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The Leeuwenhoek Lecture 2001. Animal origins of human infectious disease

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Since time immemorial animals have been a major source of human infectious disease. Certain infections like rabies are recognized as zoonoses caused in each case by direct animal-to-human transmission. Others like measles became independently sustained with the human population so that the causative virus has diverged from its animal progenitor. Recent examples of direct zoonoses are variant Creutzfeldt–Jakob disease arising from bovine spongiform encephalopathy, and the H5N1 avian influenza outbreak in Hong Kong. Epidemics of recent animal origin are the 1918–1919 influenza pandemic, and acquired immune deficiency syndrome caused by human immunodeficiency virus (HIV). Some retroviruses jump into and out of the chromosomal DNA of the host germline, so that they oscillate between being inherited Mendelian traits or infectious agents in different species. Will new procedures like animal-to-human transplants unleash further infections? Do microbes become more virulent upon cross-species transfer? Are animal microbes a threat as biological weapons? Will the vast reservoir of immunodeficient hosts due to the HIV pandemic provide conditions permissive for sporadic zoonoses to take off as human-to-human transmissible diseases? Do human infections now pose a threat to endangered primates? These questions are addressed in this lecture.

Keywords: microbes; zoonosis; epidemics; HIV–AIDS; BSE; vCreutzfeldt–Jakob disease

1. INTRODUCTION

> 'If there is any conceivable way a germ can travel from one species to another, some microbe will find it'
> (McNeill 1976).

Throughout evolution and history, humans have changed their lifestyle in ways that infectious microbes have readily exploited (Lederberg 2000). The emergence of particular diseases, like Legionnaires' or bovine spongiform encephalopathy (BSE) and variant Creutzfeldt–Jakob disease (vCJD), may be novel but the phenomenon is not. Many of mankind's social customs and taboos have had their roots in disease prevention. Pigs were domesticated over 8000 years ago separately in Europe/West Asia and in the Far East, but pork became a forbidden meat across most of southern Asia and the Middle East. Was it the tapeworms they harbour, or deadly microbes that led to this dietary prohibition among Hindus, Muslims and Jews? In Mongolia, hunters and fur-trappers were traditionally quarantined for several days apart from their community on returning from their expeditions. Whether this was to protect against the plague or anthrax is not known, but it almost certainly helped to prevent infectious disease emanating from their prey.

Epidemics or episodes of pestilence are recorded in ancient legends, though it is difficult to identify the actual diseases so tellingly portrayed by the Hebrews in exile in Egypt, Oedipus's Thebes, the epic of Gilgamesh or the Mahabharata. While divine displeasure was seen as the precipitating factor, the notion of contagion was acknowledged (Ranger & Slack 1992), including animal-to-human transmission. A Babylonian edict imposed fines if a rabid dog attacked man or slave (Oldstone 1998); early Tibetan society also had a law on dog bites (Richardson & Aris 1998).

In *The coming plague*, Garrett (1995) warned of further outbreaks of infectious disease and pestilence to be expected as we alter our environment and lifestyle. Her new book, *Betrayal of trust* analyses its acceleration as the contrast between the developed world and impoverished nations diverges further, with a falling life span and increased disease in parts of Africa and the former Soviet Union (Garrett 2000). Karlen (1995) uses historical epidemics to warn about those to come. Should we regard these premonitions as apocalyptic, millennial journalism, or as serious threats? I think the latter, although we should recall that humans have continually changed the dynamics of environment and disease. Our 21st-century global village presents a wonderful opportunity should a novel pathogen cross from animals to adapt to human-to-human transmission. Pandemic influenza and human immunodeficiency virus (HIV) are two examples from the last century. While we cannot predict the nature of the next epidemic, we can be confident that it will happen.

2. MICROBES AND HUMAN DISEASE

(a) *Antoni van Leeuwenhoek*

Antoni van Leeuwenhoek is the founder of microbiology for his discoveries in the late 17th century. He was the first investigator to describe living bacteria, protozoa and other 'tiny animalcules' (van Leeuwenhoek 1677). Van Leeuwenhoek was not a typical 17th-century academic or physician; rather he ran a draper's shop in Delft (Dobell 1932; Friedman & Friedland 1998). For a hobby he ground lenses to construct simple microscopes and then used them to observe and analyse the 'microbes' revealed under the lens. After Robert Hooke had confirmed van Leeuwenhoek's 1677 claim of myriad life forms in a drop of water van Leeuwenhoek was elected a Fellow of The Royal Society (FRS) in 1680 at the age of 48. Thereafter, until he died at the age of 91, he regularly sent letters for publication in the *Philosophical Transactions of The Royal Society* in Dutch, for this poorly educated haberdasher knew little Latin, French or English. More than 100 of his letters were duly edited and translated into English or Latin by the Society and he remains to this day the most prolific author of articles in this journal. He bequeathed his collection of home-made microscopes to the Society, and in the frontispiece to his 1693 text, *Ontledingen en ontdekkwegen* (*Dissection and discovery*), he describes himself 'lid van de Koninglyke Societeyt tot Londen' (member of The Royal Society in London). Clearly van Leeuwenhoek valued his FRS status, and it is a special honour to deliver the Society's Leeuwenhoek Lecture.

Van Leeuwenhoek's microscopes were fine instruments with superb lenses although they were not more advanced in construction than others of his time. His genius lay in using them not merely to magnify what could just be seen by the naked eye, but to reveal the invisible. Thus he was the first to depict red blood 'corpuscles' moving through capillary vessels and to demonstrate that his own semen contained millions of actively swimming 'homunculi'. When he sampled his mouth, he described what would now be called a complex biofilm of bacteria, observing that saliva was sterile, but that the scum on his front teeth was teeming with a variety of microbes (figure 1). In the same study he noted heat sterilization because living microbes were no longer apparent if he looked immediately after sipping his scalding morning coffee, although they could still be found on his back teeth (van Leeuwenhoek 1684).

Van Leeuwenhoek had a glimmering of the association between microbes and disease on discovering the protozoan parasite *Giardia lamblia* in his stool when he suffered persistent diarrhoea in 1681. But a general microbial theory of transmissible disease causation awaited the investigation of those famous French and Prussian rivals, Louis Pasteur and Robert Koch (Brock 1975). Koch's invention of cloning helped him to establish his famous postulates on specific microbes causing distinct diseases (Kellam & Weiss 2001). Yet 170 years before the great debate over spontaneous generation versus the germ theory of putrefaction and disease was firmly resolved in favour of germs (Baldacci *et al*. 1981), van Leeuwenhoek (1702) wrote scathingly:

> 'Seeing these wondrous dispensations of Nature whereby these "little animals" are created so that they may live and continue their kind, our thoughts must be abashed and we ask ourselves, can there even now be people who still hang on to the ancient belief that living creatures are generated out of corruption?'

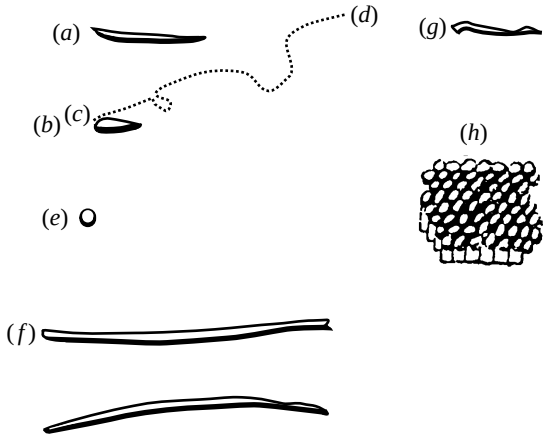

Figure 1. Bacteria drawn by Antoni van Leeuwenhoek taken from the plaque on his teeth. They include bacilli (*a*,*b*,*f*), including mobility (*c*,*d*), cocci (*e*, *h*) and a spirochaete (*g*). (Reproduced from van Leeuwenhoek 1684.)

(b) *How microbes continue their kind*

It thus seems clear that van Leeuwenhoek had deduced that microbes were living, varied in nature, and that each was able to breed true to its own kind. Van Leeuwenhoek could not, however, have discovered the most minute microbes, the viruses, because being smaller than the wavelength of visible light, even the most powerful light microscope would not reveal them. With the development of the electron microscope, viruses were visualized (e.g. HIV, figure 2*a*,*b*) and recently my colleagues (Pizzato *et al*. 1999) used confocal fluorescence microscopy to identify individual virus particles (figure 2*c*–*e*).

Today we know that bacteria 'continue their kind', like animals and plants, through replication of their genetic material, DNA. The central dogma of molecular biology is that DNA makes RNA and RNA makes protein, through a genetic code universal to all life forms (figure 3*a*). But unlike all other 'living' organisms, many viruses carry their genetic information as RNA (figure 3*b*). Viruses are parasites that can only propagate inside living cells—of bacteria, plants and animals. To do so, they use the metabolic machinery of their host, and those with an RNA genome encode special enzymes for RNA replication. However, retroviruses like HIV make a DNA copy of their viral RNA (figure 3*c*).

We now know of a form of 'self-propagating' disease that does not appear to carry any genetic material of its own. These are the prion diseases or transmissible spongiform encephalopathies, of which CJD and BSE are the most notorious. The prion protein is encoded by a host gene, and is made in largest amounts in the central nervous system. So normal prions are present in our brains all the time. The infectious agent is a prion protein with a changed shape, but one that is able over a long period of time somehow to induce native prions to adopt the new conformation (figure 3*d*). Thus the infection is a

