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# A Tick From a Prehistoric Arizona Coprolite

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ABSTRACT:    Ticks have never been reported in archaeological analyses. Here, we present the discovery of a tick from a coprolite excavated from Antelope Cave in extreme northwest Arizona. Dietary analysis indicates that the coprolite has a human origin. This archaeological occupation is associated with the Ancestral Pueblo culture (Anasazi). This discovery supports previous hypotheses that ticks were a potential source of disease and that ectoparasites were eaten by ancient people.

Nearly 1,000 coprolites from the desert west of the United States have been analyzed for parasite remains (Reinhard, 1990, 1992). Although arthropod parasites are rare, they are occasionally found in coprolites. The discovery of lice in human coprolites led Fry (1977) to conclude that arthropods were consumed to control infestation. In 2005, we commenced the dietary and parasitological analysis of coprolites from Antelope Cave in the northwestern corner of Arizona. At this site, we discovered a tick from a coprolite. This discovery has health, behavioral, and ecological implications for the Puebloan people that once occupied the cave.

Antelope Cave is a large limestone cavern sunk into the gently rolling terrain of the Uinkaret Plateau some 40 km southeast of St. George, Utah. Prehistoric Native Americans occupied it, probably intermittently, for at least 3,000 yr (2028 B.C. to A.D. 1100). The most intense habitation of the cave is attributed to Ancestral Puebloan peoples (Anasazi) who lived there 1,300 to 1,000 yr ago. Antelope Cave lies within the Virgin River Branch of prehistoric western Anasazi territory, and the great majority of artifacts (for example, woven fiber sandals, plaited basketry, Virgin series pottery) in the cave reflects Puebloan (Kayenta) affiliation. There is scant evidence of Fremont cultural influence from the north in Utah. Cultural debris left in the cave by its prehistoric inhabitants forms a 1.52-m-thick layer and contains mostly perishable artifacts, including wooden arrow shafts, basketry, string, netting, sandals, needles and thread, etc., as well as painted pottery and various lithic tools. The Pueblo people used the cave for shelter, and in the surrounding area, they grew corn and beans, gathered wild plant foods, and hunted game, mostly rabbits.

Professional archaeologists have conducted excavations in the cave, off and on, since 1954 (Janetski and Hall, 1983; Janetski and Wilde, 1989). The most extensive excavations were undertaken by the University of California–Los Angeles (UCLA) in 1959–1960 (Johnson and Pendergast, 1960). The coprolite specimen discussed here was recovered by UCLA and came from 1,000-yr-old Pueblo deposits at the rear of the cave. The date is based on cross-dated Anasazi artifacts pending C14 assay.

In 1959, archaeologists from UCLA excavated five 2 × 2 m pits into the midden deposit of Antelope Cave. The excavation units were designated AC59-1 through AC59-5. The coprolite of concern here was recovered from the 60–76-cm level below the surface in pit AC59-2. Along with the coprolite, this level yielded a wide variety of cultural debris, including fragments of Pueblo pointed-toe sandals, sandal ties, a net bag, fiber cordage, feather cordage, and pottery. No features, such as fire hearths, storage pits, latrines, etc., were exposed in this or any other level.

Eight Antelope Cave coprolites have been analyzed to date. Five are consistent with humans and 3 are consistent with canids, probably dogs. Laboratory sample 2 is the focus of this report. Its field context is AC 1516, pit AC 59-2, 60–76 cm below surface. After contextual information was recorded, the coprolite was cleaned of extraneous dirt, photographed, and weighed. Its weight, 2.67 g was then recorded. Observations relative to biological origin were made. The coprolite was then

rehydrated in 0.5% trisodium phosphate for 48 hr. It was placed in a 300-ml beaker, and rehydration solution was added until the coprolite was completely immersed. Parafilm was used to cover the beaker to prevent potential modern airborne pollen contamination. Observations were made after 24 hr of rehydration. Rehydration fluid color is sometimes useful in verifying human origin (Reinhard and Bryant, 1992). Human coprolites tend to turn the rehydration solution dark brown or black, although this is not always the case. In addition, the rehydrating coprolite was examined for a mucilage coat, which sometimes forms on dog coprolites after rehydration (Reinhard et al., 1988).

After 48 hr of rehydration, 3 *Lycopodium* sp. spore tablets were added to the coprolite to facilitate quantification (Warnock and Reinhard, 1994; Sianto et al., 2005). For this analysis, *Lycopodium* sp. spore batch 212761 was used. Previous analysis has shown that approximately 12,500 spores are present in each tablet (values presented from different analyses of tablets are 12,432, 12,489, and 12,542). The tablets were dissolved in a few drops of hydrochloric acid and added to the rehydrated coprolite.

