Communicating Equine Science

THE MICROBIOME & ANTIBIOTICS

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Early on in my academic career, I accidentally acquired some skills in an area not traditionally taught as part of a degree program in the hard sciences: public communication.

I completed my Ph.D. in 2011, and the word 'microbiome' had been buzzing around to high fanfare for at least a decade prior. Many of my young colleagues had plans to complete their doctoral studies and move to academic labs where they could study microbiomes—whole communities of microbial life associated with larger hosts—not least due to the apparent guaranteed funding prospects and recent cultivation of rockstar scientist personalities in that field. High-throughput DNA sequencing technologies were becoming (slightly) more financially accessible at that time, and people were seemingly sequencing the living daylights out of every bodily fluid and patch of soil they could find. I, alas, was still broke enough to be wrestling with the snappily named "denaturing gradient gel electrophoresis" technique. I could tell you about that, but I would have to be quite drunk first.

Scientists interrogated water, soil, bodily fluids, sewage, even the air—and generated lists and lists of genes and scraps of DNA found therein. Research laboratories spent tens of thousands of dollars producing this information. Within a matter of years we had accumulated more sequence data than we knew what to do with, and we had to invent a new field of study to analyze it all! We called that bioinformatics, and suddenly newly graduated Ph.D. biologists found themselves competing for biology jobs with computer scientists. It was all very exciting and disrupting, and people got really rather miffed when some scientists began to ask "that's great—but what does it actually mean?"

Bummer.

It was, absolutely, thrilling to widen the aperture of insight we had into the microbial world. Prior, we had approached microorganismal systems painstakingly, coaxing individuals out of their natural habitats and onto our jelly agar plates—many of them, of course, vehemently declined our invitation—and these culture-based skills were really an art form. Suddenly though, that wasn't necessary! We could suck the DNA out of a gram of soil and map microbial genes without their cooperation, and we could compare the maps of healthy soil and fallow soil and dead soil, and that would tell us which bacteria lived in dead soils and which bacteria lived in alive soils, and then we could draw conclusions accordingly.

However, some of the conclusions scientists were drawing were a little too…assertive. Biological systems are extraordinarily complex, mystifying tangles of influence and dynamicism. And, there is an unfortunate tendency to consider members of the microbial universe primitive in the most insulting sense of the word: simple, and driven by simple means, effecting simple outcomes. In this case, the word primitive, in fact, denotes a largely unknown to us but pivotally influential set of ancient relationships between single-celled and acellular creatures, and every other living thing on this planet, including the planet itself. Evolution beyond the single-celled has not progressed because it doesn't need to, these beings are able to perform gargantuan, extraordinary feats of physical, chemical, and biological prowess that dwarf our own capacities to, I don't know, invent cell phones, or pay taxes.

All that is to say, correlation does not equal causation, and microbiome research is exciting, but has fallen afoul of calculated overexcitement, and in some cases, exploitation. It won't have escaped your notice that you can buy a probiotic supplement to support almost every aspect of your own human health in almost every grocery store in town. Similarly, there are many products available for your horse, and all of them, human and otherwise, claim efficacy based on scientific research. The major limitation of most microbiome research is that it is descriptive only. It is relatively easy to generate a list of bacteria present in any given sample, but the functional implications of that list are wrapped up in scientific design, circumstance, and extremely complex biological feedback.

The word "microbiome" describes the sum of a community of bacteria, viruses, fungi, archaea, and any other microscopic organisms which live in a defined system. The system might be a lung, a lake, or a wastewater treatment facility. Arguably, contemporary research swivels around a time-point defined by the large-scale accessibility to genomics technology, which I mentioned above. Post-genomics, a lot of research went from whole-organism study to the study of nucleic acids, that is, DNA and RNA. There's plenty you can learn from a single cell by mapping its entire genome, certainly, but the context of the alive being is crucial. You could map my genome and perhaps notice that I have a predisposition to developing lots of moles on my skin. Without, however, the context of me being alive and active, you might not know that I chose to settle in New Mexico, and my increased exposure to UV versus if I had stayed in the UK means I've needed to have a bunch of those moles chopped off. So, the word "microbiome" itself is contentious among microbiologists (and we were seriously obtuse to begin with). Some suggest the suffix "-ome" should relate to the community of genomes in a sample, and that the correct term for a group of microorganisms should be "microbiota." "Microbiome" was actually a portmanteau first coined in 1988, by a chap named Whipp, and it was a smushing together of "microbe' and 'biome', used to describe all the inhabitants of an ecosystem, genomes and all. For the sake of popular modern convention, I tend to use microbiome/microbiota interchangeably.