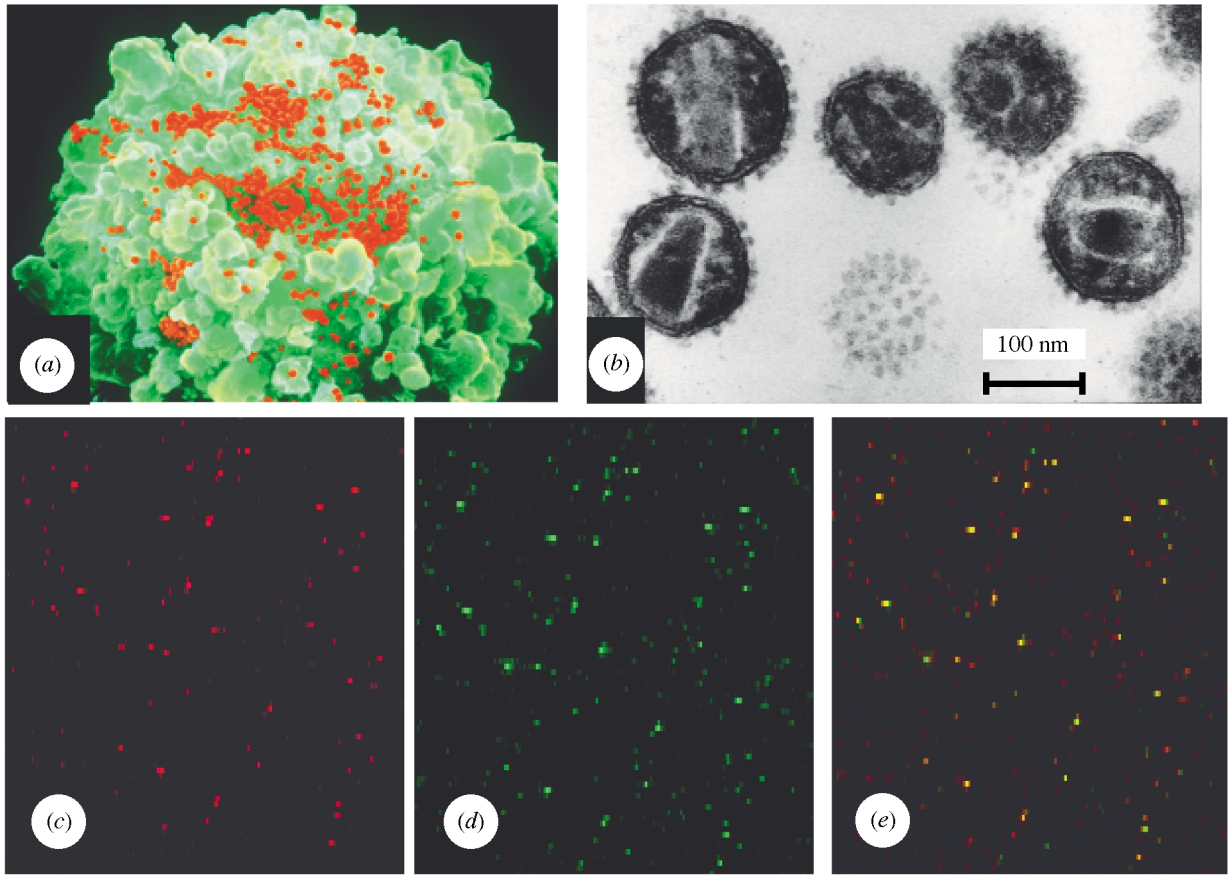

Figure 2. Visualization of HIV-1 particles (virions). (*a*) Scanning electron micrograph of a lymphocyte (green) releasing virions (red), kindly provided by D. Hockley, National Institute of Biological Standards and Control, Potters Bar, UK. (*b*) Transmission electron micrograph of virions showing envelope glycoprotein knobs on the surface and cone-shaped cores inside, kindly provided by H. Gelderblom, Robert Koch Institute, Berlin, Germany. Bottom row, HIV particles detected by confocal fluorescence microscopy, kindly provided by A. Fassati, Wohl Virion Centre, University College London, UK. Purified HIV-1 was spread onto plastic coverslips, fixed and permeabilized with 0.2% triton-X. Virions were then labelled with anti-Vpr antibody (*c*) and the fluorescent RNA dye TOTO-1 (*d*). Co-localization of Vpr and RNA is shown in (*e*).

kind of chain-reaction of protein remodelling, with ultimately fatal consequences to the infected host (Weissmann 1991).

(c) *Origin of human infections*

Given that infectious agents continue their own kind, what are the origins of human microbial pathogens? Have they coevolved with human emergence from our primate ancestors, or have they colonized us by crossing over from other host species? I think that the answer is by either route according to the specific microbe. Moreover, changing human lifestyles may encourage free-living microbes to infect humans, e.g. Legionnaires' disease where the bacterium comes from warm, aerated water in cooling systems, air conditioning and jacuzzis that can be regarded as large artificial lungs.

There is, of course, little fossil evidence for microbes, and attempts to isolate and amplify ancient microbial DNA have only been partially successful. Nonetheless, we can surmise much about the evolution of pathogens (disease-causing microbes) by comparing genetic sequences between those infecting different hosts and by analysing the genetic diversity of pathogens among human populations. History, geography and demography also come to our aid, and some of the most illuminating social histories of humankind have been written from the perspective of infectious disease (Diamond 1997; Karlen 1995; McNeill 1976; Oldstone 1998).

Early human populations were small communities with only occasional contact with other human groups. With this population structure, those pathogens that cause acute disease, and that do not persist in their hosts, would have little chance of surviving and spreading (Black 1975). For an infectious agent to maintain itself in a specific host population it must have a basic reproductive rate greater than one (Anderson & May 1992; May *et al.*, this issue). Thus epidemic diseases that leave survivors immune from re-infection need a sufficient host population size to persist in that species by infecting new individuals. It therefore appears likely that such infectious diseases colonized human populations after we developed from small, close-knit, isolated hunter-gatherers to a settled agricultural life (Tudge 1998), or to a nomadic existence dependent on husbandry of large herds of animals. That means that epidemic diseases started very recently in the evolution of microbes or humans: 'For two million years we were hunters; for ten thousand years we were farmers' (Budiansky 1997).

Some virus and parasite infections, however, may well have coevolved with the human species. These microbes

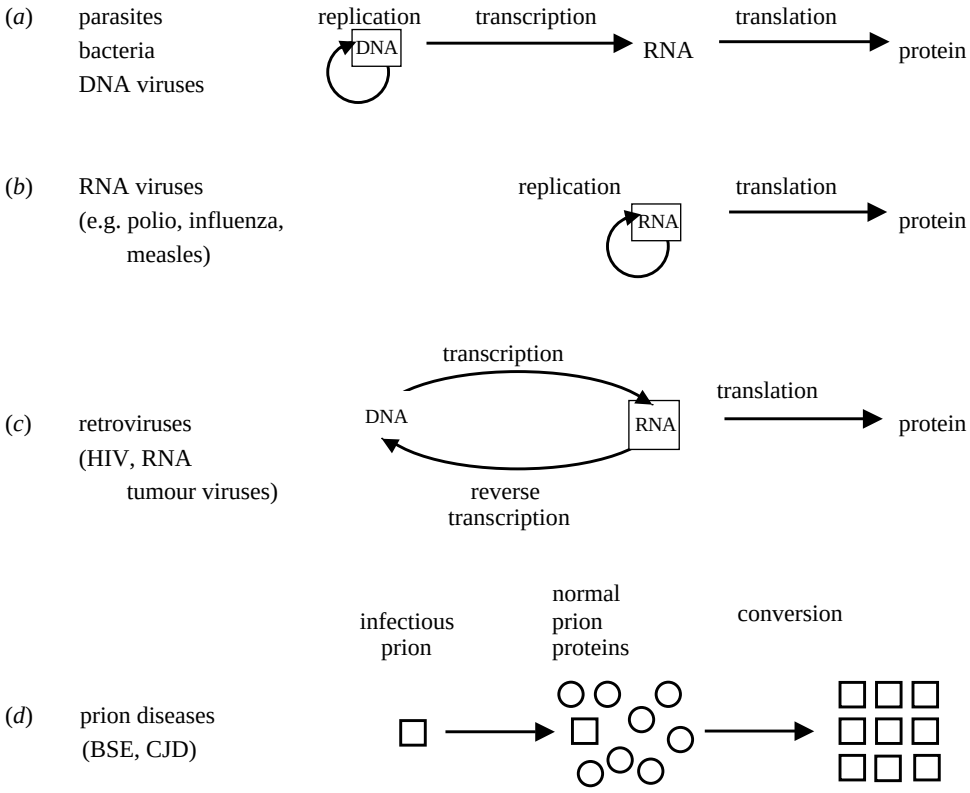

Figure 3. How different infections propagate. Boxes indicate genomes in infectious particles.

establish persistent infections that can live with their hosts throughout their life span. Such viruses include the herpesvirus family, in which the genetic sequences of individual herpesvirus species in general match those of the host species closest to us in evolution (Van Regenmortel *et al*. 2000). For instance, the γ-herpesvirus, Epstein–Barr virus, has phylogenetically related viruses in other primate species in which the relative genetic divergence between the viruses matches that of the host. Those viruses are often transmitted vertically from parents or grandparents to offspring as well as among siblings and thus can persist among small host populations. Other viruses fitting this pattern of host coevolution are the papovaviruses, including the JC virus (which causes progressive multifocal leukoencephalopathy) and human papilloma viruses like those that cause cancer of the uterine cervix.

Coevolving microbes typically exert a low mortality upon their hosts. They may cause mild disease, or severe disease in a small proportion of those infected, or cause disease from which the majority recovers, or which present late in the life span of the host. Thus herpes simplex type I causes cold sores (and type II genital sores), though on rare occasions, notably in immunosuppressed individuals, it can cause a lethal encephalitis. Varicella zoster virus causes chickenpox in infancy, from which most children recover. But like other herpesviruses, recovery from chickenpox does not mean elimination of the virus from the infected and now immune host. The virus remains latent in sensory nerve cells and can re-emerge decades later as shingles. And a grandmother with shingles can set off a new mini-epidemic of chickenpox among grandchildren and their contacts.

Table 1. *Infections shared with animals*

(Estimated from McNeill 1976 and Palmer *et al*. 1998.)

animal	number[a]
dog	65
cattle	50
sheep, goat	46
pig	42
horse	35
poultry	26
rats, mice	39
wild species	114
total	298

[a] Numbers overlap as some infections are shared among many species.

However, most human infections originally came from an animal source and have crossed the host species 'barrier' in recent times. Some continue to do so on sporadic occasions, a phenomenon known as zoonosis, while others may become established as human-to-human infections in which their animal origins have been obscured by time. I shall examine some examples.

3. ZOONOSES

There might appear to be an increasing number of 'new' zoonoses, but some of these are old infections although the microbes have been discovered recently, e.g.

Table 2. *Examples of recent zoonoses*

(DRC, Democratic Republic of Congo.)

microbe	reservoir	vector	location	year
hantavirus	rodent	none	SW USA	1996
			Argentina	1993
plague	rodent	flea	India	1993
VEE[a]	horse	mosquito	Mexico	1995
CCH[b]	sheep, hare	tick	Bulgaria	1994
typhus	rodent	louse	Bosnia	1996
			Burundi	1995
E. coli O157	cattle	food	Scotland	1996
Hendra virus	bat	none	Australia	1997
Ebola virus	rodent	none	Zaire (DRC)	2000
			Sudan/Uganda	1997
'flu H5N1	duck, chicken	none	Hong Kong	1997
Nipah virus	bat	pig	Malaysia	1999
'flu H9N2	chicken	none	Hong Kong	1999
monkeypox	monkeys	none	Zaire (DRC)	2000

[a]Venezuelan equine encephalitis virus.
[b] Crimean–Congo haemorrhagic fever virus.

