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Amber McElhinney
University of New Hampshire

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Research Article

The Heart of a Horse: 3-D Echocardiographic Analysis of the Equine Aortic Valve

—Amber McElhinney

Like human athletes, sport horses have more wear and tear put on their bodies, including on their hearts. High-performing human athletes have been known to die suddenly from cardiac events, and horses are no different. Cardiac issues like murmurs and arrhythmias can be normal in horses, but it is important to know when they are a cause for concern. This is especially true when the murmur is caused by disease or defects in the aortic valve, because this can lead to sudden cardiac death in a rare but not insignificant number of cases.

Aortic regurgitation (AR), which is leakage of the aortic valve, is one cardiac issue that can be caused by disease but can also be a normal occurrence with aging. Evaluating equine cardiac function accurately, including AR, is extremely important for the safety of both sport horses and their riders, and new methods are being developed to do just that. During the summer of 2018, I researched new ways to evaluate the equine aortic valve for AR.

As a University of New Hampshire (UNH) equine studies: equine science major on the pre-vet track, I have always had an interest in equine medicine. I needed a project for my senior thesis and wanted something heavy in hands-on research to gain experience for veterinary school. Many equine students with research-focused senior theses do projects at the UNH dairy barns, because the equine department does not conduct research, but I wanted to focus on horses. Because the United Kingdom has many areas where equestrian sports are prominent, I decided to explore summer research opportunities there.

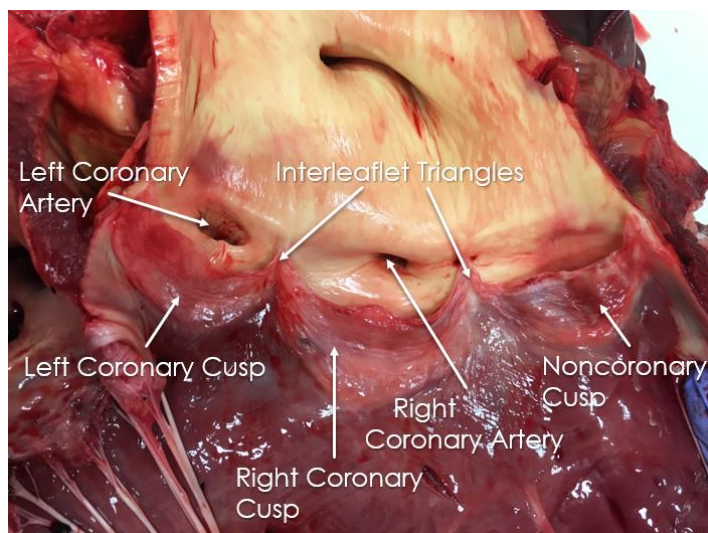


The author taking a left-side 3-D echocardiography scan of a horse. (Photo by Rachel Jago.)

My professor Drew Conroy contacted a former student of his who had studied veterinary medicine at the University of Edinburgh, and she suggested I reach out to a professor there, John Keen. Dr. Keen offered to mentor me for a project on equine cardiology, focusing on three-dimensional imaging of the aortic valve. I was awarded a Summer Undergraduate Research Fellowship (SURF) Abroad from the Hamel Center at UNH to study quantitative, direct methods to evaluate the equine aortic valve for progression of aortic regurgitation using three-dimensional echocardiography.

What Is the Role of the Aortic Valve?

To understand potential issues in the aortic valve, it is important first to understand its anatomy. The aortic valve is the last stop in the heart before blood is pumped to the body; it is the valve between the left ventricle and the aorta. As such, it is subject to very high pressure, since the left ventricle must pump blood with enough force to send it to the rest of the body. During diastole, or relaxation of the heart, the valve is closed to allow blood to fill the left ventricle. During systole, when the heart contracts to pump blood, the aortic valve opens to allow blood to leave the heart.



The author taking a left-side 3-D echocardiography scan of a horse. (Photo by Rachel Jago.)

There are three cusps, or flaps, that open and close in the aortic valve: the noncoronary cusp, the right coronary cusp, and the left coronary cusp. All cusps are attached to corresponding coronary sinuses, or enlarged cavities along the vessel wall, also called sinuses of Valsalva. The left and right coronary sinuses give rise to the left and right coronary arteries. Between each sinus is a raised triangular portion of tissue called an interleaflet triangle (Figure 1). When the cusps close in diastole, they slightly overlap to form the lunulae. The general area where the cusps overlap is called the commissures (Sutton et al., 1995).

