

Hens. Six egg-laying, red hens of Rhode Island strain of approximately 16 wks of age, obtained from a poultry farm of Lagunita town, Miranda State, Venezuela were located in individual henhouses before the beginning of the production of eggs. Hens were maintained on 12/12-hr light/dark cycle at 23°C with food and water *ad libitum* [3].

Venom

Between 7 and 15 days previous to venom extraction, the scolopendras were fed to guarantee enough venom in their glands. They were anesthetized by maintaining them at –20°C for approximately 10 min. The specimens were fixed by means of two connected stimulating electrodes (Grass model, USA). An electrode was placed in the body and the other one in the base of the claw. Once the animal was fixed, an electric stimulation was carried out by administering pulses of 60V per pulse at a frequency of 50 Hz to each claw. Each claw was given 3 to 5 pulses for approximately 15 s, with resting periods of approximately 30 s. Ejected venom through the excretory conduit in the tip of the claw was collected in glass capillaries that were placed in Eppendorf tubes and stored at –30°C until use. The volume of each extraction was calculated knowing the volume of the capillary used in the collection.

In order to avoid venom contamination with gastric juices and also avoid the capillary fracture, special mask containing holes to accommodate each claw and render them immobile were used.

Determination of protein concentration

The crude venom was resuspended in distilled water and the protein concentration was determined by means of absorbance read in a spectrometer (Spectronic® Model Genesis 2, USA) at 280 nm, assuming that 1 absorbance unit represented a concentration of 1 mg/mL.

Lethality assay

The intraperitoneal LD50 for scolopendra crude venom was determined in mice. Three of 10 mice (18-22 g) each were injected intraperitoneally with four doses of venom (0.3 µg to 0.6 µg in 0.1 mL) dissolved in saline. Forty eight hours later dead animals were recorded and the results analyzed by the Spearman-Karber method [27].

Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) of scolopendra crude venom

Scolopendra crude venom under reduced and non reduced conditions was electrophoresed using a MINI-PROTEAN II (BioRad, USA) chamber. SDS-PAGE was performed according to the Laemmli method [15] using 12% gels. Wide range molecular weight markers (Bio-Rad) were run in parallel and gels were stained with Coomassie Blue R-250.

Immunization of egg-laying hens with scolopendra crude venom

Six egg-laying, red hens were immunized with scolopendra venom in three phases: The first phase consisted in the administration of 80 µg/0.3 mL of scolopendra crude venom at three day intervals. The first doses were an equivolumetric emulsion of venom and Freund's complete adjuvant, whereas the second doses consisted of venom emulsified with Freund's incomplete adjuvant. The third and successive venom doses were mixed with saline solution.

All doses were administered subcutaneous via in the deltoid region sequentially alternating right and left. One week after the last dose of the first phase, blood was obtained for the detection of immunoglobulins that could recognize and precipitate the venom.

The second phase of the immunization protocol continued with 160 µg/0.3 mL of scolopendra crude venom intramuscular via thigh injection, in two doses, with an interval of 15 days between dose, beginning 15 days after the sixth dose of the first phase.

In the last phase a single dose of 500 µg/0.3 mL of scolopendra crude venom was administered intramuscular via in the thigh.

Isolation of eggs-yolk immunoglobulins from *Scolopendra* venom immunized hens

The eggs were collected daily, weight and stored at 4°C until use. The yolk was removed carefully and washed with tap water and wrapped in absorbent paper to remove the albumin. The yolk was processed to obtain immunoglobulins using a modified chloroform-polyethylene glycol protocol [21]. Briefly, 0.01M PBS, pH 7.6 was added to the yolk at equal volumes and mixed by vortexing. The mixture was equivolumetrically dissolved in chloroform and vigorously mixed until obtaining a homogeneous solution. After having centrifuged at 3000g for 35 min, the supernatant was decanted and treated with polyethylene glycol 6000 at 12% concentration. Following Vortex® shaking for 5 min, the mixture was centrifuged at 3000g for 30 min. The supernatant was discarded and the precipitate resuspended in equal volumes of PBS of the initial yolk volume. Then, it was dialyzed, lyophilized and kept at 5°C until use.

