entropy

Review

Deepening the Conception of Functional Information in the Description of Zoonotic Infectious Diseases

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Abstract: Infectious agents, their hosts, and relevant abiotic components are directly involved in the complex dynamic process of maintaining infectious diseases in Nature. The current tendency to focus on host-pathogen interactions at the molecular and organismal levels does not advance our knowledge about infectious diseases, as much as it potentially could, by ignoring the ecological context pivotal for understanding the biology of the diseases. A new model of investigation requires a dynamic shift of perspectives in the "simplicity-complexity" dimension: from virulence factors to multi-sided descriptions of the pathogens; from particular microbes to wide microbial communities; from clinical manifestations to a variety of infectious patterns; from findings of infectious agents to defining a natural focus of the infection as a self-regulated system; from single factors affecting host-parasite relations to the complex ecological context. Various aspects of interactions between hosts, vectors, pathogens, and environmental niches should be integrated at multiple spatiotemporal scales and at different levels of biological organization (molecular, genomic, organismal, population, and ecosystem).

Keywords: bacterial species; complexity; diversity; functional information; immune system; infection; host of infection; pathogen; infectious disease

1. Introduction

The current situation in studies of infectious diseases can be characterized by two trends. The first is exponential progress in the development of new methods for identifying and analyzing pathogens resulting in a rapidly increasing number of new publications. The second is a growing worldwide

microbial threat made evident in the appearance of novel emerging diseases and the extensive spread of pathogens not considered dangerous in previous decades. There are many factors contributing to both trends. In this article I wish to emphasize an underlying phenomenon of increasing disconnection between the accruing body of information about infectious agents, infected organisms, influence of environmental factors on epidemic processes, and our limited understanding of infectious processes. In fact, I will argue that the recent massive increase in such information is accompanied by an increase in uncertainty (entropy) in the description of all components of host-pathogen systems at the population and community level. The term "entropy" has numerous meanings, and here I adapt the definition given by Oller [1] as *"the antithesis of well-formed true reports that agree with each other and with the material facts accessible through the experience of one or more competent observers"*. The situation is paradoxical because the expectation is that new information should lead to better understanding and more accurate predictions of the distribution and dynamics of infectious diseases, and, overall, to a decrease in entropy.

The main objectives of this paper are as follows: (1) to demonstrate that increasing complexity causes uncertainty in definitions of host-pathogen relationships at the organismal, population, and community level; and (2) to define some potential approaches that can help deal with these problems. The critical point that I would like to make is that a description of host-pathogen systems based on binary logic being limited to fixed alternative conditions such as "infected" *versus* "non-infected" hosts or "pathogen" *versus* "non-pathogenic symbiont", greatly contributes to the problem.

The indicated problem will be discussed considering zoonotic infections (infections that are caused by infectious agents evolutionarily associated with animals). After all, human infections are likely to represent only a small portion of zoonotic infections associated with a single biological species (*Homo sapiens*) and many so-called "human pathogens" have their origin in other mammalian species [2]. In all descriptions of infections there are two necessary elements: an infectious agent (such as a virus, prion, bacterium, or a microscopic eukaryotic organism) causing infection by invading a multicellular or a unicellular organism. The latter is considered to be a host of the infectious agent. For obvious reasons, investigations of host-pathogen system maintenance cycles are meaningful only on the level of host populations or communities. Although disease occurrence can be studied at many different levels, including molecules, individuals, populations, and ecosystems; the population level is fundamental for epidemiology [3]. Death of an infected host frequently becomes an endpoint for surviving pathogens within this host. If pathogens can survive in the surrounding environment after the death of their animal host, then the environment should be considered to be a part of the infection reservoir (term discussed below).

It is not my objective to illustrate presented points with numerous specific examples; moreover, whenever is possible, each point will be illustrated by a single group of pathogens—namely, bartonella bacteria. The choice of these bacteria is dictated not so much by my familiarity with this group, or their public health importance, but mostly for the consistency and simplicity of the presentation. In addition, these bacteria are diverse in terms of their lifestyle, genetics, and pathogenic plasticity, making them appropriate agents for illustrating some general problems. Other groups of infectious agents are undoubtedly just as well suited to demonstrate these points. Overall, the following discussions reflect the challenges faced by scientists of different disciplines who question the view that simple accumulation of abstract information automatically leads to better understanding in research.

2. Increased Uncertainty in the Identification of a Pathogen

In scientific literature, the terms "pathogen" and "infectious agent" are commonly considered to be equivalent. Let us first look at the definition of *"infection"* given by Webster's Medical Dictionary [4]: "*Infection is the invasion and multiplication of microorganisms such as bacteria, viruses, and parasites that are not normally present within the body. An infection may cause no symptoms and be subclinical, or it may cause symptoms and be clinically apparent.*" Thus, according to this definition not every microorganism causing infection is a pathogen. I do not want to argue about these terms; I also do not want to dispute the term microorganism in the provided definition (viruses, in my understanding, are not organisms). Instead, I would mostly prefer to use the term *infectious agent* as a more neutral designation. We can define a pathogen as an infectious agent causing a specific disease characterized by a recognized set of signs and symptoms. However, the practical matter of establishing a causal link between a pathogen and a disease remains and this can be challenging. The point is that increasing information about infectious agents does not automatically lead to a better understanding of this relationship.

2.1. The Original Definition of Pathogen

At this point I wish to acknowledge a long and glorious history of defining the concept of pathogens. The association of specific microorganisms with diseases was described by the German physician Robert Koch around 130 years ago, and some of the criteria he formulated became the gold standard in the field of infectious pathology. These four criteria are known as Koch's postulates, though the last one was not actually proposed by Koch. These criteria are: (1) the same microbe must be present in every case of the disease, but not in healthy organisms; (2) the pathogen can be isolated from a diseased host and grown in pure culture; (3) the culture can cause the disease when inoculated into a healthy susceptible animal; and (4) the pathogenic microorganism can be reisolated from the experimentally infected animal, and it should be identical to the original inoculum. At the time these criteria were formulated it became evident that bacteria are highly prevalent and diverse, and that not all of them are pathogens. Koch's postulates have been widely accepted by the scientific community as a way to distinguish pathogens from other microorganisms and these postulates are still presented in virtually all introductory microbiology textbooks. However, limitations for applications of these criteria became evident even during Koch's lifetime. Koch himself abandoned the strict requirement of the first postulate after witnessing asymptomatic carriers of cholera and typhoid fever. The second postulate was called into question when the leprosy bacterium could not be grown as a pure culture.

During the following century the limitations associated with Koch's postulates multiplied. The discovery of asymptomatic carriers, the absence of animal models for many infections, the acquisition of virulence factors by previously harmless bacteria, and other observations raised questions about the credibility of the postulates. The inability, or difficulty, of many infectious agents to grow in pure culture represents a special challenge for application of Koch's requirements (third postulate). Many types of bacteria, viruses, and rickettsia cannot be cultured with currently available techniques and media.

Endospores formations were described in many Gram-positive bacteria [5]. The term of endospore for bacteria can be confusing since this is not a true spore, but a dormant form to which the bacterium can reduce itself for surviving in harsh conditions and triggered by a lack of nutrients. Roszak and Colwell [6], working with *Escherichia coli* and *Salmonella enteritidis* in aquatic systems, proposed the term "somnicells" to define those bacterial cells which were not cultivable in standard culture media but which were detectable by direct-count techniques. Similarly, L-form bacteria (the term largely applied to bacteria losing their cell walls, but surviving in modified form) were reported for many bacteria, e.g., for *Bacillus subtilis* [7]. Some bacteriologists regard such bacteria as laboratory curiosities of little or no clinical significance, but others argue that dormant bacteria in the form of nutritionally deficient organisms, or difficult-to-culture bacteria in general may serve as cryptic agents of disease in a variety of human infections [8]. Some bacteria are more prone than others to enter into this "growth stage" or survival strategy, including human pathogens such as *Legionella pneumophila*, *Vibrio cholerae*, and *Helicobacter pylori* [9]. Not-yet-cultured microbes likely constitute 99% of the microbes thought to exist in the biosphere [9]. Regardless of different mechanisms and terms, these phenomena are responsible for many of the difficulties in proving causality of infectious diseases. Kock's postulates cannot be fulfilled, because it is impossible to precisely duplicate all variables that are involved in diseases expression [8]. In addition, Koch's postulates do not allow researchers to readily address naturally occurring environmental, nutritional, genetic and other relevant factors that influence disease causation and do not consider the pathogenic complexities induced by sequential or simultaneous infection with more than one pathogenic microorganism [10].

2.2. The Use of Molecular Criteria to Identify Pathogens

Modern nucleic acid-based microbial detection methods allow detecting and identifying disease associated microbes without the need to culture these agents, and make Koch's original postulates even less relevant. In many cases bacterial culture is not possible, or the process is laborious and time-consuming. This leads to the tendency to replace culture techniques with molecular ones. An unfortunate side effect of this enthusiasm with the application of molecular techniques is a reduction in the number of specialists who have experience in culturing pathogens and studying their biological properties and the greater potential for false-positive results from the risk of contamination of samples with target DNA sequences either of amplicon or whole genomic origin.

Molecular assays, including PCR amplification and nucleotide sequencing, continue to reveal previously uncharacterized, fastidious, or non-cultivable microbial pathogens. The use of these methods has also stimulated the development of new criteria for identification of infectious agents for many diseases. Usually, genotypes are intrinsically specific, and can be quantified and standardized more easily this way than through reliance on traditional phenotypic characterizations. In particular, recent investigations of bartonelloses, ehrlichiosis, and hantaviral infections among others have relied very much on molecular identification of the infectious agents.