Borna virus which is suspected of underlying some neuropsychiatric disorders (Hatalski *et al*. 1997). Animal-to-human infections can be classed in different categories, according to the pattern of infection.

(a) *Animals as reservoirs: occasional human infection*

Accidental human infections can come from environmental contamination such as tetanus and anthrax spores, and directly from animals. Palmer *et al*. (1998) list more than 70 families of bacteria, viruses and parasites that have their natural reservoir in animals but occasionally infect humans. Numerous infectious diseases are shared by humans and domestic or wild animals (table 1). Weil's disease, for example, is a life-threatening illness caused by the *Leptospira* bacterium harboured by rats and spread through urine-contaminated water. Psittacosis is a rickettsial disease spread by birds, especially parrots and budgerigars, but also town pigeons.

Within the last ten years several outbreaks of fatal disease emanating directly from animals have caused concern (table 2). Nipah-virus encephalitis caused over 100 deaths among pig farmers and abattoir workers in Malaysia and Singapore (Chua *et al*. 1999; Paton *et al*. 1999). The natural reservoir of this newly discovered paramyxovirus is fruit-bats and the initial infection of pigs probably occurred during the clearance of the tropical rainforest to build the new international airport for Kuala Lumpur, when the bats migrated to trees around farms. Nipah virus became a pig-to-pig infection but there is no evidence as yet of human-to-human transmission. The hantavirus haemorrhagic syndrome caused by 'sin nombre' virus appeared in south-west America during a population explosion of the deer mouse (*Peromyscus*). An outbreak of a related virus in Patagonia is suggestive of onward human transmission, perhaps a virus adapting to a new host (Wells *et al*. 1997).

The H5N1 outbreak of influenza in 1998 (Osterhaus, this issue) similarly came from an unnatural disposition of ducks and chickens in the Hong Kong markets. From the chickens H5N1 then infected 18 humans of whom six died; again there is no evidence of human-to-human transmission. H9N2 is a related avian virus in Hong Kong (Horimoto & Kawaoka 2001; Webster *et al*. 2000*a*). Another avian source of human disease is the outbreak of West Nile virus in New York in 1999 (Rappole *et al*. 2000). Migratory birds brought the virus to resident birds such as crows and mosquitoes were vectors to humans. This virus has not previously been recorded in North America. Recent food-borne epidemics of *Escherichia coli* O157:H7 and vCJD have their sources in butchers and processed offal, respectively.

Thus many of these recent and novel human diseases can be attributed to changing environmental, farming or food-processing practices. Other potentially emerging diseases of animal origin are those caused by Ebola, Marburg and Lassa fever viruses (Palmer *et al*. 1998). These cause acute haemorrhagic symptoms that lead to death or recovery within a few days or weeks (Zuckerman *et al*. 2000). In these cases, human-to-human infection is evident, especially among family and health carers in close contact with the haemorrhaging patient. Nevertheless, wide-ranging epidemics have not occurred, so apparently these poorly adapted viruses are yet to become established and maintained in the human population.

An age-old zoonosis is rabies, as mentioned already. Each year hundreds of people die of rabies virus infection, yet it has never taken off as a direct human-to-human epidemic owing to its nature of transmission through bites. From the microbe's point of view, the human is a dead-end host, but rabies is an example where the symptoms of the disease it causes serves its onward transmission. This virus invades the central nervous system and induces the aggressive behaviour of the host. At the same time it replicates in the salivary gland, so that biting is very likely to result in new infections. Rabies has been widespread among wild carnivores such as foxes in Europe but is being significantly reduced by

Table 3. *Examples of human infectious diseases of animal origin*

disease	microbe	animal source	date of crossover
malaria	parasite	chimpanzee	*ca*. 8000 BCE
measles	virus	sheep or goat	*ca*. 6000 BCE
smallpox	virus	ruminant?	> 2000 BCE
tuberculosis	mycobacterium	ruminant?	> 1000 BCE
typhus	rickettsia	rodent	430 BCE
			1492 CE
plague	bacterium	rodent	541 CE
			1347 CE
			1665 CE
Dengue	virus	monkey	*ca*. 1000 CE
yellow fever	virus	monkey	1641 CE
Spanish 'flu	virus	bird, pig	1918 CE
AIDS/HIV-1	virus	chimpanzee	*ca*. 1931 CE
AIDS/HIV-2	virus	monkey	20th century

distributing edible bait laced with a strain of vaccina virus genetically engineered to carry rabies virus envelope antigen. This is an example of the successful environmental release of a genetically modified organism (the vaccine) to protect animals and humans against disease.

Rabies belongs to the rhabdovirus family of negative strand RNA viruses. Rhabdoviruses have been immensely successful in colonizing new types of host. They are widespread in fish, insects and plants (Van Regenmortel *et al.* 2000).

(b) *Animal origin of human-to-human infections*

The adaptation of a virus to become epidemic or endemic (permanently resident) in the human population occurs much more rarely than sporadic zoonotic infections that fail to take off. Thus, as discussed in papers in this issue, simian immunodeficiency viruses (SIV) from chimpanzees and from sooty mangabey monkeys have transferred on several occasions to humans to become HIV-1 and HIV-2, respectively. However, only one strain, HIV-1 group M, has successfully established itself to become the HIV pandemic, although HIV-1 group O and HIV-2 are locally epidemic. Influenza and measles, as I exemplify later, and a host of other infections, including tuberculosis, almost certainly originated from animals (table 3).

(c) *Animals as vectors*

We should not forget the more complex life cycles of infectious microbes and parasites that have a reservoir in animals or humans, but depend on a different animal species, usually an arthropod for transmission between mammalian hosts (table 2). These include some of the most debilitating diseases caused by protozoan parasites such as malaria, for which mosquitoes are vectors, and sleeping sickness, caused by a trypanosome spread by tsetse flies. European colonization of Africa in the 19th century, with forest clearance and changing agriculture, greatly exacerbated the distribution and intensity of sleeping sickness (Desowitz 1997). Flaviviruses, like yellow fever and dengue fever, have *Aedes* mosquito vectors. Typhus is spread from rats to humans and onward among humans by lice (Zinsser 1934). There is a variety of tick-borne infections for which humans are the occasional host with a reservoir in sheep, deer or dogs. Many parasites also require alternative hosts: filaria worms in blackflies and mosquitoes; leishmania in sandflies; and liver flukes in water snails.

Malaria may also be a relatively recent disease in human development. The form caused by *Plasmodium vivax* is probably older than the more severe malaria caused by *Plasmodium falciparum* and these parasites diverged in animals before independently infecting humans (Rich *et al.* 1998). The vector of *P. falciparum*, *Anopheles gambiae*, quite recently evolved to become anthropophilic, i.e. to feed specifically on humans; Coluzzi (1999) postulates that human colonization by the parasite took place no longer than about 10 000 years ago, but *P. falciparum* and *P. reichenowi* of chimpanzees possibly diverged with their hosts (Conway *et al.* 2000). Dengue virus is related to flaviviruses of East African monkeys but now infects humans exclusively. Humans created the ecological niche for the vector of Dengue fever virus, *Aedes aegypti*, to spread throughout the tropics. This mosquito has a small flight range; it breeds readily in small pools such as vehicle ruts, water butts, coconut shells, etc., and thrives in a semi-urban environment (Jacobs 2000).

4. EPIDEMICS ANCIENT AND MODERN

The pestilences of ancient myths and scriptures are difficult to identify precisely with known modern diseases. Smallpox probably dates back at least 3000 years and we do not know its animal origin. With polymerase chain reaction (PCR) amplification of DNA one can with difficulty attempt 'archaeomicrobiology' on ancient tissue specimens. But molecular genetics can help in other ways to gather information about human infections. The degree of genetic diversity of microbes can reveal the relative length of time the infectious agent has been in that population or locality. For example, HIV-1 group M (Vidal *et al.* 2000*b*; Yusim *et al.*, this issue) exhibits greatest gene sequence diversity in the Democratic Republic of Congo (Zaire), suggesting that it first flowered

there. The retrovirus, human T-cell leukaemia virus type I (HTLV-I), also shows greatest variation in Central Africa (Gessain & Mahieux 1999), as does GBV-C, a virus related to hepatitis C virus (Tanaka *et al.* 1998).

For viruses that are transmitted within families over many generations, one can attempt a kind of contact tracing of genetic variants of the microbe within defined human populations. HTLV-I is mainly transmitted from mother to child in the milk. The phylogeny of HTLV-I has also been analysed in relation to anthropological background, showing worldwide dispersion, but also independent introductions from the chimpanzee (Gessain & Mahieux 1999; Miura *et al.* 1994; Voevodin *et al.* 1997). My colleagues Natalie Wilder, Mark Thomas and Chris Boshoff have investigated familial transmission among Jewish communities of human herpesvirus 8 (HHV-8), the cause of Kaposi's sarcoma. We found that this virus commonly infects both Ashkenazi and Sephardi Jews with divergence of viral gene sequences (Davidovici *et al.* 2001). By mapping HHV-8 variants alongside human genetic traits—using Y chromosome DNA probes for paternal inheritance and mitochondrial DNA for the maternal lineage—the evolution of the virus will be tracked more precisely along the Jewish diaspora.

Speculation based on genetic divergence, however, can be a dangerous pastime without knowing the rate of divergence or the 'molecular clock' (Yusim *et al.*, this issue; Sharp *et al.*, this issue). Opinion of the origin of hepatitis B virus in humans has swung from postulating a zoonosis from New World monkeys within the last 500 years (Bollyky *et al.* 1997) to seeing dispersion of related viruses throughout Old World apes (MacDonald *et al.* 2000). It would be fascinating to see whether the satellite virus, hepatitis delta, is present in other primates. This virus cannot replicate by itself, being completely dependent on hepatitis B virus (Taylor 1999)—so where did it come from? Its nearest relatives infect plants.

Host genetics also reveals a history of past infection. If most epidemic infections are recent, it shows their powerful selective pressure on the human population, given that 10 000 years represents only 500–600 generations. The red blood cell surface antigen Duffy is used by *Plasmodium vivax* as the receptor, a means of docking onto and entering the cell. However, humans can live healthily without expressing Duffy on red cells, and where people are Duffy negative, as in West Africa, there is no *P. vivax* malaria, but we can surmise it was once widespread. Human populations in which *P. falciparum* is endemic frequently harbour mutations of haemoglobin—sickle cell anaemia and thalassaemia—that afford significant protection against malaria mortality. In the homozygous state, these mutations led to death in childhood, but such was the protective effect against malaria in heterozygotes that it paid the host population to establish and maintain these potentially deleterious genetic traits at high frequency (Miller 1994). The genetic defect causing cystic fibrosis may protect the heterozygote from cholera and other dysenteries. The mutation is common among Europeans, although cholera only arrived in 1821, so selection may have been exerted by another enteric infection such as typhoid (Pier *et al.* 1998). A deletion in chemokine receptor CCR5 that confers resistance to HIV is also frequent among Caucasians and might have been selected for protection against plague (Carrington *et al.* 1999). The infectious history of human populations can thus be revealed by the frequency of genetic traits in today's populations (Cavalli-Sforza 2000; Desowitz 1997).

