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【 ORIGINAL ARTICLE 】

Evaluation of a Treatment Algorithm for Tsushima Mamushi (*Gloydius tsushimaensis*) Snakebites, after Its Introduction to Tsushima Island, Nagasaki, Japan

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Abstract:

Objective Tsushima mamushi (*Gloydius tsushimaensis*) is an endemic species of snake inhabiting only Tsushima Island, a remote Japanese island. We conducted a multicenter, retrospective study of *G. tsushimaensis* bites for the first time and developed a treatment algorithm that unified treatment on the island and is still in use today.

Methods This is a multicenter, retrospective study comparing 72 cases from January 2005 to December 2018, before the introduction of the algorithm, and 12 cases from January 2019 to December 2020, after its introduction.

Results There was no significant decrease in the maximum grade of symptoms after the introduction of the algorithm, but there was a decreasing trend (p=0.057). Conversely, the median of the maximum creatinine kinase levels was 343.5 IU/L (interquartile range: 115.5-4,745.5) before the algorithm's introduction and significantly lower (142.5; interquartile range: 111.3-163) after the algorithm's introduction (p=0.042). After the algorithm's introduction, the disseminated intravascular coagulation merger rate and the acute kidney injury incidence both dropped to 0%, from 9.7% and 6.9%, respectively, before the algorithm's introduction. There was no significant difference in the length of hospital stay before versus after the algorithm's introduction.

Conclusion This study showed that the treatment algorithm can be safely and quickly applied. The algorithm's effectiveness is expected to be strengthened by the accumulation of more cases in the future.

Key words: *Gloydius*, mamushi, antivenom, snakebite, retrospective study, treatment strategy

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Introduction

Snakebites worldwide have the potential to be life-threatening, even in the modern age of medicine. According to the World Health Organization, 4.5 to 5.4 million people are bitten by snakes each year, and of those, 81,000 to 138,000 die of complications (1). This is a serious problem, especially in developing countries with a tropical climate (2-4). The universally accepted keys to treatment are early consultation, systemic management, and proper antivenom use.

Gloydius blomhoffii snakes are the most common venomous snakes in Japan and inhabit a wide range from Hokkaido to Kyushu. The reported incidence of bites by *G. blomhoffii* is approximately 1,000 cases with 10 deaths annually (5). The primary treatments are the administration of antivenom, cepharanthine (CEP), and a combination of these, at the discretion of physicians. Although animal experiments have shown that antivenom neutralizes *G. blomhoffii* venom, there are concerns about adverse effects, such as anaphylaxis. Alternatively, although CEP has inhibitory effects on the phospholipase A2 in *G. blomhoffii* venom, a stabilizing effect on biological membranes, and anti-

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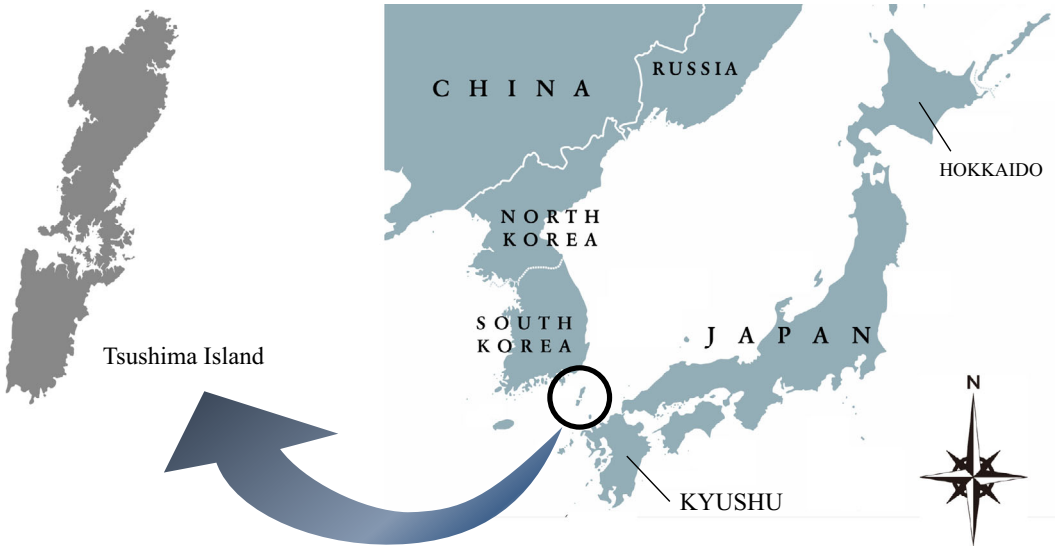

Figure 1. Location of Tsushima Island. Tsushima is an isolated island located in the northwest of Kyushu, Japan.

inflammatory effects, CEP cannot neutralize the venom (6). No reliable treatment for *G. blomhoffii* bites has yet been established.