Guidelines for defining pathogens using nucleotide sequence-based technology were proposed by Fredricks and Relman [11] as follows: (1) a DNA sequence belonging to a putative pathogen should be present in most cases of an infectious disease; (2) microbial DNA should be found preferentially in the diseased organs; (3) pathogen-associated DNA should be present only in low amounts, or should be

absent in hosts without the disease; (4) a decrease in the amount of pathogen-associated DNA should be associated with the resolution of disease; (5) DNA sequence copy number should correlate with severity of the disease; (6) the nature of the microorganism inferred from the available sequence should be consistent with the known biological characteristics of that group of organisms; (7) tissue-sequence correlates should be sought at the cellular level; and (8) these sequence-based forms of evidence for microbial causation should be reproducible. In reality, a very important point (number six) brought by Fredricks and Relman [11] about the consistency between molecular detection of pathogens and their biological characteristics is often ignored by many molecular biologists.

Though the molecular approaches have proven very effective in the detection and identification of infectious agents, they also present new challenges for answering some basic questions about the fundamental interactions between pathogens and hosts. As well, the problem of a potential detection of infectious agents in healthy hosts has not been addressed. In fact, detection of identical microorganisms in both healthy and diseased hosts has become much more common since the wide introduction and use of sensitive molecular methods. With molecular approaches becoming more prevalent in diagnostics of infectious diseases, questions about causality for specific cases of pathology in human and animal patients remain highly disputed. The only workable agreement in many cases is acceptance of an association between findings of molecular markers and clinical manifestations, rather than inferring the causality of the infection. Finally, Koch's postulates with respect to pure cultures are not truly applicable in some situations, as the vast majority of microorganisms exist as members of microbial communities. Inoculation of laboratory animals with many strains (*i.e.*, an entire community of microorganisms) is not realistic, and creates an ethical dilemma in performing animal experimentation without clear criteria for expected results.

2.3. Virulence Factors

Applications of molecular methods have not only improved the detection of infectious agents in nature, but have also dramatically changed research priorities in investigations of host-pathogen relations. The hallmarks of these new approaches are identification of virulence factors of pathogens and immunological determinants responsible for susceptibility of animal organisms to specific infections. Virulence factors can regulate a range of diverse effects: adhesion to host cells, entry into cells (in the case of an intracellular pathogen, including bartonella), interference with the host's immune response, acquisition of nutrition from the host, *etc.* Virulence factors can be bacterial capsules, flagella, endotoxins and exotoxins; and they can be encoded in the chromosome in the form of "pathogenic islands", or within extra-chromosomal elements (bacteriophages and plasmids).

Whereas the virulence of pathogenic bacteria is commonly related to the ability of the organism to produce toxins, a number of factors contribute to the pathogenicity of a particular strain of virus [12]. Pathogenicity is a complex process with stringent requirements of both the host cell and the infecting virus. Among these requirements are a port of entry into host cells, a means of replication for the virus, and a means by which infection damages host cells. Damage to the host can result from multiple mechanisms including transformation, suppression of cellular metabolism, apoptosis, and autoimmune responses directed against infected or uninfected tissues, or by molecular mimicry. Efforts to associate

specific viral infections with specific diseases may be obscured by common pathways through which viruses damage host cells in similar ways [13].

All virulence factors share in common their ability to induce a pathogenic effect in hosts. Information about the virulence factors of bacteria, viruses, fungi, and protozoa, and about genomic components encoding these factors has been accumulating very quickly. The importance of such information is unquestionable, but I wish to indicate the unintentional replacement of "pathogens" as biological organisms or non-organismal biological forms (viruses, prions) with information about specific macromolecules (nucleotides, proteins, polysaccharides) obtained at the molecular level.

2.4. Pathogen Detection in a Host Population

If the application of molecular approaches contributes to our understanding of the mechanisms of host-pathogen interactions at the cellular and organismal levels, the situation is different at the level of host populations. Detection of specific molecules in an organism (*i.e.*, a natural host) is commonly presumed to indicate the presence of specific infectious agents. Microbiologists are well aware that targeted macromolecules are NOT infectious agents, yet detection of macromolecules is commonly interpreted in terms of the spread of an infection within a host population. A similar situation is common with the interpretation of antibody responses to infectious agents as assayed using serological tests. Biologists are perfectly aware that serological test results report the presence of some proteins (antibodies) presumably produced by a host's immune the system as a response to specific molecules of infectious agents (antigens). However, during results interpretations it is frequently ignored that findings of antibodies does not necessarily indicate the current presence of the infectious agent. Selection of criteria for interpretation of serological tests results is crucially important, and ignorance of how important the interpretation criteria are can sometimes lead to mistaken conclusions. In fact, this situation more commonly leads to the generation of overwhelmingly large amounts of information, which in turn increase our uncertainty about population infection dynamics, instead of increasing our understanding. Molecular detection of microorganisms is essentially applied in a "vacuum". Conclusions about the intensity of a spread of an infectious agent, or an agent's interactions with other members of the microbial community, or with the host are purely speculative; unless or until sufficient biologically meaningful information is brought forward to augment the molecularly acquired information.

3. The Infectious Agent

3.1. Pathogen vs. Symbiont vs. Parasite

In ecology, the term *symbiosis* ("living together") describes specific and close physical associations between species, in which a "*symbiont*" occupies a habitat provided by a "*host*" [14]. The vast majority of microorganisms live in highly complex communities within which they have intensive interactions—competing, cooperating, and forming associations with one another and with their living and nonliving host environments. Complex microbial communities are present in practically all ecosystems. Microbial ecology is a fast growing scientific discipline that delivers a wealth of information about these metacommunities. Despite these observations, little is known about the factors and processes that influence these communities' assembly or stability. Listed among microbial interactions

are parasitism, commensalism, and mutualism, though some exclude parasitism from the category of symbionts. While considering different viewpoints on the question of whether parasitism is symbiosis (the naturalist's viewpoint, the germ hunter's viewpoint, the immunologist's viewpoint, and the endocytobiologist's viewpoint), Cheng [15] has given an affirmative answer on this question: yes, parasitism is a form of symbiosis. Without delving into the discussion, we can note a dramatic switch in the application of these terms in epidemiology where the term "symbiont" is often used as an opposition to "pathogen": if the microorganism is proven to cause a disease, it is called "pathogenic"; if not, it is considered "symbiotic". There are many examples where microorganisms can be defined as "pathogenic" or "non-pathogenic", but there is also a growing body of evidence that these distinctions often reflect our level of perception rather than any "objective" criteria.

Recent research has shown that many highly diverse bacteria operate along a functional continuum between pathogenicity and symbiosis in natural hosts. This functional diversity may be linked to the genomic diversity of an infectious agent, which may enhance its ability to infect a broad range of hosts. Thus, some infectious agents may "break out" of their symbiont or pathogen niche, but genetic and ecological factors driving this process are still poorly understood.

Growth of an infectious agent within an animal host commonly comes at a cost to the host, for example consumption of host nutrients, but this cost can be negated by some form of compensation due to the presence of these microorganisms. An agent can cause infection without disease in one species and be virulent for animals of another species. For example, some *Bartonella* species are responsible for illnesses in dogs; yet do not usually cause any illness in cats. The opposite can be observed for the plague pathogen *Yersinia pestis*, which commonly causes much more severe illness in cats compared to dogs. Overall, *Bartonella* species provide a good illustration of the pathogen-symbiont continuum. *Bartonella* species populate a wide range of scenarios, from endosymbiosis (there is an enormous variety of bartonella genotypes in insects within a single population), to well-defined parasitism with co-adaptation to specific vertebrate hosts, to pathogenicity causing life-threatening Oroya fever in people or endocarditis in dogs and people [16].

Confusion is also caused by the application of the term "pathogen" *vs.* "parasite". In some studies these terms are used interchangeably [17]. Though a parasite is commonly defined as an organism that lives on or in a host and gets its food from or at the expense of its host, it does not mean that it is necessarily pathogenic, as observed for bartonella bacteria parasitizing the red blood cells of their natural hosts without visible pathogenic effect [18]. On the other hand, not all pathogens are parasites (or at least not all the time). Zoonotic infectious diseases can be caused by bacteria that temporarily or permanently live in soil or water. Bacteria such as *Yersinia*, *Francisella*, *Listeria*, and *Legionella* species have a so-called "*saprophytic*" phase, which means they utilize dissolved organic material as nutrition. Thus, definitions of "parasite" and "pathogen" relate to different concepts and "parasitic" can be equated to "pathogenic" only in limited situations depending on the specific effects of the parasite at a given time point. The host recognizes microbes as infectious agents regardless of their taxonomic status or parasitic stage.

3.2. Interacting Infectious Agents

Most hosts are simultaneously or sequentially infected with several infectious agents. Importantly, spatial and temporal dynamics of any microbial species within host populations can affect the dynamics of the entire microbial community. This might lead to changes in community structure that deviate significantly from the expected pattern in closed communities (e.g., in inbred animals infected with a pure bacterial strain, which is a standard practice in the laboratory environment). Each microbial community consists of interactive species and can be designated at various spatial scales, and thus multiple communities can emerge depending on the spatial scale under consideration [19].

The scientific community is just starting to understand the complexity of outcomes possible when multiple infectious agents occupy a single animal or invade a single population. The factors involved are competition for limited resources within a host organism, varied levels of cross-immunity, and diverse immune modulation effects, some of which we might not be aware. The opposite situation is a number of host species sharing a particular infectious agent. Does a reduction in species diversity amplify or reduce the intensity of epidemic process in animal populations? This effect, widely called the "dilution effect" [20], has been discussed intensively in the last few years.