In aortic regurgitation (AR), blood leaks back into the left ventricle from the aorta during diastole, when the heart relaxes to refill with blood (Reef et al., 2014). AR can be common in older horses, and can be considered a normal function of aging in mild cases. However, when younger horses get AR, it can be a cause for concern (Reef et al., 2014). High-performance horses with AR have been known to drop dead suddenly, which, in addition to being a tragic event, can be dangerous for the rider (Reef et al., 2014). As such, finding accurate ways to predict AR severity and its potential effects on the horse is very important.

Horses have very large hearts, averaging about nine pounds, or typically about 1 percent of their body weight, and therefore pump so much blood that some leakage does not always have detrimental effects on the horses' abilities or display any symptoms at all. The primary diagnostic method for AR is echocardiography, which is ultrasound of the heart. Magnetic resonance imaging

(MRI) is used in humans to look at the heart in detail, but this is not possible with horses (they won't fit in an MRI machine), so alternative methods must be used. Various types of echocardiography used clinically include Doppler, motion mode, two-dimensional, and three-dimensional.

In three-dimensional echocardiography, the technique used for my project, a pyramid-shaped section of tissue is recorded instead of a flat, 2-D triangle of tissue. After an examination has been recorded, the cardiologist can use software on a computer to manipulate the three-dimensional image to visualize any structures within that pyramid of tissue. For my project, the software used was EchoPAC by GE, and the tools used were the GE Vivid E9 and 3VD probe. The current standard for quantification of AR severity is looking at the size of the left ventricle, flow velocity of blood through the aorta, and leakage amount and velocity of blood back through the valve in diastole (Keen, 2016). Although these are all related to the aorta, none of them directly measure anything about the aorta or the aortic valve. The aortic valve has been known to thicken in cases of AR, and part of my research included taking measurements of the valves of various horses with different levels of AR.

The goal of my research project was to determine the best approaches for evaluating the equine aortic valve and normal versus abnormal appearance using three-dimensional echocardiography. I approached this goal in three phases: (1) by describing the anatomy of the aortic valve visible in 3-D; (2) by taking novel, or new, measurements of both normal and abnormal aortic valves; and (3) by taking new images from the left side of normal horses without any AR and comparing them with previously taken images from the right.

Methods for Looking at the Aorta

The day-to-day context of my research in Scotland was fairly normal. I woke up in the morning, ate breakfast, and took the bus from my flat to the Royal (Dick) School of Veterinary Studies at the University of Edinburgh, where I conducted my research. My days at the vet school comprised reading journal articles, using the GE EchoPAC software to analyze images, recording observations, and assisting with cardiac cases in the hospital.

Phase One Methods

Cardiac scans of forty thoroughbred horses with normal cardiac function had been taken by Dr. Keen in April 2015, including right parasternal (from the right side of the sternum) 3-D images of the aorta. For the first phase of my study, I determined nine of the images from these forty horses to be of good quality, then further analyzed those nine images to get a sense of what a normal equine aorta should look like on 3-D echocardiography.

Using EchoPac, I measured the length of visible tissue between each of the cusps, which included the lunulae, or where the cusps overlap, and any visible surrounding tissue, in diastole. I also measured the thickness of each cusp from a side view, taking measurements toward the center of each cusp. I took area and circumference measurements of the valve in systole and diastole, as well as valve-diameter measurements from the front and from a side view. I repeated all diameter, area, and circumference measurements three times and calculated the averages.

In addition to these novel measurements, I obtained good views of the interleaflet triangles, coronary arteries, and cusps, when possible, from each image by zooming in, changing the coloring of the image, and rotating the image to find various structures. This was to help describe what structures can be seen on a horse with a normal heart, and therefore to give another way in which diseased hearts might appear different in 3-D echocardiography.