Quantity and purity determination of eggs-yolk immunoglobulins from scolopendra venom immunized hens

The immunoglobulin obtained from the eggs-yolk was determined by the absorbance to 280 nm, considering that the IgY extinction coefficient has been calculated to be 1.35 [16]; therefore, 1.35 UA = 1mg/mL.

The IgY purity was determined by protein polyacrylamide gel electrophoresis (PAGE) at 12% according to Laemmli et al. [15]. Wide range molecular weight markers (Biorad, USA) were used, and the gel was stained with Coomassie blue.

In gel diffusion assay using specific IgY against scolopendra venom

To demonstrate the specific IgY immunoglobulin activity, an Ouchterlony double gel diffusion test was used [19]. The scolopendra crude venom at 10mg/mL was placed in the central well, while different dilutions of IgY were placed in the outer wells and incubated at 37°C.

To explore the specific scolopendra antivenom against the immunoglobulins of the hens' sera, the sera were also assayed by an Ouchterlony double gel diffusion test as elsewhere described.

Serum protection test (ED_{50})

For antivenom potency, four groups of six mice were challenged with a mixture of three LD_{50} of venom. Scolopendra antivenom efficacy doses were calculated by mixing different amounts of antivenom (from 1.2 to 2.6 mg of immunoglobulin) with 1 mg scolopendra venom prepared at 0°C and incubated for 30 min at 37°C prior to injection. Each mouse was injected with 0.1 mL of venom/antivenom mixture into the peritoneum. The mice were observed for 48 h and the percent survival and ED_{50} was calculated. Saline controls and antivenom controls were used. Results were analyzed by Probit analysis according to the recommended WHO guidelines [32], and neutralizing capability was expressed as the 50% effective dose (the amount of antivenom that protects 50% of the population).

RESULTS AND DISCUSSION

Antibodies have been used for over a century in the treatment of envenomations caused by animal toxins. Antibodies remain a critical component for the treatment of envenoming by snakes, scorpions and spiders. Polyvalent equine antivenom represents the main therapeutic supply to remedy snakebite envenomations and it is the main source for these sera [24]. Nevertheless, undesirable effects mainly related with the capability of Fc of horse IgG to activate complement have been exemplified [9]. Due to cases of hypersensitivity to horse serum, other animals were assayed for alternative forms of antivenoms, and favorable results have been observed with sheeps (*Ovis aries*), goats (*Capra hircus*), rabbits (*Oryctolagus cuniculus*), as well as other animals [12, 13, 22].

The current study was carried out to explore, by using an experimental model, if a specific immunoglobulin raised in avian egg yolks would be effective in neutralizing scolopendra venom. To address this question, the ability of the antivenom to neutralize the venom effects *in vivo* was investigated. One hundred and ten milligrams of venom from twelve scolopendras (*S. gigantea*) were obtained from 45 successful venom extractions. The concentration of the crude venom was of 375 µg/µL. The lethal activity (LD_{50}) of scolopendra crude venom in mice was 0.3 mg/20 g mice. The venom dosages used in immunizing mice was extrapolated to hens and doses between 40 to 250 mg/kg were used to immunize the hens.

Scolopendra crude venom was subjected to electrophoresis under native conditions, and ten bands from 6 to 190 kDa were observed (FIG. 1). Under reduced conditions, new bands at 116, 60 and 45 kDa were seen, while the bands at 190, 14 and 6 kDa remained.