Co-existence of the pathogens with other microbial agents can affect ecology and microevolution of infections caused by these pathogens, but this factor has received very little attention to date. In mixed infections, complex interactions between pathogens and host may arise, so the burden of one or both of the infectious agents may be increased and the other suppressed [21,22]. The nature of any specific infection in a host concurrently infected with a particular pathogen may be very different from an infection caused by the same agent in a host co-infected with another pathogen or in a host that is otherwise uninfected [21,23]. Microbes may be affected, directly or indirectly, by cytokines or other immune system related factors, and the microbes may themselves produce factors that affect functioning of the host's immune system (Th1 and T2, as in intra-cellular *vs.* extra-cellular pathogen targeting responses). Therefore, microbes are affected when they and other members of microbial community interact with the host [21]. One area where mixed infection has attracted considerable attention in recent years is in the study of evolution of virulence [24–27]. Thomas *et al.* [22] demonstrated that avirulent pathogens, which have been given little attention and may go largely undetected in the field, can play a significant role in mediating the outcome of coupled host-pathogen interaction when these agents occur in mixed infections. Models and data indicated that mixed infection can lead to the evolution of either increased or decreased virulence. Trade-offs among the abilities of pathogens to respond to different environmental factors are often assumed to play a major role in the coexistence of microbial species [28]. The strong condition-dependency of mixed infection identified in some studies adds complexity, and points to the need for further detailed studies of the mechanisms of niche overlap between two or more pathogenic bacteria. In recent years, there has been a growing recognition that many diseases consist of multiple closely related species and interacting strains. From an evolutionary prospective, it is important to understand how the ecological interactions between related species (particularly cross-immunity) affect disease evolution and strain replacement.

A successful analysis of microbial species interaction was conducted by Telfer and her collaborators [29] focusing on a limited group of infectious agents recognized as human and animal pathogens (cowpox virus, *Babesia microti*, *Bartonella spp.* and *Anaplasma phagocytophilum*). The

authors used time-series data from individual hosts (field voles) in natural populations to analyze patterns of microbial community and detected significant positive or negative effects between the types of infections. The authors suggested there is a danger of mistaken inference if you consider parasite species in isolation rather than as a community. In his letter to Science, where the original paper by Telfer *et al.* was published, Raoult [30] commented that the history of infection cannot be summarized by a simplified scenario involving only a single host and a microbe. We can only imagine how much more complicated such an analysis would be if all bacterial symbionts were included. However, in reality, scientists understandably limit the scope of an investigated microbial community to simplify analysis. Otherwise we risk creating datasets of such complexity that description and prediction of specific pathogen dynamics becomes practically impossible. A proposal for how to approach this problem is to consider a complexity continuum between an extreme low point (one microbial species) and a high point (a whole microbial community), and address meaningful questions for selecting an appropriate scale for designing studies and the appropriate analysis. Considering the multiple spatial scales for microbial metacommunity research, Mihaljevic [19] emphasized some important questions, such as which microbial community processes are most important at different scales, how they might affect community structure, whether this structure is a reliable predictor of the disease risk, and how the level of symbiont gene transmission affects the emergence of pathogenic varieties. Veresoglou *et al.* [31] described additional complexities in microbial metacommunity analysis by stressing "*functional differences*" that exist among microbial symbionts. These functional differences could result in complementary effects on the host fitness. So, we cannot limit our analysis to only paired interactions, but if we consider a whole microbial community we have an extremely complex network with almost infinite interaction scenarios between numerous microbial participants. Analysis of such a microbial community can be fruitful only if we assign a specific functional role for each element within this system.

4. Increased Uncertainty in the Definition of Microbial Species

A species concept is central to achieve a predictive understanding of the composition and structure of microbial communities and identification of pathogenic microbes responsible for disease [32]. Defining biological species is tricky and borders on a philosophical issue. Here, we wish to present a very simple and practical question: how do we define a specific infectious agent, or, put otherwise, how can we claim that a host has a particular kind of infection?

4.1. Traditional Bacteriological Definitions

Historically, microbial species were defined based on morphology, antigenic relatedness, pathological manifestation, and other practical parameters. Currently accepted recommendations on the reconciliation of approaches for bacterial systematics were developed by a special *ad-hoc* committee of the International Committee for Systematic Bacteriology [33]. For years, these recommendations emphasized the role of DNA reassociation for defining bacterial species with a requirement of having <70% relatedness between genomic DNA-DNA to separate bacterial species. A description of some phenotypic characteristics is also recommended, but sequencing of a particular number of functionally important genes has been advised as well. Among phenotypic traits for a description of bacterial species the

ad-hoc committee of the International Committee for Systematic Bacteriology [33] recommended growth characteristics, clinical biochemical analysis, antibiotic susceptibility, cellular fatty acid analysis, light microscopic examination, and electron microscopy. In reality, the choice of such characteristics is not consistent across different bacteria groups, and in many cases is dictated by individual journals' requirements for the publication, rather than by a real usefulness for discrimination between proposed species.

4.2. Molecular Genetic Criteria for Pathogen Typing

Development of diverse molecular approaches in microbiology has resulted in a number of widely used techniques for characterization and typing of genotypes; e.g., pulsed field gel electrophoresis, multi-spacer typing, multi-locus variable number tandem repeat and sequence analyses. One of the significant problems with understanding the relationships between bacterial species based solely on analysis of some genes is a possibility of genetic recombination among bacteria.

It is now widely accepted that bacterial species diversification mainly occurs by homologous recombination between individual bacteria from different species or populations, rather than by *de novo* mutation [34]. Berglund *et al.* showed that the contribution of homologous recombination in generating diversification among *Bartonella* strains sampled worldwide [35].

The situation defining microbial species is even much more complicated in virology. The concept of quasispecies as a group of viruses related by a similar mutation or mutations has exerted great influence in virology to solve this problem. Originally coined by Manfred Eigen, the quasispecies concept has been applied to populations of a virus within its host [36]. This concept is based on observations that RNA viruses replicate in their hosts as complex mutant spectra [37].

There is a risk that by sequencing only one strain of a given microbial species, a strong bias may be introduced into any typing approach, which represents each species by a prototype strain, ignoring all variability information [38]. The application of genetic sequencing methods has permitted the tentative description of clusters of genetically similar strains within well-defined species as presumably new species. However, the molecular typing of these organisms should be coupled with defining the biological characteristics of the newly described bacteria [39].

4.3. Host-Associations of Microorganisms

One way to demonstrate that a sequence-based cluster represents a bacterial species is by description of common biological characteristics specific for a particular group preventing potential genetic exchanges between this group and other bacteria. Owing to differences in ecology between populations, each local population might endure periodic selection events by inhabiting different microhabitats [40]. Host associations can range from the level of animal species to genus to community; the common theme is that any level of association provides a certain degree of isolation for a given microbial population that can mimic "true biological isolation" [39]. Such an association defines a specific ecological niche and is based on the host behavior and interactions with other animals and the environment. Molecular (sequence) types can be used as markers for demarcation of bacterial species, but they cannot define the species.

4.4. Other Biologically Meaningful Criteria for Discrimination of Infectious Agents

The proposal for using the association of bacterial strains with a specific group of mammalian hosts as biologically meaningful criteria for discrimination of infectious agents can be challenged by an absence of host-specificity. Observations show that several distant genogroups, each potentially different from each other, can be found in a single animal species. In this situation a species cannot be defined singularly by association with a particular animal host since multiple genogroups are associated with the same animal species. The question is whether strains from genetically distant clusters should be regarded as belonging to distinct bacterial species representing different biological traits and inducing little or no cross-immunity? To answer this question, statistical methodologies have been applied using a dataset collected from a particular multi-strain bartonella system [41]. This study indicated that each cluster of genetically related variants likely constitutes a separate bacterial species consisting of minor variants, with an absence of immunity between clusters (species), but limited cross immunity against variants within a species [41]. The situations for other microbial groups can be different, and the applied approaches can differ, but the idea is that genetic similarity between strains should be used for a species definition in combination with biologically meaningful information that defines a specific ecological niche and lifestyle [39,40]. The term *ecotypes* describing populations with unique distribution along ecological gradients can be beneficial for typing microbial communities. These are populations of microbes occupying the same ecological niche and whose characteristics are shaped by the same selective factors [32,40].

5. Multiple Biological Roles of Hosts

When an infectious agent is found in an animal, especially in a vertebrate animal, the latter is frequently reported as the "host of the infection". It can be true for a particular situation, but this definition does not specify the role of the animal in the circulation and maintenance of the infection. To clarify this distinction, in the cases when an infectious agent can survive in the host population over a long period of time, the host is commonly considered to be a "*reservoir of infection*", and thus contributes to survival of this particular agent in nature [42]. Even if an infectious agent can survive in an individual host animal until the death of the animal, this period would not be sufficient for allowing the long-term survival of the agent in nature. The infectious agent needs a temporal chain of hosts, either of the same species or of different species (multi-host system), with successive events of infection. For many infectious agents, circulation among hosts involves arthropods transferring the pathogen between mammalian hosts (vector-borne infections), or depositing the pathogen in the non-biologic environment until a new mammalian host can be acquired again (sapronoses). In some instances the arthropods may act also as reservoir hosts.

The situation can be much more complicated, but the main point is a need for discrimination of these two categories: (a) a "reservoir" that is an essential part of the host-parasite system permitting long-lasting circulation of the infection in the environment; and (b) the "host" as a more general category including not only a "reservoir host", but also animals which could be infected incidentally. What is the importance of defining the role of the reservoir host? First, it is important for understanding the evolution and distribution of any infectious agent as specific animal species will

provide the environment for this agent. Second, from a practical standpoint, identification of the reservoir host is crucial for epidemiological forecasting and developing any effective prophylactic measures based on targeting and controlling specifically the reservoir host species. Unfortunately, a thoughtful attempt is rarely made to identify the reservoir host, and many investigators use the terms "host" and "reservoir" interchangeably.

