An Introduction to Microbiology

Melody A. McFarland
Yokosuka, Japan

Introduction

Microbes are life forms so small that millions of them can fit into the eye of a needle. Experts say that infectious diseases caused by microbes are responsible for more worldwide deaths than any other cause (niaid.nih.gov 2002). Yet, at the same time, our survival depends on them in a delicate and sometimes complex relationship. Microbes are ubiquitous and are found in virtually every habitat on earth, from the boiling waters of deep–sea thermal vents, to the insides of our bodies, to the very air we breathe.

But what exactly is a *microbe* and why should anyone care? The public is barraged with statements like: *hand sanitizer that kills 99.99% of most common germs that may cause disease,* (Purell.com) and *kills* Salmonella *on contact!* Newspaper headlines warn about strange diseases in animals like the bird flu, rabies, and West Nile virus. New recruits at wildlife centers find their supervisors to be almost militant about hand washing and cross contamination. As a citizen and wildlife volunteer, you are told these things, but seldom is a thorough explanation forthcoming.

Understanding some elementary concepts about microbiology is crucial for anyone wishing to be an effective wildlife rehabilitator. Although sometimes a weighty topic, it is nevertheless imperative that all team members in a wildlife rehabilitation organization have some basic knowledge of microbes and disease processes. The intent of this paper is to educate both wildlife novices and professionals on some very important, but oft–neglected, issues. This introduction is the first in a series of articles dealing with microbiology and wildlife.

Melody McFarland *holds a BS in biology from Millersville University in PA and was a research scientist with bioMerieux of Durham, NC, in the microbiology exploratory research department until recently moving to Japan with her husband's military transfer. She was also a volunteer rehabilitator with Piedmont Wildlife Center and formerly served on the board of directors of the organization.*

What is a Microbe?

A microbe is a single–celled organism not visible to the human eye without the aid of a microscope. Most microbes of concern to the health of an animal belong to one of four groups: bacteria, viruses, fungi, or parasites. They can also be grouped according to evolutionary age, with bacteria in one group called prokaryotes ('old' cells) and fungi and parasites in another, called eukaryotes ('true' cells). Eukaryotes characteristically have membrane–enclosed organelles, most notably a nucleus, whereas prokaryotes do not. This distinction is of great importance in the development of drugs to fight diseases caused by these organisms.

Bacteria

General Characteristics. Bacteria make up the largest and most diverse group of microbes and are the oldest life forms on Earth. Fossilized bacteria have been dated back 3.5 to 3.6 billion years and represent the first known cellular form of life. Since that time, bacteria have undergone considerable evolution and divergence to result in an incredible 10^7 to 10^9 (10 million to 1 billion) different species (Schloss and Handelsman 2004). The large majority of bacteria are harmless or even beneficial to animal health, and only a very small percentage are actually pathogenic or disease causing.

Bacteria vary in size, but most of them are around 1/25000 of an inch in diameter. They come in three basic shapes and two less common shapes: spheres called *cocci*, rods called *bacilli*, spirals called *spirilli* or *spirochetes* (Figure 1), comma–shaped, and, more rarely, filamentous. Many types of bacteria possess methods of locomotion by means of filaments called *flagella* (tail–like appendages), others move by a rolling or a gliding motion not yet fully understood by scientists, while still others are non–motile. Bacteria are like any other form of life in that they require food, water, and suitable living conditions in order to metabolize, respire, and reproduce.

Classification. Classifying bacteria is sometimes extremely difficult due to such staggering numbers of species, overlapping characteristics, and the fact that bacterial speciation is happening at a rate exceeding that of extinction (Dykhuizen 1998); however, they are generally classed according to microscopic appearance, staining characteristics, morphological characteristics (shape), metabolism, and more recently, molecular or deoxyribonucleic acid (DNA) characteristics. Scientists regularly reshuffle names and classifications as advancing technology reveals more information about bacterial cells and their origins.

