



RESEARCH ARTICLE

Preliminary Discussion of the Distribution Characteristics of Venomous Snake Species in One City and Four Counties in Guangxi and the Diagnosis and Treatment Strategies of Snakebite based on the Classified Evidence Method

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Abstract

Objective: From the perspective of clinical diagnosis and treatment of snakebite and combined with existing zoological data, we aimed to characterize the distribution of snake species in one city and four counties in Guangxi and make regional snakebite diagnostic charts.

Methods: Geolocation data of the research region and snake distribution were collected from the municipal and county governments and referring to authoritative zoological monographs, respectively. We characterized the distribution of venomous snake species using hierarchical evidence method.

Results: 92 species of snake (25 poisonous and 67 non-poisonous snakes) were identified according to zoological monographs, which belong to 40 genera, 4 subfamilies and 6 families. There were 15 clinical specimen evidences of grade I_a venomous snakes, which were divided into 3 families, 3 subfamilies, 8 genera and 9 species of snake, and there were 35 research specimen evidences of grade I_b venomous snakes, which were divided into 3 families, 3 subfamilies, 10 genera and 11 species. The survey covered one city, four counties, 31 towns, 13 townships and 207 villages. 1,001 pieces of grade II evidence were collected, and 20 species of distributed snake were confirmed to be venomous. In the long-term clinical practice, 233 class III

clinical picture evidences had been collected by the snakebite treatment base in north and central of Guangxi, by which 11 species of venomous snake were confirmed. Finally, confirming the distribution of venomous snake species in one city and four counties in Guangxi by Integrating level I, II and III evidence.

Conclusion This study indicated that the distribution characteristics of venomous snake species according to the counties and cities provided strategies for snakebite diagnosis and treatment.

Keywords

Distribution of venomous snake, A book of regional diagnosis of snakebite, Diagnosing of snakebite quickly and accurately

Introduction

Toxicological mechanisms of venomous snake zoology vary largely. Obvious differences existed in the clinical manifestations of snakebite and targeted treatment methods. There are a total of 240 species of snake in China, including 69 species of venomous snake and 171 species of non-venomous snake [1]. Due to the different climatic conditions and geographical features of

different regions in China, there are significant differences in the distribution of snake species, which determines regional characteristic of the treatment of snakebite. Snakebite most commonly occurs in counties and prefecture-level cities, and the patients generally seek medical treatment in county and municipal hospitals. However, the materials of snake zoology distribution in these regions are lacking, so the cure rate of snakebite is low. Consequently, it is of significant importance to diagnose accurately and treat directly of snakebite on the basis of snake zoology classification.

At present, zoology literature related to snake in China is snake distribution materials in each province. However, relatively little is known about the snake zoology distribution in each city and county [2]. The distribution of snake species varies greatly in different area of the same province. Medical staffs in prefectural and county-level hospitals have no authoritative material of the distribution of local snake species and have little knowledge of snakes, which leads to the lack of pertinence and poor curative effect in the diagnosis and treatment of snakebite. There is no objective theoretical basis for constructing a regional snakebite treatment network centered on prefecture-level hospitals. Therefore, we aim to characterize the distribution of snakes in counties and cities from the perspective of clinical diagnosis of snakebite.

Materials and Methods

Research region

The research region covered Liuzhou City and four surrounding counties of Guangxi Province (Liujiang County (district), Luzhai County, Liucheng County and Xiangzhou County), which are located in the central and northern part of Guangxi Zhuang Autonomous Region, between latitude 23°54' ~ 24°26' and longitude 108°54'~110°12', with an area of 10,624 square kilometers and a population of 2,942,700 (data of statistics bureau in 2018). The region has a subtropical monsoon climate, with an annual average temperature of 20.5 °C, with a sufficient sunlight with an annual average sunshine of 1634.9 hours and abundant rainfall with an annual average rainfall of 1450 mm. The landform of the region is characterized with typical karst landform features of many gentle hills, platforms and small plains, with a relative low altitude (85 and 105 meters) [3]. The ecological environment of the research region is excellent, with four season's evergreen and vigorous vegetation. Such natural conditions are suitable for the growth and reproduction of snakes.