I shall now briefly discuss a few known epidemics occurring within the last 1000 years.

(a) *Plague*

Although plague probably affected humans in ancient times, the Justinian plague in 541 CE is the first well documented pandemic. However, it was the Black Death arising in the 1340s that caused even greater devastation, resulting in up to 30% mortality of the populations of Asia, the Middle East, North Africa and Europe (Gottfried 1986; McNeill 1976; Ziegler 1970). Epidemic outbreaks continued for the next 300 years. The plague bacillus *Yersinia pestis* is endemic among certain species of burrowing rodents such as marmots in the steppes and deserts of Central Asia. It is transmitted from animal to animal via fleas. With the growth of the trade routes across this terrain plague jumped hosts when black rats originating from India proliferated in the towns and caravan markets along the silk road. The plague travelled east to China and westward, reaching the Black Sea in 1347, where it spread among the Tartars who were besieging the Genoese settlement of Kaffa in the Crimea. According to legend, on retreating, the Tatars catapulted corpses over the town ramparts. The Italians set sail for Italy with disease rampant aboard—docking in Messina and Genoa. Despite attempts to quarantine the ships, the rats disembarked and by spring 1348, plague had gripped Sicily and the mainland, reaching London later that year.

The traveller and meticulous diarist, Ibn Battuta mentioned nothing about disease when he journeyed the caravan route in the 1330s. However, when he returned from China to Egypt by the sea route (like Marco Polo in the previous century) he wrote 'I arrived in Alexandria in the month of Shawal in the year 748 [late 1347] amidst a terrible plague. I was told that the number who succumbed daily in Cairo amounted to one and twenty thousand' (Lee 1829). In his introduction to the *Decameron*, Boccaccio gives a graphic account of the plague in 1348 when 60% of the population of Florence perished.

The Black Death represented a combination of bubonic, pneumonic and septicaemic forms of plague. Although rats became a reservoir, septicaemic plague was spread by human flea and louse vectors from person to person and the pneumonic form was spread by the respiratory route. Between 1350 and 1420 the European population declined steadily, and the economy went into recession.

The last great plague in London flared in 1665 and ended with the fire of London the following year (Defoe 1722). Rebuilding London's dwellings with bricks and tiles relegated the rats from the rafters to the basement, so fleas leaping off from cold, dead rats had less opportunity to find their second choice host, the human. However, while the fire only destroyed central London, the plague also petered out in timbered west London. When Norwegian rats replaced the black rat in 19th-century Europe, the risk of plague disappeared, because *Rattus norvegicus* does not carry a flea that both carries *Y. pestis* and bites humans. Plague outbreaks continue sporadically

where black rats cohabitate with humans. Over eight million plague deaths occurred in India between 1895 and 1914, with a recent purported outbreak in Surat in 1994 (Campbell & Hughes 1995). In the 20th century *Y. pestis* colonized burrowing rodents in the New World such as chipmunks and woodchucks. Following the 1906 earthquake in San Francisco, it spread from the US west coast as far as the Rocky Mountains. A handful of human infections occurs each year among campers in Arizona and New Mexico, although it remains sporadic because the fleas tend to parasitize the young deep in the burrows rather than adults. Thus the subtle changes in our ecology and that of alternative hosts and vectors have reduced plague to a rare disease.

(b) *Measles*

Measles is caused by a paramyxovirus of the morbillivirus group. Measles virus needs a large host reservoir and originated through husbandry of herd animals. Related morbilliviruses of animals include canine distemper and the marine viruses discussed by Osterhaus (this issue), and rindepest of cattle and antelopes. But the nearest relative to measles is the 'peste des petits ruminants' infecting sheep and goats (Van Regenmortel *et al.* 2000), which is almost certainly its origin. The cross-species transfer may have occurred 7000 to 8000 years ago when small ruminants first became domesticated in the fertile crescent while the human population burgeoned (Diamond 1997). Since then, measles has remained a human infection and modern day animals are not a reservoir for human disease.

Measles virus relies on horizontal transmission; since survivors become immune it can only be maintained in the human population by finding previously unexposed people to infect. In populations where measles has been long established, children are its target, and before measles immunization was introduced in the 1970s periodic epidemics were seen among infants (Anderson & May 1992). In this way, measles was maintained as a human infection for millennia in Asia, Europe and Africa. Other, more isolated human populations, however, either could not sustain measles infection, or had never been exposed to it. When measles reached these virus-naive communities, it had an enormous impact with high mortality among adults. This pattern of lethality, no less devastating than the Black Death, has occurred repeatedly during the last 500 years as Europeans visited the Americas, Australasia and Oceania.

(c) *Smallpox*

Smallpox is an ancient human disease (Fenner 1988). Its relatives are so widespread among rodents, domestic animals and primates that we cannot be sure of its origin. Smallpox, with measles, was an important component of the 'Columbian exchange' that occurred when Columbus reached Hispaniola and his successors colonized North and South America. This exchange transformed the diet of the Old World, by importing maize, potatoes, tomatoes, chillies and other foods, as well as introducing Sir Walter Raleigh's 'stinking weed', tobacco, which has become the biggest non-infectious cause of mortality today. But it was smallpox that allowed Cortés and his small band of adventurers to defeat the mighty Aztec empire, with measles hot on its heels. The pestilence introduced to Tenochtitlán (Mexico City) is vividly described in the memoirs of the conquistador, Bernal Diaz (1963). McNeill (1976) estimates that the Amerindian population of North America dropped from over 30 million to approximately two million between 1520 and 1620, largely on account of infections introduced by the colonists and their African slaves. Of course, the slaves were required to man the haciendas and plantations because the indigenous population perished. Smallpox reached the Incas ahead of Pizzaro. The lesson was not lost on Sir Jeffery Amherst, who used the virus in 1758 as a biological weapon against hostile indigenous tribes during the Franco-British War in North America (Garrett 2000; Oldstone 1998). Edward Jenner's pioneering vaccine prepared from cowpox in 1798 eventually led to the eradication of smallpox in 1977 (Fenner 1988).

(d) *Yellow fever*

Another viral disease 'bartered' through the Columbian exchange was yellow fever. This virus had a natural reservoir, not in domesticated animals but in monkeys in Africa. It is transmitted by mosquitoes, though this was not proven until 100 years ago by Carlos Finlay and Walter Reed in Cuba. *Aedes aegypti* will transmit yellow fever virus from human to human, but yellow fever only invaded the Americas from Africa via the slave trade in 1641, requiring the vector to become established first. Yellow fever then found a reservoir in New World monkeys, and large parts of tropical America became no-go zones on account of 'yellow jack'. Oldstone (1998) estimates that it delayed the building of the Panama Canal for 100 years. In 1803 yellow fever killed 27 000 French troops in Haiti and helped to convince Napoleon that America was a hostile environment, so that Jefferson negotiated a real bargain over the Louisiana Purchase. Summer seasonal epidemics of yellow fever continued to plague the United States throughout the 19th century, such as the great epidemic in Memphis in 1876.

(e) *Syphilis*

If Old World diseases caused such havoc in the New World, did the Columbian exchange in microbes flow both ways? Diamond (1997) argues that pre-Columbian America had far fewer epidemic diseases because fewer animals were domesticated, the llamas, alpacas and guinea-pigs of South America being an exception. Ever since syphilis became epidemic in Europe in the 1490s there has been heated argument as to whether it had a New World origin. Tertiary syphilis affects the bones, but analysis of pre-Columbian skeletons on both sides of the Atlantic has not yielded a definitive answer (Miller 2000).

The molecular genetics of the syphilis germ, *Treponema pallidum*, is almost indistinguishable from other treponemata causing skin diseases, such as the agent of yaws. Yaws was present in Europe and Africa before Columbus (indeed, victims of yaws were sometimes confused with lepers) and pinta was present in the Americas. Syphilis is a more systemic disease than either pinta or yaws with a distinct mode of transmission. Was syphilis a more virulent strain of an Old World germ which learned a novel, sexual means of passage, or was it a New World import?

The debate is unresolved, with microbiologists remaining more sceptical than historians about its introduction. Several contemporary writers, such as the physician Diaz de Isla and the priest Bartolomé de las Casas, insist that syphilis was established in Hispaniola long before the Spaniards' first visit (Quétel 1990). The question surrounding the relationships between syphilis, yaws and pinta would merit re-analysis with modern day molecular markers.

What is clear is that syphilis was recognized as a completely new epidemic disease in Europe and Asia. Although secondary syphilis, or the great pox, does not appear until weeks after primary genital infection, it was immediately recognized as being spread by 'carnal knowledge'. Syphilis was first noted in Spain in 1494, the year following Columbus's return from his first transatlantic voyage, and became seriously epidemic during the siege of Naples in 1495 (Quétel 1990). Within a short period it spread throughout Europe; it soon set sail with Vasco da Gama for India and onward to China, reaching Japan in 1505, ten years ahead of St Francis Xavier.

(f) *Typhus*

During the Peloponnesian War the eyewitness Thucidides described an epidemic in Athens in 430 BCE that resembles louse-borne typhus. As with syphilis, typhus spread epidemically throughout Europe via Spain in the 1490s (Palmer *et al*. 1998). Ferdinand and Isabella finally expelled the Moors from Granada in 1492 despite the loss of 17 000 Spanish soldiers to typhus. *Rickettsia prowazekii* is louse-borne, and *R. typhi* is flea-borne. They can transfer from rodent to human lice and fleas and thus become a self-sustaining epidemic among humans. Typhus and war or prisons have been good partners. The disease decimated Napoleon's army on the way to and retreat from Moscow. The Eastern Front of the Great War triggered a new typhus epidemic in 1917–1925, from which my father suffered but recovered as an army recruit in Silesia. Hans Zinsser's (1934) classic *Rats, lice & history* has lost none of its verve or pertinence since it was written. During the Second World War typhus was often the harbinger of death in the Nazi concentration camps, making Zinsser's remark prescient: 'Typhus is not dead. It will continue to break into the open whenever human stupidity and brutality give it a chance.' Since 1945, no major typhus epidemics have occurred. However, the WHO considers typhus to be a potential problem (World Health Organization 1993) and local outbreaks in the 1990s again occurred in areas of conflict and refugee camps in the Balkans and Burundi (Raoult *et al*. 1997).

