BRIEF REPORT

Tetanus After White-Lipped Green Pit Viper (*Trimeresurus albolabris*) Bite

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Introduction

Snakebite is a common medical emergency in Thailand. The fatality rate due to snakebites, however, has declined from more than 200 cases per year in the 1940s¹ to fewer than 20 cases per year in the 1980s.² The green pit vipers (*Trimeresurus* spp) are responsible for the great majority of venomous snakebites in Thailand.^{3–5} Severe coagulation abnormalities from green pit viper bites are uncommon and, if present, respond readily to the administration of fresh frozen plasma, platelets, and antivenom.⁶ Almost all patients survive and some eventually develop secondary bacterial infection at bite sites. There have been a few reports of tetanus complicating snakebite.^{7–9} But no tetanus has been reported after green pit viper bites. Tetanus prophylaxis is not routinely recommended in Thailand during the early period after bites from hematotoxic snakes because there is a risk of bleeding at the injection site. We report the first case of tetanus in a patient with white-lipped green pit viper (*Trimeresurus albolabris*) bite. We also investigated whether *Clostridium tetani*, the causative agent of tetanus, are present in the oral cavity of *T. albolabris*.

Case report

A healthy 34-year-old Thai man from SamutPrakarn originally presented at Chulalongkorn University Hospital (CUH) complaining of being bitten on his right ring finger by a white-lipped green pit viper with a red tail and yellow belly while working inside his house. In SamutPrakarn province near Bangkok, the only venomous green snake with a red tail and yellow belly is *T. albolabris*. He had already received an antibiotic and analgesics from a private hospital but was admitted to

CUH because of prolonged venous clotting time without clinical evidence of bleeding. Thirty milliliters of specific antivenom against green pit viper toxin (Thai Red Cross Institute, Bangkok) was given every 6 hours for 3 times until the venous clotting time normalized. He was discharged from the hospital 3 days later, and 500 mg oral amoxicillin 3 times a day was prescribed.

Four days later (6 days after the snakebite), the patient noted purulent discharge at the bite wound, accompanied by a generalized urticarial rash. A diagnosis of drug allergy to amoxicillin was made. Prednisolone and an antihistamine were given, and he was observed in the observation room for 24 hours. Oral amoxicillin was switched to clindamycin.

Two days later (10 days after the snakebite), the patient developed pain at the neck, back, and extremities. The onset of trismus and dysphagia was noted during the next 6 hours. He was readmitted to the hospital. Pertinent findings on admission included trismus, dysphagia, and nuchal rigidity. The patient was febrile, fully conscious, and neither reflex spasm nor disturbances of the sympathetic nervous system were observed. In addition, there was dry gangrene at the tip of his right ring finger. He denied having applied any herbal or other local remedies or soil to the bite wound. There was no previous history of tetanus toxoid (TT) injection; however, almost all children in Thailand receive standard Extended Program of Immunization (EPI) that includes Diphtheria Pertussive Tetanus (DPT) vaccine.

A complete blood count showed a hematocrit of 37%, white blood cell count of 15 400 cell·mm⁻³ with 90% neutrophils, 8% monocytes, 2% lymphocytes, and a platelet count of 289 000 cells·mm⁻³. Blood sugar, urea nitrogen, creatinine, liver function tests, electrolytes, and a coagulogram were within normal limits. A chest radiograph was normal.

A clinical diagnosis of tetanus was made. Immediate specific treatment included administering 1500 units of

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human tetanus immune globulin intramuscularly, 0.5 mL of alum-precipitated TT intramuscularly, and 2 million units of penicillin G sodium (PGS) intravenously every 6 hours. Debridement of the finger wound was also performed, and aerobic and anaerobic cultures of the necrotic tissue and blood were obtained. They were subsequently negative. The patient's muscle rigidity was well controlled by oral diazepam. There was no worsening of his neurological status after admission and no adverse response to the PGS infusion. He gradually improved within 12 days and was discharged with nearly complete neurological recovery except for mild residual trismus. This case was followed up at the outpatient department, and he was seen for the last time after 8 months without any residual neurological impairment. He had received a complete course of 3 TT injections.