Contradictory definitions of infection reservoir exist [42,43]. Some investigators stress the ability of the putative reservoirs to maintain a healthy status while allowing an infectious agent to survive through multiple generations. Let me illustrate such discussions using plague infection as an example since it served as an excellent model after a hundred year history of research on relations between the pathogenic agent, *Yersinia pestis*, and numerous rodent species [44]. Among the 203 rodent species or subspecies and 14 lagomorph species reported to be naturally infected with *Y. pestis* [44], only a small proportion actually comprises significant hosts. Each of these small mammals possesses unique characteristics that influence its ability to serve as a host of plague, although most have certain features in common. Many members of important host populations, probably 40% or more, not only become infected with *Y. pestis* but also circulate sufficient numbers of bacteria in their blood ($>10^6$ *Y. pestis*/mL blood) to serve as reliable sources of infection for feeding fleas [44]. Few, if any, hosts that become heavily bacteremic will survive, but more resistant members of the same population might develop less severe illness and may live to reproduce. Major plague hosts can be often heavily infested with one or more important flea vectors, a trait that obviously promotes the spread of the disease. Finally, many significant hosts live in burrows that support large flea populations, and those that dwell elsewhere, such as wood rats (*Neotoma spp.*), often have complex nests that are also heavily flea-infested [44]. Rall [45] proposed classifying plague hosts as either primary or secondary carriers of plague, with primary hosts or their fleas able to maintain plague in natural foci without involving other potential hosts. Conversely, secondary hosts and their fleas cannot maintain *Y. pestis* in the absence of primary hosts, but might assist in dissemination of the disease. It has also been proposed that the ability of an animal to act as a primary host depends on its susceptibility to infection, abundance, distribution, and behavior. Among other factors, which determine the role of rodents either a primary host or a secondary host, were proposed colonial structure, complexity of burrow systems, resistance to a specific biotype of *Y. pestis*, ability of resistant animals to produce high antibody titers and levels of phagocytic activity, the occurrence of prolonged bacteremia in susceptible animals, and other parameters [44]. Instead, in the plague literature in North America important rodent species have been characterized as either enzootic (responsible for maintenance) or epizootic (responsible for amplifying) hosts [44]. Regardless of differences in terminology, we can accept definitions of "primary hosts" and "enzootic hosts" as referring to the concept of the reservoir host.

Haydon and his colleagues proposed a conceptual framework for defining and identifying reservoirs in the field [42]. They defined a reservoir as one or more epidemiologically connected populations, or environments, in which a pathogen can be permanently maintained and from which the infectious agent is transmitted to a defined target population. The important message is that identification of the role of a reservoir host requires meaningful biological criteria. Infectious agents can react with the system of biologically tuned signals ("*signs*") to "recognize" their reservoir hosts; in all other situations they either will not invade an "improper" host, or will be eliminated after a brief period of infection ("*incidental host*"). From the "view" of an infectious agent it is not essential whether the

incidental host develops pathology or clears the infection; in either situation this host represents a "dead end" for the circulation of the infectious agent.

In epidemic systems, animals can play different "*functional roles*" ranging from incidental host to reservoir host. The term "function" is considered here in the sense of selection-mediated biological role (adaptive or biological function) as defined by Bock and von Wahlert [46]. The biological functional role is a result of the complex interplay between main components of this system, including characteristics of infectious agents, animals, and many surrounding factors. Under different ecological conditions the role of animals of a particular species as hosts for an infection can be different.

6. Immune Status as a Regulator of Infections in a Host Population

Resistance is the ability of a host to limit an infection given a certain exposure to the pathogen, and reflects the degree of susceptibility of the host to the initial invasion, and the ability of the animal to control replication of infectious agents within itself. "*Specificity of immune defense*" describes a certain form of host-infectious agent interaction that cannot be predicted without knowledge about both the host and the infectious agent [47]. In other words, hosts differ in their susceptibility to different types of infectious agents; whereas infectious agents differ in their ability to infect and replicate on different host types. The more susceptible the hosts, the more rapid and widespread will be spread of disease. An imbalance in the relationships between animal hosts and the associated microorganisms can trigger the transformation of harmless symbionts to pathogens. Yet epidemiologists and disease ecologists have often neglected variation in host susceptibility [17].

6.1. Population Immunity and Cross-Immunity

Immunological research conducted at the molecular and cellular levels has helped to describe pathogen- immune cell interactions within a host for numerous infections, but there is no such progress in quantifying immunity at the population level. Population immunity can regulate spread and magnitude of infections, but also affects antigenic variation and emergence of resistant strains among infectious agents [48]. Though coined by Topley and Wilson [49] 90 years ago, the term "*herd immunity*" was not widely used until recent decades. Some authors use it to describe the proportion of immune individuals in a population, or to indicate a particular threshold proportion of immune individuals that should lead to a decline in incidence of infection. A common implication of the term is that the risk of infection among susceptible individuals in a population is reduced by the presence and proximity of immune individuals. Among the classic examples was the recognition that periodic epidemics of ubiquitous childhood infections such as measles, mumps, rubella, pertussis, chickenpox, and polio, arose because of the accrual of a critical number of susceptible individuals in populations and that epidemics could be delayed or averted by maintaining numbers of susceptible individuals below this critical density [50]. The concept of population immunity, or community immunity, could be developed further to measure the status of a host population, which can determines spread of the infection within this population along with other factors (transmission effectiveness, force of infection, rates of contacts, *etc.*). Other factors include degree of cross-immunity, mode of agent transmission, infectivity, and transmissibility of the agent [48]. Population immunity (herd immunity) also depends on the genetic composition of the host population. Host-microbe interactions and competition between

co-infecting agents can affect virulence and transmissibility [51]. Another phenomenon underlying the uncertainty of a specific immune response is cross-immunity. Infection by one strain of an infectious agent often modulates susceptibility to subsequent infections by related strains because of the immunity acquired by a host [52].

7. Infectious Status

Data pertaining to infectious pathogens have been generated and become available to the research community with increasing speed. Development and application of new methods, especially molecular genetic approaches, for description of the diversity of microorganisms, determination of virulence factors, understanding of immune system function, pathogenic pathways and so on have tremendously changed our knowledge. These new approaches can effectively give some answers to each specific question; but, at the same time, the abundance of new information about the structure and functions of both pathogens and infected hosts makes sometimes the role of each element of a host-pathogen system ever less certain.

7.1. Non-Dualistic Definition of Infection

Paradoxically, new scientific information, instead of defining the observed "host-agent" system as "infected" *vs.* "non-infected", often leads to multiple categories that can be defined "infected AND non-infected", depending on the criteria used for results interpretation. The answer of whether a host is "infected with a specific agent", or if a host is "free from a specific agent" is not always so simple. Indicating "fuzzy boundaries" in the identification of a disease cause, Plsek and Greenhalgh [53] see them as a result of the interplay of genetic predisposition, environmental context, and life choices.

How we can define the infection status of the host? Without a meaningful definition, how can we say that a host is really infected? From the definition of infection given by the Webster's Medical Dictionary: "*Microorganisms that live naturally in the body are not considered infections; for example, bacteria that normally live within the mouth and intestine are not infections*". Though in many cases it is easy to determine a harmful (pathogenic) effect; this is not always true. Under some circumstances differentiation between harmful and beneficial effects of microbes on the host can depend on temporal scale: the effect that may appear as deleterious for a host, e.g., consuming some host resources, might have a beneficial consequence later. All animals evolve and live in the context of a resident microbiota. Some animal groups have lost specific metabolic capabilities, e.g., sterol synthesis in insects or cellulose degradation in vertebrates [54]. As a result, animals that feed on blood (deficient in B vitamin), as expressed by Douglas [54], "can't live without" microorganisms that possess these biosynthetic capabilities.

Virus infection can present a significant challenge to host survival. The capacity of the virus to replicate and persist in the host is dependent on status of the host antiviral defense mechanisms. The study of antiviral immunity has revealed effective host immune responses controlling viral infections and enhanced our knowledge of the diversity of viral immune-modulatory strategies that undermine these defenses. There are diverse approaches that RNA viruses use to trick or evade detection and removal by the immune systems. Some of these approaches include specific blocking of the major histocompatibility complex-restricted antigen presentation pathways, inhibition of apoptosis, disruption of

cytokine function and signaling, exploitation of the chemokine system, and interference with humoral immune responses [55].

It was recently demonstrated that endosymbionts manipulate the host genome via induction of the microRNA [56]. MicroRNA is a small non-coding RNA molecule found in many eukaryotic organisms that participate in transcription and regulation of gene expression [57,58]. MicroRNAs are critical effectors in the intricate host-pathogen interaction networks, and evidence suggests that both virus and hosts encode microRNAs. The exclusive dependence of viruses on the host cellular machinery for their propagation and survival also make them highly susceptible to the vagaries of the cellular environment like small RNA mediated interference. It also gives the virus an opportunity to fight and/or modulate the host to suite its needs. Thus the range of interactions possible through microRNA "cross-talk" at the host-pathogen interface is large [59]. These interactions can be further fine-tuned in the host by changes in gene expression, mutations and polymorphisms. In the pathogen, the high rate of mutations adds to the complexity of the interaction network [60].