Scolopendra stings are common in humans, and, although they can be treated using unspecific therapies. The uses of those therapies do not solve the problems of myotoxic-

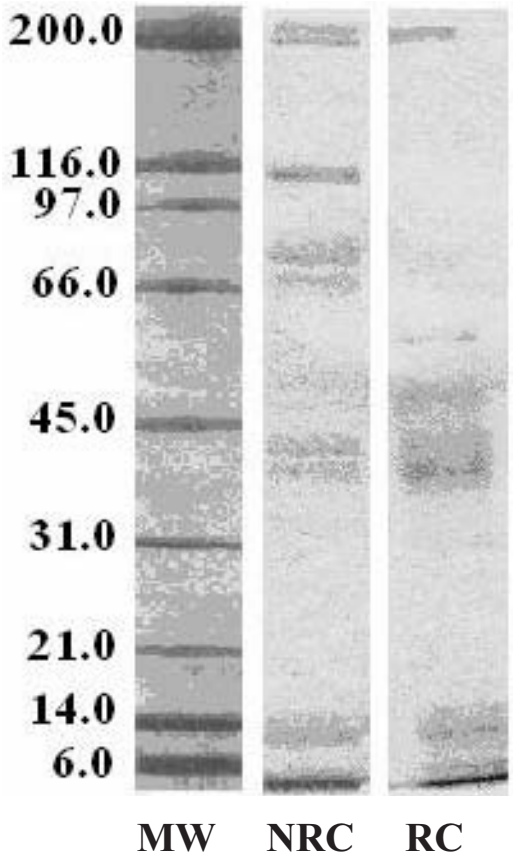

FIGURE 1. (SDS-PAGE) *SCOLOPENDRA* CRUDE VENOM UNDER NONE REDUCING AND REDUCING CONDITIONS. MW: MOLECULAR WEIGHT MARKERS; NRC: NONE REDUCING CONDITIONS; RC: REDUCING CONDITIONS. A 10-20% TRIS-GLYCINE GEL WAS USED TO RUN 6 µG OF *SCOLOPENDRA GIGANTEA* VENOM POOL. A MINI-GEL (BIORAD, EUA) ELECTROPHORESIS SYSTEM WITH TRIS-GLYCINE SDS RUNNING BUFFER WAS USED. THE GEL WAS RUN AT 130V, 90 MIN. THE MARKERS USED WERE SEEBLUE PLUS2 (INVITROGEN, EUA) / SDS-PAGE DE VENENO CRUDO DE SCOLOPENDRA BAJO CONDICIONES REDUCIDAS Y NO REDUCIDAS. MW: MARCADORES DE PESO MOLECULAR; NRC: CONDICIONES NO REDUCTORAS; RC: CONDICIONES REDUCTORAS. UN EQUIPO DE ELECTROFORESIS (BIORAD, EUA) PARA UN MINI-GEL (10-20% TRIS-GLICINA) CON TAMPÓN DE CORRIDA TRIS-GLICINA- SDS FUE UTILIZADO PARA CORRER 6 µG DE VENENO DE *SCOLOPENDRA GIGANTEA*. EL GEL FUE CORRIDO A 130V, 90 MIN. LOS MARCADORES DE PESO MOLECULAR UTILIZADOS FUERON LOS SEEBLUE PLUS2 (INVITROGEN, EUA).

ity and hepatotoxicity [12]. Only specific antivenom may be able to inhibit the development of these lesions. This study establishes that IgYs prepared from the egg yolk of hens immunized with crude scolopendra venom were effective in the treatment of scolopendrism. The first phase of immunization subcutaneous via injections did not produce visible effects in the hens in either of the six doses, while the administration of the first intramuscular dose of the second phase, caused irritation and flexion of the femoral-tibial articulation of the injected thigh in all the hens.

The isolation of egg-yolk immunoglobulins from hens immunized with scolopendra venom evidenced that the differences in the quantity of IgY from egg yolks among different hens was not significant. The IgY average concentration was 8.4 mg/mL per egg-yolk averaging a volume of 15 mL. Thus, an average of 124 mg of immunoglobulins from each egg was obtained. After 6 months at 5°C, the lyophilized antibodies still retained their activity.

