Original Article

Venom and Antivenom of the Redback Spider (Latrodectus hasseltii) in Japan. Part II. Experimental Production of Equine Antivenom against the Redback Spider

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SUMMARY: This is the first report on large-scale experimental production of an equine antivenom against the redback spider (Latrodectus hasseltii) lived in Japan. We captured 10,000 redback spiders in Japan and prepared the toxoids of crude venom extract, mixed the toxoids with a mineral oil adjuvant, and immunized healthy horses repeatedly over a period of several weeks. Thereafter, we separated the horse plasma, purified the γ-globulin fraction, and stocked it as a purified antivenom concentrate. Consequently, we manufactured approximately 6,500 vials of a single-dose freeze-dried test lot from a portion of the purified γ-globulin fraction, equivalent to the extract derived from 520 spiders. This test lot had an antitoxin titer comparable to that of a similar drug commercially available overseas (a liquid preparation), and the other quality met all quality reference specifications based on the Minimum Requirements for Biological Products and other guidelines relevant to existing antivenom drug products in Japan.

INTRODUCTION

The redback spider (Latrodectus hasseltii), named in 1870, is one of the tropical/subtropical spider species found throughout Australia (1). Currently, this spider has been identified in many other countries, with the first discovery in Japan recorded in Osaka in 1995. Since then, their habitat has expanded northwards annually, with their presence confirmed in the Kanto area, Tohoku, and as far north as Hokkaido. Human cases of bites of this spider have been reported in Japan since 1997; therefore, this spider is gaining increasing attention (2–5). The main symptom of the spider bite is severe pain at the bite location. The primary cause of pain has been thought to be the venom known as α-latrotoxin (LT), which is a protein with a molecular weight of 130,000 daltons, found in the spider’s salivary glands (6).

In Australia, where this spider species was first identified, there were risks of developing severe or life-threatening conditions following the spider bite, if not treated with equine antivenom. Therefore, an antivenom, produced from horses immunized with redback spider venom was approved for commercial manufacturing and marketing in 1956, according to personal communication from bioCSL (Seqirus; Victoria, Australia); thus, it has been clinically used for over a half-century. A comparative study on the characteristics of LT from Australian redback spiders and that of Japanese spiders demonstrated that the 2 venoms did not differ physicochemically and immunologically (7). Currently in Japan, the overseas commercial antivenom drug product (liquid type; bioCSL) has been personally imported by a doctor as the representative investigator within the framework of the clinical research to treat people with spider bites.

However, in 2014, the antivenom drug product became temporarily unavailable through import, because alternative suppliers could not be identified (8). Therefore, we, as a scientific research group of the Ministry of Health, Labour and Welfare, formulated a plan to quickly produce an equivalent domestic product and secure a supply for the safety and peace of mind of the people (9,10). In summer 2014, we collected approximately 10,000 redback spiders from the Kansai area and manufactured an equine antivenom for the first time in Japan.

MATERIALS AND METHODS

Capture of redback spiders and purification of crude LT: From June through December 2014, a total of 11,403 redback spiders, including 10,186 from 480 sites in the Osaka Prefecture and 1,217 from several sites in Nishinomiya City, were collected in collaboration with the Pest Control Organization Osaka and the Environmental Health Division of Nishinomiya City. The captured spiders were killed by keeping them in a −20°C freezer, and the frozen spiders were sent to the Department of Medical Entomology at the National Institute of Infectious Diseases (NIID). The venom glands were individually excised from 10,007 female spiders. Zirconia beads (4 mm in diameter) were added and homogenized for approximately 30 s. After centrifugation at 10,000 rpm (KUBOTA 3740; KUBOTA, Tokyo, Japan) for 3
min, the supernatant was collected and combined into 50-mL tubes. The tubes, containing beads, were rinsed with 1 mL of pre-chilled physiological saline, homogenized for approximately 30 s, and centrifuged at 14,000 rpm (KUBOTA 3740) for 15 min. The supernatants were combined with the initial supernatants in 50-mL tubes. All tubes containing venom glands were manipulated in the same manner for extraction and crude purification, yielding a single batch of LT. A portion of 236 mg from the crude purified LT solution was used to establish a mouse toxicity test system and a neutralization assay system, as well as to manufacture the equine antivenom test lot.