(g) *Influenza*

Influenza, like measles, spreads by the respiratory route and needs a large host population for sustenance as the disease is acute and the virus does not persist. But unlike measles virus, which is remarkably constant in its genetic sequence and antigens, influenza virus is highly variable. Its outer envelope glycoproteins undergo antigenic 'drift', which allows some escape from existing immunity. Thus one individual can suffer from influenza many times. A sharper change occurs after several decades called antigenic 'shift' when an entirely new strain of influenza spreads across the world (Webster *et al*. 2000*b*). The virus has a segmented RNA genome and thus the genes encoding different viral proteins can reassort in hosts where more than one strain of virus is present at a time. The 'Spanish' influenza pandemic of 1918–1919 was probably an example of such a new, recombinant strain, and it caused more deaths than all the casualties of the Great War.

Novel influenza viruses involving antigenic shift come from animal sources or represent genetic recombinants between animal and human virus strains. Webster *et al*. (2000*b*) consider that a reservoir exists in waterfowl, in which influenza viruses are maintained as chronic gut infections. The pig can be an intermediate 'mixing vessel' in which the virus adapts to a respiratory route of transmission. Where ducks, pigs and humans share homesteads, as in peasant communities in the Far East, conditions are optimal for zoonosis to occur and lead on to a human epidemic. However, recent experience shows direct avian to human zoonosis (Horimoto & Kawaoka 2001).

The H5N1 outbreak of influenza in Hong Kong in 1997 (Osterhaus, this issue) was a potential epidemic that did not quite take off. Eighteen humans were infected, among whom six died, although the virulence of the pathogen did not tally with transmissibility. All 18 cases were zoonotic chicken-to-human infections of, luckily, a non-recombinant avian strain. The source of the chicken virus appeared to be waterfowl or gamefowl held live in the Hong Kong markets. With the slaughter of Hong Kong's chickens, and subsequent separation of chicken markets from other birds, the outbreak ended. Restocking from the mainland where the birds were bred did not lead to its resurgence. Subsequent to H5N1, however, another avian influenza strain, H9N2 was directly transmitted from birds to humans in Hong Kong (Horimoto & Kawaoka 2001; Webster *et al*. 2000*a*). As was recently discussed at The Royal Society, a new pandemic strain of influenza could arise at any time (Webster *et al*. 2000*b*).

(h) *Prion diseases: BSE and vCJD*

Spongiform encephalopathies comprise a set of degenerative non-inflammatory diseases of the central nervous system (Collinge 1999) that may arise sporadically by inherited predisposition or through infectious transmission (table 4). In all cases the disease develops slowly as a result of conformational change in prion proteins (figure 3). BSE became a major epidemic in British cattle as a result of feeding these herbivores with fodder containing nervous and brain tissue extracted from other cattle. Similar cattle-feed formulations were used in other countries with high intensity farming so it could have happened elsewhere. Possibly a single elderly milk cow with incipient BSE seeded the epidemic by being introduced into cattle-feed, with those infected beasts being recycled in the same way.

As BSE had never previously been recorded, it was initially thought to have originated from scrapie-infected sheep. Scrapie is an ovine form of transmissible spongiform encephalopathy that has been present in British sheep for at least 200 years, and sheep carcasses were also recycled into cattle-feed. However, scrapie prions have different properties. They do not readily transmit infection to other species. On the other hand, scrapie is

Table 4. *Human prion diseases*

(GSS, Gerstmann–Straüssler–Scheinker syndrome; FFI, fatal familial insomnia; CJD, Creutzfeldt–Jakob disease; BSE, bovine spongiform encephalopathy.)

type	disease	origin
inherited	GSS, FFI	germline prion mutations
sporadic	CJD	somatic prion mutations?
acquired	kuru	exposure to human prions in ritual mortuary feasts
	iatrogenic CJD	exposure to human prions via transplantation or inoculation
	variant CJD	exposure to BSE prions

naturally transmissible between sheep, without requiring 'cannibalism' (Woolhouse *et al*. 1999). Indeed, pasture previously grazed by an infected flock can pass scrapie to a clean flock introduced to the same land a year or more later (Sigurdsson 1954). We do not understand the natural mode of transmission of scrapie.

There is no evidence that BSE is naturally transmitted, with the possible exception of rare vertical transmission from cow to calf. The number of infected British cattle has fallen precipitously since regulations preventing specified bovine offal in cattle-feed were introduced ten years ago (Anderson *et al*. 1996). It may therefore be possible to eliminate BSE, though the current concern over its presence in other European countries makes eradication appear a long haul effort. It seems remarkable that BSE has such a broad species host range via contaminated feed-stuff, but unlike scrapie is not naturally transmitted. It is also odd that prions, which apparently lack genetic material, possess characteristic strain specificities that breed true even across host species transfers.

The exhaustive investigation into BSE by Lord Phillips and colleagues has the benefit of hindsight in documenting the complacency, secrecy and sectionalism of government (BSE Inquiry Report 2000, available at http://www.bseinquiry.gov.uk/report/index.htm). Based on the long experience with scrapie, it was reasonably concluded by the Southwood Committee in 1989 that BSE was most unlikely to cross to other species (Report of the Working Party on BSE 1989). But when a domestic cat developed the disease in 1990, and large zoo cats and antelopes not long after, alarm bells should have sounded in the Ministry of Agriculture, Fisheries & Food, and been communicated to the Department of Health. We later witnessed the economic and political consequences of the British BSE epidemic, and the fateful evidence announced in March 1996 linking BSE to the new variant of human Creutzfeldt–Jakob disease (vCJD) (Will *et al*. 1996).

More than 80 people in the UK have developed vCJD. No-one really knows how many people are incubating the disease as the predictions have wide confidence limits (Ghani *et al*. 2000) and are based on uncertain assumptions (Bacchetti 2001). Diagnostic tests using samples of lymphoid tissue (Ironside *et al*. 2000) require further development, and central nervous tissue is not available for population screening. Because vCJD prions are expressed at low level in lymphoid cells, blood transfusions are now depleted of white cells, in the hope of preventing human-to-human transmission. But numerous other problems remain. Infectious prions cannot easily be inactivated by heat or chemical treatment, so the sterilization of hospital instruments such as endoscopes becomes a problem since doctors cannot know which patients may be incubating vCJD when they are treated for other diseases. Classic CJD has been transmitted by tissue grafting from human cadavers, e.g. of corneas and by hormones extracted from the pituitary gland in the brain. Without knowing the prevalence of vCJD infection in the British population, it is impossible to assess the risk of further iatrogenic transmission. But other nations can play safe, which is why the USA refuses blood donations from its citizens who visited the UK for over six months between 1980 and 1996.

The first transmissible form of CJD in humans, kuru, was investigated by Gajdusek among highland tribes in New Guinea in the 1950s. Kuru was spread by cannibalism and ointments prepared from the brains of revered elders at 'mortuary feasts' (Gajdusek 1977). There is no evidence to date that vCJD is transmitted from person to person, but the known routes of transmission for classic CJD must be eliminated—transplantation of tissues and other biological products. Other than this, the hygiene message is clear: eating people is wrong. Not consuming one's own species should apply to cattle too. BSE and vCJD is a peculiarly modern epidemic arising from industrialized cattle-fodder practices.

(i) *AIDS*

The recent emergence of human immunodeficiency virus type-1 (HIV-1) in the human population is one of nature's most successful adaptive radiations. HIV exemplifies Darwinian natural selection at a fast-forward pace. In the West, HIV readily exploited various niches of our late-20th-century lifestyles including air travel, narcotics dependence and steamy, promiscuous bath houses (Shilts 1987). Yet it is wreaking most havoc among the world's poorest and underprivileged communities. HIV presents a frightening yet fascinating *danse macabre* of sex, drugs and death, with no end in sight to the burgeoning pandemic (Weiss & Weiss 2001).

Acquired immune deficiency syndrome (AIDS) was first recognized when small clusters of young homosexual men in American cities were reported to suffer rare opportunistic infections (*Pneumocystis carinii* pneumonia, cytomegalovirus retinitis) and Kaposi's sarcoma (Centers for Disease Control & Prevention 1981). The underlying immune deficiency was soon shown to involve a selective depletion of $CD4^+$, T-helper lymphocytes (Gottlieb *et al*. 1981). By early 1982, reports of AIDS in recipients of blood transfusions and pooled clotting factors, as well as among injecting drug users, indicated that an infectious agent was to blame. The retrovirus causing AIDS, HIV-1, was first isolated one year later (Barré-Sinoussi *et al*. 1983). With the development of reliable serological assays (Cheingsong-Popov *et al*. 1984) we soon saw that infection was already widespread in Africa (Bayley *et al*. 1985; Serwadda *et al*. 1985). In the past 20 years, HIV has rampaged across the globe, with an estimated 58 million cumulative infections (UNAIDS 2000) (table 5).

Table 5. *Global estimates of HIV infection in December 2000*
(Data adapted from UNAIDS 2000.)

people living with HIV	36.1 million
new HIV infections in 2000	5.3 million
deaths due to AIDS in 2000	3.0 million
cumulative number of deaths due to AIDS	21.8 million
people living with HIV in Africa	25.3 million
people living with HIV in Asia	7.1 million
total cumulative HIV infection	57.9 million

About one in 800 of the global adult population is now infected by HIV, but the prevalence and rate of spread of HIV in populations varies dramatically across continents, within continents and within countries. Out of the 5.3 million new infections in 2000, two-thirds occurred in sub-Saharan Africa, and almost one-quarter in South and South-East Asia. The predominant mode of transmission is heterosexual contact and 47% of those infected are women. In some countries in eastern and southern Africa, up to 40% of young women are now infected who in turn infect their children (Piot *et al*. 2001).

Since HIV-1 was identified, understanding the molecular biology of this virus (figure 4) has been crucial for the development of antiviral drugs which, where available, have led to a greater than 60% drop in mortality. The elucidation of the cellular tropism of HIV and the cell surface receptors it uses helps to explain the pattern of immune deficiency leading to opportunistic infections, the wasting disease and the dementia that comprise the clinical manifestations of AIDS. Yet we still neither understand well why the balance of power between host and virus eventually favours HIV, nor why chimpanzees and mangabey monkeys, which are the natural reservoir of the precursors of HIV-1 and HIV-2, respectively, can harbour similar levels of virus without progressing to AIDS.