The uncertainty in assigning an effect of microorganisms as positive or negative becomes more evident at different levels of host-infectious agent system. The death of individual rodents from plague can be considered as a mechanism for regulation of rodent density on a population level. As mentioned previously, a simple presence of antibodies is not commensurate with an infection *per se*, but can serve as an indicator of either current or past infection. In fact, host contact with the infectious agent might not result in any pathogenic effect; nevertheless it can result in the production of specific antibodies, secretion of various cytokines, and numerous subclinical manifestations of an effective immune response. A functional immune system is important for survival in natural environments, where individuals are frequently exposed to parasites [60]. However, strong immune response may have fitness cost if they deplete limited energetic resources or leads to out-of-control response (anaphylactic shock or overproduction of mucus-filling lungs with liquid in pneumonia). Graham *et al.* [61] have found association between fitness and heritable self-reactive antibody responsiveness in a wild population of Soay sheep. The occurrence of self-reactive antibodies correlated with overall antibody responsiveness and was associated with reduced reproduction. All such responses can be seen as "*signals*" of the interaction between host and infectious agent.

7.2. Infectious Agents in Healthy or Chronically Infected Hosts

It is important to differentiate between the presence of an infectious agent with an ability to produce a disease, and pathology induced by this agent. There are many observations that animals are infected by pathogens without manifestation of disease [62]. A good example is provided by bartonella bacteria, which parasitize the red blood cells of many mammals without apparent harm to natural hosts, yet may cause a wide range of pathogenic manifestations in incidental hosts. In analyzing the natural history of bartonella infections in animals, Jacomo *et al.* [63] describes it as an exception to Koch's postulates. This conclusion was based on observations of high levels of bacteremia in animal reservoirs with or without any clinical manifestations. At the same time it has become apparent that *Bartonella* species are associated with many illnesses in humans, dogs, and cattle, involving a broad spectrum of manifestations and symptoms (chronic bacteremia, fever, skin lesions, endocarditis, lymphadenopathy, *etc.*). A possible explanation of the observed phenomenon is that bartonella bacteria

are well adapted to specific hosts and exploit nutritional resources of those hosts with minimal harm, whereas incidental introduction to other animals can result in the accumulation of bacteria in some tissues causing pathology. Outside of bartonellae, observations of infectious zoonotic agents in healthy animals have been reported for diverse bacteria and viruses, and should be seen as a rule, rather than an exception. Unfortunately, as was acknowledged by Koch himself, application of his postulates has serious limitations when attempting to attribute disease causation to stealth pathogens that can induce chronic, slowly progressive disease manifestations in an animal or human patients [10].

A host can develop a latent, subclinical, clinical or fatal infection; and the two former forms can be crucial for the transmission of infectious agents [64]. Bacterial and viral persistence should be considered as a regular biological phenomenon that is responsible for the overall antibody responsiveness. Infection process caused by persisting pathogens considerably differs by clinical manifestations, morphological changes and localization of pathogens from acute diseases caused by the same species of bacteria and viruses. Infection and immunity are coupled dynamic processes arising from the non-linear and multivariate interactions between a host and multiple infectious agents. Infection and host immune status within populations at any given time represent "snapshots" that do not necessarily reflect immune and infection dynamics [48].

7.3. Increased Uncertainty in the Modeling of an Epidemic Process

Many infection models treat the infectious agent as a static and homogeneous entity [65]. In real life common infectious agents often demonstrate great antigenic and pathogenic diversity and evolve rapidly. Relations between hosts and pathogens cannot be limited to simple descriptors of "epidemic" or "not epidemic". There is an entire scope of variations, within host populations with varying proportions of susceptible individuals, and among infectious agents in terms of their virulence (ability to invade the host organisms). There can be more virulent strains and less virulent strains. The proportion of susceptible animals can vary in a host population. When a circulating strain is of low virulence and many hosts are susceptible, some cases of rapid spread of the strain might happen, but this situation is not likely lead to epidemic. When the proportion of susceptible animals remains high and a virulent strain is introduced to the population, the chance of an epidemic is increased. As a result of the epidemic, many individual hosts become immune and transmission of the pathogen decreases. So, epidemic processes are cyclic. This biological system behaves as a whole, despite a large number of elements and subsystems that have their own temporal dynamics ranging from genetic mutations in bacteria to multi-year fluctuations of environmental factors.

For descriptions of host-pathogen interactions on a population level there is a widely applied "SIR model", where "S" stands for a number of "susceptible hosts", "I" for a number of "infectious hosts", and, finally, *R* for a number of hosts which "recovered" from the infection (these individuals become immune and do not participate in future dissemination of the infection, at least for some time) [66]. This is a very general model, and a number of modifications were proposed to fine-tune it. For example, some infections do not lead to an immune response sufficient to prevent a repeat infection, and the SIS model is applied in this case, where the last "S" stands for "susceptible again". Other modifications introduce "exposed hosts" (SEIR model), carrier status (SICR model), maternally-derived immunity (MSIR model), and more. There is a huge amount of literature on applications of SIR-derived

models, and whoever is interested can easily find further examples. Practically all proposed SIR models can be reduced to two simple dualistic points: (a) host is infected; or (b) host is not infected (in other words a host is currently free from the infection). In reality, the description of a host-pathogen system at the level of an animal population becomes much more complicated, reaching a situation where any deterministic model is very limited in its scope. Surely we can define some conditions when we can say that a particular host is certainly infected with a specific pathogen. We can also define other criteria to say that, to the best of our knowledge, another host is free from exactly the same pathogen. In fact, we can put both condition sets together on the scale, where presence of infection with a particular pathogen is defined as a positive OR defined as a negative. In spite of the dramatic difference between these statuses, they share something essentially common—they are definite, and have a "fixed position". We know what to do with these "fixed" variants—apply an SIR model with all appropriate modifications and improvements. The question is how to define, how to handle, and how to analyze all other situations when the status of the "host infected by the specific pathogen" is not as clearly defined. For antigenically variable pathogens (this is more of a rule rather than an exception), models become very complex and stochastic. Rhodes and Demetrius [67] conducted an epidemiological analysis based on entropy, defined as an information theoretic measure to describe the uncertainty; and, using this approach, explained empirical observations regarding the emergence of less pathogenic strains of human influenza during the antigenic drift phase.

7.4. Experimental Animal Models

Critical limitations for analysis of infectious agents and host relations can be also observed in animal models. This situation is common for many areas of experimental biology, and Bolker [68] recently warned in Nature that over-reliance on a limited number of model organisms constrains research in a number of ways that must be acknowledged and addressed. For instance, traditional models for response to many bacterial and viral agents, such as inbred strains of laboratory mice and rats, are often selected because of convenience; but these models are poorly suited to research of infectious agents that are not naturally associated with *Mus* or *Rattus* species. In addition, research with traditional inbred models clearly cannot take into account the natural variability of most animal hosts. To study the influence of population and environmental factors, it makes more sense "... *to study species in which such factors matter...*" [68]. At the same time, the effect of the typical laboratory environment, such as the details of mouse handling and housing, are often overlooked [69]. As Bolker [68] concluded, "... *if we frame a research model or system too narrowly, we cannot construct a complete picture of the mechanisms that underlie crucial variations, for example in development and disease*".

Experimental results obtained under particular conditions, or from a particular model, such as an experimental inoculation of laboratory mice or *in vitro* cell cultures, are commonly extrapolated to much more complex situations observed in the field. These studies can be extremely valuable, but the linear extrapolation of the results to the natural system can be misleading [70]. Nevertheless, many molecular biologists still prefer simple models with explanations based on a few individual factors. For example, knockout experiments—which are widely used to determine the functional role of a gene—overemphasize the role of a single gene and are of limited use for understanding complex

genetic networks. As a matter of fact, many knockout experiments have no obvious, or demonstrate rather unexpected effects because any given gene product is only a part of a complex network, often with substantial redundancy for each individual component [70]. Most infection transmission models treat the infectious agent as a static and homogeneous entity. In real life, infectious agents commonly demonstrate great antigenic and pathogenic diversity and evolve rapidly [65].

8. Uncertainties in the Analysis of Environmental Factors

Another critical component of a host-pathogen system is the environment in which a pathogen and its host come in contact, and often occupy unique conditions. Environmental factors (temperature, precipitation, *etc.*) can not only affect survival of pathogens or hosts, but also modify mechanisms of pathogen transmission between hosts over a long period.

8.1. Geography

Investigations of environmental factors required for the existence of specific infections have a long history. An important direction in the search to define the deep connections between zoonotic diseases with specific geographic areas was developed by Russian scientist Evgeniy Pavlovsky [71], and is well known under the term of "natural focality of diseases". Many other distinguished scientists continued to formulate the concept of "natural focus" as the territory where the persistence of such diseases is determined by specific parameters. According to the definition given by Kucheruk and Rositskii [72], "a natural focus as a relatively autonomous ecosystem that exist in nature in only one copy", and its boundaries "can be delimited in the field and drawn on the map". A natural focus of any particular infection essentially contains the corresponding pathogen, which is the only specific and distinctive component of this system. Hence, in elaboration of the above postulates, the term "natural focus of an infectious disease" refers to any natural ecosystem that contains the population of a pathogen as an essential component and all other components of the ecosystem that support the existence of the pathogen for a historically long period of time. These components can include populations of vertebrate hosts, arthropod vectors, and specific patterns of vegetation, soil, and water [73]. The point is that all these characteristics have direct impact on the survival and multiplication of pathogens; by other words, they are not incidental, and are biologically significant for supporting all essential components of this system.

