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Risks from emerging animal diseases

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Summary

Emerging diseases of animals pose great threats to the health of the world and could potentially cripple financial wellbeing, food security or environmental vigour. Recent history has demonstrated that factors inherent in our globalised civilisation will ensure that new diseases will continue to occur in humans, as well as in animals. Focus has been on macroscopic factors underlying disease emergence, including trade, husbandry changes and environmental damage, any of which can facilitate the transfer of a microorganism from one host species to another, with possibly deadly results. However, the events that occur at the microscopic level deserve attention. The author presents the sequence of events that a microbe undertakes as it moves from one species to another and the hurdles faced as it negotiates a new environment to perhaps cause disease.

Keywords

Commensals, Dissemination, Emerging diseases, Immune response, Pathogens, Shedding.

Rischi derivanti da malattie animali emergenti

Riassunto

Le malattie emergenti degli animali rappresentano una grande minaccia per la sanità mondiale e potrebbero potenzialmente influire sul benessere economico, sulla sicurezza alimentare o sull'ambiente. Fatti recenti hanno dimostrato che fattori propri della nostra civiltà globalizzata garantiscono che nuove malattie continueranno a susseguirsi negli esseri umani così come negli animali. Sono stati focalizzati i fattori macroscopici che sottolineano l'emergenza , fra cui il commercio, le modificazioni gestionali e il danno ambientale, ognuno dei quali puo' facilitare il trasferimento di un microrganismo da una specie ospite ad un'altra, con conseguenze a volte fatali. Tuttavia, meritano attenzione gli eventi che avvengono a livello microscopico. L'autore presenta la sequenza di eventi cui è sottoposto un microrganismo quando si sposta da una specie all'altra e gli ostacoli affrontati mentre conosce un nuovo ambiente che può essere adatto a riprodurre la malattia.

Parole chiave

Commensali, Disseminazione, Malattie emergenti, Patogeni, Propagazione, Risposta immunitaria.

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Introduction

There is growing recognition among the population of the world that a new disease could come from any quarter and create global infectious turmoil. Emerging diseases have moved to centre stage in the drama of biomedicine and human health threats. The discovery of AIDS as a novel and widespread viral disease is probably the seminal event which sparked the entire field of emerging diseases and invigorated the fading study of infectious diseases in general. Subsequent emergent threats, including, among a host of others, Ebola, variant Creutzfeldt-Jakob disease (vCJD), severe acute respiratory syndrome (SARS) and avian influenza, have only served to fuel the new discipline and underscore the importance of early recognition and control of emerging diseases that threaten public health.

It is interesting, and perhaps ironic, that there are even greater numbers of emerging diseases of animals than the more headline-making infections in humans. These emerging diseases of animals, rather than assuming the second-star billing they usually get, should, instead, be highlighted for their importance to both humans as well as the animal populations they impact. Direct human health impact as a result of emerging animal diseases is of three occasionally overlapping types. First, if a primary source of animal protein is eliminated, there could be considerable compromise of food available for humans. Highly pathogenic avian influenza (HPAI) is doing this today in areas where the disease has occurred; many backyard chickens that supply eggs for daily cooking and meat for local populations in many regions are no longer available. Second, it has been shown that emerging diseases of animals often presage a similar event in humans. Monkeypox, Rift Valley fever and Nipah virus all emerged to cause significant problems in animals prior to being recognised in human populations (24). The occurrence of these infections can be considered as a proverbial canary in the coal mine, with the disease in animals serving as a harbinger of infection in humans. Maybe bird flu is also this kind of canary and is a warning of what could happen in humans. It is reasonable to assume that the global public health effort to control bird flu would not be necessary if the disease had been adequately controlled in poultry. A third reason that emerging diseases of animals may deserve more scientific, media and policy interest has to do with the animal populations themselves. As disease emerges to impact a population, especially a wild population, morbidity and mortality may create an altered ecological landscape, with irreparable and reverberating impacts. Chytridiomycosis in frogs provides a heartbreaking example. As this emerging fungal disease moved to new frog populations around the world, it is estimated that dozens of species of frogs may have suffered extinction, resulting in a dismaying decrease in biodiversity (19). Similarly, Ebola virus, known primarily for its effects on humans, has had a more significant logarithmic impact on great ape populations, threatening extinction in some regions (14). Permanent loss of these magnificent creatures is an ecological disaster that is painful to imagine.