The situation is more complicated in Tsushima, which is an isolated island that requires treatment for a venomous snake specific to this region. Tsushima is located in the northwest of Kyushu, Japan (Fig. 1), and the venomous snake that lives on Tsushima is not *G. blomhoffii* but instead *G. tsushimaensis.*

In 2011, Takahashi et al. (7) studied the difference in virulence between the venoms of *G. tsushimaensis* and *G. blomhoffii* using mice and rabbits as test subjects. The authors reported that the 50% lethal dose of *G. tsushimaensis* venom was approximately half that of *G. blomhoffii* venom, whereas the minimum hemorrhagic dose of *G. tsushimaensis* venom was approximately 1/100 that of *G. blomhoffii* venom. In addition, the study suggested that *G. blomhoffii* venom was almost completely neutralized by the antivenom, whereas the *G. tsushimaensis* venom was only partly neutralized. It is not realistic to develop an antivenom specific to *G. tsushimaensis* snakes, which live only on Tsushima Island; therefore, the antivenom for *G. blomhoffii* is still employed to treat patients with *G. tsushimaensis* bites.

No studies have developed and evaluated treatment algorithms from retrospective studies for rare venomous snakes. Therefore, we conducted the world's first multicenter, retrospective study of *G. tsushimaensis* bites, clarified the clinical features of these bites, and developed a therapeutic strategy algorithm based on cases that occurred on the island from 2005 to 2018 (Fig. 2). We clarified the criteria for hospitalization and discharge. We were also challenged to unify the indications and methods of use for multiple therapeutic agents such as antivenom, CEP, tetanus toxoid, tetanobulin, antibiotics, and premedication for the prevention of anaphylaxis. Two years have passed since the algorithm was applied in a unified manner on the island, and additional cases have since accumulated; thus, we evaluated the effectiveness of the algorithm.

Materials and Methods

Study population

Tsushima Island has a population of approximately 30,000 people. No major changes have occurred in the population or lifestyle from before to after introduction of the algorithm. Approximately 90% of Tsushima Island is deeply forested, thus the only endemic viper species, *G. tsushimaensis*, which inhabits forests, rice fields and the edges of swamps, is often encountered. This was a multicenter, retrospective study comparing 72 cases from January 2005 to December 2018 (before introducing the algorithm), with 12 cases from January 2019 to December 2020 (after introducing the algorithm). The clinical data were collected from the medical records in Tsushima Hospital and Kami-tsushima Hospital. The Tsushima Hospital Ethics Committee approved the study protocol.

Systemic management

Instructions regarding the treatment algorithm were provided to all doctors and nurses involved with the treatment of patients in the emergency departments of both hospitals. Furthermore, we established the order of diagnostic tests and drugs so that anyone could execute the algorithm. Four vials of antivenom are always available at Tsushima Hospital, and three vials are always available at Kami-tsushima Hospital. Antivenom has never been out of stock; it can be transferred between hospitals, and a system for next-day delivery from outside the island is available. To ensure accurate implementation of the algorithm, we gave a lecture on *G. tsushimaensis* bites in the spring season, when medical staff are re-