Distribution of snakes

We obtained the geographic information, including longitude and latitude, vertical distribution and distribution province of each species of snake from authoritative monograph *Chinese Snakes*, consisting of 205 species of snake [4]. Referring to province, geography, latitude,

longitude and average altitude of the research region, we framed the zoological distribution of snake species in this area. In this study, sea snakes were excluded owing to the absent information of the geographical features of the sea region.

Survey map and questionnaire of snake species distribution

We obtained the appearance and morphology information of each snake and then accomplished a survey map of snake species in the research region by referring to the monograph of *Chinese Snakes* and searched pictures of snake species on the Internet.

The questionnaire was designed according to the name of the snake species. The questionnaire included the information of the interviewees and the snake species in the corresponding research area.

We applied and obtained the snake catching license from Liuzhou forestry administration department for the reason of clinical scientific research of snakebite. Live snake specimens were collected and identified in Liuzhou city and counties and one snake of each species was preserved as the evidence material.

Collection of Grade I evidence: In the clinical practice of the snakebite treatment base in north and central of Guangxi, we collected those snake specimens which were killed and brought to the hospital by snakebite patients as clinical specimen evidence I_a. The clinical information included snake name, patient name, bite time, bite site, bite address, bite reason and telephone.

After consulting local professional and semi-professional snake catchers in the process of carrying out questionnaire survey in districts, towns and townships under the jurisdiction of cities and counties, we collected contact information of the snake catchers to build snake catching relationship network through We Chat platform. The snake catchers were requested to collect one wild snake from each species in research area where he or she lived. Live snakes we collected saved as scientific research specimens of evidence I_b. Relative information were recorded including specimen collection address, altitude, longitude and latitude, and specimen collection time.

Collection of Grade II evidence: A questionnaire survey was conducted in the research region to collect the information of the snake species that they had seen. The interviewees were mainly local farmers, professional snake catchers and semi-professional snake catchers. Snake pictures for the reference snake species were provided as visual aids. The information of collected questionnaires were summarized as grade II evidence of snake species distribution.

Collection of Grade III evidence: After being bitten by snakes, some patients would use their phones to take pictures of the offending snakes to the snakebite base to identify their species. The specialist of snakebite

Table 1: Family, Genera and Species in the Research Region.

Family (Sub)	Genus	Chinese Name	Common Name(E)	Latin Name	Type of tooth	Toxicity
Viperidae						
Azemiopinae	Azemiops	白头蝰	White-headed Burmese Viper	Azemiops Kharini	Solenoglyphic tooth	Highly toxic
Crotalinae	Deinagkistrodon	尖吻蝮 (五步蛇)	Five-pacer Viper	Deinagkistrodon acutus	Solenoglyphic tooth	Highly toxic
	Ovophis	山烙铁头蛇	Chinese Mountain Pit Viper	Ovophis monticola	Solenoglyphic tooth	Highly toxic
	Protobothrops	原矛头蝮	Brown spotted pitviper	Protobothrops mucrosquamatus	Solenoglyphic tooth	Highly toxic
		菜花原矛头蝮	Jerdon's Pitviper	Protobothrops jerdonii	Solenoglyphic tooth	Highly toxic
	Trimeresurus	白唇竹叶青蛇	White-lipped Pitviper	Trimeresurus albolabris	Solenoglyphic tooth	Highly toxic
	Viridovipera	福建绿蝮	Chinese Green Tree Viper	Viridovipera stejnegeri	Solenoglyphic tooth	Highly toxic
Viperinae	Daboia	泰国圆斑蝮	Eastern Russel's Viper	Daboia siamensis	Solenoglyphic tooth	Highly toxic
Elapidae						
Elapinae	Bungarus	金环蛇	Banded Krait	Bungarus fasciatus	Proteroglyphic tooth	Highly toxic
		银环蛇	Many-banded Krait	Bungarus multicinctus	Proteroglyphic tooth	Highly toxic
	Naja	舟山眼镜蛇	Chinese Cobra	Naja atra	Proteroglyphic tooth	Highly toxic
		孟加拉眼镜蛇	Monocled Cobra	Naja kaouthia	Proteroglyphic tooth	Highly toxic
	Ophiophagus	眼镜王蛇	King Cobra	Ophiophagus hannah	Proteroglyphic tooth	Highly toxic
	Sinomicrurus	福建华珊瑚蛇	Kellogg's Coral Snake	Sinomicrurus kelloggi	Proteroglyphic tooth	Highly toxic
		中华珊瑚蛇	MacClelland's Coral Snake	Sinomicrurus maclellandi	Proteroglyphic tooth	Highly toxic
Colubridae						
Ahaetuliinae	Ahaetulla	绿瘦蛇	Oriental Whipsnake	Ahaetulla prasina	Opisthoglyphic tooth	Low toxic
Colubrinae	Boiga	广西林蛇	Guangxi cat snake	Boiga Guangxiensis	Opisthoglyphic tooth	Low toxic
		繁华林蛇	Many-spotted Cat Snake	Boiga multomaculata	Opisthoglyphic tooth	Low toxic
		绞花林蛇	Kelung Cat Snake	Boiga kraepelini	Opisthoglyphic tooth	Low toxic
Natricinae	Rhabdophis	红脖颈槽蛇	Red-necked Keelback	Rhabdophis subminiatus	Duvernoy's gland	Highly toxic
		虎斑颈槽蛇	Tiger Keelback	Rhabdophis tigrinus	Duvernoy's gland	Highly toxic
Homalopsidae						
	Hypsiscopus	铅色蛇	Boie's Mud Snake	Hypsiscopus plumbea	Opisthoglyphic tooth	Low toxic
	Myrrophis	中国沼蛇	Chinese Mud Snake	Myrrophis chinensis	Opisthoglyphic tooth	Low toxic
		黑斑沼蛇	Bennett's Mud Snake	Myrrophis bennettii	Opisthoglyphic tooth	Low toxic
Lamprophiidae						
Pseudaspidinae	Psammodynastes	紫砂蛇	Common Mock Viper	Psammodynastes pulverulentus	Opisthoglyphic tooth	Low toxic