The genetic and phenotypic evolution of HIV proceeds at a prodigious rate, both within the infected individual (Haase 1999) and across the worldwide pandemic (Piot *et al*. 2001). Each infected individual possesses an immense pool of HIV variants, or quasi-species, allowing substantial genetic and antigenic draft to occur within each infected individual. It is this high rate of virus replication that provides the conditions for numerous immune escape and drug-resistant mutants to be generated (Phillips 1999). The evolution and selection of variants is complex, allowing both colonization of new cells and antigenic variation. For example, a mutation in gp120 selected for escape from antibody neutralization can also result in a change of cell tropism, and vice versa (McKnight *et al*. 1995). There is, however, a phenotypic resetting of the evolutionary clock upon transmission from one person to the next. The type of HIV variants that emerge in late stage infection at high viral load appear to be less fit for transmission. They may be viewed as opportunistic variants exacerbating progression to AIDS once the host immune system has become sufficiently damaged to favour their propagation (Weiss & Weiss 2001).

As HIV has spread, it has generated genetic subtypes that have bloomed in different countries and risk groups. The most complex HIV-1 variation is seen in Central Africa, the hearth of the pandemic (De Cock, this issue; Yusim *et al*., this issue). Where cross-infection of strains occurs, HIV frequently recombines to generate yet more variants (Vidal *et al*. 2000*a*; Sharp *et al*., this issue), even between HIV-1 groups M and O (Takehisa *et al*. 1999). This high rate of HIV genetic variation impedes vaccine development because it is reflected in antigenic variation (Weiss *et al*. 1986).

As discussed in this issue, reporting the Discussion Meeting on the Origins of HIV and the AIDS epidemic, both HIV-1 and HIV-2 represent novel, zoonotic introductions into the human population within the past 100 years. The animal lentivirus most related to HIV-1 is SIVcpz of chimpanzees. Humans harbour three major groups of HIV-1, named M, N and O, with group M representing all the subtypes or 'clades' A–H that have spread to cause the worldwide pandemic. The gene sequences of groups M, N and O are as distinct from each other as they are from SIVcpz (Gao *et al*. 1999). This finding implies that they are derived from three separate introductions from chimpanzees to humans, yet only one of them has become pandemic (Hahn *et al*. 2000).

HIV-2 is endemic in West Africa, but has spread to Europe (especially Portugal) and to India. Like HIV-1, HIV-2 can be subdivided into a number of groups, which appear to represent separate zoonoses from a primate host (Gao *et al*. 1992). In this case the original primate reservoir is not a great ape but the West African monkey, sooty mangabey (*Cercocebus atys*), which is infected with SIVsm. The infection of captive Asian macaque species by SIV also appears to be a cross-species transfer of SIVsm that occurred in captivity. Many species of African monkey harbour other types of SIV, but there is no evidence that any of these SIV strains have infected humans.

When, how and why HIV came into human populations and took off epidemically was the topic of The Royal Society Discussion Meeting reported in this issue. As a virus with a long incubation period between infection and disease, during which it is actively replicating, HIV had every opportunity to spread before it was recognized. But its adaptation to natural transmission via sexual intercourse and from mother to child may initially have been helped by the widespread medical reuse of needles and syringes in mid-20th-century Africa (Marx *et al*., this issue), just as HIV later colonized patients with haemophilia and intravenous drug abusers in the West. When considered in the light of many cross-species transfers of primate retroviruses (Weiss, this issue), the mobility of other retroviruses during mammalian evolution (to be discussed next), and of the numerous other infections reviewed here, the origins of HIV-1 and HIV-2 appear less unique and remarkable than when viewed alone. The daunting problem facing mankind today is to bring this pandemic under control. A safe, efficacious vaccine against HIV-1 remains a pious target rather than a reality (Nabel 2001).

5. ENDOGENOUS RETROVIRUSES

The human retroviral pathogens arose from animal sources. As already discussed, HIV-1 and HIV-2 transferred from primates within the last 100 years. The human T-cell leukaemia viruses types I and II are older

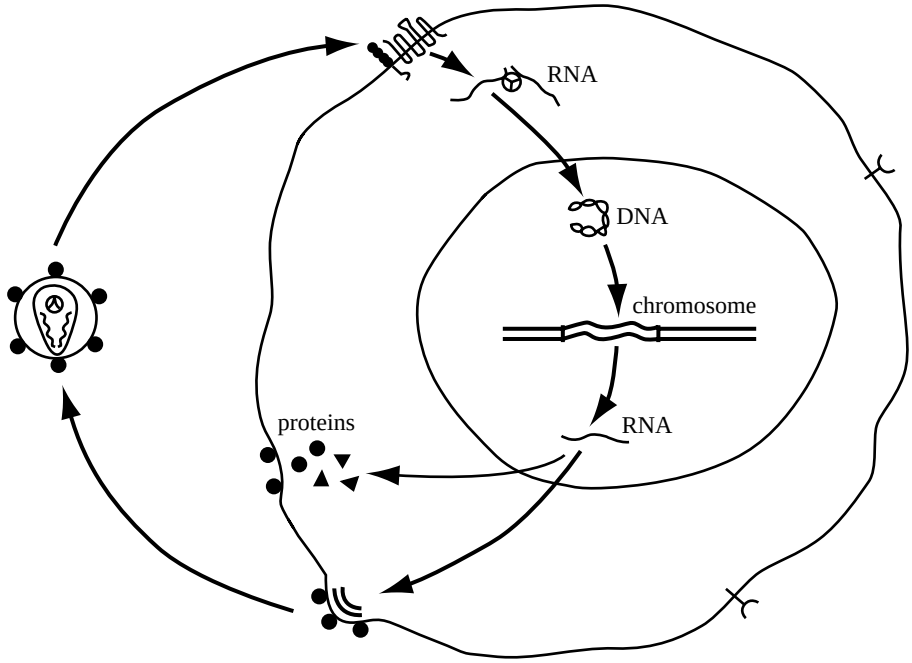

Figure 4. Replication cycle of a retrovirus. The virion on the left contains two RNA copies of the genome. Upon entry into the host cell, the viral enzyme reverse transcriptase makes one copy of a double-stranded DNA genome, which integrates into the DNA of the host chromosomes. Transcribed RNA is then translated into viral proteins and full-length RNA transcripts are packaged into progeny virions. Current anti-HIV drugs block virus replication at two steps: reverse transcription of RNA to DNA; and cleavage of large, precursor proteins into smaller components during virus assembly. Future drugs may block interaction with cell-surface receptors and integration into host DNA.

human infections, although recent, local transfer from primate sources may have added to the worldwide strains. Other groups of retrovirus have crossed host species many times. The story of host–virus relations of retroviruses is particularly intriguing. My stumbling upon endogenous retroviruses when I was a research student led me into virology (Weiss 1967).

The phenomenon of integration of the DNA 'provirus' into chromosomal DNA (figure 4) allows retroviruses to establish long-term persistent infections, sometimes silent when the provirus is simply carried as a gene sequence that is reproduced with the host DNA if the cell divides. This has allowed retroviruses to establish the most intimate host–parasite relationship of all by infecting the germline, the cells destined to become the eggs or sperm. In this way retroviruses obtain a free ride to the next generation. These inherited 'endogenous' retroviruses have invaded almost all vertebrate species including humans (Patience *et al*. 1997*b*). However, the retrovirus subfamilies that include HIV and HTLV have not become endogenous.

Figure 5 illustrates three distinct modes of transmission of avian retroviruses. Figure 6 illustrates how a virus inherited in a Mendelian way in one species can re-emerge, infect another species and re-integrate into its germline. By analysing retroviral sequences in animal DNA one can build up a picture of these distinct episodes of transmission among mammals. Figure 7 shows the evolutionary relationships of two sets of endogenous sequences related to murine leukaemia virus. Type I viruses have been mobile across phylogenetically unrelated hosts (Martin *et al*. 1999, 2001), whereas type II viruses have strictly coevolved with their hosts. Only type I viruses have maintained full-length genomes potentially recoverable as infectious virus, and it is these that pose a risk in using animal tissues for medical purposes. The evolutionary relationships of endogenous retroviruses to their exogenous, infectious counterparts and with their hosts is surely ripe for analysis by the techniques applied to other infections (Nee 2000; Nowak & May 1994).

6. IATROGENIC ZOONOSES

One major concern in modern times is that medical treatments themselves might inadvertently lead to introduction of animal viruses. Diseases introduced as a result of 'healing' practices are described as iatrogenic.

(a) *Vaccines and biologicals*

Medicines prepared in animals or from animal cells will carry a risk, if only a remote one, of contamination with animal viruses. Who knows, for instance, what viruses were harboured by leeches in the 18th century when every patient of standing was treated by blood letting? Later, in the 20th century, antitoxins were prepared in horses and, aside from anaphylactic shock due to prior sensitization, the serum preparations could at best be said to derive from healthy animals. Today, such biologicals as monoclonal antibodies and recombinant proteins are prepared in animal cell cultures, which can also release potentially harmful viruses, as I pointed out in respect to hybridomas releasing xenotropic retroviruses