Polyclonal antibodies against natural toxins are produced in domestic animals, such as horses and goats that require multiple bleedings to acquire the antibodies. A less invasive method could be the production of antibodies in avian egg yolks [8, 25]. The main classes of antibodies in hens are IgYs. The use of hens for the production of antibodies instead of mammals signify a decrease in the number of animals because hens produce higher quantities of antibodies in egg yolks (1500 mg of IgY per month) than that of small mammals (200 mg of IgG per month) [9]. Therefore, hens are an outstanding selection for the development of mammalian antibodies [2, 6, 30]. Furthermore, the recovery of IgY does not require bleeding procedures, because laying eggs is a hen's natural process. Hens can be considered an efficient source and an ideal alternative for the development of scolopendra antivenom as well as other antivenoms.

The isolation of IgY from egg yolk using the chloroform-polyethylene glycol method was uncomplicated and resulted in high yields. The results are in accordance with a study conducted by Polson et al. [21] whom evaluated extraction methods of IgY and demonstrated that the chloroform-polyethylene glycol method was efficient, rapid and easy for antibody purification. The lipids are soluble in organic solvents such as chloroform, which allows the isolation of IgY from the watery phase of the egg yolk [18]. The chloroform method was carried out in this study allowing the recovery of 8.4 mg of IgY/mL per yolk.

In this study, 80 to 500 µg of crude scolopendra venom in 0.1 mL of Freund's complete and incomplete adjuvant were used for initial and reinforced immunizations, respectively, followed by final immunizations with venom and saline. The used antigen concentrations and volumes were inside the scope of 10 ng to 1 mg in 0.1 mL, which are the recommended dosages for immunizing hens [24].

The results demonstrate that antibody titers were considerably higher in yolk as compared to hen´s sera tested after 26 days. These results are analogous to Kuhlmann et al. [14] whom revealed that IgY generated by hen was 18 times higher than IgG produced by rabbits. This elevated level of antibody titer may be due to the steady existence of the venom-adjuvant mixture in the subcutaneous tissue of hens, which probably activates the immune cells over a long period of time. Moreover, the immune response when challenged with mammal antigens is increased in hens compared to mammals, which is due to the hen's distant phylogenetic differences [10]. Hens also produce antibodies against highly conservative mammalian pro-

teins and the amount of antigen needed for immune response is very low. On the other hand, the sera of hens immunized with scolopendra venom presented a poor amount of precipitant immunoglobulins. The sera was obtained a week after the last dose of the immunogen during the first immunization phase, revealing that an important immune response had not yet taken place.

Purity determination of eggs-yolk immunoglobulins from hens immunized with scolopendra venom was analyzed by SDS-PAGE. The IgY has two major bands, the molecular weight of the heavy chain is about 65 kDa and that of the light chain is about 25 kDa (FIG. 2). The efficiency of this immunoglobulin production method is illustrated by the cleanliness of IgY in SDS-PAGE reducing conditions, which is presented by the heavy and light bands representing the antibody. Immunoglobulin Y has two structural differences compared with mammalian IgG, the molecular weight of the heavy chain of IgY is larger than that of its mammalian counterpart, while the molecular weight of the light chain of IgY is smaller [29].

An in gel diffusion assay was carried out against scolopendra venom and IgY, resulting in the development of prominent precipitin bands in the immunodiffusion experiments (FIG. 3). As a result of the immunization protocol, precipitin bands were formed against both dilutions of scolopendra antivenom. Further more, scolopendra venom did not show any precipitin bands with the polyvalent antivenom against *Crotalus durissus cumanensis*, *Bothrops atrox*, *B. colombiensis* and *C. vegrandis* venoms indicating significant differences in venom toxins of these snakes and scolopendra.

Mice were used to test the efficacy dose (ED_{50}) of the scolopendra antivenom. The ED_{50} for the scolopendra antivenom calculated for scolopendra venom is 2.6 mg/20 g mice (TABLE 1). Six control mice injected with 1 mg/0.1 mL of scolopendra venom (3 LD_{50}) died after 48 hr. Meanwhile, the group injected with 0.1 mL of venom (1mg)/antivenom (2.6 mg) mixture, protected the mice from the venom-induced death. Animals injected with the antivenom (2.6 mg/0.1 mL of immunoglobulin) 5 min after venom injection survived beyond 24 h. The antivenom also protected all mice when injected before administration of three LD_{50} of the venom. It also rescued all mice injected with one LD_{50} of the venom, either immediately before antivenom or 5 min after venom injection. The antivenom neutralized not only the lethal effects of venom, but also other toxic effects. For instance, it entirely neutralized the macroscopic necrotizing effects [11] induced by scolopendra venom.