The coprolite was then disaggregated. It was transferred to a 600-ml beaker along with the rehydration solution and dissolved *Lycopodium* sp. tablets. A magnetic stir rod was added to the beaker, which was placed on a stir plate. The coprolite in the solution was then stirred for 45 min until it was completely disaggregated.

Microscopic remains were separated from macroscopic remains by pouring the disaggregated coprolite through a 300-μm mesh screen. A stream of distilled water under pressure was used to thoroughly wash the microscopic remains through the screen and into a 600-ml beaker. The macroscopic remains on top of the screen were dried on cotton filter paper. The microscopic remains were sedimented by centrifugation in 50-ml tubes.

The microscopic remains were then analyzed for parasite eggs and microscopic dietary evidence such as plant cells, phytoliths, and starch grains. Nine microscope preparations were made for helminth eggs or protozoan cysts. The dietary residues were categorized and counted. Next, using the following formula, the numbers of each category of dietary residue per gram of coprolite were calculated: concentration = $([i/m] \times n)/w$, where $i$ is items counted, $m$ is marker *Lycopodium* spores counted, $n$ is marker *Lycopodium* spores added, and $w$ is weight of coprolite in grams.

Subsequent to this analysis, about 10 ml of sediment were processed in hydrofluoric acid and acetolysis solution for pollen following the methods of Reinhard et al. (2006). Pollen grains were counted, and the numbers of pollen grains per gram were calculated using the above formula. When the analysis of microscopic remains was completed, the dried macroscopic remains were examined for food residues and arthropods.

One fragment of a tick was found. Images of the tick were made with a Syncroscopy Auto-Montage digital microscope system at the University of Nebraska State Museum Biodiversity Synthesis Laboratory, Lincoln, Nebraska. This system eliminates depth of field limitation problems by automatically capturing the in-focus regions from a range of focal planes and combining them into a single, fully focused, high-resolution image. Adobe Photoshop was used to reconstruct the complete view of the dorsal posterior of the tick so that festoons could be more accurately counted.

The specimen was not cleared or mounted. We are saving the specimen for future study, including molecular analysis. Therefore, we felt that it was best to preserve the specimen with no further chemical treat-
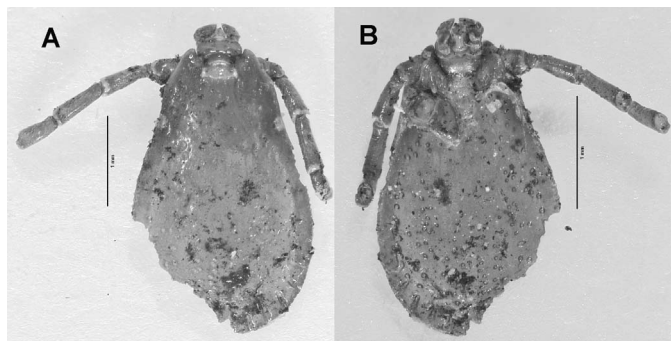
FIGURE 1.   Dorsal (**A**) and ventral (**B**) views of the whole specimen. A dorsal scutum is evident. However, the posterior ventral portion of the tick was fractured away and was not discovered in extensive examination of the coprolite residues.



FIGURE 2.   In this close-up of the dorsal (**A**) and ventral (**B**) aspects of the tick, one can see the capitulum from both sides. The palpi are short, about as long as the basis capituli. Also, a coxal spur is visible.

ment. A temporary mount was used for microscopy that did not utilize adhesive.

Coprolite Lab 2, FS 1516, is most likely human in origin. Its initial appearance was consistent with human feces, it had a dark rehydration color, and there was no evidence of the mucilage that we often observed in dog coprolites (Reinhard et al., 1988). Moreover, there were no dog hairs in either the macroscopic or the microscopic remains. In contrast, the 3 dog coprolites analyzed so far from the site contained abundant animal hair, lizard bones and scales, and oddities such as fragments of rabbit hide with attached fur. The dogs appear to have hunted small animals, including horned lizards, and supplemented their diet by eating human feces and trash in the cave.