It is apparent that greater importance needs to be given to the understanding of emerging animal diseases. Much has been written about the underlying reasons for disease emergence, such as globalisation, altered lifestyles, and habitat disruption. But these are all reasons seen from a macroscopic perspective. In fact, all these reasons boil down to one main microscopic element – a microbial agent crossing species boundaries to infect a novel host and cause disease.

Of human emerging diseases, which are the most thoroughly documented, it is well recognised that

the majority are due to a microbe invading a new and decidedly human niche. Three-quarters of these new entities have been shown to be zoonotic in nature, that is, with defined animal origins. With respect to emerging diseases that occur in animal populations, virtually all occur as a result of a virus, bacteria, protozoa, fungus or higher organism moving from one animal species, where often it is just carried subclinically and perhaps not even recognised, to a novel animal host. Studies usually focus on this final blow, that is, the outbreak of the new disease, the impact on the new host and the efforts at containment and control. But in reality, this is only the final outcome in a fairly long sequence of events that occurs between the microbe and the two different hosts. This paper attempts to step back from the larger or end-stage effects and instead to direct the magnifying lens to these intimate interactions.

Crossing species boundaries – the steps in progression

From a microbial perspective, a number of hurdles must be successfully negotiated as an agent moves from one species to another. According to traditional infectious disease paradigms, there are at least six essential steps in order for a microorganism to cause disease – attachment, local spread, multiplication, evasion of host defence, shedding, and ability to cause damage (16). However, today, when we are dealing with emerging diseases and the propensity for crossing species boundaries, an additional factor needs to be added at the outset, and that is exposure of the microbe to a new host, making seven critical steps, as displayed in Table I. Each of these stages will be considered below.

Exposure of host to a new microbe

Global trade has guaranteed that new populations of animals will have contact with each other. The advent of the World Trade Organization, in concert with and partially responsible for booming economies in many regions, has resulted in a massive surge in international trade. Some of this trade is in animals. In 2004, figures for export of live agricultural animals from all countries in the world include 37 million head of livestock (cattle, buffalo, sheep, goats, pigs, horses, camels and donkeys), and 818 million chickens, ducks, geese, and turkeys (10). This is a monumental load of travelling fur and feathers, each individual animal potentially hosting literally trillions of commensal microbes.

The international transport tapestry prominently features humans as well. Data from the United States Bureau of Transportation Statistics chronicles

Table I

Steps in the sequence of events as a novel microbe enters a new host

Step	Phase of infection	Phenomenon
1	Exposure	Geographic proximity to a new host
2	Infection	Attachment and entry into the body
3	Local spread	Establishment at initial site
4	Successful colonising	Multiplication or replication
5	Evasion of host defence	Evade phagocytic and host immune responses, at least temporarily
6	Shedding (exit) from body	Transmission – leave body from a place and in the right numbers to allow spread
7	Cause damage in host	Sufficient compromise of bodily function to be recognisable as clinical disease

that in 2004 there were 54 million people entering the United States by air and at land border crossings, there were 49 million pedestrians entering the country, 133 million passenger vehicles and 11.4 million trucks (25). If we underestimate that each passenger vehicle and each truck has only a single occupant, then the total number of people entering the United States from another country each day is a minimum of 802 000, certainly many more than could be adequately screened for the possibility of carrying animals or animal products that undoubtedly harbour microorganisms. People will travel with their pets, they may carry infectious agents on their clothing, or they will transport animal products. Any and all of these can introduce microbes to new areas. One very bizarre but memorable example of illicit international transport was the confiscation of 345 kg of bologna at the US/Mexico border in November 2003, when rolls of the lunch meat were discovered as a poorly-disguised back seat in an extended-cab pickup truck (6). A recent report of an individual attempting to board an international United Nations flight with a dead chicken in a plastic bag sparked considerable concern about the potential transport of HPAI across country borders, prompting new regulations for screening that include mandatory examination for dead birds within carry-on baggage (J. Sarn, USAID, Kabul, Afghanistan, personal communication).