The idea of a natural focus of the infection can be formulated in terms of an ecologic niche that combines a set of conditions under which a population of infectious agent can persist in nature without a need for continuous invasion from other areas. In many cases, occurrences of diseases or of species participating in disease transmission (e.g., vectors, hosts, pathogens) can be defined by association with specific ecological parameters [74]. The application of remote sensing (acquisition of information from satellites), and GISs designed to capture, manage and present all types of geographical data has dramatically changed our ability to analyze associations between pathogen-host systems and a range of environmental factors. With the tremendous progress in obtaining and storing geographic information, application of these new approaches increases uncertainties in our understanding of specific elements playing a key role in the relations between pathogens and hosts. Spatial heterogeneity refers to differences between host populations or to differences in host-pathogen relations at different

geographic locations and different spatial scales. Though spatial heterogeneities are common, they are frequently ignored because of the extra complexity they introduce.

8.2. Effect of Seasonality and Climate

Temporal changes in transmission rates are widely recognized for many infectious diseases [75]. Seasonality is just one, though the most visible, cause of temporal dynamics of epidemic processes observed in animal populations. Among important factors we can mention are seasonal changes in host behavior, migratory patterns, physiological and immunological characteristics, behavior, and other stress factors. The influence of seasonality and other climatic factors on the dynamics of infectious diseases is well recognized. The complexity of these interactions has required development of new categories to develop even a basic understanding of how seasonality may affect multiple processes. These affected processes include host behavior and ecology, level of herd immunity, and infectious agent transmission and survival in the environment [76]. For example, investigations of the effect of environmental factors on plague activity may involve models that include flea activity, host specificity of fleas, susceptibility of rodent species to the pathogen, survival of rodent species, ability of fleas to support plague bacteria within their bodies, *etc.* However, such factors further depend on other factors such as temperature and precipitation [77]. Such research requires a systems-based approach that not only integrates information from several fields of research in order to address the population context in which infectious disease occurs, but also considers the interactions and feedback loops between components of the system [3,78].

9. Concept about Exogenetic Information within an Animal Population

A significant step in switching the perceptions of viruses and microorganisms as not only sources of infections but also as carriers of genetic information of their hosts is attributed to Howard Temin's discovery of protoviruses and his concept of dual nature of viruses [79]. On one hand, protoviral DNA is an essential component of a genome that determines the normal development and functioning of the host organism. On the other hand, protoviruses are able to "break loose" from the host genome and emerge as viruses circulating among natural host and potentially playing the role of infectious agents. Such direction of thinking has led Russian virologist Vadim Agol to consider many viruses as "runaway protoviruses" [80].

For our discussion, it is important to note that each animal population is exposed to a diverse array of bacteria and viruses, which carry specific genetic information and that circulation of such information, within a particular host population, can contribute to significant and direct changes in this population [81]. Empirical observations of interactions between animal populations with microbial communities associated with these animal populations require introduction of new conceptual approaches. One of the approaches was proposed more than 20 years ago, the category of "exogenetic information" as the entirety of genetic information encoded in a microbial community which is in turn associated with a particular animal population [82]. The idea behind this concept is the recognition of two different dynamic processes represented by circulation of viral, bacterial, fungal, and parasitic genetic material in an animal population. The first process represents changes in communities composed of diverse microorganisms which occupy animals of a single local population. Each

microbial species has specific genomic information adapted to its specific environment, such as its particular animal host. There are different mechanisms of lateral (horizontal) exchange of nucleotides, genes or larger genetic elements, such as plasmids, between microscopic organisms, but nevertheless this combination of nucleotides is specific to particular kinds of microbes. The second process can be defined as a combination of genetic information that is alien for animals since it is carried by the microbes, nevertheless the sum of the genetic information shared by all internal microparasites is highly specific to a particular animal population and can be responsible for a specific manifestation in the ecology, behavior, physiology, and overall fitness of animal hosts [81]. The ability of parasites to change behaviors of the infected hosts has been documented when the changes in behavior increase rates of infection transmission and more sophisticated manipulations of the host's behavior repertory [82,83]. There is accumulating evidence that interaction of animal hosts with microbes have resulted in the coordinate evolution and that coevolved animal-microbial partnerships represent a common and fundamental theme in animal development. McFall-Ngai [84] distinguished two types of potential influences of bacteria on animal developmental programs: (1) the nonspecific influences of microbes as ubiquitous and critical components of the environment; and (2) the specific influences of the bacterial cells that have coevolved with animals in tight associations that are maintained across generations.

As it was indicated above, not all symbiotic microorganisms are harmful for an animal host and can be vital for their metabolic, developmental, and behavioral patterns and therefore contribute to signaling networks that shape host functional status. Douglas [54] emphasized that animal dependence on microbes have multiple bases: a requirement for specific capabilities of bacteria, the "signature of interactions past" in which an animal function requires input from the microbes, and "addiction" to the manipulative traits of microorganisms. Measuring the effect of cowpox virus on survival in natural rodent populations, Telfer *et al.* [85] came to conclusion that the pattern of the effect is not simple, and is likely to be the result of subtle and changing interactions with other processes.

Importantly, intensity and character of the circulation of exogenetic information carried by viruses and microorganisms is tightly regulated by structure of animal populations. Animals that live in dense populations, large social groups, and with promiscuous mating systems can provide conditions, which facilitate transfer of exogenetic information among animals owing to the close proximity and higher contact rates among individuals [86,87]. Different transmission modes reflect host traits and should influence spread of exogenetic information. Increased sociality and greater host population density are predicted to increase infection rate for viruses and bacteria transmitted through direct contact [88], whereas infectious agents spread by arthropod vectors or exposure to contaminated environment (e.g., soil or water) may be less sensitive to changes in host contacts and density [86,87]. Host population organization and behaviors influence not only microparasites diversity and prevalence but may also determine the fitness advantage of different transmission strategies to carries of exogenetic information [87]. Thus, exogenetic information is meaningful from the "point of a host population" and represents an independent and important characteristic of the animal population. While exogenetic information in its physical structure (encoded in nucleotides) is similar to hereditary (genetic) information, the mechanisms of its exchange have fundamental similarities with the so-called "*signal biological information*", such as visual, acoustic, and olfactory information transmitted through direct or indirect contact between animals. The intensity of transmitted exogenetic and signal information

among animals depends on animal population conditions (density, social behavior, frequency of contacts, stress level, *etc.*) and environmental factors (geography, seasonality, substrates, *etc.*). Functional expression of both exogenetic and signal information can be amplified or suppressed during "*passages*", a series of transfers from one animal to another. Significantly, information encoded in microorganisms' DNA ("genetic information") has very different "biological meaning" depending whether it is reflected in functions of microbes, at the level both of a single microbial species and a whole microbial community, OR it is reflected in functions of the host animal population. As Nijhout [89] indicated, "*it is necessary to identify the sender, the receiver and the information channel (or means by which information is transmitted)*". The same physical content of microbial DNA can be measured in very different ways based on its effect on microbes or animal hosts. Here is one more quote, this time from the prominent geneticist Lewontin [90]: "*A deep reason for the difficulty in devising causal information from DNA messages is that the same 'words' have different meanings in different contexts and multiple functions in a given contexts, as in any complex language*".

10. Complexity

The problem of growing uncertainty in the description of complex pathogen-host species is certainly not unique to epidemiology and is relevant to all complex adaptive systems. The "*Science of complexity*" has influenced many scientific fields, but its application to biology of infectious diseases has been limited, though it was recognized to be important [3]. From a very general perspective, it is not enough to analyze separate properties of elements of infectious system, e.g., molecules and cells within either microbes or hosts, as it would be not possible to comprehend the complex organization as an indivisible whole system. A new model of scientific investigation to understand complex systems; such an organization of interacting infectious agents, the hosts, and environmental factors, would require shifting the perspective from the whole to the parts and back again [70].

Infectious agents are not just random collections of cells or molecules and populations of hosts are not just random collections of individual organisms. Risk factors for infectious systems do not operate in isolation, but occur in a particular population context [3]. A complexity-based approach to infectious diseases involves different types of questions than "does agent *A* cause disease *B*?" or "what risk factors are associated with the transmission of infection?" [3]. These questions "*can be answered using straightforward methods (e.g., the relative risk of transmission of infection in those exposed compared with those not exposed to a particular factor) ... but may in practice be insufficient for the control of infection in a particular population*" [3]. Reductionism favors the removal of one particular object of investigation from the normal contexts. For example, commonly experimental results are obtained from an artificially simplified model, such as an inbred mouse for inoculation with a singular strain or *in vitro* cell culture; and the obtained results are often extrapolated to more complex situation. As Mazzocchi [70] noted, such extrapolation is at least debatable and at worst misleading or even hazardous.

Though the characteristics of infectious agents and the hosts are very variable, the concept of complexity is more specific. According to the definition provided by Plsek and Greenhalgh [53]: "*a complex adaptive system is a collection of individual agents with freedom to act in ways that are not always totally predictable, and whose actions are interconnected so that one agent's actions changes*

the context for a variety of other agents." Systems are complex when they represent many apparently independent agents interacting with each other, but the richness of these interactions allows for the system as a whole to undergo self-organization [91]. They are also characterized by the involvement of non-linearity and feedback loops in which small changes can have striking effects that cannot be understood simply by analyzing the individual components, operate under conditions far from equilibrium, and are embedded in the context of their own histories [92–94].

It is easy to recognize that the systems of varies microbes associated with diverse hosts with their non-linear relations regulated by population immune status and changeable environment conditions represent classical complex systems. Beldomenico and Begon [17] illustrate the mechanism by which host susceptibility and disease can act in synergy, generating "*vicious circles*" at both the individual and population levels. Individuals in poor physiological condition might be more susceptible to infections, which further weakens their conditions, increasing the risk of further infections.