**Quantitative assay of venom and antivenom:** The quantity of the redback spider venom was determined using the BCA Protein Assay Kit (Thermo Fisher Scientific, Waltham, MA, USA). The total amount of protein in the single batch of LT was determined to be approximately 235 mg, which provided a protein concentration of 1.392 mg/mL. A total of 170 mL of it was used for horse immunization.

The neutralizing antibody titer (potency) of the antivenom was determined using a method reported by Matsumura et al. (11). As the reference antivenom was not available in Japan, the commercially available redback spider antivenom (bioCSL) with known potency was obtained and used as a reference in calculating the relative potency. Five different amounts (4.0, 3.2, 2.56, 2.05, and 1.64 U per mouse) of bioCSL antivenom were mixed with an equal volume of Japanese redback spider venom solution containing a fixed amount of venom (40 μg per mouse, equivalent to 4 × LD50). Each mixture was incubated at room temperature for 1 h and administered intravenously to 3 ddY mice per group. The sera from the immunized horses were initially diluted to 3 levels (5-, 25-, and 125-fold) and assayed as above to estimate the approximate potency. Any dilution showing a high potency was further diluted to 5 levels at intervals of 1.5-fold and assayed again. Individual vials of the test lot were diluted to 5 different levels at intervals of 1.25-fold and assayed. Following administration, survival of mice was observed for up to 10 days, and the neutralizing antibody titer was determined using Probit analysis.

This assay using mice was performed according to institutional guidelines and with the approval of the NIID Animal Care and Use Committee (Permit Number: 115035).

**Preparation of antigen for horse immunization:** The native LT (redback spider venom gland extract) was aliquoted into small containers without dilution and stored in a freezer at −80°C in single-use portions to avoid potential degradation because of thermal stress, low protein concentration, and repeated freeze-thaw cycles. A portion of 235 mg of LT was used for horse immunization.

For the toxoid preparation, 21 mL of LT was supplemented with l-lysine hydrochloride to a concentration of 1 mg/mL and with formalin to 0.18%, followed by incubation at 5°C for 7 days. Formalin was further added to the mixture to 0.36%, and the mixture was incubated at 37°C for 14 days, followed by dialysis to yield the LT toxoid preparation for initial immunization (hereinafter, LTtd[1]). The antigen (LTtd[1]) was prepared according to a previous report, in which, a commercial manufacturing method for a domestic equine antivenom drug product (antibodies against bacterial toxin) in Japan. Formalin was added to 8 mL, 12 mL, and 32 mL aliquots of LT to achieve 0.2%, and the mixture was incubated. Formalin was further added to 0.4%, and finally to 0.6%. Each incubation was conducted at 36.5°C for 2 days. The final preparations were dialyzed against saline to obtain the LT toxoid preparation (hereinafter, LTtd[2]) for booster immunization. The antigen (LTtd[2]) was prepared according to a commercial manufacturing method for the snake antivenin in Japan.

**Immunization of horses:** Healthy horses (thoroughbred, n = 4; ages 3–4 years) were acquired domestically and introduced to stables at Aso Branch of KAKETSUKEN. During 1 week of acclimatization, the horses were treated once with an anti-parasitic (Equivalen Gold; DS Pharma Animal Health, Osaka, Japan) and administered a combined vaccine (KAKETSUKEN) for equine influenza, Japanese encephalitis, and tetanus toxoid. For approximately 1 month following stable introduction, their health status (body temperature, gait, appetite, movement, water intake, and discharge) was recorded to confirm their wellbeing.

The 4 horses were immunized according to the 4 protocols described below (Table 1). The interval between the end of the initial immunization to the beginning of the booster doses was 9 weeks, and the interval between immunizations was 1 week, as previously reported (12). Four immunization protocols used were as follows: Protocol 1 used aluminum adjuvant (immunization with venom), and the initial immunization consisted of 6 ascending doses of LT; followed by booster immunizations of 4 ascending doses of LT. This served as the control protocol. Protocol 2 used Freund’s incomplete adjuvant (FIA) (immunization with toxoid, with fixed booster doses) and the initial immunization consisted of 3 fixed doses (3 mg) of LTtd[1], followed by booster immunizations with 6 fixed doses (3 mg) of LTtd[1]. Protocol 3 used FIA (immunization with toxoid, in ascending booster dose) and initial immunization consisted of 3 doses (3 mg) of LTtd[1], followed by booster immunizations with 6 ascending doses of LTtd[2] (from 1 to 24 mg in approximately 2-fold increments). Protocol 4 used FIA (immunization with toxoid and venom) and initial immunization consisted of 3 fixed doses of LTtd[1] (3 mg), using the material prepared according to a previous report describing commercial manufacturing conditions for a domestic equine antivenom drug product (antibodies against snake venom) in Japan (13) followed by booster immunizations with 6 ascending doses of LT (from 1 to 24 mg in approximately 2-fold increments). However, Protocol 4 was not followed as planned because the immunized horse developed abdominal colic after the second booster dose, resulting in discontinuation of the subsequent procedure, including the third and subsequent doses.