Phase Two Methods

For the second phase of my research, I followed the same procedure as in phase one but on images from horses that had AR. These images were from cases from the last few years in the Royal (Dick) School of Veterinary Studies equine hospital database. I found usable 3-D images on file for fourteen horses that had previously received a diagnosis of AR and selected one good-quality image for each horse. I separated the images by diagnosed severity of AR: mild, moderate, or severe. Six horses had mild AR, six had moderate AR, and two had severe AR from endocarditis, which is infection of the heart. I took the same measurements for each image as for the images of normal aortas. Likewise, I manipulated each image in the software to get good views of various anatomical structures, as well as any pathology of the aortic valve. I compared the averaged measurements for mild and moderate AR with the normal values I had determined in phase one. I also compared the values from the two horses with endocarditis to normal valves and to those with mild and moderate AR.

Phase Three Methods

For the third and final phase of my research, I analyzed 3-D echocardiography images taken from the left sides of nine racing thoroughbred horses with normal aortas. I assisted PhD candidates in taking these left-side scans, which are important to get clear views of the aortic valve and cusps. I compared the left views with the right views of normal aortas previously taken, because the current standard for evaluation of the aorta is to take images from the right-hand side of the horse. Images taken from the left appear different from those taken from the right, and therefore in theory could show different aspects of the progression of disease for a more accurate diagnosis of equine cardiac issues relating to the aorta.

Results: What I Learned About the Aorta

For phase one of my research, I found that three-dimensional images of the aorta taken from the right side of the horse can be manipulated in a variety of ways to show many of the structures in the aortic root, which includes the aortic valve and surrounding structures. From an en-face view in diastole, meaning the front of the image is the top of the valve, the cusps themselves were not widely visible, but small sections of tissue, likely the lunulae between each cusp, were visible. The left coronary cusp was generally more visible than the others, having a more opaque appearance throughout the center of the cusp. This may have been because of the technique or approach used.

When the en-face image was rotated to either side, cross sections of the valve cusps could be viewed, but the left coronary cusp still had a more uniform, opaque appearance, whereas the noncoronary cusp and right coronary cusp were not as visible. Right-side images of normal aortas could also be rotated to view the sinuses of Valsalva, interleaflet triangles, and coronary arteries. These observations are important, because 3-D echocardiography is really the only available imaging

technique that can show these minute anatomical structures in horses. This could be very helpful for accurately identifying the exact area of disease in disorders affecting the sinuses of Valsalva or other aortic structures (Figure 2).

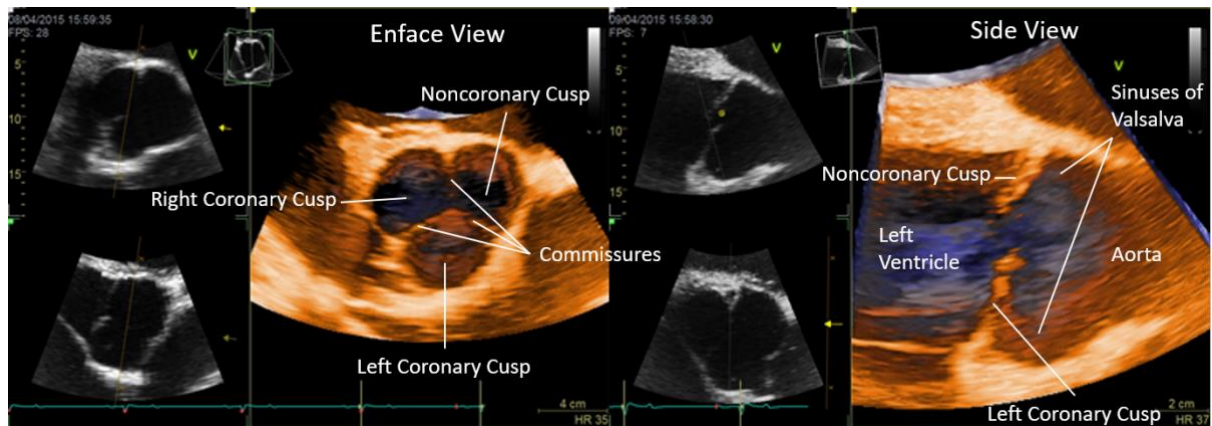


Figure 2. A 3-D echocardiography scan of a normal equine aorta in diastole, taken from the right side of the horse. The image to the left is an enface view, or looking down at the valve from the aorta. The image to the right is a side view, or a cross section through the aortic valve. The cusps are closed, and thin tissue can be seen where they meet in the left. These are the commissures. Cross sections of the closed cusps can be seen in the right image.