Table 2: Clinical Specimen Evidence I_a

Latin Names	Specimen NO.	Patient Occupation	Bite Site	Bite time	Bite Address	Bite Reason
<i>Azemiops kharini</i>	LYRJZ 2	Farmer	The root of left index finger	2016/5/9	Tianhe Town, Luocheng County, Hechi City	Mowed beside the field
	LYRJZ 70	Farmer	Right foot	2017/10/20	Rongshui, Miao Autonomous County	On the farm
<i>Ovophis monticola</i>	LYRJZ 14	Farmer	Left ring finger	2016/8/8	Miaohuang Town, Xiangzhou County	On the farm
	LYRJZ 89	Farmer	Left heel	2018/12/8	Zhongdu Village, Luzhai County	Walked up the hill looking for honey and stepped on a snake and getting bitten
<i>Protophrops mucrosquamatus</i>	LYRJZ 3	Farmer	Second toe of left foot	2016/10/3	Pingshan Town, Luzhai County	Watered in garden and stepped on poisonous snakes
	LYRJZ 17	Soldier	Left instep	2016/10/10	38417 Troop, Luzhai County	Bitten in sleep in dorm of 38417 Troop
<i>Trimeresurus albolabris</i>	LYRJZ 11	Farmer	Right heel	2016/9/3	Liutang Town, Liucheng County	Bitten in the bathroom early in the morning
	LYRJZ 67	Farmer	Right thumb	2018/8/25	Zhongping Town, Xiangzhou County	Bitten while working in a corn field
<i>Viridovipera stejnegeri</i>	LYRJZ 13	Farmer	Left foot	2018/7/9	Jiaguan Town, Duan County, Hechi City	Bitten in the bathroom early in the morning
<i>Daboia siamensis</i>	LYRJZ 1	Worker	Root of left index finger	2017/2/21	Luzhai County urban	Bitten while setting up a phone pole
	LYRJZ 70	Farmer	Root of right thumb	2018/9/8	Zhongping Town, Xiangzhou County	On the farm
<i>Bungarus multicinctus</i>	LYRJZ 60	Farmer	Right foot thumb	2018/5/24	Liutang Town, Liucheng County	Bitten while catching fish beside the pond
	LYRJZ 34	Farmer	Right thumb	2014/4/20	Pingshan Town, Luzhai County	On the farm
<i>Naja atra</i>	LYRJZ 81	Farmer	Right elbow	2018/7/28	Baisha Town, Liujiang County	Bitten in sleep
	LYRJZ 68	Citizen	Right midfinger	2018/8/26	Yufeng District, Liuzhou City	Bitten while removing the stone
<i>Myrrophis chinensis</i>	LYRJZ 12	Five years old child	Left thumb	2016/9/10	Liuhou Park, Chengzhong District, Liuzhou City	In autumn outing
Total	9 species of snakes	15 specimens				

base identified the snake species according to the pictures and geographical location of snakebite as clinical grade III evidence of snake species distribution.

