

## Forensic DNA test conclusively links snake bite marks on people to species

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Starting with a simple DNA swab taken from fang marks on people bitten by snakes, an international research team correctly identified the species of the biting snake 100 percent of the time in a first-of-its-kind clinical study, according to data presented today at the American Society of Tropical Medicine and Hygiene's (ASTMH) Annual Meeting.

The study, conducted at three medical facilities in Nepal, found that if snake DNA could be isolated from the bite wound, the test identified the species of snake responsible every time.

"These findings represent a significant step toward improving care for patients in areas of the world where snakebites constitute a massive but neglected health risk," said François Chappuis, MD, PhD, chief of the division of tropical and humanitarian medicine at Geneva University Hospitals, Switzerland, who directed the study and presented the results. "This DNA test may hasten more effective bedside diagnostics for snakebite victims, giving them a better chance of surviving and making a full recovery."

Bites from venomous snakes are common in many parts of the world and an especially serious unresolved health problem to millions of people living in South and Southeast Asia, as well as Africa and Latin America. Although there are no reliable numbers at the global scale, a study published in 2008 estimated at least 421,000 cases of envenomation and up to 94,000 deaths occur worldwide from snakebite each year. However, experts warn that these figures may underestimate the real



problem, which is believed to affect several million people bitten by venomous snakes annually and hundreds of thousands who die or survive disabled, suffering from amputation or deformed limbs as a result of unavailable or delayed treatment.

In some villages of the study area of southeast Nepal reported today, a community-based survey in 2002 revealed an estimated 1,162 snakebites and 162 snakebite deaths per 100,000 people per year, one of the highest rates ever reported. Likewise, a recent survey of more than one million deaths in India, published in the journal *PLoS Neglected Tropical Diseases* in 2011, found that 46,000 people die every year in India from snakebites. This figure is about 20 times the official death toll recorded in Indian hospitals, most likely because less than one in four fatal snakebite cases had received any hospital treatment, the study revealed.

"People bitten by snakes in South Asia often do not seek treatment at a medical facility, and if they do, the vast majority don't take the snake to clinics, although it is often killed, and can't identify the species that bit them," Chappuis said. "Yet knowing the species of snake is critical to determining the best course of treatment."

In Nepal, for example, cobras and kraits are among the most common venomous snakes. Their venoms attack the nervous system and can be fatal. Patients bitten by either snake have similar symptoms, such as nausea, headache, drowsiness and limb as well as respiratory paralysis. However, the toxins of these snakes and their mechanisms of action differ, and so does the response to antivenom and other drugs. For example, the locally available antivenom has limited or no efficacy against krait envenomation, which often leads to useless repeated doses resulting in increased cost and—possibly—adverse side effects, explained Chappuis. Also, bites by cobras and kraits lead to different complications that the attending physicians need to prevent or be prepared for. Therefore, positive identification of the biting species



becomes important to effective treatment.

Preliminary results presented today show that among 194 bite-site DNA samples collected in Nepal during the study, 87 were from a venomous species. The spectacled cobra accounted for 42 bites and the common krait for 22. In 21 cases, the patients brought the dead snake with them, and in those cases expert examination of the snake and the DNA test, performed by different investigators, independently provided the correct identification. Snake DNA could be obtained from about one in four bite wounds. According to the researchers, one factor that excluded samples was if the patient attempted folk or home remedies tampering with the bite site prior to arriving at the medical clinic.

The research team also correlated clinical symptoms with the species biting patients. They found that krait bites happened more often at night, indoors, or while people were sleeping, while cobra or pitviper bites were more likely to cause swelling at the bite site. The technique also identified several species of non-venomous snake involved in bites.

"The proportion of non-venomous snakes that bite people is actually rather high," said Ulrich Kuch, PhD, of the department of tropical medicine and public health in the Institute of Occupational, Social and Environmental Medicine of Goethe University (Frankfurt, Germany), and developer of the snake DNA identification test. "Snakebite patients are kept in the hospital for 24-hour observation, which strains healthcare resources in many countries. By identifying species of nonvenomous snake, it is also possible to assist the local people and avoid hospital stays for those not at risk of envenomation."

The research team is currently developing a rapid diagnostic "dip-stick" test similar to a pregnancy test that could be used to rule out certain common <u>venomous snakes</u> and help physicians more quickly decide the best course of treatment. Conversely, if for example, krait venom is



detected, doctors could quickly give antivenom instead of waiting for clinical signs of envenomation, as is current practice. They would also accelerate the transfer of patients to referral hospitals with intensive care units able to ensure adequate respiratory support. Such a test would be easy to administer in rural healthcare settings with limited resources, said Chappuis.

"That's where the recently developed DNA test could be most valuable," he added. While the DNA test is labor intensive and time consuming, making it impractical for day-to-day clinical use, its high accuracy could allow it to become a "gold standard" in clinical studies of rapid diagnostic tests under development. In addition, the DNA test could become a powerful epidemiological tool to determine which species of snake bite people in various tropical regions.

"Knowing the distribution of snakes and the number of snakebites within a region could help improve allocation of scarce antivenoms to areas where they are most needed" said Chappuis.

The researchers have completed small clinical studies in Nepal and Bangladesh and are preparing a larger multi-center clinical study using the DNA test in Myanmar and Nepal, countries with among the highest rates of snakebite deaths. The current study is funded by the UBS Optimus Foundation and the Swiss National Fund.

"We need new tools in order to save more lives," said Sanjib K. Sharma, MD, professor of medicine at the B.P. Koirala Institute of Health Sciences in Nepal, and the principal investigator of the study. "There is gross disparity in the management and outcome of snakebites since most occur in rural, agricultural areas while the great majority of healthcare workers are in urban locations."

"Most people are unaware that snakebite is a very real and serious threat



both to the health and economic vitality of rural communities throughout much of the developing world," said Alan J. Magill, MD, FASTMH, president of the American Society of Tropical Medicine and Hygiene. "This innovative research may be enabling for the development of a point of care test to positively identify biting snakes. Accurate diagnosis would go a long way toward documenting the impact of snakebite and improving medical care for this often neglected yet pervasive problem."

## Provided by Burness Communications

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