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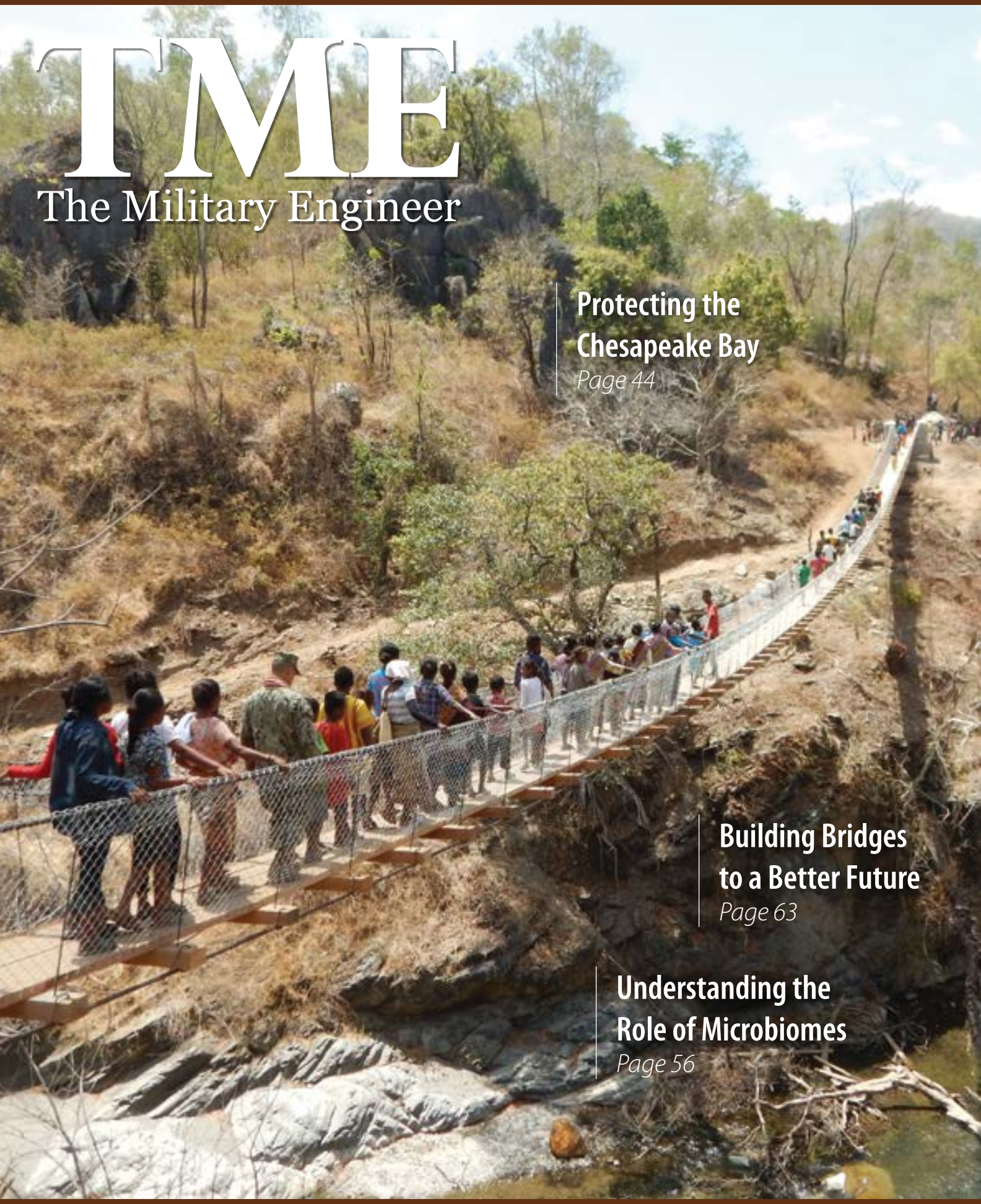