Results

Distribution of snakes

Theoretically, there were 92 species of snake in 40 genera, 4 subfamilies and 6 families in the research region, including 25 species of venomous snake and 67 species of non-venomous snake. Venomous snakes were classified as follows: 7 species of venomous snake with front hook teeth, 8 species of venomous snake with tubular fangs, 2 species of venomous snake with Duvernoy's glands [5] in the upper palate, and 8 species of venomous snake with back groove teeth, which were summarized in Table 1 [6].

Classified evidence of snake species distribution

Grade I evidence: There were 90 pieces of grade I evidence, including 20 pieces of clinical specimen evidence I_a and 70 pieces of scientific research specimen evidence I_b. We identified 14 species of venomous snake and 18 species of non-venomous snake, in which venomous snake accounted highly for 43.75% (14/32). The venomous snakes were classified into 7 species with tubular fangs, 4 species with front hook teeth, 1 species with Duvernoy's glands in the upper palate, and 2 species with back groove teeth.

The snakebite treatment base in north and central Guangxi collected 15 clinical specimen evidence of grade I_a

in their five-year clinical practice, which were classified into three families, three subfamilies, eight genera and nine species (Table 2). The farmers in snakebite patients contributed to 73.33% (11/15). Snakebite mainly occurred when the farmers were on the farm, which indicated that the main target of grade II evidence questionnaire were farmers.

A total of 70 scientific research specimen evidence I_b were obtained in 3 years. The 70 scientific research specimen evidence I_b were divided into 28 species of snake, with 21 genera, 3 families, 3 subfamilies. Among them, 11 species venomous snake and 35 species of venomous snake were identified, which were summarized in Table 3. Most species of venomous snake in scientific research specimens are edible snake, and non-edible species such as the white-headed viper, mountain tip and viridovipera stejnegeri only appeared in clinical specimens, which indicated that the main injury group of non-edible poisonous snake species were farmers and field workers, but not active snake catchers.

Grade II evidence: According to the activity rules of snake every year, we selected the two periods with the most frequent snake activity (i.e., the end of hibernation (from March to May) and the early period of hibernation (from August to November)). During the this two periods, the questionnaire investigation was carried out in the towns and villages for five years. Finally, we integrated the questionnaire information to form grade II evidence of snake species distribution, which were listed in Table 4.

Table 3: Research Specimen Evidence I_b.

Family(Sub)	Genus	Chinese Name	Latin Name	Number of Specimens	Specimen NO.
Viperidae					
Crotalinae	<i>Protobothrops</i>	原矛头蝮	<i>Protobothrops mucrosquamatus</i>	2	LRYJZ 83.84
	<i>Trimeresurus</i>	白唇竹叶青蛇	<i>Trimeresurus albolabris</i>	2	LRYJZ 7.54
	<i>Deinagkistrodon</i>	尖吻蝮 (五步蛇)	<i>Deinagkistrodon acutus</i>	1	LRYJZ 28
Viperinae	<i>Daboia</i>	泰国圆斑蝰	<i>Daboia siamensis</i>	1	LRYJZ 76
Elapidae					
Elapinae	<i>Bungarus</i>	银环蛇	<i>Bungarus multicinctus</i>	6	LRYJZ 15.16.35.46.53.69
		金环蛇	<i>Bungarus fasciatus</i>	2	LRYJZ 39.90
	<i>Naja</i>	舟山眼镜蛇	<i>Naja atra</i>	12	LRYJZ 22.24.41.43.61.62
					63.64.65.66.71.74
	<i>Ophiophagus</i>	眼镜王蛇	<i>Ophiophagus hannah</i>	2	LRYJZ 8.57
Homalopsida					
	<i>Myrrophis</i>	中国沼蛇	<i>Myrrophis chinensis</i>	4	LRYJZ 4.5.6.9
Colubridae					
Natricinae	<i>Rhabdophis</i>	红脖颈槽蛇	<i>Rhabdophis subminiatus</i>	1	LRYJZ 38
Ahaetuliinae	<i>Ahaetulla</i>	绿瘦蛇	<i>Ahaetulla prasina</i>	2	LRYJZ 36.75
Total 3 families and 10 generas 11 species			35 species 3 subfamilies		

Table 4: Grade II Evidence.