Reproduction. Bacteria reproduce by an asexual (without sex) method called binary fission, i.e., by simply dividing in half. It is a simple and effective method of propagation: the cell duplicates its DNA and organs, swells to accommodate its newly doubled innards, and then splits in half, each half with a set of organs and DNA. It is that simple. And it can result in staggering numbers of organisms in a very short time. Given optimal conditions, and estimating that a cell can replicate itself every 20 minutes, one bacterial cell can create a population of over one million cells in seven hours and one billion cells in 24 hours! This type of reproduction is called logarithmic growth and is a very important factor in disease prevention and progression.

Even though bacteria reproduce asexually, they are still able to obtain new DNA from other organisms. If a bacterial cell ruptures or dies, its DNA is released into the environment and the broken pieces can sometimes be absorbed by other cells. Many bacteria contain small pieces of DNA, in addition to their large circular DNA, called *plasmids*. Plasmids can replicate and often supply their host cell with extra information and abilities, such as antibiotic resistance. Plasmids can be transferred from cell to cell through the environment or by direct contact. Each of these gene transfer methods allows bacteria to absorb new information and gain novel defenses and survival properties.

Nutrition and Growth. All life requires basic nutritional elements that include the following:

- An energy source
- Nitrogen, carbon, and oxygen
- Minerals and vitamins
- Water

How bacteria absorb and make use of these elements is as varied as the species themselves. Bacteria can metabolize everything from gasoline, antibiotics, and plastics to elemental sulfur and manganese, but many species important to animal health have requirements much less demanding and are able to live anywhere there is water and a food source. Generally, any food source suitable for an animal is also suitable for many bacteria.

Bacteria have environmental requirements as well, meaning they need proper light, temperature, humidity, and air (or lack thereof) in order to grow. Every microbe has a preferred environment, whether it is boiling saltwater or the inside of a human's mouth, in which its nutritional and environmental requirements are best met. A basic principle of microbial growth is that each type of microbe has optimal growth conditions and, in order to propagate microbes in the laboratory, these growth conditions must be met. Some microbes have a fairly broad range of temperatures and conditions under which they will grow, while others are very selective and cannot tolerate even slight changes in their environment. While most human pathogens can be grown in a laboratory, usually less than one percent of the bacteria from natural communities can be propagated in a lab (Dykhuizen 1998).

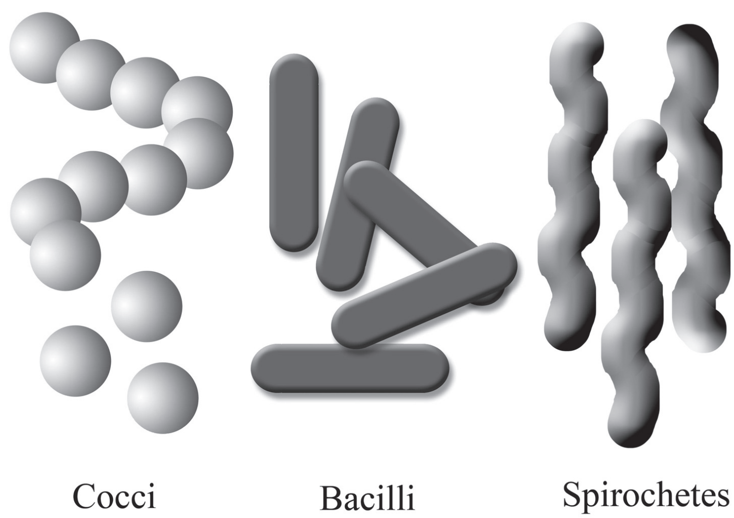

Figure 1. Bacteria.

Defenses and Pathogenicity. Bacteria have an impressive arsenal of self–preservation methods that they must employ to ensure their survival. Dessication (drying) is a problem for such small organisms and some bacteria combat this by producing a waxy coating, while others group together and secrete a protective slime layer. Still other species are able enter a kind of dormancy when they detect less than optimal environmental conditions and form a hard, impenetrable coat called a spore. Spores are resistant to drying, cold, and even boiling water, and they allow an organism to stay in this seed–like state for many years. When they sense that conditions are again appropriate for growth, they shed the protective spore coat and once again begin metabolizing.