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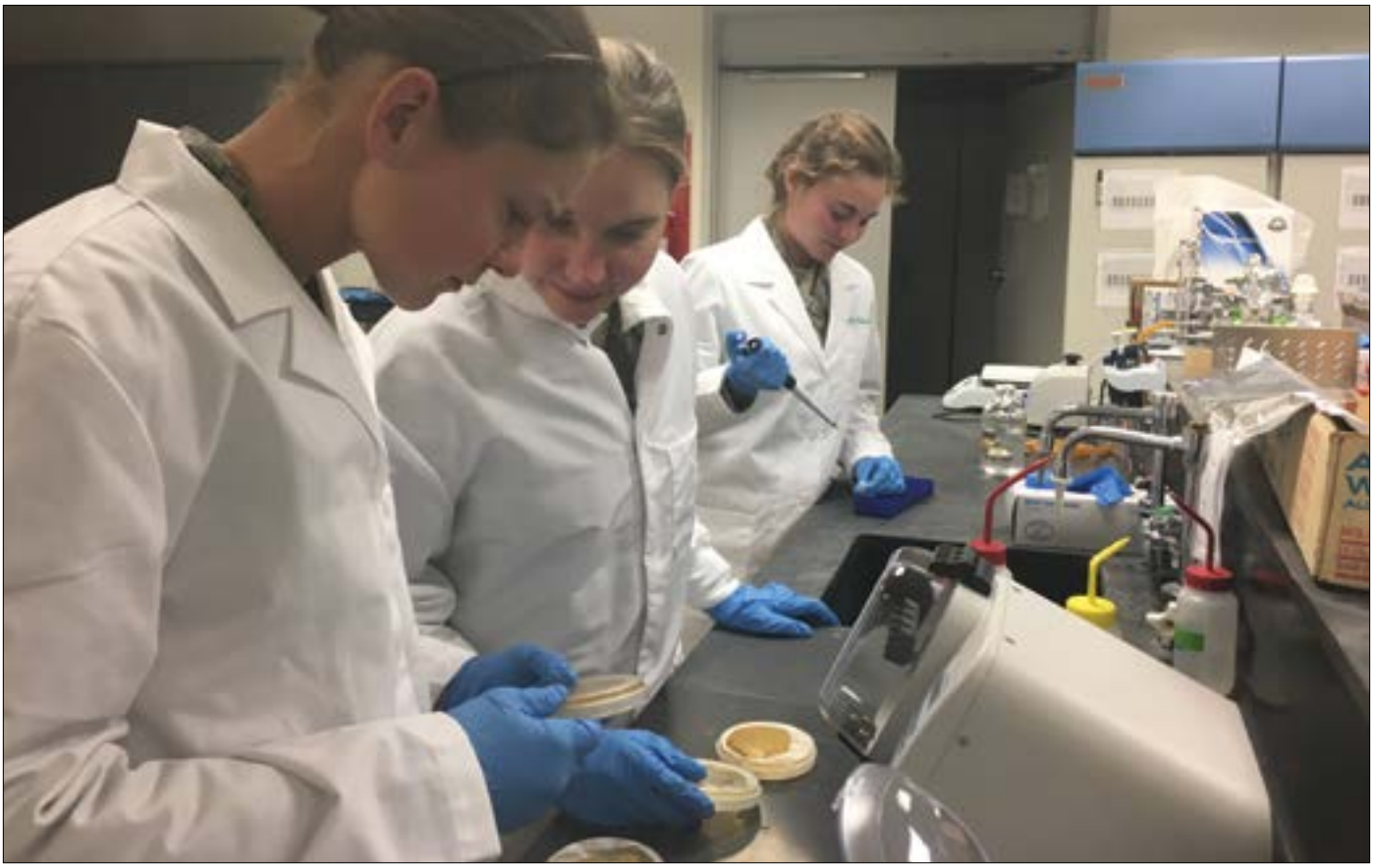


(From near to far) Cadet 2nd Class Amanda Underhill, Cadet 2nd Class Amanda Elliott, and Cadet 1st Class Amelia Roddenberry analyze microbiome samples while conducting research at the U.S. Air Force Academy. Microbiomes are found on and inside the human body, in buildings, and the outdoor environment.
PHOTOS COURTESY U.S. AIR FORCE ACADEMY

Understanding the Role of Microbiomes

The establishment of the Military and Veteran Microbiome Consortium for Research & Education is helping to unlock future discoveries that will greatly increase scientific knowledge of the microbiome to ultimately benefit individuals and the organizations they support.