Current research suggests that microbiomes within living bodies should be considered as functionally important as any other organ: livers, brains, or skin. The microbial worlds inside us don't just influence, but constantly interact with, our immune systems, our gastrointestinal tract, our gut-brain axis, and our general states of health and capacity. We have every reason to believe this is true of every other being in possession of a microbiome. Considering that a standard mammalian host may be in possession of a microbiome comprising trillions of microbial cells, we can see why microbiome research is so challenging. These communities don't come from nowhere—they've been developed over the course of an individual's lifetime, as a result of bazillions of microscopic interactions since the beginning of that branch of the tree of life. A study which examined the gut microbiome of semi-feral horses found connections and relationships to spatial and social factors. Foals do not have a community of symbiotic gut microbes present in their gastrointestinal tract (GIT) as they develop in their mother's belly—colonization begins at birth and lasts until weaning. They receive a majority of their microbiome through external transmission from their dam's vagina, udder, skin, and hair. The wild mares were shown to have a microbiome more similar to the dominant stallion, than the other mares in the herd. Microbiota composition in the wild is structured according to the local geography, the equine group, and the equine individual—similarities between mare-offspring and mare-stallion notwithstanding, every individual could be most strongly identified via having a unique microbiome. Further to the thread of evolutionary influence, research has demonstrated that the domesticated horse microbiome looks very different from samples collected from the only breed considered to have never been tamed: Przewalski's horse.

The microbiome is made of many players, and is dynamic enough that some of those players change roles, every now and then. Most of a microbiome is symbiotic, which means it either has a neutral or beneficial interaction with the host with which it lives in close association with. Some members of the community, however, may be pathobionts—symbionts who, in certain circumstances, may switch over to a pathogenic lifestyle (e.g., some bacteria may overproliferate and cause diarrhea in response to an antiobiotic treatment). Generally speaking, however, a stable microbiome speaks to a healthy host. Defining "healthy" however, is a huge challenge, due to the aforementioned issues of complexity and complicatedness. It's also no small feat to study microbial communities in situ—once you remove a group of bacteria from a lung or the soil, they immediately begin to behave and grow differently. Despite these difficulties, one desirable aspect we can perhaps define is that a diverse microbiome tends to be more robust and resistant to change than one which contains only a few different genera or species of flora. Correlation does not equal causation, and a descriptive study offers us little in the way of understanding the global view. For example, while we may observe a dramatic reduction in bacterial diversity in the gut of a horse post-colic, we cannot draw conclusions a to whether a drop in diversity caused the colic, or colic caused the drop in diversity. As scientists, we observe and report what is, to a statistically robust degree. Experimental design is important: it seems clear that diet is the biggest driver of microbial diversity in the equine gut. A study comparing the microbiota found in lean horses versus obese horses that does not control for diet, then, is flawed. Not useless by a long shot, but limited.

With all this said, the current research is fascinating. The bulk of equine research in this area invariably relates to the gut microbiome. One of the most frequently employed proxies for microbiome research is the ubiquitous fecal sample, used to represent the microflora of the GIT. While not necessarily an accurate representation of GIT flora in situ, due to the fact that horses readily produce manure, fecal samples can be used to develop a baseline descriptive understanding of a stable community versus an unstable one, or track gross changes over time. The equine gut microbiome mostly (not entirely) inhabits the hind gut (cecum and colon). Research has associated microbial species composition and diversity in fecal samples to matters of nutrition, management, medications, stress, age, and pathology, to name a few. The nascent biota developing around

our aforementioned foals comprises bacteria that are competing with one another for the right to exist in such a highly beneficial environment. Therefore, the microbiome is more diverse and dynamic in a youngster than that of an older horse, which may be responding to increased digestion time, energy requirements, or disease.