Pathogenic (disease causing) bacteria have developed a multitude of ways to outwit a host organism's immune system. A bacterium that is not part of a body's normal microbial flora is considered an invader, and upon entry, the body's immune system defenses will be dispatched. However, millions of years of evolution have allowed microbes to outmaneuver their host and employ some remarkable evasive tactics:

- Secretion of toxins that kill tissues and defensive cells
- Ability to mimic host cells and go unrecognized as an invader
- Persistence at sites in the body inaccessible to the immune response of the host
- Depressing the host's entire immune system, rendering it less effective
- Constantly changing to avoid recognition by the host.

Antibiotic and Chemical Resistance. Perhaps one of the greatest concerns among health care professionals and scientists is the ability of bacteria to become resistant to antibiotics to which they were previously sensitive. Resistance to antibiotics is a natural outcome of evolution—in any population of organisms there are going to be variants, and in the case of bacteria, individuals that are different enough to withstand the deadly effects of an antibiotic. The surviving individuals are the only ones left to propagate; therefore, their genes, with coding for antibiotic resistance, are passed on to successive generations. Resistance genes are transferred to susceptible populations by plasmids and in a short time, all bacteria in those populations are resistant to certain drugs.

Although antibiotic resistance is a natural phenomenon, the situation has been exacerbated by societal factors and is an increasing public health problem. Inappropriate antibiotic use is the primary reason for the emergence of resistant strains of bacteria. Some research has shown that antibiotics are given to patients more often than recommended in guidelines set by federal and other healthcare organizations. For example, patients sometimes ask their doctors for antibiotics for a cold, cough, or the flu, all of which are caused by viruses and do not respond to antibiotics. Also, patients who are prescribed antibiotics but fail to take the full dosing regimen can contribute to resistance. A patient who takes only a portion of a prescribed course of antibiotics kills only the weakest bacteria and allows the strongest and most resistant bacteria to survive. The problem of antibiotic resistance is so bad that about 70 percent of bacteria that cause infection in hospitals are resistant to drugs that were once effective. Some organisms are actually resistant to *all approved antibiotics* and must be treated with experimental drugs (FDA.gov).

Viruses

General Characteristics. Viruses are so small that they cannot be seen with a regular microscope; they can only be seen with a very high–powered electron microscope. They have no organs or cellular structures and consist only of a tiny bundle of genetic material carried in a shell called a *viral coat*. In fact, scientists are uncertain if they should even classify viruses as living organisms because they are only active when inside another organism's cells—outside those cells, they are completely inert. Their evolutionary history is largely unknown because no fossil record has ever been found, yet viruses live everywhere and have evolved to infect every life form on earth, including bacteria.

Classification. Viruses are classified according to physical properties, constituent components, and antigenic and biologic properties, but the three most important taxonomic criteria are host species, morphology, and genomic (DNA) properties.

Reproduction. Without access to the cells of its host species, viruses are helpless. They do not eat or metabolize, and their only function is reproduction. They are completely dependant upon a host cell for survival and are sometimes referred to as intracellular parasites. A virus reproduces by finding a host cell and entering it either by 'tricking' the host cell into absorbing it as it would a nutrient, fusing itself to the host cell and transferring its genetic material to the cell, or injecting its DNA directly into the cell. Once the viral DNA is incorporated into the cell, it commandeers the host cell's own DNA replication mechanism to make more copies of itself. When the new viral parts are produced, new viruses are assembled and they can either leave the cell unharmed, or the cell may explode under the sheer volume of new viruses.

Defenses and Pathogenicity. Viruses are able to replicate widely throughout the body without any disease symptoms as long as they do not cause significant damage or alert the host's immune system.