During the development of IgY isolation protocols for the production of scolopendra antivenom, the current work maintained constant quality of IgY production, and required low venom dosages and volumes. Furthermore, a multi-site immunization protocol, the number and interval of immunizations along with the hens' age are all required parameters for such aim [28]. It has been demonstrated in hens that, after the first immunization with other immunogens (e.g. bacteria), the IgY

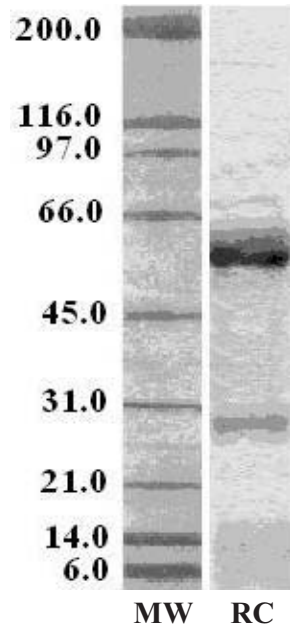

FIGURE 2. SDS-PAGE OF EGG-YOLK IMMUNOGLOBULINS FROM *SCOLOPENDRA* ANTIVENOM UNDER REDUCING CONDITIONS. MW: MOLECULAR WEIGHT MARKERS; RC: REDUCING CONDITIONS. A 10-20% TRIS-GLYCINE GEL WAS USED TO RUN 6 µg OF *SCOLOPENDRA GIGANTEA* VENOM POOL. A MINI-GEL (BIORAD, EUA) ELECTROPHORESIS SYSTEM WITH TRIS-GLYCINE SDS RUNNING BUFFER WAS USED. THE GEL WAS RUN AT 130V, 90 MIN. THE MARKERS USED WERE SEEBLUE PLUS2 (INVITROGEN, EUA) / SDS-PAGE DE IMUNOGLOBULINAS DE YEMA DE HUEVO DE ANTIVENENO DE *SCOLOPENDRA* BAJO CONDICIONES REDUCIDAS MW: MARCADORES DE PESO MOLECULAR; RC: CONDICIONES REDUCTORAS. UN EQUIPO DE ELECTROFORESIS (BIORAD, EUA) PARA UN MINI-GEL (10-20% TRIS-GLICINA) CON TAMPÓN DE CORRIDA TRIS-GLICINA- SDS FUE UTILIZADO PARA CORRER 6 µG DE VENENO DE *SCOLOPENDRA GIGANTEA*. EL GEL FUE CORRIDO A 130V, 90 MIN. LOS MARCADORES DE PESO MOLECULAR UTILIZADOS FUERON LOS SEEBLUE PLUS2 (INVITROGEN, EUA).

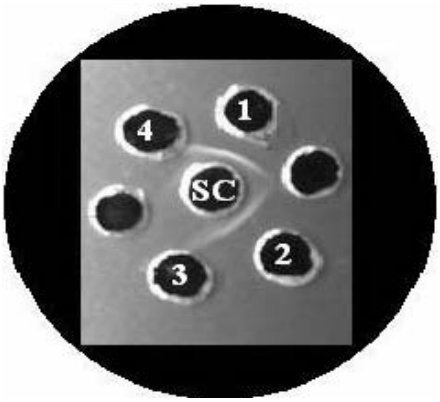

FIGURE 3. IN-GEL-DIFFUSION ASSAY USING SPECIFIC IGY AGAINST *SCOLOPENDRA* VENOM. SC: *SCOLOPENDRA* VENOM; 1 AND 2: IgY; 3 AND 4: ANTI-OPHIDIC SERUM (CONTROL) / ENSAYO DE DIFUSIÓN EN GEL USANDO IgY ESPECÍFICAS CONTRA VENENO DE *SCOLOPENDRA*. Sc: VENENO DE *SCOLOPENDRA*; 1 Y 2: IgY; 3 Y 4: SUERO ANTI-OFÍDICO (CONTROL).