The macroscopic dietary residues from Lab 2, FS 1516, are derived mostly from ground maize, ground sunflower, unknown plant epidermis and fiber, bone, and possibly a female flower from cottonwood. Grass stem/epidermis fragments and maize starch dominate microfossils. For example, 34,300 maize starch grains, 18,724 Poaceae (grass family) cells or phytoliths, and 17,164 vascular bundle fragments were found per gram of coprolite. The pollen has some cottonwood-type grains, but we are uncertain of this identification because fossil cottonwood pollen can resemble many other spores and pollen from other taxa.

A well-preserved tick was found in the microscopic remains (Figs. 1–3). Examination of the tick showed that it was a species of *Dermacentor*. This diagnosis is based on number and size of the remaining festoons, as well as on the fact that the capitulum is visible from dorsal and ventral aspects, a dorsal scutum is present, and the palpi are short (about as long as the basis capituli). Originally, the tick had 11 festoons. Unfortunately, the spiracular plates are not present on this fragmentary specimen, so species-level diagnosis is not possible. However, the basis capituli have short cornua, which is consistent with *Dermacentor andersoni* (Stiles). The white dorsal markings typical of this species are not evident. This is probably a result of passage through a digestive tract.

The incomplete nature if the specimen leaves some doubt as to the identification of species and developmental stage. We believe the tick is an adult or nymph of *D. andersoni* (Stiles). It is 3.4 mm long and 2.0 mm wide. This is in the range for adults and nymphs. The reconstruction of the tick (Fig. 3) shows that it originally had 11 festoons. The ventral surface is fractured posterior to the first left coxa and second right coxa. The outline of a broken-off third coxa is visible, but it is impossible to ascertain if there was a fourth. Three coxa would be consistent with a larva, and 4 would be consistent with a nymph or adult. There are no visible spiracles. If this was an adult, the spiracle plates had to have been present just posterior the fourth coxa. Larvae have no spiracles. We believe that spiracles have been simply fractured away from the specimen since the region of the fourth coxa is missing. To support our identification, there is a distinct spur on the first coxa that is characteristic for larvae and nymphs of *D. variabilis* and *D. andersoni*. Relative to the three-legged larval stage, the general morphology of the scutum is consistent with an adult or nymphal male. The larval scutum is not as elongate as adults and nymphs. We cannot determine whether *D. variabilis* or *D. andersoni* is represented by this
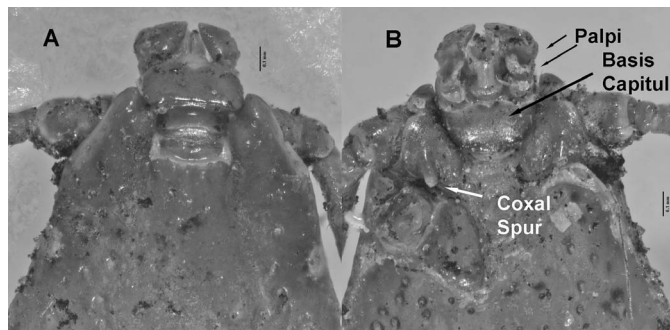
specimen. The key anatomical elements, i.e., spiracular plate goblets, are not present. *Dermacentor variabilis* is not present in the Rocky Mountain region and is not endemic to Utah due to climate (Longstreth and Wiseman, 1989). However, Antelope Cave is within the southernmost range of *D. andersoni*. Therefore, in all likelihood, the tick discovered at Antelope Cave is an adult or nymph *D. andersoni*.