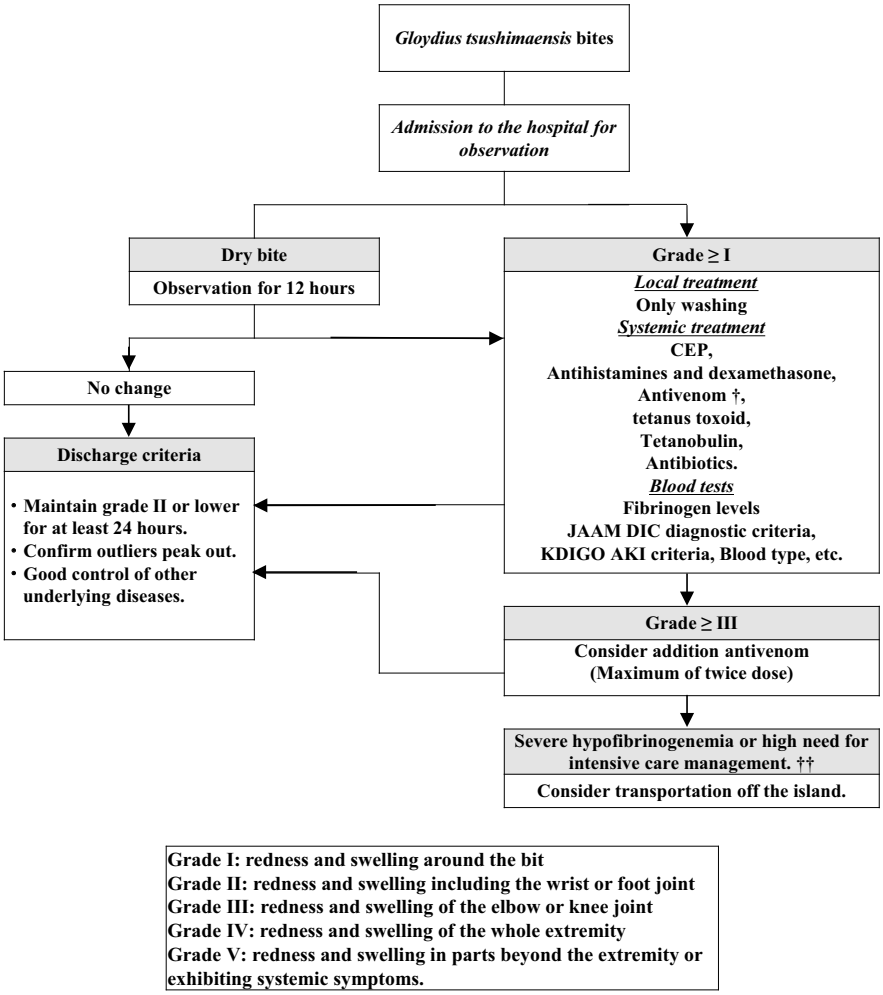

Figure 2. **Original *Gloydius tsushimaensis* treatment algorithm. † Six thousand units of antivenom are administered to patients who have reached, or are expected to reach, Grade II within less than 6 hours of injury. †† When intensive care management, such as hemodialysis, ventilation, strict observation and monitoring is required.**

placed due to transfer. In addition, because the purpose of the algorithm was to unify treatment over the entire island, it was necessary to avoid surgical procedures; thus, local treatment was limited to washing the wound.

Records

The following information was retrieved from the clinical records: demographic data (number, sex, mean age, and age range of patients), time to hospital visit, bite location, symptoms of envenomation (local swelling, diplopia, blurred vision, and nausea and vomiting), laboratory data [white blood cell count, platelet count, blood urea nitrogen concentration, creatinine concentration, creatine kinase (CK) concentration, fibrinogen degradation product concentration, prothrombin time - international normalized ratio, and fibrinogen concentration], treatment (CEP, antivenom, combination of CEP and antivenom, and symptomatic treatment), time from injury to administration of antivenom, adverse effects of antivenom (anaphylaxis and other), duration of hospital stay (days), and severity (grade) of the *G. tsushimaensis* bite symptoms. Dry bites were defined as those that had no symptoms. The symptom grades were evaluated using the classification method proposed by Sakio (8), as follows: Grade I, redness and swelling only around the bite; Grade II, redness and swelling including the wrist or ankle joint; Grade III, redness and swelling extending from the hand to the elbow, or from the ankle to the knee; Grade IV, redness and swelling of the whole extremity; and Grade V, redness and swelling beyond the extremity, or with any systemic symptoms.

Diagnoses and treatments

No diagnostic biomarkers or treatment methods specific for *G. tsushimaensis* bites are currently available in clinical practice. Based on the fact that there are no venomous snakes, other than *G. tsushimaensis* in Tsushima Island, we diagnosed *G. tsushimaensis* bites when a patient presented with a swollen limb after a bite from an unidentified snake or with characteristic bite marks (a pair of bite scars approximately 1 cm apart).

Antivenom therapies were performed using *G. blomhoffii* antivenom, which has manufactured by immunizing horses

Table 1. Comparison of Baseline Characteristics for Cases from 2005-2018 (before Algorithm) and from 2019-2020 (after Algorithm).