In horses with AR, whose images I analyzed in phase two of my research, the valve cusps tended to be more visible in diastole, and more opaque throughout, indicating that they are thickened. This opacity worsened with the degree of AR. Likewise, the commissures, or sections of overlapping tissue between the cusps in diastole, had more visible tissue, especially in affected areas (Figure 3).

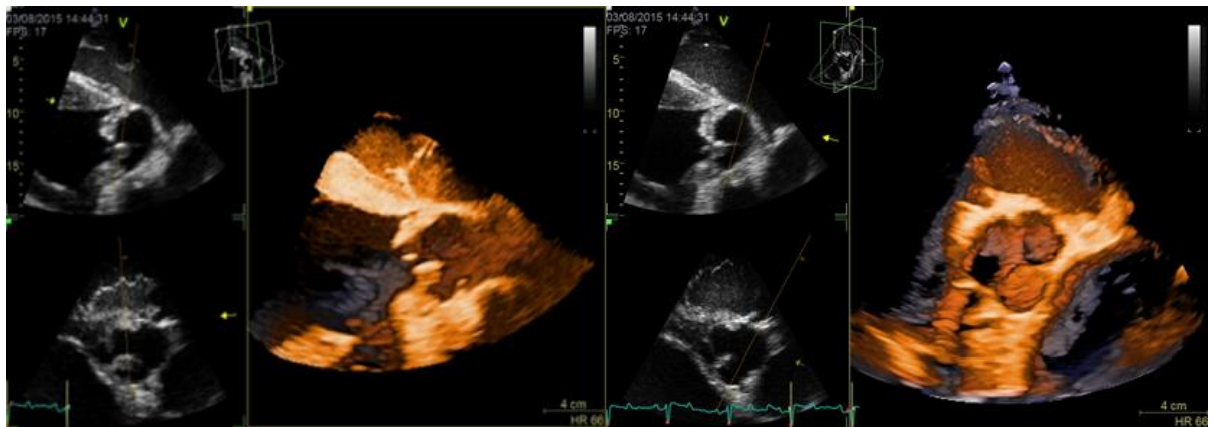


Figure 3. A 3-D echocardiography scan of an equine aorta with severe AR from endocarditis, or infection of the heart, in diastole. These images were taken from the right side of the horse. Here the left image shows a side or cross-sectional view of the aorta, while the right side is an enface view from the top of the aortic valve. On the right, tissue is visible throughout the cusps, indicating they are thickened. On the left, the cross sections of the cusps show that they are severely thickened from AR.

In some cases, I could see regurgitation of blood in the image. I observed that in general, the more severe the AR, the greater the various valve thicknesses. The diagnosed AR severity was scored from 1 to 4, with 1 being normal and 4 being severe.

The severe cases were excluded from statistical analysis because they were both from endocarditis and therefore not representative of normal disease progression. The normal images were included because they are the control of the study. In Figure 4, each dot represents one horse, showing 21 horses in all (9 normal, 6 with mild AR and 6 with moderate AR). Predicted AR score was determined by an equation in the Statistical Analysis Software (SAS) that includes only statistically significant measurements of the left coronary cusp taken from the side, and of the commissure between left coronary cusp and noncoronary cusp as taken from the front. The AR score given to each horse was determined by an equine cardiologist. I found the measurements taken of both normal and diseased valves to be significantly correlated with the degree of AR (Figure 4).