Research Region	Administrative Areas Covered by Research	The Number of Questionnaires	Snake Species
Liuzhou City	Four areas: Liubei, Chengzhong, Yufeng, Liunan	232	14
Liujiang County	11 towns, 1 township, 89 villages	248	17
Luzhai County	4 towns, 5 townships, 33 villages	136	20
Liucheng County	9 towns, 3 townships, 46 villages	164	13
Xiangzhou County	7 towns, 4 townships, 39 villages	221	16

Table 5: Grade Evidence of Venomous Snakes Distribution in the Research Region.

Viper species	Toxicity	City of Liuzhou	Liujiang County	Luzhai County	Liucheng County	Xiangzhou County
		I II III	I II III	I II III	I II III	I II III
Proteroglyphic tooth						
<i>Naja atra</i>	Highly toxic	√ √ √	√ √ √	√ √ √	√ √ √	√ √ √
<i>Oph- hannah</i>	Highly toxic	√ √ √	√ √	√ √	√ √	√
<i>Bun- fasciatus</i>	Highly toxic	√ √	√	√ √ √	√ √	√
<i>Bun- multicinctus</i>	Highly toxic	√ √ √	√ √ √	√ √ √	√ √ √	√ √ √
<i>Sin-maccllelandi</i>	Highly toxic		√	√		√
Solenoglyphic tooth						
<i>Azemiops Kharini</i>	Highly toxic		√	√		
<i>Dei-acutus</i>	Highly toxic			√ √		
<i>Ovophis monticola</i>	Highly toxic	√	√	√ √	√	√ √
<i>P-mucrosquamatus</i>	Highly toxic	√ √ √	√ √ √	√ √ √	√ √ √	√ √ √
<i>Tri-lbolabris</i>	Highly toxic	√ √	√ √	√	√ √ √	√ √
<i>Vir-stejnegeri</i>	Highly toxic	√	√ √	√ √	√	√
<i>Daboia siamensis</i>	Highly toxic			√ √		√ √
Duvernoy's gland						
<i>Rha-subminiatus</i>	Highly toxic	√ √ √	√ √	√	√	√
<i>Rha-tigrinus</i>	Highly toxic		√	√		√
Opisthoglyphic tooth						
<i>Achaetulla prasina</i>	Low toxic	√	√ √	√	√	√
<i>Boi-multomaculata</i>	Low toxic	√ √	√ √	√		
<i>Boiga kraepelini</i>	Low toxic			√		
<i>Myr-chinensis</i>	Low toxic	√ √ √	√ √	√ √	√ √	√ √
<i>Hyp- plumbea</i>	Low toxic	√	√	√	√	√
<i>Psa- pulverulentus</i>	Low toxic	√	√ √	√	√	√
Total		14	17	20	13	16

Grade III evidence: In the long-term clinical practice, the snakebite treatment base in north and central Guangxi collected 233 pictures of snakes that caused snakebite and identified 11 species of venomous snake, which were summarized in Table 5.

Distribution of venomous snakes

The research confirmed that there were 20 species of venomous snake distributed in one city and

four counties in Guangxi. The distribution of venomous snakes in the research region and the classification of evidence were shown in Table 5. Luzhai County had the richest natural distribution of venomous snake, with 20 species, followed by Liujiang County (district) and Xiangzhou County. Overall, these three counties were the worst-hit areas of venomous snakebite in Liuzhou region of Guangxi. *Daboia siamensis* is a famous blood venomous snake, whose bite could