By Cadet 1st Class Amelia Roddenberry, M.SAME, Cadet 1st Class Jacob Holland, 2nd Lt. Tabitha Sprankle, M.SAME, USAF, Katherine Bates, Ph.D., and Lt. Col. Andrew Hoisington, Ph.D., P.E., M.SAME, USAF

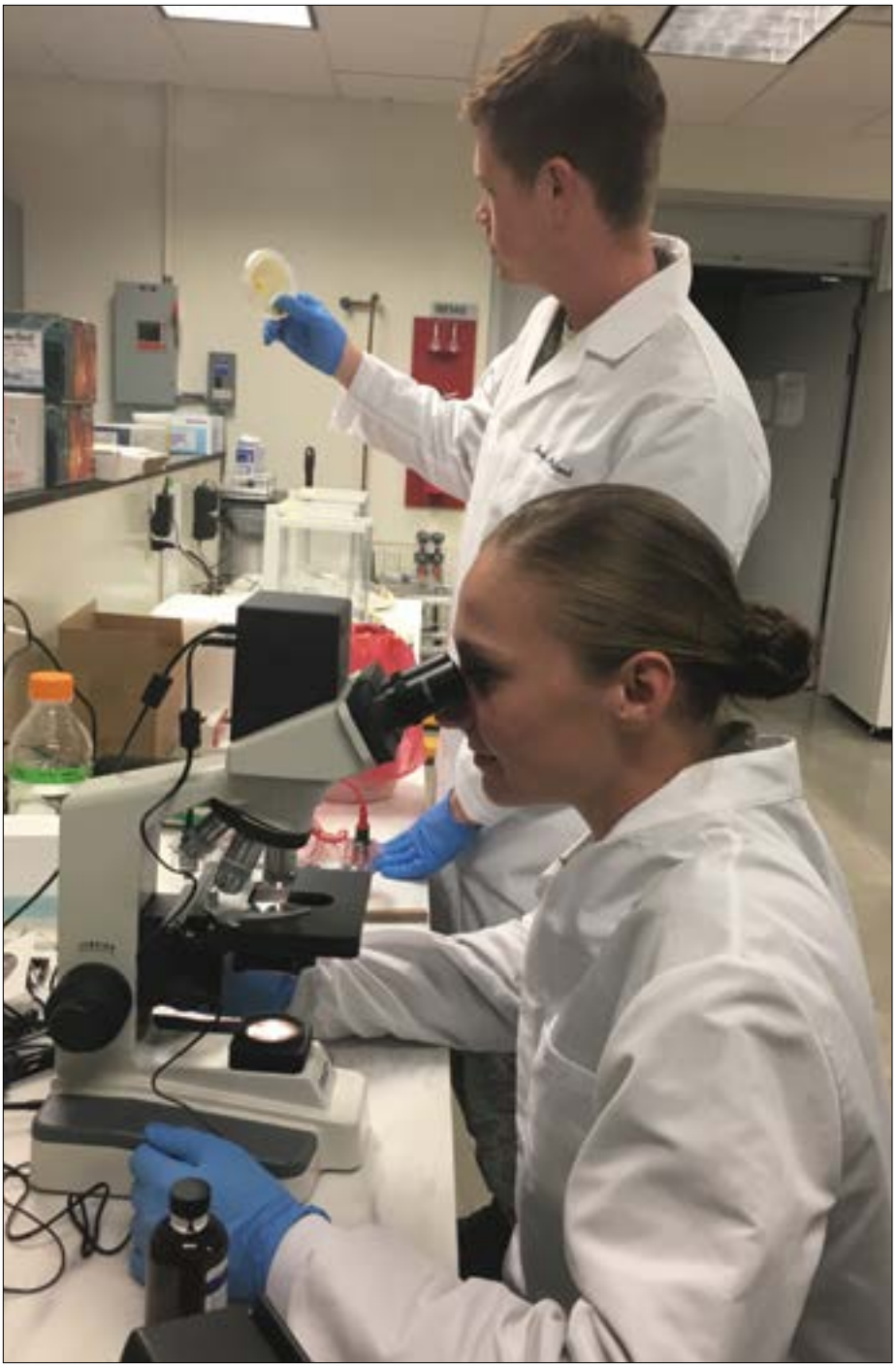
Microorganisms may have a tremendous impact on human health and behavior. These bacterial and fungal communities, collectively known as a microbiome, are found on and inside the human body, in our buildings, and in the outdoor environment. Approximately 4-lb of an individual's body weight can be attributed to the microorganisms living on that individual, and human cells are outnumbered 10 to 1 by personal microbial cells.