BACTERIOLOGICAL STUDIES OF THE ORAL CAVITIES OF WHITE-LIPPED GREEN PIT VIPERS

Several studies, dealing with the bacterial flora of snake venom, oral cavities, and bite wounds, have been published.^{8,10-15} A large variety of potentially pathogenic aerobic and anaerobic bacteria have been cultured. These organisms are capable of causing gas gangrene and tetanus and may be implicated in secondary infection. It is apparent from the literature that patterns of bacterial flora vary geographically and with different species of snakes.⁷ In Thailand, the white-lipped green pit viper is widely distributed and is the most common cause of venomous snakebite.³⁻⁵ There has been only 1 microbiological study of green pit viper bite wounds.¹⁰

Materials and methods

BACTERIAL ISOLATES

Samples of the oral bacterial flora were collected from 20 recently captured white-lipped green pit vipers at the snake farm of the Queen Saovabha Memorial Institute (Thai Red Cross Society), Bangkok. They were all healthy and had been observed in captivity for at least 1 week. They were fed live frogs or mice. No killed meat products, antibiotics, or antiseptics were used in feeding. Each snake's mouth was held open by a professional handler. No snake displayed any evidence of injury or infection. The oropharyngeal cavities were swabbed with 2 sterile cotton-tipped applicator sticks. One swab was then plated immediately on the following aerobic culture media: 5% sheep blood agar, MacConkey agar, and 5% sheep blood Staph/Strep agar. These specimens were incubated aerobically at 37°C for up to 48 hours. Another swab was incubated anaerobically at

37°C for up to 48 hours, using AnaeroPack (Mitsubishi Gas Chemical Co Inc, Tokyo, Japan), on 5% sheep blood Brucella agar containing 10 µg·mL⁻¹ vitamin K and 5 µg·mL⁻¹ haemin (BK) (Becton Dickinson and Company, USA), *Bacteroides* bile esculin agar, and phenylethyl-alcohol (PEA) agar. Specimens were also placed into 20-mL tubes containing 10 mL of thioglycollate broth medium. Cultures of these specimens were carried out at the Department of Bacteriology, CUH, Bangkok. Aerobic and facultative aerobic bacteria were identified by standard bacteriologic, biochemical, and serologic methods. The API system was used to identify the species of some bacterial isolates which were not detected by standard methods. Anaerobic bacteria were identified by standard procedures and the BBL Crystal Identification System (Becton Dickinson and Company, USA). In addition, soil of the laboratory, where the snakes were housed and fed, was also cultured anaerobically.

Results

The oropharynx of white-lipped green pit vipers contained a wide range of bacteria, including 6 species of aerobic gram positives (Table 1), 16 species of aerobic gram negatives (Table 2), and 10 species of anaerobic bacteria (Table 3). Each snake yielded at least 1 species of aerobic gram-negative bacilli and anaerobic bacteria. Aerobic gram positive bacteria were not recovered from every snake's oropharynx. Coagulase-negative *Staphylococci* (11 isolates) were the most commonly isolated gram-positive bacteria. Only 4 isolates of *Corynebacterium* spp, *Micrococcus* spp, or *Nocardia* spp were recovered. No isolation of *Streptococcus* spp and *Staphylococcus aureus* was observed. *Morganella morganii* (9 isolates), *Bordetella avium* (8 isolates), and *Acinetobacter baumannii* (6 isolates) were the 3 most common aerobic gram-negative bacteria. *Aeromonas caviae*, *Providencia rettgeri*, *Pseudomonas putida*, and *Salmonella* spp were recovered from 3 snakes. *Proteus mirabilis*, *Pseudomonas aeruginosa*, and *Stenotrophomonas maltophilia* were recovered from 2 snakes.

Among anaerobic bacteria, *Clostridium perfringens* and *Bacteroides fragilis* (11 isolates) were the most common. Eight isolates of *Clostridium hastiforme*, 4 isolates of *Clostridium innocuum*, 3 isolates of *Clostridium beijerinckii*, and 2 isolates of *Clostridium septicum* were observed among *Clostridium* spp. No *C. tetani* was recovered from any of our snakes. Furthermore, no other species of *Bacteroides* were isolated.

Two duplicate sets of anaerobic cultures of soil yielded *C. hastiforme* and *C. perfringens*. Again, no *C. tetani* was recovered.

