A crash course in veterinary microbiomics

Candace Lyman
Oklahoma State University

Abstract

Veterinary microbiomics has arrived and is thriving in today's "Omics Era." Investigating microbial diversity within veterinary species will allow researchers to determine the physiologic state of the microbiome in healthy animals as a first step in developing treatments for animals with ailments resulting from dysbiosis. What impacts on clinical practices will arise as a result of the genomic information steadily accumulating in laboratory settings? As those answers come to fruition, it is our job as medical professionals to be capable of interpreting data and results provided to us. In order to develop and utilize a skill set, veterinarians should become acquainted with basic foundational concepts of veterinary microbiomics. Building on these terms and definitions, we will explore, compare and contrast the clinical relevance of currently available information from those species for which this work has been performed. Understanding these concepts will ultimately provide veterinarians with tools to analyze data and integrate information into clinical practices when appropriate.

Keywords: Metagenomics, 16S rDNA, 16S rRNA, microbiota, next generation sequencing

Introduction

Not long ago we entered an interval coined "Omics Era" due to our improved ability to collect large amounts of information on organisms at the molecular and protein levels and filter that information utilizing newer computational and statistical tools. Previously, utilization of next-generation sequencing techniques was cost prohibitive. However, with improvements in equipment and automated efficiency, this burden has been lifted and information pertaining to the bacterial population in various areas of the body is now accumulating with impressive momentum. Investigating microbial diversity within veterinary species will allow researchers to determine the physiologic state of the microbiome in healthy animals as a first step in developing treatments for animals with ailments resulting from dysbiosis. Whereas the day of veterinary microbiomics has successfully arrived, we as clinical veterinarians must now catch up by delving into a subject matter that has yet to even be integrated into veterinary school curriculums.

In order to develop and utilize that skill set, veterinarians will need to become acquainted with basic foundational concepts of veterinary microbiomics. Building on these terms and definitions, we will explore, compare and contrast clinical relevance of currently available information from those species for which this work has been performed. Understanding these concepts will ultimately provide veterinarians with tools to analyze data and integrate information into clinical practices when appropriate. What viable treatments or practices will arise as a result of the stockpiles of information being shared with veterinarians? With a basic understanding of veterinary microbiomics, veterinarians will be able to answer this question for themselves in clinical settings.

Terms to define, understand, and utilize

Metagenomics: Study of genetic material recovered directly from environmental samples.

Microbiome: Microorganisms in a particular environment (including body or a part); combined genetic material of microorganisms (not limited to only bacteria) in a particular environment.

Microbiomics: This term is used frequently in lay press but it is not frequently utilized in the microbiome community. Refers to the study of microbiome, the totality of microbes in (particular) environments; a study of microbes by considering all constituents collectively.

Microbiota: Commonly misused to refer to only bacteria, this is an ecological community of commensal, symbiotic, and pathogenic microorganisms (including bacteria, archaea, protists, fungi and viruses) present in and on all multicellular organisms.

Human Genome Project: An international scientific research project with the goal of determining the sequence of nucleotide base pairs that comprise human DNA, and of identifying and mapping all genes of the human genome from both a physical and a functional standpoint; although it started in 1990 and took 13 years and $1 billion to complete, today the human genome can be sequenced in 1-2 days for ~ $3,000-5,000.[1,2,3]

Operational taxonomic units (OTUs): An operational definition used to classify groups of closely related individuals; refers to clusters of organisms grouped by DNA sequence similarity (usually defined as 97% similar). Sequencing results are often reported in OTUs (e.g. "Firmicutes had 74 OTUs").

Phylum: A principal taxonomic category (e.g. Proteobacteria).

Genus: A principal taxonomic category, denoted by a capitalized Latin name in italics (e.g. *Escherichia*).

DNA sequencing: A process to determine the order of the 4 chemical building blocks (i.e. "bases") that make up a DNA molecule; these sequences tell scientists what kind of information is carried in a particular DNA segment and changes in a gene sequence can highlight a gene that may cause disease.

Next generation sequencing: A fast and efficient DNA sequencing technology capable of deciphering entire genomes (e.g. human genome).

A read: In DNA sequencing, an inferred sequence of base pairs corresponding to all or part of a single DNA fragment (e.g. Over 4 million reads of the V4 region of the 16S rDNA gene were obtained).

SILVA or Greengenes Database: Comprehensive web resources for up-to-date information, quality-controlled databases of aligned ribosomal RNA (rRNA) gene sequences from *Bacteria*, *Eukaryota*, and *Archaea* domains. When comparing sequenced results, it is imperative that identified sequences are *not* compared against a bank of *known* pathogens for a particular species, since these banks will not consider all possible normal, nonculturable bacteria and therefore are inherently biased and limiting.

16S rRNA vs 16S rDNA: rDNA, or "ribosomal DNA": DNA sequence that codes for ribosomal RNA. rRNA is the transcribed product of rDNA whereas rRNAs combined with proteins make up ribosomes.

PCR 16S rDNA sequencing process: Isolation of an unknown bacteria, bacterial DNA extraction, amplification of the 16S rRNA gene (i.e. PCR), sequencing of a portion of the 16S rRNA gene, comparison of sequenced gene with previously logged database sequences to identify a match (and therefore, bacterial identity).

Richness: A measure of the number of species represented in an ecological community; does not take into account abundance of individual species.

Diversity: Takes into account bacterial species richness and bacterial species evenness.

Metagenomics vs 16S rDNA profiling: "Shotgun metagenomics" surveys the entire genomes of all the organisms present in a sample rather than only those genomes in the 16S sequences; therefore, all organisms (bacteria, viruses, fungi) will be captured.[4]

Conclusion

Understanding these terms and concepts will enable veterinarians to better grasp forthcoming literature and presentations reporting results pertaining to veterinary microbiomes, whether it be describing the canine gut or reproductive tract. Having provided explanations and definitions of these commonly utilized terms, veterinarians can now make practical and effective use of this knowledge in a way that will allow them to analyze data and integrate information into daily clinical practice.

References

1. Lewis T 2013, Human genome project marks 10th anniversary, Live science health, viewed 12 Feb 2019, <https://www.livescience.com/28708-human-genome-project-anniversary.html>.
2. www.genome.gov 2010, National Human Genome Research Institute: frequently asked questions, viewed 20 Feb 2019, <https://www.genome.gov/11006943/human-genome-project-completion-frequently-asked-questions/>.
3. www.genome.gov 2015, National Human Genome Research Institute: DNA sequencing, viewed cited 20 Feb 2019, < https://www.genome.gov/10001177/dna-sequencing-fact-sheet/>.
4. www.blog.genohub.com, 2018, 16S sequencing vs. shotgun metagenomics: which one to use when it comes to microbiome studies, viewed 20 Feb 2019, <https://blog.genohub.com/2018/04/12/16s-sequencing-vs-shotgun-metagenomics-which-one-to-use-when-it-comes-to-microbiome-studie/>