	Before (2005-2018)	After (2019-2020)	p value
Variable	n (%), Median [IQR]	n (%), Median [IQR]	
Total number of patients	72	12	
Sex of patients, female (%)	33 (46)	4 (33)	0.536
Mean patient age in years (range)	61.2±8.9 (range: 14-92)	73.1±8.3 (range: 61-86)	0.041
Number and percentage of patients in each of 8 age ranges			0.437
10≤Age≤19	3 (4.2)	0 (0)	
20≤Age≤29	3 (4.2)	0 (0)	
30≤Age≤39	4 (5.6)	0 (0)	
40≤Age≤49	7 (9.7)	0 (0)	
50≤Age≤59	10 (13.9)	0 (0)	
60≤Age≤69	17 (23.6)	4 (33.3)	
70≤Age≤79	16 (22.2)	4 (33.3)	
Age≥80	12 (16.7)	4 (33.3)	
Time to hospital visit (min)	60 [50-116.3]	60 [53.8-80]	0.464
Bite location			0.841
Upper limb	44 (61.1)	9 (75)	
Lower limb	27 (37.5)	3 (25)	
Other	1 (1.4)	0 (0)	

IQR: interquartile range

against *G. blomhoffii* venom (manufactured by KM Biologics, in Kumamoto, Japan; https://www.kmbiologics.com/en/). CEP, antihistamines, antibiotics, tetanus toxoid, and tetanobulin were administered in some cases. Before the introduction of the algorithm, the timing of antivenom administration was irregular, but after the introduction, it was administered to all patients with Grade ≥II symptoms. Disseminated intravascular coagulation (DIC) was diagnosed using the acute DIC diagnostic criteria (9). Acute kidney injury (AKI) was diagnosed using the Kidney Disease Improving Global Outcomes criteria (10). Patients with a fibrinogen level below 100 mg/dL were considered to have severe hypofibrinogenemia.

Statistical analyses

Descriptive statistics were expressed as median (interquartile range; IQR) for continuous variables, and as frequency (percentage) for categorical or ordinal variables. We compared cases from 2005-2018 (before algorithm) and from 2019-2020 (after algorithm) with regards to age, sex, time to hospital visit (min), maximum symptom grade during hospitalization laboratory data, treatment method and the period of hospitalization. Mann-Whitney U-tests were used for continuous variables and Fisher's exact tests for categorical or ordinal variables. Two-sided $p<0.05$ was considered to be statistically significant. All data were analyzed with the SPSS Statistics software program for Windows, Version 25.0 (IBM, Armonk, USA).

Results

Baseline clinical characteristics

Table 1 compares the demographic characteristics of patients and the profile of the snakebites between cases before (2005-2018) and after (2019-2020) the introduction of the treatment algorithm. There was a significant difference between groups in the average age ($p=0.041$) but no significant differences in the sex ratio, age distribution, time to visit, or injury site.

Symptoms, laboratory data, treatment method and duration of hospital stay

As shown in Table 2, "dry" (i.e. non-toxic) bites were observed both before and after the introduction of the algorithm, but no worsening of symptoms was observed. Before the introduction, 10 cases of diplopia (13.9%), 3 of blurred vision (4.2%), and 2 of nausea and vomiting (2.8%) were observed as systemic symptoms (each in a different individual), but no systemic symptoms were observed after the introduction. There was no significant difference in the maximum grade before versus after the introduction, but after the introduction, the percentage of patients with a maximum of Grade ≥III tended to be lower than before the introduction. The median of the maximum CK levels was 343.5 (IQR: 115.5-4,745.5) IU/L before the introduction and was significantly lower [142.5 (IQR: 111.3-163) IU/L], after the introduction ($p=0.042$). The DIC complication rate, incidence of

Table 2. Comparison of Clinical Features for Cases from 2005-2018 (before Algorithm) and from 2019-2020 (after Algorithm).