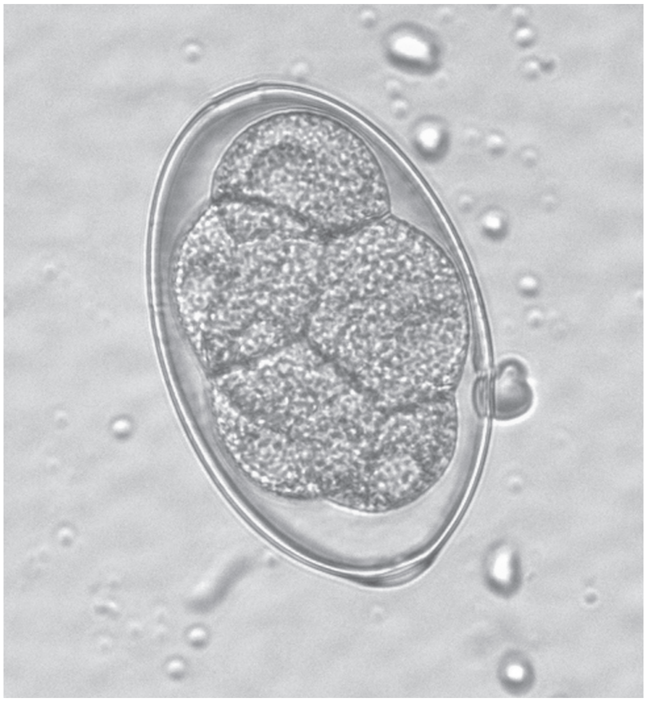
Figure 2. Syngamus *(avian gapeworm parasite).*

The most important virulence factor is the ability to replicate in host tissues and the more rapid the rate of replication, the more likely the success of the virus in producing its disease syndrome. The ability of a virus to proliferate in a host depends on being able to replicate undetected in host tissues while not stimulating host defense mechanisms which would otherwise kill or remove them. Riding this fine line is not easy for any kind of pathogenic organism, but it is particularly difficult for viruses because of the absolute parasitism involved (Smith 1972).

Some viral diseases are more communicable than others. Communicability depends on the ability of a virus to escape one host, survive in the environment, and subsequently infect another host. Some viruses enter host tissues directly by trauma or insect bite, but most infections start on the mucous membranes of the respiratory and digestive tracts.

Although viruses typically have a preferred host species, they sometimes not only acquire the ability to switch hosts and produce disease, but also to establish the ability to propagate between hosts (Taubenberger and Morens 2006).

Antiviral Drugs. Antiviral drugs are available to treat only a few viral diseases. The reason for this is the fact that viral replication is so intimately associated with the host cell that any drug that interferes significantly with viral replication is likely to be toxic to the host. Since most viral diseases are not susceptible to antimicrobial therapy, the best way to combat them is through vaccination.

Fungi

General Characteristics. Contrary to popular belief, fungi are not plants, but rather are a more primitive life form in a category all their own. Fungi come in many forms and some of the more familiar are mushrooms, molds, yeasts, lichens, rusts, and truffles. Fungi range in size from single–celled yeasts to the largest organism on earth, the fungus called a honey mushroom covering over 2200 acres in Oregon (Discover.com 2004). These organisms grow as *saprophytes*, meaning they grow on and derive nourishment from dead or decaying organic matter. Fungi are ubiquitous in nature and most people are exposed to them every day.

Classification. There are between 100,000 and 200,000 species, depending on how they are classified, 300 of which are presently known to be pathogenic to animals (pathmicro.med.sc.edu 2005). Fungi were formerly classified based on physical and reproductive characteristics, but advances in molecular science are causing scientists to rethink previous classifications.

Reproduction. Fungi have a diverse repertoire of reproductive strategies, including both sexual and asexual methods, but all of them result in the creation of enormous numbers of offspring in the form of spores.

Defenses and Pathogenicity. Many fungi have the potential to cause disease in animals but are usually prevented from causing profound disease by the animals' optimally functioning immune systems. Most common fungal health problems for humans involve dermatophytes or skin–loving fungi. Otherwise, fungi have to be inhaled, injected into, or lodged in susceptible tissues of individuals with reduced immune system function. For the great majority of animals, fungi will not cause more than an irritating reaction that is either self–limiting or easily treated with anti–fungal medications (usyd.edu.au 2004).

Unfortunately, diseases caused by fungi are becoming more common. There are a number of reasons, but some include the use of antibiotics that suppress natural competitors of fungi, the use of drugs that suppress the immune system after tissue transplant operations, and viral diseases that compromise healthy immune responses such as human immunodeficiency virus (HIV). A healthy body normally suppresses fungal growth, but when immunological mechanisms fail, fungi become a serious and sometimes life–threatening problem in humans.