cause acute refractory recurrent renal failure and collapse of coagulation system [7]. At present, there is no targeted antivenom in China. The research confirmed that the *daboia siamensis* was only naturally distributed in Luzhai County and Xiangzhou County of Liuzhou, which was of great guiding significance for the medical staff of these two counties to publicize the prevention and treatment of snakebite of *daboia siamensis*. *Deinagkistrodon acutus* was only naturally distributed in Luzhai County. The author collected a wild *deinagkistrodon acutus* specimen in the mountain of Lago township, Luzhai County. The border between Lago and Guilin indicated that there was no natural distribution of *deinagkistrodon acutus* in Liuzhou region, and *deinagkistrodon acutus* only distributed in the border between Liuzhou and Guilin. The snakebite treatment base of northern and central Guangxi had never accept a patient confirmed bitten by *deinagkistrodon acutus* in Luzhai County, suggesting that the difference of in distribution of the regional treatment of snakebite was determined by the distribution of snake species.

Discussion

Snake venom is a kind of antigen. In the past many years, immunologists have been trying to develop various immunological diagnostic methods for snakebite based on the principle of antigen and antibody reaction.

There are three kinds of immunological diagnostic method for snakebite in China, i. e. convection immunoelectrophoresis, enzyme-linked immunosorbent assay and natural latex agglutination inhibition test [8]. Convection immunoelectrophoresis is a qualitative diagnostic method, with a sensitivity of 300~800 ng/mL, which is characterized with a low positive rate and a small number of covered species of venomous snake. Li and Teng reported that only 5 species of snake venoms can be detected by convection immunoelectrophoresis [9]. It is still yet no report about the successful case that the method of convection immunoelectrophoresis was used to identify bite from other snake species. Therefore, the significance to develop a quick and accurate diagnosis of snakebite in regions with rich venomous snakes using the method seems to be little. For Enzyme-linked immunosorbent assay (ELISA), it generally takes more than 3 hours to test snakebite. ELISA is not regarded as ideal for timely rescue of patients owing to the relative long time for the diagnosis, but it can be considered as a method of retrospective diagnosis. At present, there is only one report in 1981 that bite of *Bungarus multicinctus* was successfully identified using ELISA in China [10]. But due to its high demand for laboratory conditions, the widespread use of this method has not been developed. In the past 30 years, many scholars in the world have tried to improve ELISA for the diagnosis of snakebite. But their trying were all failed due to various factors, such as the large interference of non-specific reaction [11],

reduced sensitivity of reaction when shortening the detection time [12], and cross-reaction influencing specificity [13]. A third method that natural latex agglutination inhibition test can be obtained within 3~5 minutes, but it requires potential snakebite people take along snake species diagnostic kit when bitten by a snake. After snakebite, it also requires to gather around the wound poison or squeezing the wound for poison tissue fluid to do diagnosis experiment but not cleaning the wound in the first place, which was not in accordance with the principle of first aid for snakebite (China consensus group of snakebite treatment experts, 2018) [14]. There are only 8 species of snake venoms can be detected by natural latex agglutination inhibition test. However, due to extremely high cross reaction rate, false positive rate, and poor specificity between different snake species [8], the method developed in the early 1970s and ended in the late 1980s, and rarely used in the diagnosis of snakebite at present [12].

This study confirmed that there were 20 kinds of venomous snake distributed naturally in Luzhai County alone of Liuzhou, Guangxi, and the existing immunological technology has little significance in the clinical application of quick and accurate diagnosis of snakebite. Quick and accurate diagnosis of snakebite is the premise of targeted treatment. To diagnose snakebite quickly and accurately, it is necessary for clinicians to possess profound professional knowledge of snakebite, master and apply the knowledge and skill of six aspects of snakebite, including distribution of snake species, snake appearance, morphology and living habits, classification and name of snake, clinical manifestation discrimination of snakebite, bite mark discrimination and snakebite epidemiology. Therefore, quick and accurate diagnosis of snakebite has always been a problem which urgently need to be addressed.