Blood was collected 1 week after each dose. Sodium citrate was added to the blood as an anticoagulant to a final concentration of 1% (w/v), and then the plasma was separated to determine the neutralizing antibody titer.

Horse immunization was performed with the approval of the KAKETSUKEN Institutional Animal Care and Use Committee. (Approval number: C15-03P).
Experimentally Production of the Equine Antivenom

Preparation of antivenom:
Horse plasma with the highest titer level (Protocol 3; 14.8 L, 3,772 U/mL) was selected as the starting material for purification of the \(\gamma\)-globulin fraction according to the present production method for antivenom purification (13). Namely, the horse plasma was first digested with pepsin to remove the Fc portion from the \(\gamma\)-globulin molecule, and the F(ab'\(^2\)) fragment-rich fraction was purified through ammonium sulfate fractionation, dialysis, alum adsorption (removal of pepsin), and sterile filtration, and the final material was used as the purified antivenom concentrate. After a preliminary study confirming that the titer was maintained at small scale of bulk, the final bulk solution was prepared from approximately one quarter (1 L) of the purified antivenom concentrate by diluting it with saline to an appropriate concentration and adding sodium L-glutamate as a stabilizer (excipient) to a final concentration of 1.54\(^{(w/v)}\). After a preliminary study confirming that the moisture content was not increased by our setup conditions, a test lot of the freeze-dried redback spider antivenom was prepared from the final bulk solution by filling 2-mL glass vials with 0.65 mL antivenom solution, stoppering, and freeze-drying the vials (Lot No. 001, approximately 6,500 vials).

All unused portions of horse plasma and the purified antivenom concentrate were stored frozen at –20°C for production in the future.

Quality control test:
Both the purified bulk of the redback spider antivenom concentrate and the test lot of freeze-dried antivenom were tested for the specifications regarding immunoglobulin content, sterility, pyrogen, potency, moisture content, pH, appearance, weight variation, foreign insoluble matter, insoluble particulate matter, osmotic pressure ratio, and freedom from residual proteolytic enzymes, adventitious virus, and abnormal toxicity, in accordance with the personal communication from bioCSL regarding redback spider antivenom, Minimum Requirements for Biological Products in Japan (14), Japanese Pharmacopoeia (15), and other published information (16) relevant to the present equine antivenom product (anti-snake venom). The potency test was carried out as described in the Materials and Methods.

Table 1. Summary of immunization protocols

| Protocol 1: immunization with venom | Protocol 2: immunization with toxoid, in fixed booster dose | Protocol 3: immunization with toxoid, in ascending booster dose | Protocol 4: immunization with toxoid and venom |
|------------------------------------|-------------------------------------------------------------|-------------------------------------------------------------|------------------------------------------------|
| Wk  | Immunization Dose (mg) | Adjuvant  | Wk  | Immunization Dose (mg) | Adjuvant  | Wk  | Immunization Dose (mg) | Adjuvant  | Wk  | Immunization Dose (mg) | Adjuvant  |
|-----|------------------------|-----------|-----|------------------------|-----------|-----|------------------------|-----------|-----|------------------------|-----------|
| 0   | 0.01 Al\(^{11}\)      | 0         | 0   | 3 FIA\(^{12}\)        | 1         | 0   | 3 FIA                  | 1         | 0   | 3 FIA                  | 1         |
| 1   | 0.05 Al                | 1         | 1   | Initial immunization (toxoid) | 1         | 1   | Initial immunization (toxoid) | 2         | 1   | Initial immunization (toxoid) | 2         |
| 2   | 0.1 Al (venom)         | 2         | 2   | 3 FIA                  | 2         | 2   | 3 FIA                  | 2         |
| 3   | 0.3 Al                 | 3         | 3   | 3 FIA                  | 3         |
| 4   | 0.8 Al                 | 4         | 4   | 4 FIA                  | 4         |
| 5   | 2.0 Al                 | 5         | 5   | 5 FIA                  | 5         |
| 6   | 6                      | 6         | 6   | 6 FIA                  | 6         |
| 7   | 7                      | Resting period | 7   | Resting period | 7  | Resting period  |
| 8   | 8                      |          | 8   | 8 FIA                  | 8         |
| 9   | 9                      |          | 9   | 9 FIA                  | 9         |
| 10  | Resting period         | 10        | 10  | 10 FIA                 | 10        |
| 11  | 11                     | 11        | 11  | 11 FIA                 | 11        |
| 12  | 12                     | 12        | 12  | 12 FIA                 | 12        |
| 13  | 13                     | 13        | 13  | 13 FIA                 | 13        |
| 14  | 1                      | 14        | 14  | 14 FIA                 | 14        |
| 15  | Booster immunization (toxoid) | 15  | 15  | 15 FIA                 | 15        |
| 16  | 2                      | 16        | 16  | 16 24 -                | 16        |
| 17  | 6 Al                   | 17        | 17  | Blood collection       | 17        |
| 18  | Blood collection       | 18        | 18  | 18                     |
| 19  |                         | 19        | 19  | 19                     |