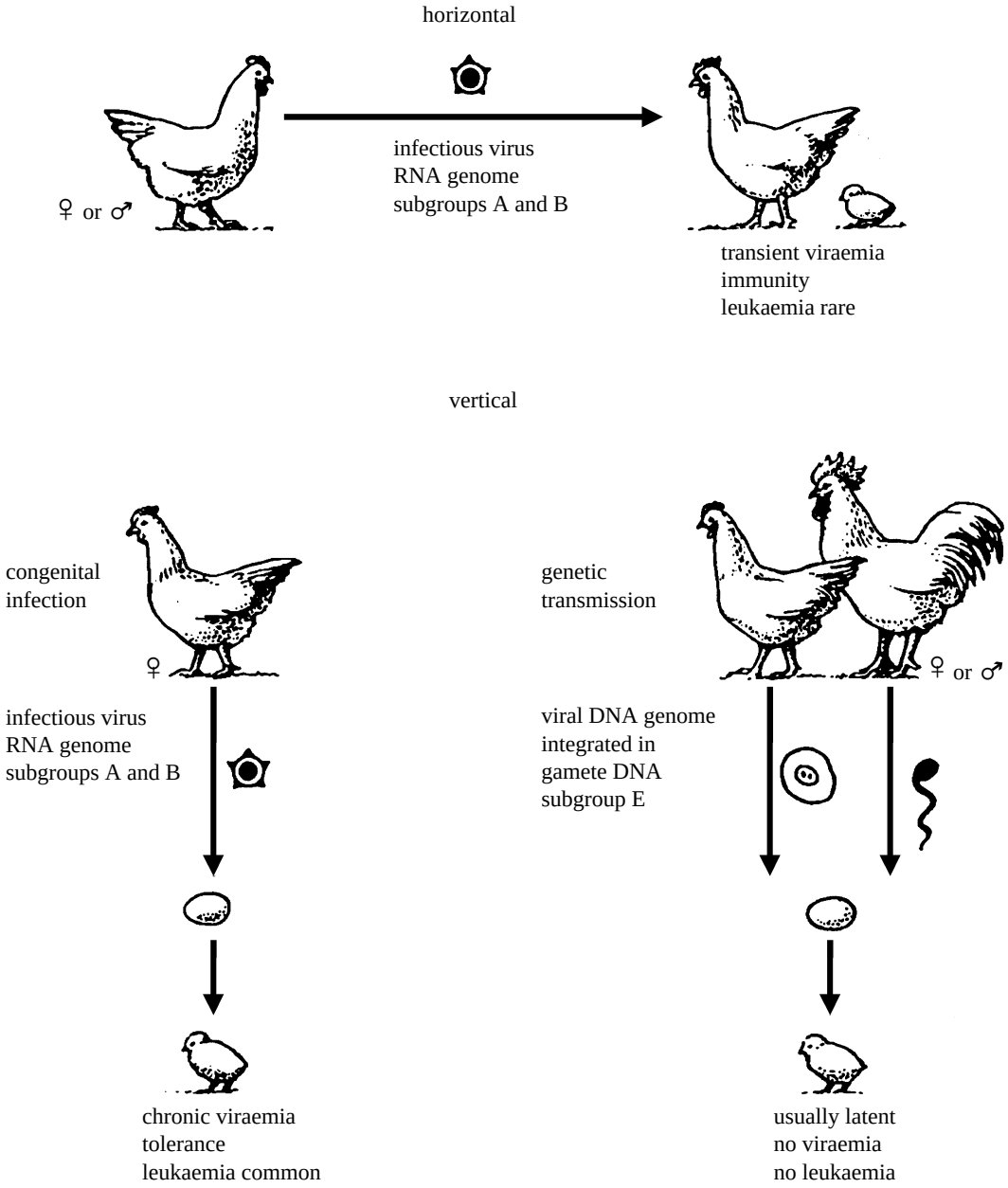

Figure 5. Transmission of avian leukosis viruses. The mode of transmission affects the virulence of the retrovirus. Vaccines prepared in chick embryo cells are at risk of contamination by vertically transmitted viruses (Weiss 2001*a*). While genetically transmitted subgroup E viruses seldom re-infect chicken cells, they are 'xenotropic' as they readily infect cells of other galliform species such as quail, turkey and pheasant (Weiss 1975).

(Weiss 1982). The widespread use of bovine serum albumin and gelatin in pharmaceuticals and vaccines can be questioned in the light of BSE (Birchard 2001).

Vaccines grown in animal cells are administered to the general population including infants. Much of the discussion published in this issue concerns the question of whether HIV might have been introduced into mankind via contaminated live attenuated polio vaccines. While that now appears most unlikely, the situation might have been quite different had the simian immunodeficiency virus (SIV) of African green monkeys been readily transmissible to humans. However, Beale & Horaud (this issue) argue that SIV would not survive the preparation of oral polio vaccine.

There are, however, documented cases of contamination by animal viruses of vaccines via the cellular substrates used to propagate them. Polio vaccines became contaminated in the early days of vaccine development. Primary kidney cells from rhesus macaques were used to propagate polio virus for vaccine preparation. The kidneys were sometimes infected by simian vacuolating virus 40 (SV40) (Sweet & Hilleman 1960), a papovavirus related to human JC and BK viruses. The culture techniques used for optimal poliovirus propagation also encouraged activation and replication of SV40. No-one knows how many doses of the Salk and Sabin polio vaccines contained SV40 but there could have been millions before its elimination by changing the substrate from macaque to African green monkey kidneys in 1962. SV40 is not associated with cancer in monkeys, but it is highly oncogenic in hamsters. Nevertheless, an early investigation of polio vaccinees did not reveal an increased risk of

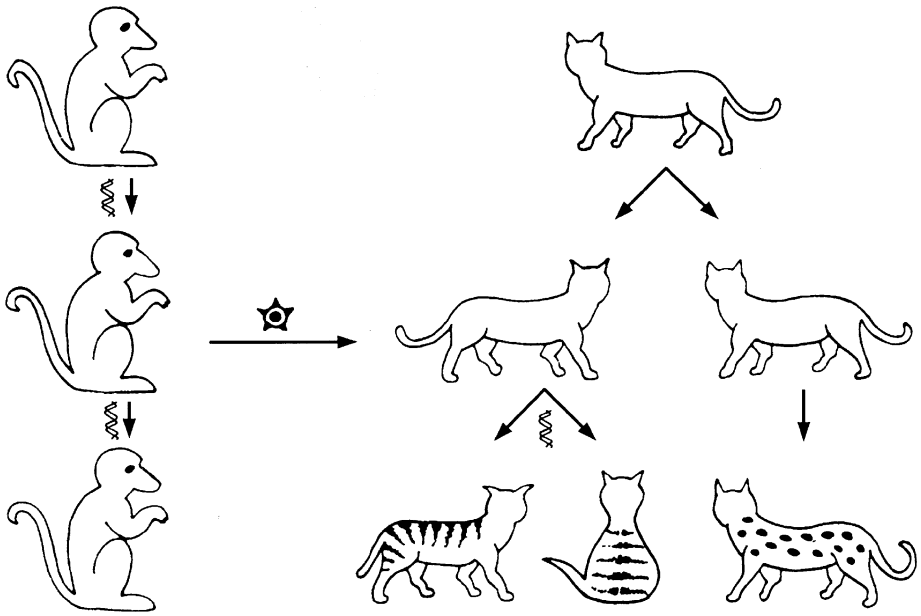

Figure 6. Transmission of retrovirus from the baboon germline to that of cats. Baboon endogenous virus (BaEV) is expressed at high load as infectious virions in the placenta, which perhaps was once scavenged by a cat. The virus colonized the germline of cats after the Asian spotted species of *Felis* diverged from tabby Mediterranean and European species (Benveniste & Todaro 1974). BaEV itself is a recombinant between a β-retrovirus (its *env* gene) and a γ-retrovirus (its *gag* and *pol* genes) and the cat has inherited the chimeric virus.

cancer (Shah & Nathanson 1976). More recently, however, evidence has been claimed of SV40 in human tumours of a similar type to those found in hamsters, namely ependymoma and mesothelioma (Butel & Lednicky 1999). Since ependymomas affect infants, the SV40 detected in cases from the 1990s cannot have been be derived directly from the polio vaccine. So has SV40 become a human transmissible virus? The question remains controversial because reliable detection of SV40 DNA remains fraught with the possibility of false-positive PCR results, owing to the presence of SV40 sequences in many molecular biology reagents used in research and diagnostic laboratories (Griffiths *et al*. 1998). A blinded, controlled study did not detect SV40 sequences in mesothelioma (The International SV40 Working Group 2001).

Yellow fever virus, like measles, mumps and rubella, is administered as a live, attenuated vaccine, propagated in eggs or in chick embryo fibroblasts. Before screening for avian leukosis virus (ALV) was introduced, many eggs used for vaccine preparation must have been contaminated, and ALV was reported in yellow fever and measles vaccine preparations (Harris *et al*. 1966). Luckily, neither ALV nor the endogenous retroviruses still present in vaccine substrates (figure 5) appear to infect human cells, and there is no evidence of increased risk of leukaemia or other tumours in those administered the vaccines (Weiss 2001*a*). However, a genuine outbreak of disease did occur when a yellow fever vaccine batch was contaminated by hepatitis B virus from human serum used to stabilize the vaccine.

(b) *Xenotransplantation*

Xenotransplantation involves the grafting of animal tissues or organs into humans (Platt 2001; Weiss 1998*a*). Pigs are the favoured animal source for a number of practical husbandry, ethical and safety reasons. Although there remain immunological hurdles for preventing organ rejection, dispersed tissues such as foetal pig brain cells have already been xenografted into patients with Parkinson's disease. In theory, a pig from a specific pathogen-free herd should be a cleaner source of tissue for transplantation than a 'free-range' human who has suddenly died. But pigs can only be screened for viruses that are already known. The number of porcine viruses newly identified in the last five years (table 6) leads me to think that more remain to come to light.

Table 6. *Recently identified viruses in pigs*

virus	year
porcine endogenous retroviruses A, B, C	1997
porcine hepatitis E	1997
porcine circovirus 2	1997
porcine torovirus	1998
nipahvirus	1998
porcine γ-herpesviruses	2000

What, then, are the risks of animal-to-human infections via xenotransplantation? Paul Herrling, Director of Research and Development for Novartis, was quoted (Butler 1998) as saying, 'Animals have transmitted infections to humans throughout history. The additional risk of successful xenotransplantation might be minimal.' My counter to that argument is that xenotransplantation provides nearly ideal conditions for cross-species transfer of viruses: first, the animal tissue will be placed directly inside the human body; second, the patient will be heavily immunosuppressed to avoid xenograft rejection; and third, transgenic pigs bearing human genes may aid virus transfer (Weiss 1998*b*). Viewed from the predicament