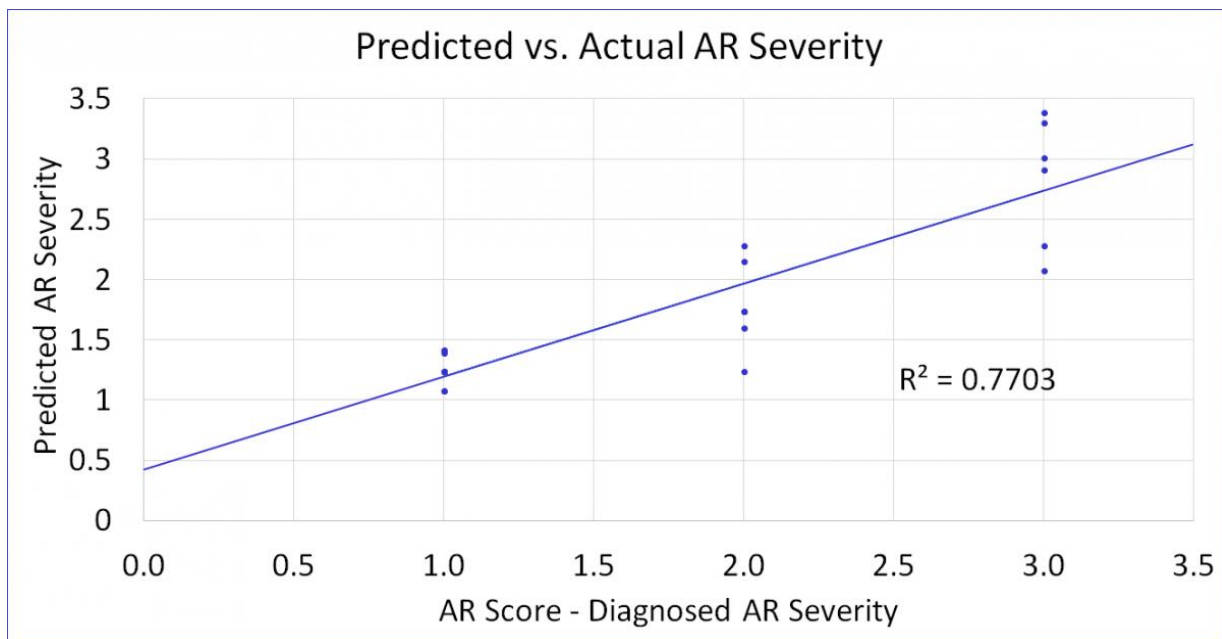


Figure 4. There was a positive linear correlation between diagnosed AR score and predicted AR score, with an R^2 of 0.7703. R^2 is a measure of the strength of the model, and can range from 0 to 1 (with 1 being 100%). The closer to 1, the more closely linked the dependent and independent variables, or X and Y values, are.

For the third part of my research, I observed differences in the parts of aortic anatomy visible in images taken from the right versus those taken from the left side of the horse. Images taken from the left side of the horse showed an orientation of the aortic valve opposite from those taken from the right. As a result, the noncoronary cusp could be visualized clearly, whereas the other two cusps were not generally as clear. More of the tissue throughout each cusp could be visualized in the left view than in the normal images taken from the right. The borders of the right coronary cusp and left coronary cusp were not always visible, but when the sinuses of Valsalva were visible, the coronary arteries were visible as well. Left-sided images were generally of worse quality than right-sided images, as the aorta is closer to the right side of the horse and there is less space between the

transducer and the aorta on the right. However, in some images the valve cusps were more visible throughout, so taking images from the left could provide a good method to evaluate valve damage when good images can be obtained.

Discussion: Why I Care About the Aorta

As previously noted, AR can be dangerous in horses, but it can also be considered normal. Accurate diagnosis of severity is very important, as it may indicate one risk factor for sudden cardiac arrest in horses. I was able to see a strong, positive, linear correlation between AR severity and thickness of the aortic valve cusps. Measuring aortic cusp thicknesses from 3-D images could potentially be another way to evaluate severity of aortic regurgitation and predict what kind of an effect it will have on the horse, which, combined with current methods, could paint a more complete picture and make diagnosis more accurate. This study helped to outline what is visible on 3-D, and could provide a basis for others to use in their cardiac evaluations. Human medicine has the ability to use cardiac MRI, but horses are too big for this technology to be possible. Cardiac evaluation using 3-D echocardiography is not at all standard in horses because of the novelty of the technology and cost of new equipment, but it provides a good alternative to MRI, because it allows visualization of soft tissue from many angles, as well as showing volumes of chambers.

During my project, I learned a lot about the anatomy and physiology of the equine heart in general, even though my focus was on the aorta and aortic valve. I had the opportunity to work with residents, PhD students who had already completed both veterinary school and internal medicine residencies, and other board-certified specialists in equine medicine at the University of Edinburgh equine hospital. I assisted in a “summer camp” for internal medicine residents from all over the world, and was able to look at pre-dissected, preserved hearts from real horses, both normal and abnormal. I was even able to dissect a heart from a horse that had been euthanized for noncardiac issues to get a better sense of the anatomy of a normal horse heart. I observed and assisted with numerous heart scans, practiced listening to hearts with a stethoscope and identifying murmurs, and went to a racetrack to assist veterinarians during Thoroughbred flat races. I really enjoyed working with the vets and learning more about equine sports medicine.