\(^{11}\): Aluminum adjuvant.
\(^{12}\): Freund’s incomplete adjuvant (oil adjuvant).

The 3rd and subsequent doses were not administered because the horse developed colic and seemed not to tolerate further immunization.

Preparation of antivenom: Horse plasma with the highest titer level (Protocol 3; 14.8 L, 3,772 U/mL) was selected as the starting material for purification of the \(\gamma\)-globulin fraction according to the present production method for antivenom purification (13). Namely, the horse plasma was first digested with pepsin to remove the Fc portion from the \(\gamma\)-globulin molecule, and the F(ab'\(^2\)) fragment-rich fraction was purified through ammonium sulfate fractionation, dialysis, alum adsorption (removal of pepsin), and sterile filtration, and the final material was used as the purified antivenom concentrate. After a preliminary study confirming that the titer was maintained at small scale of bulk, the final bulk solution was prepared from approximately one quarter (1 L) of the purified antivenom concentrate by diluting it with saline to an appropriate concentration and adding sodium L-glutamate as a stabilizer (excipient) to a final concentration of 1.54\(^{(w/v)}\). After a preliminary study confirming that the moisture content was not increased by our setup conditions, a test lot of the freeze-dried redback spider antivenom was prepared from the final bulk solution by filling 2-mL glass vials with 0.65 mL antivenom solution, stoppering, and freeze-drying the vials (Lot No. 001, approximately 6,500 vials).

All unused portions of horse plasma and the purified antivenom concentrate were stored frozen at –20°C for production in the future.

Quality control test: Both the purified bulk of the redback spider antivenom concentrate and the test lot of freeze-dried antivenom were tested for the specifications regarding immunoglobulin content, sterility, pyrogen, potency, moisture content, pH, appearance, weight variation, foreign insoluble matter, insoluble particulate matter, osmotic pressure ratio, and freedom from residual proteolytic enzymes, adventitious virus, and abnormal toxicity, in accordance with the personal communication from bioCSL regarding redback spider antivenom, Minimum Requirements for Biological Products in Japan (14), Japanese Pharmacopoeia (15), and other published information (16) relevant to the present equine antivenom product (anti-snake venom). The potency test was carried out as described in the Materials and Methods.

Immunoglobulin content was tested by cellulose acetate membrane electrophoresis. The test sample was diluted with sodium diethylbarbiturate buffer solution (pH 8.6) to render the protein concentration of approximately 5% and then electrophoresed using a cellulose acetate membrane that was equilibrated in the above buffer solution as support medium. After electrophoresis, the membrane was stained with Ponceau 3R. Protein constituents and relative concentrations were analyzed by densitometry.

The mouse potency test was performed according to institutional guidelines and with the approval of the NIH Animal Care and Use Committee (Permit Number: 115035).