Complexity science emphasizes the importance of the concept of "levels of analysis" [95]. Emergent phenomena that occur, for instance, at the level of the organism cannot be fully explained by observations made at the level of cells or macromolecules [70]. Complex systems of infectious diseases exist at different levels of organization ranging from molecular to ecosystem levels, and include molecules, cells, tissues, organisms, communities, and ecosystems. All these levels share common properties, such as (1) self-regulation in supporting an existence of the whole system of a particular infection; and (2) emergence phenomena expressed in high increase of intensity (epidemics). Both the infectious agents and the hosts can express emergent properties that arise from interactions both among their components at each level, and in accordance with the environment factors. Self-organizing systems spontaneously arrange their components and their interactions into adaptive structures with emergent properties [70,96]. Complexity of infectious agents-hosts systems can be expressed on two separate levels: one with a wide range of genetic variability within microbial communities occupying specific ecological niches, including a transit stage in incidental hosts, and another with a range of variable characteristics of pathogen populations adapted to long-term survival and circulation within a population of their reservoir hosts. Hosts within a population differ in their susceptibility to infections due to a number of different factors and processes, including genetic variability, environmental factors, behavioral and social differences, demography, and more factors [51].

Agents can reach a high level of sustained endemic circulation by developing antigenic diversity or expressing immune-suppressive traits, thus escaping immune responses. The immune system responds by increasing its own complexity, e.g., through a built-in random generator (REG1 and 2-driven recombination in TCR and BCR/antibodies) to allow for a vast potential pool of recognizable structures. The diversity of the major histocompatibility complex (MHC) system likely arose for the same reason of creating a large antigen recognition pool in populations [65]. The evolutionary trend is for the agent and hosts each to develop a high level of diversity in adaptation to each other.

On the one hand variability of genetic characteristics of infectious agents represents a big challenge for explaining the emergence of new pathogen-host patterns. On the other hand, time measurements of bacterial diversity and of the degree of host-specificity can provide some workable criteria that can be used in modeling to define some functional stages in "microbe- vertebrate animal-arthropod vector" relations. As an example of possible applications of science of complexity for the analysis of microbial diversity, the dynamics of antigenic structure of pathogens under different degrees of cross-protection

was expressed over a wide range of intermediate levels of selection and exhibited proportions of the different strains through very complex, and, often, chaotic dynamics [97]. Infectious agents exhibit a range of population structure and life-history strategies, including different transmission modes, life-cycle complexity, and off-host survival mechanisms [98].

In an editorial note for the *Journal of Epidemiology and Community Health*, Materia and Baglio [99] emphasized that "integration" and "context" became key terms in the multidisciplinary approach in the fields of biomedical research and clinical medicine. The authors sited Susser and Susser [100] that the modern approach focusing on the decontextualized association between exposure and the outcome in single individuals is "similar to the physical (theoretical) sciences in its search for the highest level of abstraction of universal laws". The traditional approach is determined at an individual, rather than at a population, level, whereas ecological context is pivotal for understanding biology of infectious diseases at all levels. The same authors also call for the re-evaluation of the ecological studies, and advocate the use of hierarchical analysis for the multilevel studies. Another related note was made by Vineis [101] about "... *mistakes made in the past by underestimating the effect of environment and overestimating the effect of genes*".

In epidemiological statistics, re-evaluation of Bayesian methods can be also considered as a shift since these methods take into account the context and prior knowledge [102]. The assessment of the quality of evidence should focuses not only on the study design and internal validity but also on consistency and transferability of the results to the context of interest [99].

11. Biosemiotic Concept

The ability of animals to acquire infection, and ways of reacting to the invading microbes invading, depends on numerous factors, with their immune status being one. A much more complicated situation is observed on the level of an animal population: some animals remain resistant to an infection; some animals are invaded by microorganisms for a short time until the infection is completely cleared up; some animals can be carriers for identical microbes for a long time, theoretically the whole lifespan, and only a proportion of the animals apparently become sick, some of which can die. Of course, this is a much generalized picture and not applicable for all infections. There are numerous potential outcomes in the development of an infectious process in animal populations.

The situation that we touch on here is certainly not limited to biology of infectious diseases only, but represents a fundamental challenge currently experienced by all branches of biology. The systems of infectious agents at the population and community levels are so complex, that the paradox of the growth of uncertainties due to the accumulation of information at the molecular level becomes very evident. The scientific discipline, commonly known as "*biosemiotics*", makes a clear statement about this paradox: interactions at the molecular level and exchange of genetic information cannot fully explain biological processes, one must acknowledge the meaning of the transmitted information. The central notions of biosemiotics are "*sign*" and "*signal*". Neuman [103] defined a signal as meaningful, if it involves the communication of something that is not directly expressed. For example, being an antigen is not an attribute directly expressed by a molecule (a signal). The meaning of being an antigen is the result of a complex process ("*meaning-making*" process according to Neuman), which is finally evident in the specific immune response [102]. Similarly, an infectious agent is not just a microbe that

has a molecule recognized as a "virulent"; it is infectious because of its ability to cause infection in the appropriate host; and the "host" is characterized by its ability to be infected, not by the presence of a "susceptibility" gene. The "lock-key" model is a popular metaphor for presenting immune recognition; however, living systems at different levels of analysis present a much more flexible interpretation of signals than the lock-and-key model suggests [102].

Brier formulated principles of "cybersemiotics" as an evolutionary approach going beyond entropy and information into the question of meaning [104]. The term "information" is often used in a narrow sense as a degree of non-randomness or as a sequence of characters in DNA, but Sharov [105] cites the words of Bateson, "… information is a difference which makes a difference".

If we imagine the field of biology of infectious diseases as a play, the actors are infectious agents, their hosts, and vectors. These actors can play different roles. An infectious agent can play different roles, ranging from a neutral symbiont or a beneficial player hiding in tissues of the host to a lethal pathogen. The vertebrate host can play the role of an incidental receiver of the infection, or an effective spreader of the infection to other animals, or a long-term reservoir host, and so on. People can also participate in this play, either as a quiet and passive audience or as a prompter repeating the text, as a suffering victim, or as an active participant of the play, assuming different roles in the investigations of infectious diseases. This is not a poetic image, but rather an attempt to illustrate the reality of this complex process where DNA and other molecules provide text, but the dialogue is performed on higher levels (organismal, population, and communities).

The question can be formulated as to how participants of infectious systems in natural settings can recognize each other? How can an infectious agent "recognize" the animal organism as "reservoir host" or "incidental host"? How can a potential host "recognize" a microorganism as a "symbiont" or a harmful "pathogen"? For decades, investigators concentrated on elucidating some detailed mechanisms, eventually coming to the molecular level of the research. A fruitful approach for analysis of communication between elements of the infectious system on population levels should take into account not only the interactions between specific molecules, but also recognition of complex "images" characterized by specific population structures and life histories.

Characteristics of the life-history of infectious agents are important determinants of the interaction of infectious agents with their hosts [98]. Functional representation of such roles can be exemplified as "infectious agent causing death of an immune suppressed host", "infectious agent causing death of local subpopulations of a host species", "infectious agent causing a reduction of breeding functions of host organisms detectable on population level", "host population supporting circulation of infectious agent during a period of many generations", "host species supporting a dispersal of the infectious agent over geographic barriers", and so on. The list of such roles can be long, but major common scenarios can be defined. Genetic information encoded by DNA/RNA of microorganisms can be interpreted in very different ways depending on the role of the infectious agents, animal hosts, ecological factors, *etc.* Describing dynamic host-parasite relations, Horwitz and Wilcox [106] use the analogy with dancing: "*much like a waltz, the 'partners' are constantly adapting to each other's 'moves' in responses to the presence, or potential presence, of each other.*" This adaptive response can be behavioral, genetic or phenotypic, implicating each of the partners, and the interaction between them, environmental surrounding and structure of populations.

12. Concept of Functional Information

While contemporary molecular biology describes cells and organisms in terms of parts (e.g., proteins, nucleic acids, *etc.*), biosemiotics define living systems on the basis of their function rather than composition [105]. There is a general agreement that DNA/RNA molecules carry important information that codes responses both of microbes, potentially manifesting as pathogens, and of larger animals that can serve as hosts or vectors. However, biological entities communicate between themselves using not only primary genetic information, but also by exchange of signal information. In a similar context, Alexei Sharov [105], at NIH, defines *functional information* as collections of signs that help agents to preserve, control, and disseminate their function [105]. The functional meaning of information was originally introduced by MacKay [107] and Bateson [108] as *a difference that makes a difference*. In this interpretation, any piece of information is defined through its interaction with information from other sources to create a non-random effect, hence it is context dependent [109]. Microbes can enter an animal, but the host organism can react through the immune system in specific and, sometimes, very different ways based on the recognition of certain signs or signals, which are biologically informational for both microbes and hosts. When an animal becomes infected it means that the introduced microbe has caused a specific and complex reaction in this animal, and the entire animal population can react accordingly to "*signs*" presented by this infected individual. As a result, this individual infected animal can either represent a dead end for the spread of the pathogen (if the animal is excluded from the animal population, as happens with prairie dogs infected with plague), or it can become the starting point of an epidemic in this population (if the infected animal is allowed to continue spreading the infection). In other words, microorganisms become infectious agents when they function in a manner that theirl hosts recognize them as infectious agents and react correspondingly at cellular, tissue, or organismal levels. We can also say that microorganisms become infectious agents when they express functions that prevent an animal host from displaying appropriate functional signs to prevent a further spread of infection.