TABLE I
EFFICACY OF AVIAN SCOLOPENDRA ANTIVENOM / EFICACIA DEL ANTIVENENO AVIARIO PARA ESCOLOPENDRA

Venom	*Scolopendra* venom $3LD_{50}$ (mg/0.1 mL)	Scolopendra antivenom concentrations (mg/0.1 mL)	Scolopendra antivenom ED_{50} (mg/20g mouse)
Scolopendra gigantea	1	1.2 to 2.6	2.6

In this study the ED_{50} was the amount of antivenom that protects 50% of the mouse population.

against bacteria reaches a peak in serum in week 4, and weeks 6 and 8 in egg yolk [7]. The development of antibodies facing the stimulus of an antigen is a genetic inheritance for each immunized animal [26]. To assure the development of IgY against crude scolopendra venom following the scheme proposed here, it is recommended to immunize a minimum of three hens.

In this study, IgY against scolopendra venom reached its peak production in week 8, which corresponded to the response of the third immunization. In agreement with this result, it is therefore recommended to immunize the hens with crude scolopendra venom at week 7, applying one immunization with an interval of two weeks between each one of them.

The additional therapeutic advantages of this antivenom are the laborious elimination of the complement-reactive FC fraction encountered in mammalian immunoglobulins, which is usually performed by pepsin digestion, thus reducing the yield and potency of the resulting antivenom. Where as, hen antibodies do not fix human complement [4, 5, 23] and for that reason the risk of anti-complement responses is avoided.

The low amounts of venom antigens required for immunizing allowing for the utilization of animals, such as hens, with low body weights, the quantity of egg yolk produced per animal, the ease of gathering eggs and most importantly, the low cost and effort of isolating the IgY from the egg yolk all contribute to the low cost efficiency of the hen model.

In reviewing the scientific literature, many works producing antibodies in avian egg yolks exist, but it is apparent that there is no antivenom available in the market capable of neutralizing the lethal effect of *S. gigantea* venom with the exception of the one produced in this study. To the best of the author's knowledge, this work is the first report where IgY purified antibodies against scolopendra venom is proposed as a therapeutic agent. The IgY was subjected to a series of purification and toxicological steps, sterilized by bacterial filtration and filled in ampoules under aseptic conditions. Scolopendra antivenom will soon be (once the clinical trials for tolerability and safety are completed) accessible for human use in the Tropical Medicine Institute of the Universidad Central de Venezuela,

CONCLUSIONS AND RECOMMENDATIONS

These results sustain the necessity to thoroughly continue investigating the possibilities of antivenoms manufacturing with this novel methodology. It is ratified here that this is the first antivenom produced in avians, against the toxins of the scolopendra venom. This study remarks that the antibodies of hen egg can be a valuable therapeutic instrument to treat scolopendrism in humans and domestic animals. It also opens a field for the production of other antivenoms against various natural toxins, which could also be used as possible diagnostic tools.

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BIBLIOGRAPHIC REFERENCES

[1] ACOSTA, M.; CAZORLA, D. Centipede (Scolopendra sp.) envenomation in a rural village of semi-arid region from Falcon State, Venezuela. **Rev. Invest. Clin.** 56: 712-717. 2004.

[2] ALARCÓN, C.E.; HURTADO, H.; CASTELLANOS, J. Anticuerpos aviares: alternativa en producción y diagnóstico. **Biomed**. 20: 338-43. 2000.

[3] NATIONAL INSTITUTE OF HEALTH. **Principles of laboratory animal care**, USA. Pub.85 Nº 23. 1-112 pp. 1985.

[4] BENSON, H.N.; BRUMFIELD, H.P.; POMEROY, B.S. Requirement of avian C'1 for fixation of guinea pig complement by avian antibody-antigen complexes. **J. Immunol**. 87: 616-622. 1961.