It is a well-known fact that agricultural animals, dead or alive, as well as humans, are continually crossing borders and heading to new areas. What about wildlife? International wildlife trade is almost impossible to quantify, largely because much of it is done illegally. Some estimates include 4 million live birds, 640 000 live reptiles, 350 million live tropical fish and at least 40 000 live primates that are exported from one country to another each year (13). This means that there is exposure of millions of potential new hosts to the trillions of commensals from all these wild-caught animals. Then there are the domestically raised wild animals, such as those that might be raised for food or the pet trade. For instance, in Costa Rica, approximately 10 000 green iguanas are harvested every year from ranches for food and the pet trade (5). In Brazil, tons of capybara (*Hydrochoerus hydrochaeris*) meat move from rural areas to urban centres for sale as food. Also in Brazil, at least 43 turtle farms produce hundreds of thousands of animals in captivity for shipment to meat markets in cities within the country and beyond (5). The United States imports almost 2 million reptiles per year from more than ten countries on three continents. Many of these imported reptiles are lizards destined for the pet trade. Surprisingly, though, the United States is a net reptile exporter, with approximately 10 million red-eared slider turtles (*Trachemys scripta elegans*) exported to countries in Asia and Europe for use as food (26). Non-domesticated animals and their products are moving around the world at an unprecedented rate. Each introduction carries with it the potential for a microbe to become exposed to a host in a new geographic area, always with the possibility of engendering disease.

There are also other non-trade situations within the industrialised world that foreshadow the emergence of new disease. Modern agriculture has focused on intensive production. For the most part, this means concentrations of single species of animals reared under conditions that maximise productivity and efficiency of operations. As these operations have become financially successful, many of the smaller farming situations have decreased correspondingly. The backyard type of family farm with small numbers of a variety of species is now much less common, and so it would seem intuitive that emerging disease threats involving animal agriculture would be reduced due to decreased cross-species contact. However, some of the intensive industries have moved

beyond single-species husbandry to conditions that allow for utilisation of one species to augment production of another. Although many of these innovations are laudable for their efficiency and utilisation of all available components, there is concern about infectious agents from one species crossing over into another. In parts of Asia, pigs or chickens are reared on grates suspended over fish ponds, allowing the nutrients from the faecal material to supply nutrition to the fish. In North America, chicken litter is spread onto pastures to improve the quality of grasses eaten by cattle.

Mixing of species has become common in slightly less intensive husbandry situations as well. Of 13 000 game farms in South Africa, 4 000 integrate cattle with the game animals (5). The rearing of African ostriches in barnyards and pastures in North America, South America and parts of Europe demonstrates how commercial operations can bring non-native species into contact with indigenous species. In the Pantanal region of Brazil, ecotourism and cattle ranching exist side by side and both efforts are increasing. So, capybara are routinely exposed to all the flora of their bovine neighbours.

However, it is not just commercial food production that is resulting in the mixing of species. Iguanas from Central America can be found sharing living rooms with cats and dogs all over the world. Gambian giant rats mix with North American prairie dogs. The pocket pet trade is thriving, with degus (*Octodon degus*) from South America, sugar gliders (*Petaurus breviceps*) from Australasia and hedgehogs from Africa sharing homes with animal-loving humans in the wealthier nations of the world. 'Pocket pets', the term given to these small, non-domesticated, often foreign, often furry critters, can include snakes as well. The West African country of Togo exports 50 000 royal pythons per year, destined for pets (5). In Japan alone, the value of the exotic pet business is estimated at US$7.8 billion (2). With values such as this, it is easy to understand how trafficking of modern-day alternatives to the traditional Fluffy and Fido will continue.