From the perspective of the function of a tick as a disease vector, specific identification matters. Tickborne diseases have been suggested as potential health threats for Ancestral Pueblo people (Stodder and Martin, 1992, p. 62). The discovery of *Dermacentor* sp. at Antelope Cave is the first empirical evidence that ticks and humans were in contact. Which diseases were potential threats for the Ancestral Puebloans of Antelope Cave? The fact that we found a *Dermacentor* sp. tick limits the number of disease possibilities (Roberts and Janovy, 2000; Bowman, 2003; PAHO, 2003). Lyme disease is transmitted by *Ixodes* spp. and *Amblyomma* spp. ticks. Ehrlichiosis is transmitted by ticks of 2 genera, i.e., *Ixodes* and *Amblyomma*. Tickborne relapsing fever is transmitted by *Ixodes* spp. and *Ornithodoros* spp. ticks. These diseases probably were not potentialities for Antelope Cave.
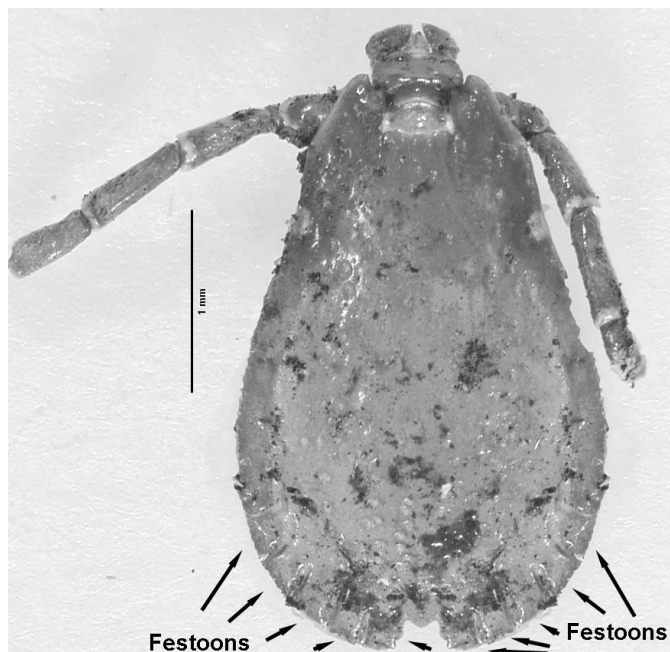


FIGURE 3.   Adobe Photoshop reconstruction of the dorsal aspect of the tick. This was made by copying, reversing, and inserting the right lateral edge of the tick over the missing region on the left side. Note that 10 festoons are reconstructed. The posterior-most festoon is lost and could not be inserted in this reconstruction.

Rocky Mountain spotted fever is spread to humans by *Dermacentor* sp. ticks. Tularemia is caused by the bacterium *Francisella tularensis*, which is transmitted by species of *Amblyomma* and *Dermacentor*. Therefore, these 2 infectious diseases could have been transferred by ticks at Antelope Cave. Tick paralysis is a condition that results from neurotoxins secreted by ticks during their feeding process, so tick paralysis or serious skin reactions such as dermatosis, inflammation, swelling, ulceration, and itching are possible.

The question might be raised as to whether this tick was accidentally consumed inadvertently in grain or some other food product. The maize and sunflower found in the coprolite was thoroughly ground. Had the tick been a contaminant of the grain, it would have been ground into powder. It is not likely that ticks would have been in the cave, since *D. andersoni* feeds on small animals in brushy areas. The human coprolites from Antelope Cave contain bones of *Sylvilagus* sp. Rabbits, and the trash deposits contained bones from a variety of small vertebrates. Thus, humans came into contact with the ticks when they hunted small mammals. The fact that this *Dermacentor* sp. was found in an apparent human coprolite and the finding that it was partially crushed indicate that the tick was pinched between the teeth and swallowed. This action reflects a response on the part of one ancient person who chose to remove the tick, bite it, and swallow it, thus suggesting a prehistoric behavior pattern of eliminating arthropod pests by eating them.

We are currently examining more human and dog coprolites from Antelope Cave and anticipate reconstructing the parasite ecology of this site.

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# Observations on *Cryptosporidium* Life Cycle Stages During Excystation

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abstract:    *Cryptosporidium parvum* (HNJ-1 strain, genotype 2) merozoites were released from oocysts directly during an incubation and excystation procedure without bleach treatment. They were polymorphic, mostly spindle-shaped; others were bean shaped, actively motile, and underwent division. Merozoites survived for short time–period in an in vitro culture system, but could not be established in a subsequent cultivation effort in RPMI medium.

Many vertebrates, including humans, are hosts to the intestinal protozoan parasite, *Cryptosporidium parvum*. Species in this genus have a worldwide distribution and in the last 3 decades have become an increasingly important public health problem. *Cryptosporidium parvum* has been reported as the causative agent of a number of waterborne outbreaks of diarrheal disease and is considered one of the most important contaminants of drinking water (Karanis et al., 2007). The general life cycle and biology of *C. parvum* are comprised of an exogenous stage (oocysts with 4 sporozoites) and an endogenous phase (trophozoites, merozoites, and sexual stages) as extensively described in Fayer et al. (1997). Oocysts of *Cryptosporidium* spp. can be exposed to different media to produce sporozoite excystation. In vitro techniques for sporozoite excystation from oocysts have been reported by several investigators (Upton, 1997). Exposure to an acid pH during in vitro excystation protocols for *Cryptosporidium* spp. mimic host–derived signals, but some of these host–derived triggers seem to be unessential. There is still an insufficient understanding of the hierarchy or synergism of specific