But the gut microbiome is studied so much for reasons other than sample abundance. The gut impacts the immune system, brain function, nutrient absorption, and drug metabolism. The stability of the GIT biota can be markedly disrupted by diet, particularly. Sudden ingestion of high starch feeds can lead to an over-proliferation of lactic acid bacteria in the hindgut (thereby reducing overall microbial diversity), which has been linked to colic and laminitis. Some studies suggest that a very high carbohydrate diet leads to some starches overstepping the chemical and mechanical digestion processes of the foregut, which leads to more entering the hindgut where those bacteria produce lactic acid. This lowers the pH, hindering the growth of acid-intolerant beneficial bacteria. Scientific authors suggest that microbial manipulation of reward pathways, production of neurochemicals, and changes in taste receptors may lead to overall changes in behavior. There is a clear association between microbiome composition and obesity and equine metabolic syndrome in horses, and there also seems to be a difference in gut biota communities when comparing hard and easy keeping horses.

A day-to-day gut, then, is enough of a moving target for researchers. Unsurprisingly, disruption to gut communities during times of crisis are widely described. Both oral and intramuscular administration of antibiotics in one study demonstrated significant impact, and research suggests that it can take up to 30 days for the belly to settle back down. This also applies in the use of NSAIDs, such as phenylbutazone and firocoxib. Colic, colitis, and laminitis are all associated with gross changes to the gut microbiome. Similarly, research suggests that the complex host-bacteria-parasite interactions which take place in response to helminthic (parasitic worm) infestations are another area to examine, with clear but currently not-understood roles in inflammation and immunity. Helminths may alter production of proteins in the equine gut, which change the nutritional environment for microorganisms.

While harder to collect samples from the foregut, studies have also been performed on the gastric microbiome, largely in support of research related to pathology. The equine stomach is not sterile, and in fact contains an abundance of microbial life which is quite variable between horses in response, again, to diet and environment. In studies focused on equine glandular gastric disease (EGGD) and ulcers, the research cautiously suggests evidence of alteration to the gastric mucosal microbiome in horses with the disease, versus those in a healthy population. This aligns with research into human microbiomes and gastritis. The authors point out, however, that no single bacterial species is associated with equine lesion biopsies, which is not true of humans. In humans patients suffering from Helicobacter pylori related gastritis, antibiotics are employed in order to wipe out the causative organism. Because there is no clear causative pathogen in EGGD, additional specific research cautions against the use of antibiotics in cases where omeprazole and sucralfate do not lead to improvement. This highlights, again, that microbiome research does not represent a silver bullet in management of pathologies such as these. Furthermore, researchers were unable to identify if the differences in bacterial communities at the lesion level were beneficial, i.e., mitigating the impact of pathology, or otherwise.

Aside from the gut, the most popular topics since 2021 during my literature review were studies examining the uterine and endometrial microbiome in mares, the periodontal microbiome and the skin microbiome. Few appear to expand beyond the descriptive metagenetic studies which allow us to compare community composition between different sample populations. So, while we should be reticent to make sweeping statements regarding what all this research means, we can agree at least that the horsie-ome, in its richness and diversity, probably has a really big impact on equine health and function. This is one of the main reasons why responsible stewardship of the use of antibiotics is so important—we've already learned that the gut microbiome can take up to 30 days to recover from treatment with antibiotics. However, antiobiotic resistance is one of the most unnerving problems that modern medicine currently faces—equine and otherwise. You may have heard about antibiotic resistance—and it's looming specter—promising disaster in the not-so-distant future. Our current reality, however, is that antibiotic resistance is not looming, it's here, and the promise of cataclysm that comes with lurks just round the corner.