[5] CARROLL, S.B.; THALLHY, B.S.; THEAKSTON, R.D.G.; LAING, G. Comparison of the purity and efficacy of affinity Purified avian antivenoms with commercial equine crotalid antivenoms. **Toxicon.** 30: 1017-1025. 1992.

[6] ERHARD, M.E.; SCHMIDT, P.; HOFMANN, A.; STANGASSINGER, M.; LOSCH, U. Production and purification of mouse IgG subclass specific chicken egg yolk antibodies using a new indirect affinity chromatography method with protein G sepharose. **ALTEX**. 13: 66-69. 1996.

[7] ERMELING, B.L.; STEFFEN, E.K.; FISH, R.E.; HOOK, R.R. JR. Evaluation of subcutaneous chambers as an alternative to conventional methods of antibody production in chickens. **Lab. Anim. Sci.** 42: 402-407. 1992.

[8] GARCÍA, D.A.; NICHOLLS, R.S.; ARÉVALO, A.; TORRES, O.; DUQUE, S. Obtención, purificación y caracterización de anticuerpos policlonales IgY desarrollados en gallina, dirigidos contra aislamientos colombianos de *Giardia duodenalis.* **Biomed. (Bogot**á). 25: 451-463. 2005.

[9] GARCIA, M.; MONGE, M.; LEON, G.; LIZANO, S.; SEGURA, E.; SOLANO, G.; ROJAS, G.; GUTIERREZ JM. Effect of preservatives on IgG aggregation, complement-activating effect and hypotensive activity of horse polyvalent antivenom used in snakebite envenomation. **Biol.** 30: 143-51. 2002.

[10] GASSMANN, M.; THOMMES, P.; WEISER, T.; HUBSCHER, U. Efficient production of chicken egg yolk antibodies against a conserved mammalian protein. **FASEB. J.** 4: 2528-2532. 1990.

[11] GONZÁLEZ. A.; GASSETTE, J.; GHISOLI, M.; SANABRIA, E.; REYES-LUGO, M.; RODRIGUEZ-ACOSTA, A. Aspectos Bioecológicos de la Escolopendra (*Scolopendra gigantea* Linnaeus 1758) y la actividad histopatológica de su veneno. **Rev. Cientif. FCV/LUZ**. X(4): 303-309. 2000.

[12] HOWES, J.M.; THEAKSTON, R.D.; LAING, G.D. Antigenic relationships and relative immunogenicities of isolated metalloproteinases from *Echis ocellatus* venom. **Toxicon**. 45: 677-80. 2005.

[13] KARLSON-STIBER, C.; SALMONSON, H.; PERSSON, H. A nationwide study of *Vipera berus* bites during one year-epidemiology and morbidity of 231 cases. **Clin. Toxicol (Phila)**. 44: 25-30. 2006.

[14] KUHLMANN, R.; WIEDEMANN, V.; SCHMIDT, P.; WANKE, R.; LINCH, E.; LOSCH, U. Chicken egg antibodies for prophylaxis and therapy of infectious intestinal diseases. 1. Immunization and antibody determination. **J. Vet. Med.** 35: 610-616. 1988.

[15] LAEMMLI, U.K. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. **Nature.** 227: 680-685. 1970.

[16] LESLIE, G.A.; CLEM, L.W. Phylogen of immunoglobulin structure and function. 3. Immunoglobulins of the chicken. **J. Exp. Med**. 130: 1337-1352. 1969.

[17] MACHADO, A.; RODRÍGUEZ-ACOSTA, A. Animales terrestres. En: **Animales venenosos y ponzoñosos de Venezuela.** Consejo de Desarrollo Científico y Humanístico de la Universidad Central de Venezuela. Caracas, Venezuela. 69-70pp. 2005.

[18] MERCKX, A.; LE ROCH, K.; NIVEZ, M.P.; DORIN, D.; ALANO, P.; GUTIERREZ, G.J.; NEBREDA, A.R.; GOLDRING, D.; WHITTLE, C.; PATTERSON, S.; CHAKRABARTI, D.; DOERIG, C. Identification and initial characterization of three novel cyclin-related proteins of the human malaria parasite *Plasmodium falciparum*. **J. Biol. Chem**. 278: 39839-39850. 2003.