RESULTS

The redback spider antivenom preparation had a
neutralizing antibody titer (potency) of 1,055 U/mL in Protocols 1 and 2, and 3,772 U/mL in Protocol 3 at the end of the booster immunizations (Fig. 1). We collected blood from the horse that exhibited the highest titer with Protocol 3, purified the γ-globulin fraction using the same procedure as for our present antivenom drug product and obtained a purified antivenom concentrate (5,660 U/mL, 4.2 L). The recovery rate of antivenom in

Fig. 1. Production of venom-neutralizing antibodies under 4 horse immunization protocols.
- : neutralizing antibody titer; and ı: immunization.

Fig. 2. Analysis of redback spider antivenom by cellulose acetate membrane electrophoresis. Immunoglobulin peaks (γ-globulin and f(ab')2 fragment) are shown in the purified antivenom concentrate. (A) Plasma from immunized horses. (B) Purified antivenom concentrate.

Table 2. Recovery of potency in the purification process of redback spider antivenom

| Purification step | Volume (L) | Protein Conc. (mg/mL) | Recovery (%) | Potency (antibody titer) |
|------------------|------------|-----------------------|--------------|-------------------------|
|                  |            |                       |              | U/mL                    | Recovery (%) |
| Plasma           | 14.8       | 75.3                  | 100.0        | 3,772                   | 100.0        |
| Purified\(^1\)   | 4.2        | 62.1                  | 23.4         | 5,660                   | 42.6         |

\(^1\): Purified antivenom concentrate.
this process was 42.6% (Table 2). The quality test of this purified concentrate using cellulose acetate membrane electrophoresis demonstrated a high purity of γ-globulin (Fig. 2), and albumin was not detected. In addition, the material met all the pre-specified reference specifications (Table 3). The freeze-dried redback spider antivenom had a potency of 541 U/vial and met all the quality specifications, including reference specifications we had detailed (Table 3).

**DISCUSSION**

There is no previous report on equine immunization against spider venom in Japan, therefore we were uncertain about the most effective procedure for immunization of horses with LT. Various horse immunization methods reported globally have been inherited and have evolved into unique procedures at antivenom manufacturing sites in each country, based on researcher’ experience with each type of immunizing antigen (e.g., snake venom, bacterial toxin). The World Health Organization (WHO) and other researchers have reported various methods for horse immunization with snake venom, but no consensus has yet been reached (16–23). Furthermore, very limited information is available describing the immunization of mammals with spider venom, and the relevant information on redback spider venom was found in only a single report by Wiener in 1961 (12). The author used a mixture of native LT with aluminum adjuvant as the immunizing antigen. In the present study, we used the immunization protocol reported more than 50 years ago, as a control protocol, where the horse was given an initial immunization with 3 doses, followed by booster immunizations of up to 4 doses of native LT with aluminum adjuvant (Protocol 1), and obtained antibody production (1,055 U/mL) that was equivalent to the level reported in each country, based on researcher’ experience with each type of immunizing antigen (e.g., snake venom, bacterial toxin). The World Health Organization (WHO) and other researchers have reported various methods for horse immunization with snake venom, but no consensus has yet been reached (16–23).

| Product | Test | Specification | Reference | Test result |
|---------|------|---------------|-----------|-------------|
| Purified concentrate | Freedom from residual proteolytic enzyme | Not significant residual enzyme (≤ 3,200-fold in serial dilution) | Freeze-dried mamushi antivenom, equine | 100-fold |
| Immunoglobulin content | ≥ 95% of total protein<sup>1</sup> | | | 95%<sup>1</sup> |
| Sterility | No bacterial growth | | | No bacterial growth |
| Pyrogen | Total pyrogenic response of 3 tested animals ≤ 1.3°C | (14) General test | | 0.0°C |
| Freedom from adventitious virus | No adventitious virus-related cytopathic effects | Manufacturing and marketing approval application: Freeze-dried mamushi antivenom, equine | | No abnormal findings |
| Potency | ≥ 330 U/mL | Personal communication from bioCSL | | 5,660 U/mL |
| Protein content | (–) | In-house; Freeze-dried mamushi antivenom, equine | | 62.1 mg/mL |
| Aluminum content | (–) | | | 0.708 ppm |
| Test lot | Moisture content | ≤ 3.0% | | 0.3% |
| pH | 6.8–7.4 | | | 7.0 |
| Appearance | Clear slightly white turbidity, colorless to light yellow-brown liquid | | | Slightly opalescent colorless liquid |
| Sterility | No bacterial growth | | | No bacterial growth |
| Freedom from abnormal toxicity | No abnormal findings in any test animals | | | No abnormal findings |
| Pyrogen | Total pyrogenic response of 3 tested animals ≤ 1.3°C | (14) General test | | 0.1°C |
| Weight variation | 0 or 1 sample with a variation > 7% AND no sample with a variation > 2-fold | | | 0 sample with a variation > 7% |
| Foreign insoluble matter (Method 2) | No apparent foreign insoluble matter | | | No foreign insoluble matter |
| Insoluble particulate matter (Method 2) | ≥ 10 μm, ≤ 3,000 μm/vial | (15) General test | | 0 |
| | ≥ 25 μm, ≤ 300 μm/vial | | | 0 |
| Potency | ≥ 500 U/vial | Personal communication from bioCSL | | 541 U/vial |
| Osmotic pressure ratio | 0.8–1.7 | | | 1.2 |
| Protein content | (–) | In-house; Freeze-dried mamushi antivenom, equine | | 8.1 mg/mL |
| Endotoxin | (–) | | | < 0.020 EU/mL |
| Dissolution time | (–) | | | 12 s |