	Before (2005-2018)	After (2019-2020)	p value
Variable	n (%), Median [IQR]	n (%), Median [IQR]	
Clinical manifestation			
Dry bite	8 (11.1)	1 (8.3)	1.000
Local swelling	64 (88.9)	11 (91.6)	1.000
Diplopia	10 (13.9)	0 (0)	0.344
Blurred vision	3 (4.2)	0 (0)	1.000
Nausea and/or vomit	2 (2.8)	0 (0)	1.000
Max symptom grade			0.057
I	26 (36.1)	2 (16.7)	
II	10 (13.9)	6 (50.0)	
III	8 (11.1)	1 (8.3)	
IV	4 (5.6)	1 (8.3)	
V	16 (22.2)	1 (8.3)	
Laboratory data			
Acute Kidney Injury	5 (6.9)	0 (0)	1.000
Disseminated Intravascular Coagulation	7 (9.7)	0 (0)	0.586
Maximum creatinine kinase IU/L (includes dry bites)	343.5 [115.5-4745.5]	142.5 [111.3-163]	0.042
Severe hypofibrinogenemia (<50 mg/dL)	5 (6.9)	0 (0)	1.000
Treatment			
Cepharanthine (CEP) only	23 (31.9)	3 (25.0)	0.746
Antivenom only	12 (16.3)	0 (0)	0.200
CEP and antivenom combination	11 (15.3)	8 (66.7)	<0.001
Symptomatic treatment only	26 (36.1)	1 (8.3)	0.092
Time from injury to antivenom injection (hours)	3 [1.5-8.0]	2 [1.9-2.8]	0.246
Time from injury to antivenom injection (≥6 hours)	8 (34.8)	0 (0)	0.0746
Adverse effect of antivenom			
Anaphylaxis	1 (1.4)	0 (0)	1.000
Other	2 (2.8)	1 (8.3)	0.374
Outcome			
Death in hospital (double bites)†	1 (1.4)	0 (0)	1.000
Period of hospital stay (days)	3.0 [2.0-8.0]	4.5 [2.0-5.3]	0.845

IQR: interquartile range

† A patient whose legs were bitten twice by one *Gloydius Tsushimaensis* at a time. He died on the 5th of hospitalization due to multiple organ failure caused by renal failure, severe rhabdomyolysis, severe hypofibrinogenemia, and disseminated intravascular coagulation.

AKI, and incidence of severe hypofibrinogenemia were 9.7%, 6.9%, and 6.9%, respectively, before introduction, and all were 0% after introduction. Before the introduction, CEP alone was used in 31.9% of cases, antivenom alone in 16.3%, CEP and antivenom combination therapy in 15.3%, and symptomatic treatment in 36.1% of cases. However, after the introduction, combination therapy was predominant, at 66.7%, with CEP monotherapy used in 25% of cases and symptomatic treatment in 8.3%. No significant reduction was obtained in the time from injury to antivenom administration. However, before the introduction, 8 out of 23 (34.8%) patients received antivenom more than 6 hours after the injury, but after the introduction, all patients were treated within 6 hours of the injury. The median time from injury to antivenom injection was 3 (IQR: 1.5-8.0) hours before the introduction of the algorithm and 2 (IQR: 1.9-2.8) hours after introduction of the algorithm (p=0.246). The first antivenom injection tended to be earlier after the introduction, but the difference was not statistically significant.

No anaphylaxis occurred in any patients who underwent premedication to prevent anaphylaxis. Side effects after the administration of antivenom, other than anaphylaxis, were pruritus and tachycardia before the introduction and systemic erythema after the introduction, but all were transient. There was no significant difference in the length of the hospital stay before and after the introduction. The 1 patient (1.4%) who died was an 84-year-old man whose legs had been bitten twice by a *G. tsushimaensis*. Due to the slow progression of local swelling and the systemic symptom of vomiting being overlooked, the first administration of antivenom was significantly delayed by 22 hours after injury. He had severe hypofibrinogenemia, a maximum CK value of

50,973 IU/L, DIC, and AKI. He died on the fifth day in the hospital due to multiple organ failure.

Discussion

The purpose of this study was to establish a treatment strategy for *G. tsushimaensis* bites. In general, snake venom is a cocktail venom with multiple toxins, and antivenom is also a multivalent antibody. Therefore, antivenom is expected to be effective against multiple closely related species. For example, only three species of the genus *Gloydius* (*G. brevicaudus*, *G. saxatilis*, and *G. ussuriensis*) are found in Korea. A freeze-dried antivenom (manufactured by Korea Vaccine; http://www.koreavaccine.com) has shown some efficacy despite being developed in horses against the Chinese species *A. halys* (11). Therefore, in developing the algorithm, Korean reports and animal experiments (7) were used as a basis for expecting *G. blomhoffii* antivenom to be effective against *G. tsushimaensis* bites.