Zoological collections also provide ample opportunities for microbes to move between ecologically distinct species. There are over 1 200 professionally managed zoos and aquaria throughout the world. Inherent in the pledge to familiarise the general public with animal life in far-flung areas of the world is the potential, no, the probability, of mixing microbes from different regions. For instance, African elephants (*Loxodonta africana*) carry an endotheliotropic herpesvirus subclinically. When this virus finds its way into young Asian elephants (*Elephas maximus*), fatal disseminated haemorrhagic disease can ensue (11). In the absence of aeroplanes and cross-country trucks, these two pachyderm species would never have co-mingled. In a zoological collection, there can be ample opportunity for a virus from one continent to make the very short trip to the adjacent paddock and infect an unsuspecting species from another continent. Some zoological collections have recognised this and raise their own replacement animals purchased from other collections within the same country and quarantine all new animals.

Exposure to new hosts has expanded exponentially with global travel and trade. Perhaps no-one benefits more from globalisation than infectious agents. The burgeoning and mobile human population, with its desires for production of adequate animal dietary protein, economic success, and curiosity for observing or living with unusual species, have all created recurring scenarios of geographic proximity allowing microbes from one species to make novel forays into new hosts.

Entry into the body

Simply putting two species into geographic proximity does not dictate that microbes might

abandon one host for another. The next essential step in disease causation is that the infectious agent from one species needs to make intimate contact with the other at a portal of entry.

Agents that are commonly present at normal body orifices are, of course, the ones that most easily find their way to portals of entry in the recipient host. Examples abound. Nipah virus-laden urine of fruit bats dripping into feed troughs on swine farms in Malaysia resulted in an emerging disease of colossal proportions. Ascarid egg-containing raccoon faeces deposited in playgrounds wind up in the mouths of toddlers, events which have caused untold misery for affected children and their families, due to the very virulent form of visceral larva migrans that is characteristic of this raccoon parasite.

There are some organisms that will remain permanently separated because of the ecological niche that they occupy, or because of the respective behaviours of the two species. The spongiform encephalopathies are instructive in this regard. For instance, bovine spongiform encephalopathy (BSE) will not spread from one infected cow to another simply by virtue of two bovines sharing a common pasture or feed bunk. Neither will it emerge in humans by simple geographic proximity of a teenager to an affected cow. The BSE prions are sequestered within the nervous tissue and unless and until this neurological tissue of the affected animal is ingested by a new host, there will be no establishment in the new host. However, chronic wasting disease of deer, another prion-caused entity, offers a different scenario. In this case, the prions are shed, perhaps in saliva, perhaps in faeces, and hence become available to other species in close proximity.

With vector-borne diseases, the two host animals may be in very close contact, but the feeding pattern of the vector (i.e., on one species as opposed to another) might just dictate whether or not the microbe will come into contact with a new species. On the other hand, the only capable vector might be geographically restricted. This is why the African animal trypanosomoses are not of great concern outside of the host range of the tsetse fly insect vector. Although *Trypanosoma brucei* and *T. congolense* are very debilitating diseases for African livestock, the possibility for these protozoan parasites to move to a new area and possibly infect ecologically distinct species is minimal because effective spread and maintenance requires the efforts of the tsetse fly.

Local spread (applies predominantly to extracellular organisms)

At this point, the two hosts have made contact with each other, e.g. the geographic proximity issue has been achieved, and the microbe from one has been allowed access to a novel host. The next step in the progression is if the microbe can survive at this portal of entry. Here, there is a definite distinction between intracellular and extracellular organisms. For intracellular organisms, most notably viruses, to survive, they must have access to the machinery of the host cell. This means they have to gain entry to the intracellular milieu and, because for viruses this is so closely tied to replication and survival, many of these mechanisms are covered in the next section ('Multiplication').