Fungicides. Like bacteria, fungi can develop resistance to fungicides over a period of time. While a range of compounds have been found that act against fungi, problems arise with their use in human disease because of biosynthetic pathways common to both fungi and host, resulting in toxicity to the host. The continued use of fungicides, especially in chronic conditions, will increase levels of resistance as has happened to bacterial pathogens.

Parasites

General Characteristics. A parasite is an organism that simultaneously injures and derives sustenance from its host. This designation includes a variety of species including some insects. Some organisms called parasites are actually *commensals*, meaning that they neither benefit nor harm their host. Parasites can live either on (ectoparasite) an animal, like fleas and ticks, or inside its body (endoparasite) like tapeworms and roundworms (Figures 2 and 3). Infections of humans caused by parasites number in the billions and range from the relatively innocuous to fatal. Diseases caused by parasites constitute major animal health problems throughout the world.

Reproduction. Since the term parasite applies to an array of different types of animals, it encompasses a wide variety of reproductive techniques; however, all parasites are dependant upon their hosts for survival, and as a result are tethered to them for the entirety of their lives. That means they cannot invest much parental care and their only recourse to ensure the survival of their genes is usually the indiscriminate scattering of prodigious numbers of eggs. Because the chance that an individual parasite offspring will find a new host is very slim, parasites are much more fecund than their free–living relatives (Calow 1983).

Life cycles of parasites range from very simple to sometimes extremely complex involving transmission between two and three different host animals and environments. Parasites range greatly in size and form from microscopic to several feet in length in the case of some worms.

Defenses and Pathogenicity. Because it is not in the interest of the parasite to kill the host, an infection sometimes goes undetected for years, if not a lifetime. The level of pathology caused by parasites is highly varied and can include physical trauma or cell–tissue destruction from worms migrating through the body, nutritional diversion, blood loss, ulceration of intestinal wall and liver by enzymes, displacement