<sup>1</sup>: In case of no albumin recognized by cellulose acetate membrane electrophoresis, we determined that it meets the reference specification of immunoglobulin content.
in the original paper (680 U/mL), demonstrating reproducibility. To induce higher titer levels, it is important to investigate in detail the immunization protocol, including type of antigen (venom vs. toxoid), antigen purity, type of adjuvant, dosage of immunization, and frequency and intervals of immunization, while taking into account the sensitivity (resistance) of the horse against the venom. However, in reality, these various conditions are not easily assessed in horses. Instead, based on our experience with the improvement of antivenom manufacturing methods, we used FIA-containing toxoid as the antigen for all doses (Protocol 3), which allowed for safe frequent (up to 6 times) ascending toxoid booster administrations, while causing little pain, stress, or agitation to the horse. A relatively higher antibody titer (3,772 U/mL) was obtained by this protocol. These results suggest that our conventional horse immunization procedure for bacterial protein toxins was applicable even to horse immunization with snake venom.

Subsequently, we purified the γ-globulin fraction and manufactured the test lot of antivenom (approximately 6,500 single-dose vials, 541 U/vial) according to the current manufacturing process (13) from a portion of the plasma with a higher neutralizing antibody titer. This test lot was equivalent to be derived from the amount of 520 spiders. We successfully confirmed that this test lot had a quality similar to the present antivenom drug product (bioCSL), meeting the Minimum Requirements for Biological Products in Japan and other guidelines. These results indicated that the current manufacturing process is applicable to the purification and formulation of antivenom against redback spiders with highly efficient productivity that is capable to support mass production of antivenom against redback spiders on an industrial scale.

This test lot had a potency (541 U/vial) equivalent to the only commercial product in the world (bioCSL) along with the safety profile and physicochemical properties similar to those of the existing antivenom drug products commercially available in Japan. As explained above, our test lot of antivenom against redback spider venom appeared to be of quality that was fully comparable to existing commercial antivenom drug products against bacterial toxins and snake venoms.

Further studies are warranted to improve the manufacturing process to provide better yield, considering recent reports, describing a new non-mineral adjuvant that can minimize burden on the horses (24,25) and describing a stabilizer for freeze-drying of antivenom (26).

Stability monitoring has been ongoing, and once long-term stability monitoring of the test lot shown to be at a similar level as that of the existing antivenom drug products (shelf life of 10 years), and thus, the nation’s stockpile of antivenom of redback spider will be ensured for at least 10 years. From now on, it is necessary to continue the stability test over time. Following pre-clinical studies to sufficiently confirm product safety and approval by the ethics committee, we can proceed with clinical research for the treatment of human victims of redback spider bites that have occurred domestically.

With regard to clinical safety issues (adverse reactions), we are aware of several cases of adverse events that have been reported on the current commercial antivenom product derived from horse (27), and we also expect serum sickness and other issues that are unique to antivenoms. From an efficacy perspective, there are reports that the overseas commercial product was effective in patients with bites from not only redback spider but also other spider species (28–35). However, there are also conflicting reports that the use of antivenom is not significantly effective in treating patients with bites when an analgesic is used in combination or when an analgesic is the primary treatment choice (36,37). Therefore, future clinical applications should take these aspects into account.

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Conflict of interest S. Mori, A. Horita, A. Ginnaga, and Y. Miyatsu are employees of KAKETSUKEN.

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