We agree that education in Nepal has likely decreased rates of AMS, high altitude pulmonary edema, and high altitude cerebral edema. However, trekkers and climbers in the Himalaya still succumb to AMS and other altitude illnesses at a higher rate than would be expected with this additional education. The high altitude research community does not have good data on the current rate of these altitude maladies, and this topic would serve as an excellent future project.

We agree with Dr. Bennett that a single study does not provide evidence to change practice. We have taken the first step to further explore alternate dosing regimens. We encourage Dr. Bennett and other high altitude scholars to join our efforts and design and execute additional studies to help elucidate the optimal dose of acetazolamide to prevent AMS.

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DNA from Tooth Embedded in Man's Foot Resolves Quarter-Century-Old Shark Bite Mystery

To the Editor:

Shark attacks are rare, but when they occur they generate substantial media, public, and other interest. The aftermath of an attack has the potential to influence public attitudes toward sharks and policy decisions surrounding shark attack mitigation and conservation. After an attack, the media often generate interest in determining the species of shark involved. This information can fulfill curiosity, complete medical and case records, and inform shark attack mitigation strategies. However, determining the identity of the species responsible is not an easy task. Currently, there are over 500 described shark species.¹ Many of them (eg, requiem sharks of the genus *Carcharhinus*) are difficult to identify to the species level, even for trained professionals. This is especially true when there is only limited information available, as is usually the case after a bite. Bite marks vary by species, although clear classifications are rare, making precise species identification difficult. In some rare cases teeth or tooth fragments may be left in the wound. The shark species is unlikely to be identified even through examination of these teeth/fragments because of similarities in tooth morphology across species. Here, we present a novel case study that retrieved DNA from a shark tooth fragment that had been embedded in a victim's foot for 24 y and successfully used it to identify the species involved.

In September 1994, a man was bitten on his right foot while paddling on a surfboard near Flagler Beach in Flagler County, Florida. The victim received dorsal lacerations to his toes and joint damage to his fifth digit. The shark involved approached from behind and was not observed during the incident. About 10 y ago, 1 tooth fragment came out of the area between 2 of his toes but was discarded. In the summer of 2018, a second tooth fragment was expelled from the same area. According to the victim, both of these tooth fragments had remained in his foot since the incident. The victim sent the second tooth fragment to us for identification of the shark species involved. We examined the tooth but could not determine the species responsible for the bite based on external morphology alone. Thus, we decided to subject the sample to DNA analysis.

Considering the tooth fragment had been buried in the victim's foot for over 2 decades and that it may have since been handled by multiple persons, it was first soaked in a 5% bleach solution for 5 min and then rinsed twice with sterile water to remove possible contaminants. The lower surface of the tooth fragment was debrided to obtain pulp tissue from the tooth interior. DNA extraction was then conducted, and an Illumina sequencing library was prepared using the "with-bead" method,^{2,3} following the protocol for ancient or fragmented (<100 bp) DNA samples in a 2013 study.⁴ A gene capture approach followed by next-generation sequencing on an Illumina MiSeq platform were then used to obtain the whole mitochondrial genome (mitogenome) sequence.^{4,5} After a basic local alignment search tool search of the assembled sequence against an independently curated reference database of 1251 mitogenome sequences from cartilaginous fishes, we determined that the tooth fragment was from a blacktip shark (*Carcharhinus limbatus*). As a complementary identification approach, we also built a neighbor-joining tree using the mitochondrial NADH dehydrogenase 2 (ND2) gene sequence from this sample and over 14,000 other samples of cartilaginous fishes of known species identity.⁶ Results from this analysis further confirmed the species to be a blacktip shark.

Carcharhinus limbatus is common year round in the coastal waters of southern areas of the United States, especially Florida owing to its long coastline. These sharks use coastal bays and estuaries as nursery grounds.⁷ Blacktips are relatively small sharks that mainly feed on small fishes. They are normally wary of humans but can become aggressive in the presence of food. When blacktips follow a group of small fishes near the beach, occasional interactions with humans become inevitable. According to the International Shark Attack File (www.floridamuseum.ufl.edu/shark-attacks), blacktip sharks have been involved in approximately 20% of unprovoked shark bites in Florida from 1926 to 2018.

The tooth fragment was buried in the victim's foot for many years, and, unsurprisingly, we noted evidence of DNA degradation in the tooth during this time. The mitogenome sequence we obtained from the tooth fragment is 16,729 bp in length but with as many as 456 ambiguous nucleotides. The mean coverage is 406× but is uneven across the full length of the mitogenome. This contrasts with the case reported in a 2018 study, in which we obtained good-quality mitogenome sequence from a shark tooth fragment retrieved from a victim's body only several weeks after the incident.⁸ Nevertheless, it is surprising that the shark's DNA would persist intact for such a long period of time at mammalian physiological temperatures and in the presence of enzymes and an immune system optimized to target and break down foreign tissue. Despite some DNA degradation, the sequences we obtained in this case were still sufficient to assign the tooth fragment to a species. The mitogenome and ND2 databases used in this study, which we have spent years sampling, sequencing, and maintaining, are the key factors that made this identification possible.

In this study, we used a genetic approach to identify a requiem shark species that was responsible for a bite that happened decades ago. This effort, our past effort,⁸ and our ongoing efforts (identifying species from wound swabs, clothing, flotation devices) coupled with case reports curated by the International Shark Attack File will contribute to a better understanding of the circumstances surrounding shark bites worldwide and may inform mitigation of such incidents. The techniques we used here may also be applied to investigate other wild animal incidents, even those that occurred quite some time ago.

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