It is also important to understand the similarities and differences between *G. blomhoffii* and *G. tsushimaensis*. There is no marked difference in the case fatality rate between *G. blomhoffii* and *G. tsushimaensis*. *G. blomhoffii* venom has more hemolytic activity than neural activity. Therefore, it causes local swelling and pain via hemorrhagic necrosis and vascular permeability enhancement (12-14). Clinical symptoms suggest that there are similar constituents in *G. tsushimaensis* venom. In addition, cases with systemic symptoms are very severe because of the impact of the circulating venom. In a study by Chiba et al. (15), a significantly higher CK concentration was found in patients with Grade V symptoms than Grade I to IV. Severe rhabdomyolysis is associated with AKI. Our previous study showed a significant difference in the relationship between the maximum CK concentration and the duration of hospitalization for each grade; patients with Grade V had the highest maximum CK concentration and the longest hospital stays (16).

One difference between the two snakebites is that *G. tsushimaensis* venom causes severe hypofibrinogenemia sometimes, which is likely to cause pro-coagulation, resulting from a specific toxin. Severe hypofibrinogenemia was observed in 5 of 84 cases (6.9%) in our previous study, all of which were complicated by DIC (16), and the only death was the result of severe hypofibrinogenemia. There is no specific treatment for severe hypofibrinogenemia, so treatment is limited to symptomatic management. Strict monitoring is required, as a fatal condition, such as cerebral hemorrhaging, can suddenly develop. Therefore, off-island transport should be considered for severe hypofibrinogenemia.

There are various opinions about the timing of antivenom administration. In recent years, antivenom administration to cases exceeding Grade II in severity has increased in Japan and South Korea (11,14,17). In addition, administration of antivenom within 3 hours reportedly leads to a decrease in the incidence of systemic symptoms and the severity of organ damage (18, 19), and administration within 6 hours can be expected to shorten the duration of hospitalization (20). Therefore, early administration of antivenom is expected to lead to a desirable outcome.

The most notable adverse effect of administering antivenom is anaphylaxis. In the present study, anaphylaxis did not occur in any of the patients who were administered antivenom after premedication (antihistamines and steroids), so a beneficial effect of premedication can thus be expected.

There are three signs of the effectiveness of the algorithm after its introduction. First, after the introduction, the algorithm was applied safely. Second, all patients who received antivenom after the introduction of the algorithm were given the antivenom within 6 hours, and the median duration of time until the initial injection was decreased by 1 hour. Shortening the timing of antivenom injection may have contributed to the downward trend of the maximum grade. Third, the maximum CK value, which correlates with severity, was significantly reduced.

Although the algorithm has been introduced and the clinical guidelines have been clarified, education for hospital staff members as well as the general public is also important. We also recognized the importance of fostering citizen awareness and conducted educational activities using the mass media (21).

However, several limitations associated with the present study warrant mention. First, the sample size was small, particularly for the after-introduction group, and treatments and laboratory examinations were inconsistent. Caution should be practiced when generalizing the study findings to other institutes or other countries. Second, various factors, such as the sex, age, and diet of a snake, are said to affect the composition and pathophysiological impact of snake venom (22). However, these factors were not considered in this study.

There are various recommendations for treating bites from venomous snakes and administering antivenom, but evidence concerning the effectiveness of treatment of *G. tsushimaensis* bites in clinical practice is still weak. Larger and more-rigorous prospective, multi-institutional studies on the appropriate management of venomous snakebites are necessary. Nevertheless, we believe this report will prove a useful resource for physicians in the field who have been uncertain regarding the correct diagnosis and treatment of *G. tsushimaensis* bites. We hope that the effectiveness of the algorithm will be strengthened by accumulating more cases in the future. We believe that this report is the world's first study to evaluate the correct diagnosis and treatment of rare *G. tsushimaensis* bites and that it will prove a useful resource for the treatment of bites by *Gloydius* species that are widely distributed in Asia, including Russia.

Conclusion

This is the first study to evaluate an algorithm for the treatment of *G. tsushimaensis* bites, which has now been employed for the past two years. The algorithm was applied

in all recent cases, and antivenom was administered in 8 of 12 cases. There were no cases of anaphylactic reaction after the administration, and antivenom was administered safely and promptly. Although no significant reduction in hospital stay was achieved, the complication rates of AKI, DIC, and severe hypofibrinogenemia all dropped to zero, and the maximum CK value was significantly reduced. Thus, we confirmed the efficacy and safety of the treatment algorithm, which we plan to continue studying.

The authors state that they have no Conflict of Interest (COI).

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