I will be able to use the knowledge I gained from this project in my professional field as a future equine veterinarian. I am most interested in sport horse medicine, and through this research experience I was able to work with and learn about sport horses and their common cardiovascular issues. My research gave me insights into the limits for normal in equine cardiovascular issues in more than just the aorta, which I know I will be able to apply in my future career.

First and foremost, I would like to thank my faculty mentor Drew Conroy for telling me about the Summer Undergraduate Research Fellowship (SURF) Abroad and helping me with everything from putting a proposal together to actually writing up my research. Thank you to Dr. John Keen, who allowed me to work with him in Scotland and taught me about equine cardiology. Thank you to PhD candidates Jenny Brown and Rachel Jago for taking me under their wings while abroad and teaching me practical skills while I assisted on their study. Thank you to my adviser Sarah Rigg for being supportive of everything I do and writing me countless recommendation letters throughout my time at

UNH. To my friends and family—thank you for listening about my research, even when you had no idea what I was talking about. Finally, thank you to everyone at the Hamel Center for Undergraduate Research for making my research possible, especially Georgeann Murphy for organizing everything and keeping in touch throughout the summer, and Mr. Dana A. Hamel and Mr. Benjamin Marcek for funding my research.

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Author and Mentor Bios

Amber McElhinney came to the University of New Hampshire (UNH) from Abington, Pennsylvania, just outside Philadelphia. Her work for an equine studies major on a pre-vet track, with a minor in dairy management, has led her—so far—to two countries: first to Ireland for an animal science course, Integration of Culture and Agriculture in Ireland, and immediately after that to Scotland. In Scotland Amber assisted PhD students at the Royal (Dick) School of Veterinary Studies in their field research and also conducted her own research under the mentorship of Dr. John Keen, who specializes in equine cardiology. “I learned everything I now know about aortic regurgitation and three-dimensional echocardiography during my research project,” Amber said. It was an effective learning curve. By the end of the summer, she was explaining to vet students what they were seeing during a 3-D echocardiography scan of a horse. Her research resulted in statistically significant findings related to heart valve thickness and aortic regurgitation in horses. The experience also helped her prepare for veterinary school. Amber has been accepted at four top veterinary schools and knows that wherever she ends up, she will arrive with a solid background knowledge of equine cardiology.

Andrew Conroy, a professor of animal science and sustainable agriculture and food systems in the Department of Agriculture, Nutrition, and Food Systems, has taught at the University of New Hampshire (UNH) since 1990. Dr. Conroy’s specialty is cattle, but he has global experience with other livestock, animal handling, and applied animal behavior. He has conducted research in Africa, including research with pastoral people in Tanzania and Namibia. Dr. Conroy spent 2008 in Namibia and 2016 in Rwanda as a Fulbright Scholar. While abroad, he investigates organizations where UNH students could do internships or supervised research in wildlife management and wildlife conflict. He has worked with many UNH undergraduates conducting research abroad, including projects with

cheetahs, elephants, and black rhinos in Africa and with koalas in Australia. By reaching out to a former UNH student who had also studied in Scotland, Dr. Conroy helped Amber find her research site at the University of Edinburgh. Encouraging his students to publish their research in *Inquiry* is yet another way in which Dr. Conroy helps them acquire valuable skills for their future careers.

John A. Keen is a member of the Royal College of Veterinary Surgeons, a diplomate of the European College of Veterinary Internal Medicine, senior lecturer in equine medicine, and director of equine hospital and practice at the Royal (Dick) School of Veterinary Studies in Edinburgh. He specializes in equine medicine, with a particular interest in equine cardiology. Amber approached Dr. Keen after being referred by a former University of New Hampshire (UNH) student who spent time in Scotland, and he agreed to supervise her research on 3-D echocardiography. Dr. Keen was impressed by Amber's "fantastic job getting to grips with the project and integrating with the team" in Scotland. Amber was the first UNH student mentored by Dr. Keen.

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