[19] OUCHTERLONY, O. Diffusion-in-gel methods for immunological analysis. **Prog. Allergy.** 15: 1-78. 1958.

[20] OZSARAC, M.; KARCIOGLU, O.; AYRIK, C.; SOMUNCU, F.; GUMRUKCU, S. Acute Coronary Ischemia Following Centipede Envenomation: Case Report and Review of the Literature**.** **Wild. Env. Med.** 15: 109-112. 2004.

[21] POLSON, A. Isolation of IgY from the yolk of eggs by a chloroform-polyethylene glycol procedure. **Immunol. Invest.** 19: 253-258. 1990.

[22] RICHARDSON, W.H.; TANEN, D.A.; TONG, T.C.; BETTEN, D.P.; CARSTAIRS, S.D.; WILLIAMS, S.R.; CANTRELL, F.L.; CLARK, R.F. Crotalidae polyvalent immune Fab (ovine) antivenom is effective in the neutralization of South American Viperidae venoms in a murine model. **Ann. Emerg. Med.** 45: 595-602. 2005.

[23] ROSE, M.E.; ORLANS, E. Fowl antibody: III. Its haemolytic activity with complements of various species and some properties of fowl complement. **Immunol**. 5: 633-641. 1962.

[24] SANCHEZ, E.E.; GALAN, J.A.; PEREZ, J.C; RODRIGUEZ-ACOSTA, A.; CHASE, P.B.; PEREZ, J.C. The efficacy of two antivenoms against the venom of North American snakes. **Toxicon**. 41: 357-365. 2003.

[25] SCHADE, R.; HLINAK, A. Egg Yolk Antibodies, State of the Art and Future Prospects. **ALTEX.** 13: 5-9. 1996.

[26] SHIN, J.H.; YANG, M.; NAM, S.W.; KIM, J.T.; MYUNG, N.H.; BANG, W.G.; ROE, I.H. Use of egg yolk-derived immunoglobulin as an alternative to antibiotic treatment for control of *Helicobacter pylori* infection. **Clin. Diagn. Lab. Immunol**. 9: 1061-1066. 2002.

[27] SPEARMAN, C.; KARBER, G. Alternative methods of analysis for quantal responses. In: Finney DJ, Ed. **Statistical Methods in Biological Assays**. Charles Griffin and Co Ltd: London. 1-78pp. 1978.

[28] SRIPARPAT, S.; AEKSOWAN, S.; SAPSUTTHIPAS, S.; CHOTWIWATTHANAKUN, C.; SUTTIJITPAISAL, P.; PRATANAPHON, R.; KHOW, O.; SITPRIJA, V.; RATANABANANGKOON, K. The impact of a low dose, low volume, multi-site immunization on the production of therapeutic antivenoms in Thailand. **Toxicon** 41: 57-64. 2003.

[29] SUN, S.; MO, W.; JI, Y.; LIU, S. Preparation and mass spectrometric study of egg yolk antibody (IgY) against rabies virus. **Rapid. Commun. Mass. Spectrom.** 15: 708-12. 2001.

[30] TERZOLO, H.R.; SANDOVAL, V.E.; CAFFER, M.I.; TERRAGNO, R.; ALCAIN, A. Agglutination of hen egg-

yolk immunoglobulins (IgY) against *Salmonella enterica*, serovar *enteritidis*. **Rev. Arg. Microbiol**. 30: 84-92. 1998.

[31] WELSH, J. H.; BATTY, C. S. 5-Hydroxytryptamine content of some arthropod venoms and venom-containing parts. **Toxicon**. 1: 165-173. 1963.

[32] WORLD HEALTH ORGANIZATION. Progress in the characterization of venoms and standardization of antivenoms**. Who Offset Public**. No. 58. Geneva. 58. 1-44pp. 1981.