In general, bacteria are much less host-specific than viruses, because there is no need for specificity of all cellular machinery for reproduction. For some bacteria, requirements are not very host-restricted at all. For instance, *Salmonella enterica* var Typhimurium has an extremely wide host range and can cause disease in any number of animal host species. In contrast, two other salmonellae, *S. typhi* and *S. choleraesuis*, are notably species-specific, for humans and swine, respectively. What needs to be elucidated are the exact factors that make an extracellular organism specific for one species, or that allow for adequate growth in

one host and not another. For some organisms we have a good grasp of the factors that might be responsible for survival in a novel host and some of these are detailed below.

Capnocytophaga canimorsus is a fastidious Gram-negative bacteria found in the oral cavity of 15-25% of all dogs and cats. It causes no problems for the carnivore but may cause massive septicaemia and death when inoculated, usually via a bite, into humans with defined susceptibilities, which include asplenia, alcoholism, or tobacco smoking. The common thread in these three conditions is higher than normal levels of circulating iron. So we understand that cross-species transfer of this organism only occurs when local conditions of iron availability are present (28).

Brucella abortus causes disease in both cattle and humans, although the disease has a very different clinical presentation in the two species. It is well known that *B. abortus* is a prominent cause of abortion in cattle, however, when *B. abortus* infects humans, abortion is not part of the repertoire of this organism. Studies have shown that bovine foetal erythritol is a strong growth stimulant for *B. abortus* and this substance is present in abundance within the bovine uterine environment, allowing the organism to thrive and cause severe damage to the tissues that comprise the placenta. In contrast, this substance is not found in placental tissues of humans, explaining the specificity of one clinical aspect of the infection (21).

Baylisascaris procyonis, the ascarid of raccoons, demonstrates how a eukaryotic organism may behave in a novel host. The eggs of this roundworm, when they find their way into a human host, migrate much more widely than in the raccoon host, finding the milieu of the brain especially appealing. This malignant form of visceral larva migrans is proving to be an especially horrific emerging disease in children. Understanding the reasons for extensive migration in a novel host is urgently needed.

However, it is not just the extracellular milieu that determines if a bacterium will be able to gain a foothold. The understanding of the adhesion and invasion molecules that allow pathogenic bacteria to exploit various niches will provide great insight. Major advances have been made concerning pili, fimbriae and invasions, and new adhesive structures continue to be described (18).

Multiplication (with focus on intracellular organisms)

For obligate intracellular organisms, which require using host cell machinery in order to survive and multiply, the possibilities for successful infection become much more limited, as entry into a host cell often requires almost a lock-and-key kind of mechanism. The virus-host cell membrane interaction is a field experiencing rapid discovery, with daily delineation and definition of various binding factors. It is also true that we are discovering that viruses often use more than one receptor and also that the receptors accept more than one virus, or as eloquently stated by Baranowski *et al.* (3), there is 'a receptor for several viruses and a virus for several receptors'. So the lock-and-key analogy might be more accurately imagined as the extensive collection of door-opening devices hanging from the belt of a custodian responsible for several buildings.

In general, cellular receptors for virus adhesion and/or attachment fall into two broad biochemical categories – proteins and carbohydrates. The protein receptors tend to be somewhat more host-restricted than the carbohydrate variety.

Quite a few protein receptors for specific diseases have been well identified. Some of the more definitively characterised receptors include CD4 for HIV-1, intercellular adhesion molecule (ICAM)-1 for numerous rhinoviruses and CD46 for measles (33). The species specificity of the receptor in turn may define the range of animals that can be infected. For instance, CD46 is similar enough among most primates that measles can easily pass among

primate species. In contrast, the measles virus cannot infect cells from more evolutionarily distant species, such as the dog or cat, because the similarity of the receptors is not sufficient to allow viral binding.

Viruses that use carbohydrates for binding to a cell are often much less species- or group-specific and therefore tend to pose a greater risk for crossing species boundaries. The carbohydrates are usually components of cell surface glycoproteins or glycolipids and can be found on cells over a wide host range. The influenza A viruses and canine parvovirus, both examples of viruses that have had their share of spotlight time as emerging diseases, bind to sialic acid-containing oligosaccharides and represent prominent models for this category.