In the past, most research efforts have been focused on destroying or managing microbial pathogens. However, new information suggests that our microbiome is essential for good health and may be linked to emotional behavior, asthma and allergy treatments, and cognitive function. Given that bacterial and fungal communities seem to play a large role in personal health and have even altered the instinctive nature of animals, researchers have begun to ask whether microbial exchanges can affect individuals' behavior over time.

IMPACTS ON INDIVIDUALS

The U.S. Air Force Academy, in collaboration with the Department of Veterans Affairs, the Universities of Texas and Colorado, and Maryland's School of Medicine, is working to collect and analyze thousands of aerobic and anaerobic microbiome samples through multiple studies.

A research group, the Military and Veteran Microbiome Consortium for Research & Education (MVM-CoRE) has embarked on a large-scale longitudinal study of the homogenization of the microbiome in U.S. Air Force Academy cadets. More than 40 third-year cadets



Cadet 1st Class Karen Wolf and Cadet 1st Class Jacob Holland use traditional biological methods to analyze microbiome samples.

sampled their gut and skin microbiomes twice a week during the first five weeks of school, and will sample again bookending the winter break.

To better understand stress levels in the cadets, saliva cortisol levels and self-reported stress levels are being measured through surveys. The study is also collecting data for both indoor and outdoor air quality and dust samples from the participants' rooms and public areas within the dormitories. This data will be used to assess the microbiome of the built environment.

This is the largest known study of both the built environment and the personal/building microbiome. In just the first five weeks of the program, hundreds of surveys, thousands of microbiome samples, and nearly a million data points on the indoor and outdoor air quality were collected.

The study will address multiple questions:

- Does proximity to others increase the transfer of microbiomes and disease?
- Will isolation from urban society result in increased negative health outcomes?
- Is the microbiome responsive to stress, and if so, can this change increase the potential for disease?
- Does diet make cadets and airmen more resilient?
- Are there any long-term effects of microbiome shifts?

ASSESSING VARIABLES

With the expansion of microbiome-related sampling and the ease with which individuals can collect and submit their own microbiome sample, it is important to understand the impact shipping conditions may have on the microbiome collected.

To investigate this, 12 people volunteered to provide four microbiome samples from skin and gut swabs. Each of the four samples was delivered from the Air Force Academy to the University of Colorado by a different method: standard shipment through the U.S. Postal Service; standard shipment with a stop in Austin, Texas; next-day Federal Express shipping; and hand-delivery to the destination on dry ice.

Though researchers are still in the process of analyzing the microbiome data, the results from the temperature and relative humidity monitoring throughout the shipping time are available. Temperature and relative humidity changes may alter the microbial communities during shipping, especially when shipping from or to a different climate. These differences may provide the conditions for growth of select microbes during the shipment process and may alter the microbiome of interest.

Additionally, sharp increases in temperature or relative humidity could make moisture available to the bacteria and thereby allow growth of select bacteria.

UNIQUE IDENTIFICATION

Because an individual's microbiome is more unique than DNA, it is worth exploring its use for identification purposes.

(Left) Cadet 1st Class Justin Jones, foreground, and Cadet 1st Class Jacob Holland collect microbiome samples from the floor of a dorm room at the U.S. Air Force Academy. (Right) Cadet 2nd Class Amanda Elliott swabs a desk to help determine the microbiome transfer from occupants to their built environment.

Since humans shed approximately one million bacterial cells per hour, people are constantly exchanging portions of their microbiome. Indeed, 99.9 percent of human DNA is identical, yet the human microbiome might be as little as 10 percent similar.

To determine if it would be possible to exploit these differences for identification, a small-scale sampling of faculty members and their families was done to determine the biofingerprint that individuals leave behind and the relationship of those microbial communities with family members.