Table 1. Species of aerobic gram-positive bacteria isolated from the oropharynx of 20 green pit vipers

Organisms	Specimen number																				Total
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	
Aerobic gram-positive bacteria																					
1. <i>Bacillus</i> spp				/																	1
2. <i>Corynebacterium</i> spp							/	/									/	/			4
3. <i>Enterococcus faecalis</i>							/	/			/										2
4. <i>Micrococcus</i> spp											/	/					/				4
5. <i>Nocardia</i> spp											/	/		/	/	/					4
6. Coagulase-negative <i>Staphylococci</i>			/	/			/				/	/	/	/	/	/	/	/	/	/	11
Total	—	—	1	2	—	—	2	2	—	—	3	2	2	2	2	2	1	2	2	1	26

Discussion

The white-lipped green pit viper (*T albolabris*) has been found in every province of Thailand but is most common in the central part of the country, including Bangkok. Reports concerning tetanus complicating snakebite are sparse, probably because of the absence of *C tetani* in the oropharynx or venom of snakes or the presence of some antibacterial activities in the venom.^{8,15,16} Willson presented, from a group of 740 cases of rattlesnake bite, 1 case of tetanus that was fatal within 2 days.¹⁷ Evans and Farrell found reference to tetanus complicating snakebite.¹⁸ Parrish reported a documented case of tetanus complicating a cottonmouth moccasin bite.¹⁹ Arroyo and colleagues isolated *C tetani* from wild *Bothrops asper* venom in Costa Rica.²⁰ Theakston and colleagues cited a patient who recovered from tetanus after a rattlesnake bite.⁸ In Southeast Asia, there were 2 reports of 2 fatal cases and of tetanus complicating Malayan pit viper bites (Thailand and Malaysia).^{21,22} To our knowledge, no tetanus after *T albolabris* bite has ever been reported. This may be because of the absence of severe tissue necrosis complicating *T albolabris* bites, compared with other venomous snakebites. A clinical diagnosis of generalized tetanus was made in our patient based on trismus, rigidity of muscles, and reflex spasm in the presence of full consciousness. These criteria are well accepted because laboratory studies are of little value in the bedside diagnosis of tetanus.²³ The origin of *C tetani* in a victim could be the snake's venom or oropharynx, the patient's skin and clothing, materials which the patient applied to the wound as first aid and self treatment, or unsterile medical instruments used to incise the wound. In our case, no contaminated materials or unsterile instruments were used to apply to the wound that was not incised. The patient was bitten inside his house on his finger (not the toe), and the possibility of contamination was small. He had not incurred any other injuries within months before his illness. Thus, we believe the source of *C tetani* was from the snake's venom or oropharynx.

Microbiological studies were carried out to determine whether *C tetani* are present in the oropharynx of *T albolabris*. Venom was not studied because of the small amount and difficulty in aseptically collecting it from the small fangs of *T albolabris*. *C tetani* are present in the soil, especially on the surface,^{24,25} and often colonize the intestinal tract of some animals such as mice and horses.^{25,26} Thus, we cannot exclude the possibility of contamination of the snake's oropharynx by *C tetani* from such sources, including the live prey of the snake. In our study, the snakes were removed from their normal habitat and were kept in laboratory captivity on soil for

Table 2. Species of aerobic gram-negative bacteria isolated from the oropharynx of 20 green pit vipers

Organisms	Specimen number																				Total
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	
Aerobic gram-negative bacteria																					
1. <i>Acinetobacter baumannii</i>							/	/					/	/		/	/		/		6
2. <i>Aeromonas caviae</i>	/								/	/											3
3. <i>Burkholderia cepacia</i>														/							1
4. <i>Bordetella avium</i>		/	/	/	/	/			/					/	/						8
5. <i>Citrobacter freundii</i>										/											1
6. <i>Klebsiella pneumoniae</i>								/													1
7. <i>Morganella morganii</i>			/	/	/					/	/	/	/				/		/		9
8. <i>Proteus mirabilis</i>									/												1
9. <i>Proteus vulgaris</i>							/				/										2
10. <i>Providencia rettgeri</i>											/				/			/			3
11. <i>Pseudomonas aeruginosa</i>		/														/					2
12. <i>Pseudomonas putida</i>	/									/										/	3
13. <i>Shewanella putrefaciens</i>															/			/			2
14. <i>Salmonella</i> spp					/	/								/							3
15. <i>Stenotrophomonas maltophilia</i>											/	/									2
Total	2	2	2	2	3	2	2	2	3	4	4	2	2	3	3	2	2	2	2	1	47