The clinical manifestation discrimination of snakebite, bite mark discrimination and snakebite epidemiology are helpful to the diagnosis of snakebite, but their accuracy is poor. Neither the clinicians nor the patients are zoologists, and there are serious obstacles in the communication of snake species name, appearance and morphology. Therefore, a book of regional diagnosis of snakebite would play a key role in the early quick and accurate diagnosis of snakebite. In most cases, the patients could diagnose the snakebite quickly and accurately through guidance of a book of regional diagnosis of snakebite by the clinicians. It is necessary to take the distribution of snakes in prefecture-level cities and counties as the objective theoretical basis in order to make the book of regional diagnosis of snakebite pointed, suitable and reasonable. The snakebite treatment base in north and central Guangxi developed to carry out research on snake distribution in prefecture-level cities and counties based on existing zoological data in the perspective of clinical diagnosis and treatment of snakebite. The research was conducted in four dimen-

sions, which were clinical specimens of snakebite (grade I_a evidence), scientific specimens of wild snake species (grade I_b evidence), questionnaire survey (grade II evidence), and clinical pictures (grade III evidence).

The research confirmed the natural distribution of venomous snakes in one city and four counties of Guangxi, the specific distribution of them in each county of the prefectural city, their toxicity characteristics and fangs type and the relationship between the genera and species of venomous snakes. The research provided an objective theoretical basis for the scientific production of the book of regional diagnosis of snakebite, and facilitated clinicians to carry out clinical research on snake distribution, which was of great guiding significance for the clinical diagnosis and treatment of snakebite.

There are four kinds of snake antivenins in China now, which are *naja* antivenin, *bungarus multicinctus* antivenin, *agkistrodon halys* antivenin and *agkistrodon acutus* antivenin. Antivenom is recognized as the most effective drug in treating snakebite in the world, and it is also an important part of targeted treatment for snakebite. This research identified 20 species of venomous snake distributed in one city and four counties in Guangxi, among which 14 species were highly venomous snake, accounting for up to 70% (14/20). The number of domestic antivenom is obviously lower than the species of venomous snake. Therefore, selecting and using of combined antivenoms is the key to clinical treatment of snakebite. Snake venom often has the same antigenic components with those belonging to the same family and genus. Therefore, the antivenom made from one snake venom can be used to neutralize the snake venom from other poisonous snakes in the same family and genus to some extent.

Generally speaking, a certain kind of snake antivenin has the strongest neutralization specificity to the antigens used to prepare the antivenom of its species, followed by other venomous snakes belonging to the same family and genera. The specificity is poor when using in other venomous snakes belonging to different genera of the same family, and that is even worse to snake venom of different families, even without specificity [8]. For example, the precipitation lines generate in agp immunodiffusion reaction when using the antivenin from *deinagkistrodon acutus* against other viperidae species venom from the same family, such as *gloydius brevicaudus*, *trimeresurus albolabris*, *protobothrops mucrosquamatus* and *ovophis monticola*, however, no precipitation line generates when it is used against the venom of different families of *naja atra* [15]. Based on the principle of similarity theory, precipitation lines generate when using *naja* antivenin to neutralize the venom of *ophiophagus hannah*, *bungarus fasciatus* and *bungarus multicinctus* in the same family [16]. The *bungarus multicinctus* belongs to elapidae family and *bungarus* genus, *bungarus multicinctus* antivenin has obvious strong precipitation

line to the same family and genus of *bungarus fasciatus* venom, but Zheng and his colleagues did not find precipitation line and cross neutralization when using the antivenin to the venom of the species from same family and different genus, such as *naja atra* and *ophiophagus hannah* [17].

The zoological classification of injury-causing snake (family, genus and species) would provide objective theoretical basis for the pointed selection of antivenoms and the combined use of antivenoms. The book of regional diagnosis of snakebite according to the distribution of snakes from clinical perspectives would be expected to play a role in the more quick and accurate diagnosis after snakebite. In addition, this paper confirmed the specific distribution of venomous snakes as well as the relationship of family, genus and others in prefecture-level cities and counties, which hope to provide an objective theoretical basis for the establishment of the regional snakebite treatment network centered on the prefecture-level hospitals.

Conclusion

The research confirmed the natural distribution of venomous snakes in one city and four counties of Guangxi, the specific distribution of each venomous snake in each county of the prefectural city, the toxicity characteristics and fangs type of each venomous snake and the relationship between the genera and species of venomous snakes. The research provided an objective theoretical basis for the scientific production of the book of regional diagnosis of snakebite, and provided an objective idea and method for clinicians to carry out clinical research on snake distribution, which was of great guiding significance for the application of the book of regional diagnosis of snakebite in the clinical diagnosis and treatment of snakebite.

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