The host immune system can be mistaken in its recognition of some viruses and bacteria. A good illustration of the misrecognition was provided by investigation of biology of Acanthamoeba. Acanthamoeba is a free-living protist pathogen, capable of causing a blinding keratitis, granulomatous encephalitis, and other diseases in humans and animals [110]. Free-living amoebae are the dominant bacterial consumers and are responsible for up to 60% of the total reduction in bacterial population [111]. There is a significant emphasis on Acanthamoeba as a "Trojan horse" of other microbes including viral, bacterial, protists and yeast pathogens. The majority of Acanthamoeba isolates harbor endosymbionts which may include viruses, yeast, protists and bacteria, some of which are potential human pathogens. The exact nature of symbiosis and the benefit they represent for the amoeba host are unknown, but it is suggested that such interactions may help transmit microbial endosymbionts to the susceptible hosts and/or endosymbionts may contribute to the pathogenicity of Acanthamoeba [112]. Another interesting example of mistaken recognition related to Acanthamoeba is their predation on the largest known virus, Mimivirus, initially mistaken for a parasitic bacterium with a particle size of 400 nm and genome size of 1.2 million base pairs [113].

Analyzing the functional dependencies of parasites, Cheng [15] introduced the term "*attraction signals*" and emphasized its importance for defining parasite-host relations: "*Ample evidence indicates*

that host-emitted attraction signals are usually involved in those species of parasites that actively make contact with their hosts" (page 23). The point about an "*attraction signal*" can be very helpful for making a distinction between parasites and infectious agents (pathogens). Whereas a parasite recognizes attraction signals expressed by the host, a pathogen IS RECOGNIZED by the host as the pathogen through developing specific reactions. Whereas some pathogenic factors can be defined in microorganisms at the molecular level, infectivity itself does not make sense outside the host organism. As Eduard Korenberg indicated [114], "microorganisms are originated not for causing diseases, but for their own existence".

Let me illustrate this style of reasoning by using some simple scenarios postulated from the world of bartonella bacteria…. A flea sucks the blood of a rat infected with bartonellae, and the level of bacteremia (concentration of bacterial cells in blood) can be sufficient for enough bartonella cells to be consumed by the flea, or not. If the bacterial density does not reach a particular threshold level, the flea is not likely to acquire the infection (no sign: the flea cannot "recognize" the pathogen) …. The level of bacteria in rat blood can be high enough, but so is the level of the bartonella-specific antibodies developed by the rat immune system, and the bacteria received by the flea with blood could be neutralized by so-called immune complexes (the flea cannot "recognize" the pathogen) … bartonella bacteria enters the flea and is met antagonistically by other bacteria already present in biofilm formations in the digestive systems of the fleas, and the bartonellae thus are eliminated (bartonella cannot "recognize" the flea environment; other bacteria recognized bartonella); … bacteria successfully reaches the flea gut, but having no appropriate environment for colonization and multiplication (wrong flea species), all the bartonellae fail to establish in the gut and are flushed out from flea in the latter's feces (bartonella cannot "recognize" the flea); … bartonellae multiplied significantly in the flea gut and are excreted with feces (bartonella "recognized" the flea); … flea feces with bartonella bacteria are deposited in soil, and were consumed by a soil inhabiting insect (bartonella cannot "recognize" the environment; soil insect recognized bartonellae); … infected flea defecated on skin of another rat, and by scratching the irritated spot this animal introduces bartonella to its capillary system (the rat "recognized" bartonella and bartonella "recognized" the new host); … bartonella bacteria occupies the rats' endothelial cells via the VirB/VirD4 molecular system (the rat "recognized" bartonella and bartonella "recognized" the new host) or bartonella bacteria fails to occupy the endothelial cells because of defective VirB/VirD4 system (bartonella cannot "recognize" the host); … bartonellae are released in the blood stream (the rat "recognized" bartonella and bartonella "recognized" the host); … bartonella invade red blood cells (the rat "recognized" bartonella and bartonella "recognized" the host). I can continue expending this list (though not endlessly) and I may be wrong in some of the assumptions. However, all actors in this play (bartonella bacteria, flea vectors, and rat hosts) are not frequently mistaken because their systems of recognition have been adapted to specific signals through evolutionary time (e.g., bacteria have evolved to utilize the flea and rat). The main criterion for the satisfactory outcome (from bartonella point of view) for each separate event in the interactions between infectious agents, vectors and host is completing the function.

13. Conclusions

Despite the enormous success of molecular biology, it becomes clear that a discrete biological function can only infrequently be attributed to an individual molecule. Analysis of biological systems may require additional measurements beyond those currently applied in the biology of infectious diseases. The interactions between the variables that determine the transmission of infection in populations are generally complex and non-linear [3,115].

Network models can be more appropriate for descriptions of infectious agent-host-environment systems than a dualistic "infectious-noninfectious" model. Help is provided by the rapidly developing network biology that, in the past decade, has made advances toward uncovering the organizing principles that govern the formation and evolution of complex biological systems [116]. Neuman [103] defines a network of parameterized units that with certain values yield a unique response. The role of the scientist is to identify the dynamics that may lead to a specific response in a given context. It is a context that may turn microorganism into pathogen; that is, its stability is assured by a qualitative set of relations that defines its unique identity [103]. Similarly, an infected host is defined not only by the presence of a microbe, but by also by its context of hierarchical relationships that can explain diverse manifestations resulting from a host-agent interaction, such as a resistant organism, a short-term carrier, a reservoir host, or a sick animal.

Conventional treatments of epidemics oversimplify the process, starting from an arbitrary beginning and proceed in a linear fashion. An alternative approach that emphasizes complexity utilizes six phases in epidemic progression: (a) emergence of critical elements of a pathogen-host system, such as the appearance of new characteristic in a microbial population, or introduction of a new strain (variant); (b) extension of limits for potential variability in a microbial community, including pathogenic, commensal, and saprophytic forms; (c) natural selection of strains with significant biological modifications, e.g., virulent strains or antibiotic resistant strains; (d) specialization of strains to a new ecological niche (hosts, vectors, and environment); (e) distribution of pathogens, as adapted units, among potential hosts across diverse geographic biotic associations; (f) development of a specific phase in a pathogen-host system where the status of "infected" or "susceptible" carriers of the infection can be defined with a high degree of certainty; and (e) a stage where relations between components of the pathogen-host system are balanced in a self-regulated regime during long periods determined by the host's demography that can last until catastrophic environmental changes or anything that tilt the system out of balance occur.

The main question asked in this paper is how not to drown in the ocean of arriving information about infectious microorganisms that interact with animals and people. Biology of infectious diseases has benefitted from an enormous, and, overall, very positive influence of molecular biology. Unfortunately, generation of all these data has not been accompanied by corresponding development of theoretical methods and concepts to effectively process the newly available information. Development of such approaches can benefit from modern scientific disciplines, such as the science of complexity and biosemiotics. The ecologically informed view is a paradigm shift away from the conventional "one-agent—one-disease" perspective in the biology of infectious disease. Investigations of infectious agents with their relations with the natural hosts in the ecological context have begun to illuminate emerging properties of infectious disease system. The new observations have revealed a

complex and dynamic network of interactions across the spectrum of "infectious agent", "host", and "environmental niches" that may influence interaction of these components at different levels of biological organization (individual organisms, populations, species, and ecosystems).

The previously indicated paradox about complex and unpredictable relations between molecular determinants (virulence factors) and development of an epidemic situation in populations is analogous to observations made for diseases not caused by infectious agents as well. As Hoffmeyer [117] indicated, "*the conception of genes as unambiguous or autonomous function units does not even come true in these monogenetic diseases that originally served as models for our ideas of gene functions*". In the article entitled "Monogenic traits are not simple", Scriver and Waters [118] provided an example of phenylketonuria (an inherited disorder commonly known as PKU) as exhibiting unexpected manifestations: not all untreated carriers of the "disease gene" develop disease, probably because of the influence of unknown factors. Hoffmeyer's conclusion was that "genetic information does not simply cause things to happen" [117].

The current tendency to investigate host-pathogen interactions at the molecular and organismal levels is limited by ignoring the ecological context pivotal for understanding biology of infectious diseases at all levels, including the population, community, and ecosystem levels. It is not enough to analyze individual components of an epidemic system separately, nor would it be possible to analyze the entire system as a whole. A new model of investigation requires dynamic shift of the perspectives from separate components (the agents, the hosts, the environmental factors) to the whole system and back. The perspectives within the "simplicity-complexity" dimension might change from separate virulence factors to the multi-sided descriptions the pathogen; from well-defined pathogens to wide microbial communities; from clinically manifested disease to a variety of infectious patterns ranging from a latent infection to a deadly epidemic; from occasional findings of an infectious agent to stable natural foci of the infection; from single factors affecting a host-parasite relation to the ecological context, and so on. Infectious diseases systems are not simple, with their complex interactions between infectious agents, their reservoir and incidental hosts, and the surrounding environment. There are no simple recommendations for resolving the situation. The warning I would like to provide is that expectations that simple information gathering through application of modern molecular approaches can automatically lead to better understanding of evolutionary potential of infectious microorganisms and epidemiological patterns are illusory, considering the complexities of these systems.

Infectious agents, their hosts, and some abiotic components are directly involved in the complex dynamic process of maintaining specific infectious diseases in natural condition. These interactions require specific signals for a mutual recognition. This system of signals ("signs" in biosemiotics), however, is not fixed and is very sensitive to the ecological context. The call for "going back to the field, to nature" might sound naïve and naturalistic, but we can do so equipped with all the latest technology and also with a new perspective. Research designed to define specific biological roles in infectious processes at the population and community levels, and to evaluate biologically functional information that allows communication between participants of this system is an emergent and critical task for all biologists working in this research area.

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