Table 3. Species of anaerobic bacteria isolated from the oropharynx of 20 green pit vipers

Organisms	Specimen number																				Total
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	
Anaerobic bacteria																					
1. <i>Bacteroides fragilis</i> group	/	/					/	/	/	/	/	/	/	/	/	/	/	/	/	/	11
2. <i>Clostridium beijerinckii</i>				/								/					/				3
3. <i>Clostridium butyricum</i>																		/			1
4. <i>Clostridium difficile</i>																			/		1
5. <i>Clostridium histiforme</i>	/	/	/	/	/	/		/							/						6
6. <i>Clostridium innocuum</i>	/	/	/			/					/										4
7. <i>Clostridium paraputrificum</i>				/										/							1
8. <i>Clostridium perfringens</i>			/	/	/		/	/	/	/	/	/	/	/	/	/	/	/	/	/	9
9. <i>Clostridium septicum</i>																	/				2
10. <i>Clostridium</i> spp			/																		1
Total	3	1	4	3	1	1	1	2	2	1	2	2	2	2	1	2	2	2	3	2	39

at least 1 week, even though they naturally live in bushes and trees. They were fed live frogs or mice to imitate the natural behavior of *T albolabris* and to possibly increase chances of recovering *C tetani* in their oropharynx. But we failed to find any *C tetani* in our sample snakes.

To our knowledge, this is the first documented microbiological study of the oropharynx of *T albolabris*. One previous report of the bacteriology of *Trimeresurus* spp bites was carried out by isolation of microorganism from infected bite wounds.¹⁰ Only 4 out of 19 patients had infected wounds. Of these, only 1 patient had available microbiological data and *C perfringens* was isolated from the infected wound. Our results suggest that both aerobic and anaerobic bacteria are present in the oropharynx of *T albolabris*. A wide variety of aerobic gram-negative organisms were isolated, compared with a relatively narrow variety of aerobic gram-positive bacteria which were mostly coagulase-negative *Staphylococci*. These results are similar to other studies which were carried out with other species of snakes.^{7,8,10-15} Of aerobic gram-negative bacteria, Enterobacteriaceae (mainly *M morganii*) were the most common isolates. Surprisingly, *B avium* was the second most common aerobic gram-negative bacterium. It is a small aerobic gram-negative coccobacillus or short rod.²⁷ *B avium* is a respiratory pathogen of a variety of birds, especially turkeys.²⁸ There have been no reports of *B avium* causing disease in snakes. The source of oropharyngeal contamination is likely live prey.

Anaerobic bacteria are the most common isolates, compared with aerobic gram-positive or gram-negative bacteria. Of these, both *B fragilis* and *Clostridium* spp were commonly recovered. *B fragilis* was the only isolated species of *Bacteroides*, compared with a wide variety of species of *Clostridium*. This finding is also consistent with other studies that were carried out in the oropharynx of Malayan pit vipers⁸ or rattlesnakes^{7,12} and cobra bite wounds.¹⁴

No *C tetani* were recovered from our oropharyngeal and soil specimens. Only 1 study reported isolation of *C tetani* from the venom of wild *B asper* in Costa Rica.²⁰ There probably are qualitative and quantitative differences in the oral bacterial flora of captive and natural *T albolabris*. But this observation was the subject of controversy. Some reported sparse oral bacterial growth from freshly caught snakes when compared with numerous growth of oral bacteria from captive snakes.²⁹ Others showed heavy growth of bacteria from freshly caught snakes.⁷ There may be differences in the fecal flora of laboratory and wild rodents that are fed to snakes, and it is also probable that the flora of wild rodents varies according to geographic area.¹² This might

explain our failure to detect *C. tetani*. But this study did not exclude the presence of *C. tetani* in *T. albolabris*, and it may well be a very rare oral organism in this species. The number of samples in this study may also have been too small. A larger study carried out in a greater number of freshly caught snakes would be needed to elucidate this issue.

Tetanus prophylaxis is not routinely recommended during the early period after *T. albolabris* bite because of the risk of bleeding at the injection site. This is the first reported tetanus case after a *T. albolabris* bite; nevertheless, it supports the use of tetanus prophylaxis as soon as any coagulopathy has been corrected.

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