Antibiotics occur in the natural environment, everywhere, and that's where we accidentally discovered them and began gathering them up in earnest. The discovery of penicillin saved thousands of lives during World War II, compared to war deaths before and during World War I, which were more numerous as a result of infectious disease rather than direct military action. "Antibiotic" and 'antimicrobial' are words often used interchangeably to describe antibacterial agents (they also cover antifungals, antivirals, etc.). Antibacterials are used to treat infections by killing bacteria, and stopping bacteria from growing. Some antibiotics kill broadly, and can be used against several genera or species of bacteria. Some kill narrowly and work against fewer. Some medically-significant bacteria are now able to escape the bactericidal effects of antibiotics entirely, and some of their names have entered into the contemporary vernacular: MRSA (methicillin-resistant Staphylococcus aureus), VRE (vancomycin-resistant Enterococcus), and resistant C. diff (Clostridium difficile). Estimates suggest that 36 million deaths have been caused due to antimicrobial resistance since 1990. Forecasting predicts that, without comprehensive policy

intervention, that number will rise to 39 million between 2025 and 2050, which is the equivalent of three deaths every minute. And this is a burden shared by us all—antibiotic resistance is phenomena which smudges across human and veterinary fields alike.

Resistance occurs when bacteria are able to evade the effects of an antibacterial agent. The biological mechanisms by which this can occur are numerous, and the prevalence of resistance is largely driven by antimicrobial overuse. Within a given population of bacteria which are sensitive to an antibiotic, a small percentage of individuals will possess natural, that is innate, immunity to that antibiotic. When it is applied to the population and kills the sensitive bacteria, all that remains is the resistant portion, which persists in the environment to pass on the genes required to be resistant. Inefficient use of antimicrobials can also lead to resistance—a dose small enough to challenge a microbe, but not enough to kill it, can develop an evolutionary response in that microbe, which then produces a resistant family.

A study performed on hospitalized horses treated with trimethoprim-sulfadimidine (TMS)—commercial names include Equisul-SDT and Uniprim—found that "oral treatment with TMA has a rapid and long-lasting effect in faecal microbiota composition….making the equine hindgut a reservoir and potential source of resistant bacteria posing a risk to animal and human health through transmission." The presence of different antibiotic resistant genes (ARGs) differs geographically and according to dosage—in cattle, subtherapeutic doses leads to higher levels of ARGs collected in fecal samples. Scientists highlight the importance of studying the equine hindgut as a reservoir for ARGs because the microbiome is so dense—as well as the close contact horses have with humans, and because similar antibiotics are used for the treatment of infections in both.

Antibiotic resistance is a growing problem in human and veterinary medicine, characterized by the level playing field of who is going to suffer as a result. The One Health approach highlights the need to balance human, animal, and ecosystem health if any of us are going to make it through the next hundred years or so unscathed—and it also applies to zoonotic and vector-borne disease, food safety, and the environment. So what can we do, as horse owners and humans, to mitigate the impact of antibiotic resistance in our sphere? The most important thing we can do is to not share antibiotics prescribed for one horse with another, and not use them at all unless it is under the specific guidance of a vet. Giving your horse antibacterials when they are unlikely to have a positive effect on the treatment of an infection means you get none of the benefits while still also dealing with the side-effects, and these can include colic and other gastro-intestinal problems. If you throw antibiotics at every scratch and bruise as a preventative, you may find that those meds do not work against a problem that actually presents a serious health risk—such as cellulitis.

Microbiome research represents an extraordinary potential for how we understand systems biology, and how to keep those systems running optimally. Wonderful discoveries are just around the corner for humans and horses alike. These discoveries, however, will pale in the dark of the catastrophe of running out of reliable options to treat infectious disease. Judicious use of antibiotics is crucial in maintaining both the day-to-day health and long-term wellbeing of your horse—as well as the horses and humans who will have far, far fewer antimicrobials in their toolkit in the decades to come.

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