This study involved 35 participants and included both human and built environment microbiome sampling in the office and at home. Microbial biofingerprinting could be useful in determining who might have been in an occupied space and how long it has been since they were last there.

OTHER NOTABLE STUDIES

Another study being conducted at the Air Force Academy is focusing on Toxoplasma gondii (*T. gondii*), a parasitic protozoan that is the most widespread parasite to infect humans, including approximately 15 percent of Americans. The protozoan is one of the few organisms with the ability to penetrate the blood-brain barrier, but in humans it has long been considered benign for most. However, in pregnant women, *T. gondii* infections cause toxoplasmosis, with serious complications including stillbirth and birth defects. This parasite can also alter the mental state of birds and small mammals, making the animals bolder and reducing their fear of predators, including domesticated cats. Recent research has shown that *T. gondii* might have an impact on the mental health and cognitive abilities of humans, although that field of study is still developing. Presently, the only known method of human infection involves ingestion of the spores, generally from cleaning the litter box of a house cat. The Air Force Academy research project is investigating if *T. gondii* could be detected in the built environment outside of a cat litter box.

In another effort, MVM-CoRE is investigating a probiotic to reduce fear and

(Left to right) Cadet 2nd Class Amanda Underhill, Dr. Katherine Bates, Cadet 2nd Class Amanda Elliot, Cadet 1st Class Jacob Holland, Cadet 1st Class Karen Wolf, Cadet 1st Class Amelia Roddenberry, 2nd Lt. Tabitha Sprankle, USAF, and Lt. Col. Andrew Hoisington, USAF (not pictured, Cadet 1st Class Justin Jones).

anxiety in veterans suffering from post-traumatic stress disorder. Research has shown a strongly suggested connection between the composition of the human gut microbiome and mental health and behavior. Additional research has linked probiotic use to a reduction in fearful behavior in a mouse model; this pilot study of 40 veterans will determine if this same phenomenon is seen in humans.

Finally, MVM-CoRE has begun sampling oral, skin, and gut microbiome of veterans in conjunction with their mental health data to explore connections between mental health and the microbiome on a large scale. Up to 200 veterans are expected to be sampled in this effort.

PROMISE FOR THE FUTURE

The initiative of MVM-CoRE has led to the first Department of Defense Microbiome Symposium, which will be held at the Air Force Academy in April 2017. Microbiome experts from academia and the military will gather to discuss the needs and future directions for microbiome research to benefit our military, veterans, and their families.

This study involved 35 participants and included both human and built environment microbiome sampling in the office and at home.

The possibility for collaborative research across disciplines is exciting. We believe bringing together a diverse team of researchers will be the key to unlocking future discoveries that will greatly increase our knowledge of the microbiome to ultimately benefit both individuals and the organizations they support.

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RESEARCH TAKES ACTION

In the January-February 2015 issue of *The Military Engineer*, the article "Microbes Among Us" by then-Maj. Andrew Hoisington, Ph.D., USAF, was published. The article discussed the microbiome of the built environment and the role it might have in facility design, occupancy, and specific considerations for Department of Defense facilities. "Microbes Among Us" prompted several discussions and eventually led to the establishment of the Military and Veteran Microbiome Consortium for Research & Education (MVM-CoRE).

MVM-CoRE is a multi-disciplinary research team that focuses microbiome research for the benefit of military, veterans, and their families. Founding members include Lisa Brenner, Ph.D., (Director, Veteran Affairs Rocky Mountain Mental Illness Research Education and Clinical Center and Associate Professor, University of Colorado Denver), Christopher Lowry, Ph.D., (Associate Professor, Department of Integrated Physiology, University of Colorado Boulder),Theodore Postolache, Ph.D. (Professor of Psychiatry, University of Maryland School of Medicine), and now-Lt. Col. Andrew Hoisington, USAF (Assistant Professor and Environmental Engineering Division Chief at the U.S. Air Force Academy).

In less than two years, this group has received more than $800,000 in grant funding. It has successfully collaborated on multiple projects, including a longitudinal survey of microbiome homogenization; the effect of shipping conditions on the composition of the microbiome; biofingerprinting with the microbiome; use of probiotics to reduce fear and anxiety in veterans suffering from post-traumatic stress disorder; detecting *T. gondii* in the environment; and surveying the microbiomes of military veterans.