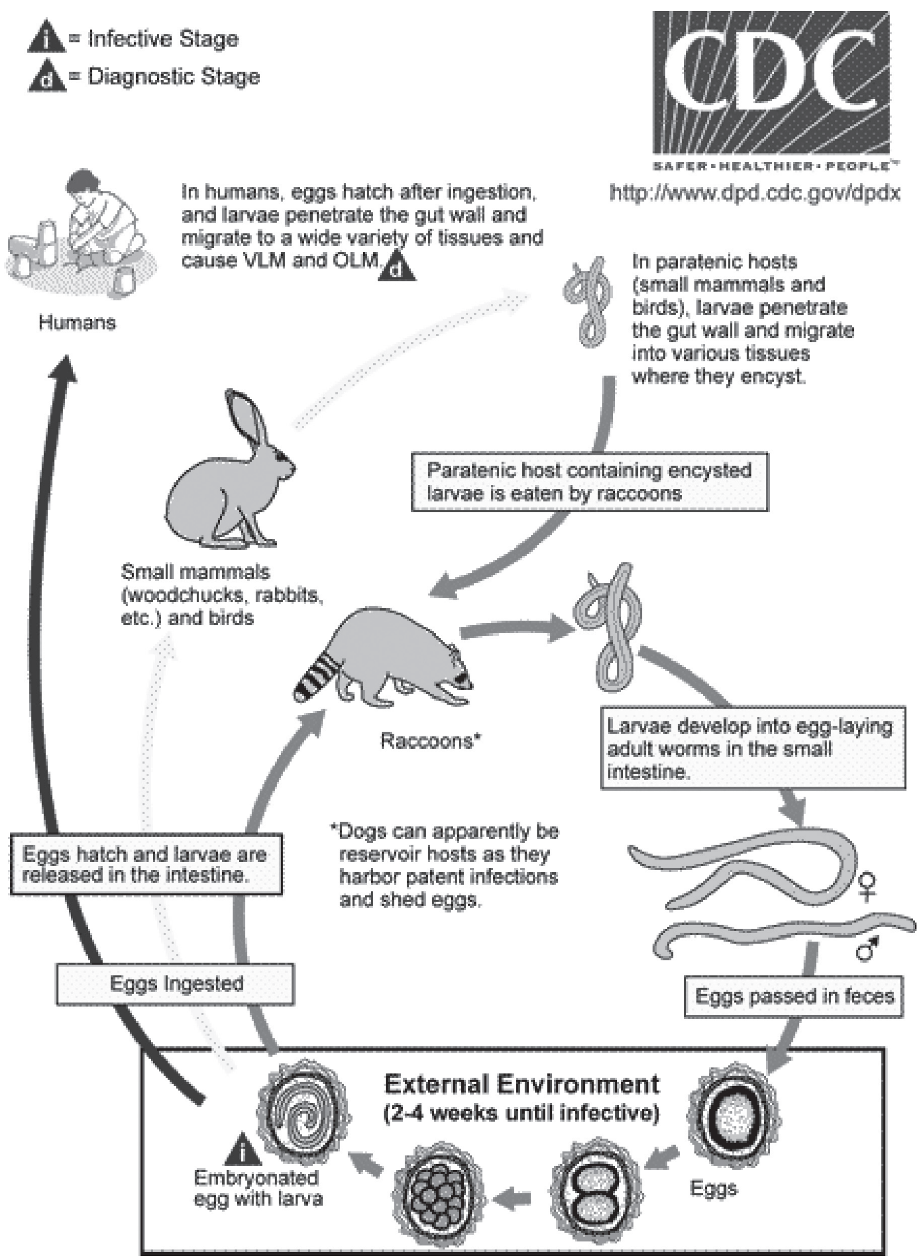

Figure 3. Baylisascaris *(Raccoon* [Procyon lotor] *roundworm) (CDC.gov).*

of tissue or structures by encysted larvae, enzymatic digestion of skin cells, or ulceration due to insertion of hooks or spines into the intestinal wall.

Parasites and their hosts evolve together, meaning that it is a constant battle between each to stay ahead of the other, evolutionarily speaking. Parasites have evolved the following ways to avoid being ejected from their host:

- Antigenic variation—some trypanosome species have evolved methods of constantly changing their surface antibodies to go undetected by their host's immune system
- Tough armor–like exoskeletons
- Hiding inside cells
- Antigen acquisition—some species of schistosomes have the ability to coat themselves with antigens from the host's immune system, rendering them invisible to the host
- Prevention of the body's defense cells from migrating to the site of the parasite
- Encystment (encasement in a shell within tissues)
- Ability to destroy host's antibodies and chemical defenses
- Ability to trigger certain arms of the immune response, which may in turn damage host tissue enough to facilitate parasite invasion.
(k–state.edu 2004)

Microbial–Host Relationships

Most of the time, bacteria are associated only with decay, dirt, disease, and death, and a common misconception is that all bacteria are bad. Popular media would have one believe that the only good bacterium is a dead one, but the fact remains that we could not exist without them. In reality, the human body is typically not a hospitable place for most microorganisms and it is only when the host is compromised that pathogenic invaders can gain a foothold.

In a healthy animal, internal tissues such a muscle and bone are free from microorganisms, but surfaces continuous with the external environment, including the digestive tract, skin, and mucous membranes, are colonized by a complex amalgam of bacterial species termed *normal flora*. More than 700 bacterial species have been detected in the oral cavity alone (Aas 2005), and it has been estimated that there are over 500 species in the human colon (Mai and Morris 2003). Normal flora colonize the body during birth or shortly thereafter and remain throughout life. The composition of normal flora varies between individuals and is affected by factors such as sex, age, health, stress level, and diet. The normal flora exist in a mutualistic relationship (benefits both parties) with its host. Some important areas of microbial communities in the body of animals are the skin, nose, mouth, digestive tract, and vagina. Some of the benefits of indigenous flora are as follows:

- Production of nutrients. Many bacteria synthesize and excrete vitamins in excess of their own needs that are then absorbed by the host.
- Aid in digestion. Some food is either wholly, or in part, digested by bacteria that then renders nutrients available for the host.
- Prevention of colonization by pathogens. Normal flora fend off invading pathogens by competing for space or food sources, thereby making it an inhospitable environment for pathogens.
- Defense against pathogens. It is thought that normal flora actually produce toxins that inhibit or even kill non–indigenous species.
- Stimulate the production of antibodies. Normal flora are constantly stimulating the body's immune system, keeping it on its toes in the event of an attack by a pathogen. Since normal flora have many pathogenic relatives, low levels of antibodies produced in response to normal flora are then able to cross–react with invaders.