Influenza A, in particular, has become a sort of poster child for crossing species boundaries to generate global public health panic. Two recent studies highlighted the importance of receptors for HPAI H5N1, elucidating the nature of cross-species interaction (23, 27). It is known that the avian influenza viruses use sialic acid linked to galactose by an alpha-2,3 linkage and this receptor is widely present in birds. In comparison, the human influenza viruses bind preferentially to a sialic acid linked to galactose by an alpha-2,6 linkage and this receptor is present in the respiratory tract from the nose to the lungs in humans. The alpha-2,3 sialic acid receptor was not thought to be present in humans. However, it is now known that cells lining the alveoli of the human lung are susceptible to H5N1 because they possess the alpha-2,3 sialic acid receptor. This explains why the number of humans infected with H5N1 is relatively low, as successful replication requires that the virus make its way deep into the respiratory tract to the alveolar structures. It also explains why the virus is not spread readily from human to human.

Following binding, there is a linked series of events that consists of viral uptake, uncoating, replication, transcription, translation, assembly and release. In general, less is known about the host-specificity of these events than is known about binding.

Evasion of host defence

In most circumstances, a microbe that finds itself within a new host sooner or later will be challenged by the host defence system. A concerted and specific attack on the new microbe (what we know as the immune response), is designed to halt the invader and keep it from going any further to inflict damage.

In some cases, technological innovations have abrogated the role of the immune response and greatly facilitated cross-species transfer of microbes and resultant emergence of disease. The following is one example: several years ago, there were reports of pregnant dogs dying subsequent to vaccination with a certain vaccine product. Viral isolation from tissues of the affected dogs revealed that they were infected with a strain of bluetongue virus, an agent previously known for infecting ruminant species only (30). Close examination of the vaccine in question determined that there was a strain of bluetongue that had been incorporated into the vaccine which was used to protect dogs against several (unrelated) agents of disease. Subsequent animal infection experiments with dogs showed that this particular strain of bluetongue could infect and kill dogs, fulfilling Koch's postulates (4). This bluetongue virus was probably a contaminant in the foetal bovine serum included in the media used to bathe the canine cell cultures in which the vaccine stocks were grown. It is likely that the bluetongue virus, permitted such close, prolonged and unfettered *in vitro* proximity to canine cells, was able to invade and adapt to the cells, becoming a canine pathogen as well as a ruminant pathogen. A similar event may not have been possible *in vivo* because the host response

would have diligently dislodged the invader prior to its establishment.

Xenotransplantation is installing organs from one species into another. The demand for human organs far exceeds the supply and in an effort to respond to this demand, several biotechnology companies have investigated the use of alternate species, specifically pigs, as a potential supply of hearts and kidneys for human use. As one of the major factors in cross-species organ rejection is the acute response, which is driven in large part by the complement reaction, lines of pigs genetically modified to contain human complement receptors were developed. Specifically, swine with human decay accelerating factor (DAF)/CD55 and monocyte chemoattractant protein (MCP)/CD46 were created (22). Organs from these pigs could theoretically be transplanted into humans and the rejection response effectively controlled. However, this xenotransplantation procedure involves placing organs from one species into very intimate contact with body fluids of another. The same receptors that were cloned into the pigs were also the same receptors for some diseases of humans – measles for CD46; echoviruses and Coxsackie B viruses for CD55. Questions arose. Would the genetically modified pigs then be susceptible to measles? If Coxsackie B virus entered the body of the pig, could it create a new disease that might then subsequently spread among swine? There was also discussion regarding heart-lung transplants, which generated fascinating and frightening queries about disease transfer in the other direction. The respiratory tract is the portal of entry for many viruses. Would a human being with a pig-origin respiratory tract then be susceptible to infection with foot and mouth disease, swine influenza, or pseudorabies? Would one of those viruses, allowed such red carpet access to the inner sanctum of a *Homo sapiens*, then become a human disease? Once the genie is out of the bottle, it might be exceedingly difficult to coax it back in. Fortunately or unfortunately, these questions became academic as the existence of porcine endogenous retrovirus was described. As the more or less ubiquitous presence of this retrovirus among swine was delineated, the momentum for xenotransplantation lagged considerably because the public health implications of placing a novel retrovirus within the human population presented an insurmountable conundrum.