Maintaining the balance of the body's microbial communities is crucial for good health, and when this balance is upset, disaster can ensue. The constituent normal flora organisms keep each other in check, meaning that they prevent each other from unbridled reproduction by limiting space and nutrients. When one of the constituents falters, the others move in to take its place resulting in population explosions termed *opportunistic infections*. Opportunistic infections happen when the host is somehow compromised by sickness, lowered immune function, changes in pH, or antibiotic use. A yeast infection after administration of antibiotics is a common example of an opportunistic infection. A person whose immune system is compromised through disease, drugs, or medical treatment is susceptible to sickness by their very own normal flora.

Microbial Benefits

The benefits provided by microbes are incalculable. Here are just a few:

- **Production of drugs**—Penicillin, one of the first and most widely used antibiotics, is made from the fungus *Penicillium.* Scientists have engineered the genetic blueprints of bacteria and yeasts to turn them into medicine–making factories. They insert genes for medicines they want to make into the microbial cells, as if adding new building information to the microbe's blueprint. Scientists then grow the microbes in huge containers called fermenters and harvest the desired by–products.

- **Food production**—Bread and beer are made with yeasts, yogurt and sour cream are made with bacteria, and a variety of cheeses are made with molds.
- **Decay**—Bacteria and fungi degrade leaf litter that enriches soil; they also recycle dead animals by the same means.
- **Industry**—Microbes make compounds called enzymes that we use in making hundreds of products. Bacteria are grown in large quantities and then broken apart to extract enzymes to make soy sauce, soda, beer, wine, cheese, infant formula, chewing gum, leather goods, paper, laundry detergent, the stone–washed look on blue jeans, etc.
- **Janitors**—Researchers are using bacteria that eat methane gas to clean up hazardous waste dumps and landfills. These bacteria, called methanotrophs, make an enzyme that can break down more than 250 pollutants into harmless molecules. By piping methane into the soil, we can increase growth of the methanotrophs that normally live in the polluted soil. More methanotrophs means pollution is broken down faster.
- **Pest control**—A bacterium called BT or *Bacillus thuringiensis* is a popular agent used in non–toxic garden pest control.
- **Nutrient absorption**—Some species of fungi, called *mycorrhizal fungi*, live on or in the roots of many plants to facilitate absorption of nutrients and moisture (Microbe.org).

Conclusion

In conjunction with dramatic changes in the global society and the environment, the spectrum of infectious disease is mirroring that change. Despite historical predictions to the contrary, people and animals today remain vulnerable to a wide array of new and resurgent diseases such as multi–drug resistant tuberculosis and staphylococcal infections, avian influenza, rabies, and West Nile virus. With an estimated 10^{30} (that's 1 followed by 30 zeroes!) (Curtis et al 2002) microorganisms on the planet at any given time and a world population of 6.5 billion (Census.gov) humans, it is important that we keep a balanced and informed perspective of the role we play in our environment. Bacteria, and even their associated diseases, are essential parts of our existence and it behooves us to view bacteria as more than just pathogens. As wildlife enthusiasts, educators, and rehabilitators, it is important that we are able to make that distinction.

Acknowledgments

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Continued on page 25.

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(Miller, continued.)

It is hoped that this will encourage wildlife rehabilitators to look for other applications of some of these ideas to provide psychological and environmental enrichment for infant wild creatures, whether in a home based setting or in a rehabilitation center. In particular, the surrogate mother figure was so successful with the beavers that the author would be interested in hearing from others if they successfully make use of the device for other mammal species.

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[***Editor's Note:*** Rehabilitators may have difficulty locating a copy of the Marcum publication cited for this article. The *NWRA Principles of Wildlife Rehabilitation, The Essential Guide for Novice and Experienced Rehabilitators* contains much of Debbie Marcum's information in the Mammal Nutrition chapter.]

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