Shedding

An essential, often overlooked, step in disease causation is that the organism must be released from the body. For a disease to be established, there must be dissemination to new hosts. If there is disease, but no shedding of the organism to affect others, the initial case dead-ends.

The most time-tested means of dissemination are those in which there is shedding from body surfaces. Saliva, skin, respiratory secretions and diarrhoea are all evolutionarily validated methods for transfer of disease. If a novel host happens to be in the vicinity of any of these secretions or excretions, transfer is facilitated.

Some patterns of shedding can be augmented by behavioural, environmental or technological factors. In these cases, the dissemination of smaller numbers of organisms can be facilitated. For instance, members of the camelid family have a proclivity for spitting which makes expulsion of microbes from the respiratory system or oral cavity much more efficient. Environmental factors can facilitate or prolong the viability of organisms that might be shed in small numbers. For instance, organisms that can survive in water can find their way to numerous new hosts even if shed in relatively low numbers. Leptospirosis has moved to novel species very effectively in this manner. Microbes released into milk provide another example. The shedding may not be in great quantities, but the efficiency of mixing in a bulk tank and bottling

for cross-country and massive distribution ensures that the shed organisms might have an enhanced chance of finding novel hosts.

Some organisms are not shed naturally but conditions exist for effective dissemination. Blood-borne microbes in bush meat, although perhaps not shed in significant numbers through any orifices in the ante-mortem state of the host, once exposed on the butcher's block are available in the environment for novel hosts. Many of the Ebola outbreaks have begun in this manner.

Will there be disease?

The last but by no means least step in the progression of the microbe-host encounter is whether or not disease will result. Here it is important to remember that the pathogen of one organism can be the symbiont of another, and vice versa (32). Those of us who specialise in infectious diseases tend to forget that the vast majority of microbes with which we share our bodies are not pathogenic but rather live in a quiescent, perhaps even a beneficial way, within or on us. However it is also important to remember that there are many instances when commensals can become pathogens in an immunocompromised individual. There are many cases when pathogens are carried but do not cause disease, such as *Helicobacter* carriage in a high percentage of humans and *Pasteurella multocida* in healthy cattle (9). So the situation is really very complex.

It is generally accepted that microorganisms far outnumber any eukaryotic populations on the earth. They are undoubtedly the 'unseen majority' in almost every ecosystem, including the human body (29). The approximate total number of bacteria that humans have on their skin is 10^{12}, in the mouth 10^{10} and in the gut a whopping 10^{14}. In comparison, the total number of human cells in the average person is 10^{13} (16). We are very much outnumbered by our prokaryotes and it is a big party that we have with them every day. Even the term 'commensal' is instructive, coming from the Latin and meaning 'at the same table'. These are the organisms with which we share all our meals.

Microbes comprise nearly half of all biomass on earth (15). Similarly, the diversity of that microbial life is only just beginning to be understood. Wilson declared that studying microbial diversity was futile because it is 'beyond practical calculation' (31). Yikes.

Recent advances have allowed scientists to determine the microbial diversity that might be present in various niches, many of them previously thought to have been well-characterised with respect to their organismal components. Using small subunit ribosomal RNA gene sequence (SSU rDNA) analysis, it is possible to survey bacterial populations, identify dominant members of a population and discover new species. In addition, with DNA microarray technology, using thousands of rDNA sequences, high-level overviews of microbial community compositions can be undertaken, with the possibility of determining species that are present at concentrations less than 1%. In a recent study of 359 bacterial phylotypes identified from human intestine, only 244 (62%) are organisms previously unidentified. Furthermore, 80% of the sequences were from organisms that have never been cultivated (8).

In a related study, preliminary results on samples of human colon contents from six different sites indicate that populations differ significantly from individual to individual but that there is remarkable similarity from one site to another within the same person (17). Could this mean that there is a 'signature' bacterial composition for each person? Probably. Alterations in intestinal flora are associated with some diseases that are presumed to be non-infectious, including autism and ankylosing spondylitis. Is it possible that there are some as yet unidentified pathogens associated with these diseases? Undoubtedly. As commensals

from one host become established in another, that event might foretell the initiation of some chronic disease that was previously thought to be non-infectious.

Moving outside the well-studied microbial community of the human intestine to more external environments, including soil and sea water, less than 0.4% of the bacteria identified by the 16srDNA techniques are organisms with which we are familiar and/or that can be cultivated. Some authors speculate that, overall, less than 1% of naturally occurring microorganisms can be cultivated by standard techniques and that the vast majority of the organisms present in natural environments, such as sea water or soil, are not yet identified (1, 20).

In short, we have only just barely perceived the tip of the proverbial iceberg that represents all the bacterial life that is teeming around and within us. Then there are the viruses. Much has been written about RNA viruses and a precautionary comment is necessary here. It is well established that because they lack a proofreading function, RNA viruses have a mutation rate per genome replication that is 300 times higher than the DNA-based replication system (7). Thus, there is a 'genetic lottery' of virus mutations that are inevitably and unpredictably generated as RNA viruses replicate (3). The result has been likened to typing on a keyboard without the benefit of using the backspace key. If mistakes cannot be corrected as they are generated, the end result could be a message that is very different in meaning from the original. In considering cellular receptor specificities, the concept of lack of fidelity in replication becomes somewhat alarming and so for RNA viruses, the concept of species specificity may be very fluid indeed. With each virus replicating to become a kind of mutant viral swarm, it is understandable how novel surface proteins that can utilise alternate cellular receptors could be selected and incorporated into populations, making infection of new species possible. Examples abound of viruses that are quiescent in one species but when transferred to a new host create clinical disease – HIVs in sooty mangabeys, chimpanzees and humans; Nipah or Hendra viruses in bats and people; HPAI viruses in waterfowl and poultry; and SARS-associated coronavirus (CoV) in civet cats and humans. What will be next?

Conclusion

There is no doubt that we will continue to see emerging animal diseases. We are at great risk of agents moving from one species to infect novel hosts. What will determine whether or not there will be a 'take' in the new host and result in massive morbidity, mortality and disruption? Over the last few years, we have moved remarkably fast in developing surveillance and detection systems to monitor the potential emergence of a new disease. Perhaps now it is time to take the technology to the next most focused area. We need to turn on the high power lens of the microscope and examine the exact reasons that microbes can become successful in a new host. We need to study the factors in the seven-stage sequence of infectious disease causation and to consider the journey from the viewpoint of the infecting agent.

One is reminded of the science fiction movie *Fantastic Voyage* released in 1966 (12). This cinematic feature took the viewer on a journey through the human body. A miniaturising process has been perfected and a tiny submarine, 'Proteus' with several scientists is sent into the body of a high level free-world scientist in order to dissolve the blood clot in his brain suffered as a result of an assassination attempt by Communist forces. As this very tiny aquatic vessel courses through all parts of the body, the passengers marvel at microscopic features and events – the surface of a red blood cell, the cavern-like grandeur of the

heart ventricles, an attack by marauding leukocytes and a shock-filled jolt at the crossing of the blood-brain barrier. As an analogy, perhaps we are now due for similar cerebral machinations regarding a microbe's trip through the seven stages of disease causation. Will there be exposure? What happens at the portal of entry? Is multiplication possible given the resources and receptors present in the new host? Can the host defences be evaded? Will the microbe be successful enough to have offspring that have an exit strategy? And, last but not least, will there be enough damage from the invader that the host becomes clinically ill? Only when we begin to examine cross-species transfer in this kaleidoscope of multiple-phase and microscopic events can we adequately evaluate the true risks of any new disease emerging.

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