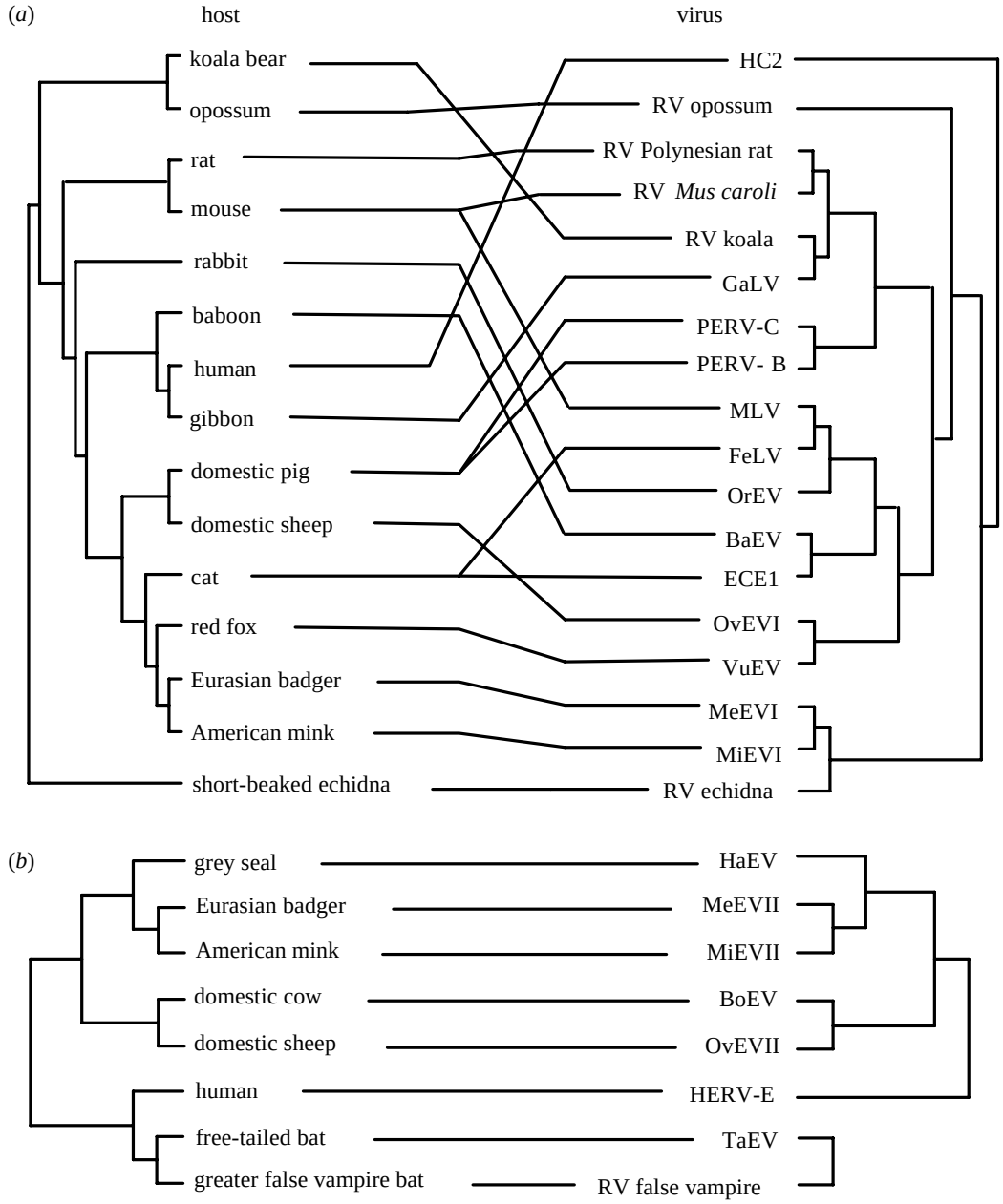

Figure 7. Treemap 'tanglegrams' showing the relationship between host and virus phylogenies for the *pol* gene of mammalian murine leukaemia virus (MLV) related retroviruses. (*a*) Type I MLV genomes show little co-speciation indicating horizontal, infectious transmission between hosts; indeed, some of the viruses listed (FeLV, GaLV) represent exogenous infections. (*b*) Type II MLV genomes are entirely congruent with their host's phylogeny, indicating co-speciation. (Adapted from Martin *et al*. 2001.)

of a desperately ill individual, a risk–benefit analysis may still favour proceeding with clinical xenotransplantation; viewed from a community health stance, one has more misgivings, in case a zoonotic infection triggers a novel epidemic. While that seems a remote possibility, so were AIDS and BSE before they happened.

In order to prevent hyperacute rejection of pig organs transplanted to primates, genetically modified pigs have been bred (Platt 2001). These express certain human genes (*CD55*/*DAF*, *CD46* and *CD59*) that help to suppress hyperacute rejection by blocking the complement cascade. My concern has been that these human proteins expressed on the surface of pig vascular lining cells also happen to serve as receptors for viruses (Weiss 1998*b*). *CD46* is a receptor for morbilliviruses like measles, and *CD55* is a receptor for certain small RNA viruses like Coxsackie B3, B5 and Echo 7. Swine vesicular disease virus is closely related to Coxsackie B5 virus and can infect humans in a non-transmissible manner (Brown *et al*. 1976). If porcine viruses 'learned' by mutation to use the human form of the *CD* receptors expressed in transgenic pigs, they would be preadapted to take off in humans.

The major zoonotic concern over porcine xenotransplantation has been a set of viruses called porcine endogenous retroviruses (PERVs), which are closely related to the endogenous γ-retroviruses already discussed (figure 7). We recently showed that they have been transmitted as Mendelian traits in pigs and their ancestors for some 30

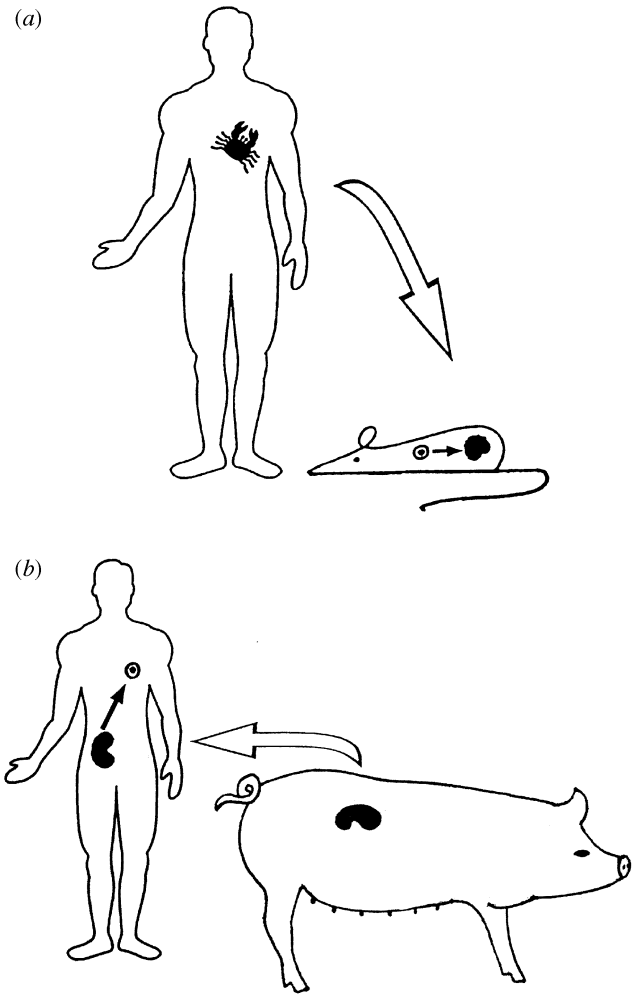

Figure 8. (*a*) Approximately 1% of human tumour xenografts in immunodeficient mice become infected by endogenous, xenotropic strains of murine leukaemia virus. (*b*) Will pig-to-human xenografts allow the transfer of pig endogenous retroviruses to humans?

million years (Patience *et al*. 2001), yet some of these genomes (PERV-A, -B and -C) have the propensity to re-emerge as infectious viruses and to infect human cells in culture (Patience *et al*. 1997*a*; Takeuchi *et al*. 1998). We know that human tumour xenografts in mice occasionally become infected by murine xenotropic virus (figure 8), so will pig xenografts introduce PERVs to humans?

Fortunately, a retrospective survey of 160 patients exposed to living porcine cells or tissues has not shown evidence of PERV infection (Paradis *et al*. 1999), so PERVs do not appear to be highly contagious. Nevertheless, PERVs remain an issue of concern regarding xenotransplantation. Regulatory bodies such as the UK Xenotransplantation Interim Regulatory Authority (UKXIRA) and the US Food and Drug Administration recommended stringent clinical monitoring of xenograft recipients and their intimate contacts following our investigation of PERVs (Boneva *et al*. 2001; UKXIRA 2000). Whether such surveillance can be upheld in practice remains to be seen. We have also suggested that appropriate antiretroviral drugs be available in case a patient becomes infected by a PERV (Qari *et al*. 2001).

7. ZOONOSIS AND VIRULENCE

As Anderson & May (1992) pointed out, 'The received wisdom, set forth in most medical texts and elsewhere, is that "successful" or "well-adapted" parasites are relatively harmless to their hosts'. They go on to show that while the long-equilibrated infectious agent may well become attenuated, the general assumption is not valid because the chances of onward transmission will be affected by its virulence. Anderson & May (1992) provide a simple formula for the basic reproductive rate (R_0) of a microbe:

$$R_0 = \frac{\beta(\alpha, N)N}{\alpha + \mu + \nu(\alpha)},$$

where α is the disease-induced host mortality rate, β is the transmission coefficient, ν is the recovery rate, μ is the mortality rate for all causes, and N is the total population size. If neither transmission rate nor recovery rate depend on virulence, then R_0 is maximized by making α infinitesimal, that is non-pathogenic.

Epidemics in new populations do tend to diminish in virulence over time (Lederberg 2000), as was seen following the introduction of myxoma virus in Australian rabbits (Anderson & May 1992; Frank 1996). Even Girolamo Frascotoro, who wrote on syphilis in 1530 and the first treatise on contagion in 1546, noted the lessening mortality in the syphilis epidemic among afflicted persons between 1495 and 1530 (Porter 1997; Quétel 1990). However, some evolutionary biologists have swung so far away from the conventional wisdom as to interpret almost all pathological symptoms in the host as adaptive features of the microbe to promote its transmission (Ewald 1994).

As discussed earlier, rabies pathogenesis is indeed precisely geared to onward transmission; on the other hand its close relative in bats, lyssavirus, is a persistent infection exhibiting different transmission dynamics and is consequently less virulent. Some symptoms of disease may aid transmission of the microbe, e.g. coughing and sneezing, diarrhoea, skin pocks and genital sores. Some, such as fever, are part of the host's innate response to overcome infection, or in hepatitis, may result from the adaptive immune response. Other symptoms, such as paralysis in polio or dementia in AIDS, play no role in transmission, yet they are the consequences of bearing a high pathogen load, which does help transmission.

The analysis of virulence requires consideration of multiple factors of immune status, time and dose of infection, and genetic variation of both host and parasite. As in measles, infection of a naive population can lead to severe mortality in adults, whereas in an experienced population, epidemics restricted to infants may be less severe. Delayed infection by Epstein–Barr virus and poliovirus also tends to be more virulent than infection in infancy, which may explain the emergence of glandular fever and paralytic polio as mid-20th century, largely middle-class epidemics. The danger of neurovirulent polio variants emerging from the attenuated vaccine has recently been highlighted (Clarke 2001). With bacterial infections, the emerging knowledge based on comparative genomics between different strains and species of bacteria (Weinstock 2000) and the genetic mobility of

pathogenicity islands (Hacker & Kaper 2000) demands a radical review of transmission dynamics in relation to virulence.

Changes in virulence upon crossing host species can be difficult to predict (Frank & Jeffrey 2001). Several animal pathogens that can infect humans seldom cause disease except in immunosuppressed individuals, e.g. Sindbis virus from birds. Others are pathogenic in the original and the new host. The H5N1 avian influenza virus was both deadly and highly transmissible among chickens in the crowded conditions of Hong Kong's markets; it was lethal without onward transmission among the crowded human residents. Microbes are frequently non-pathogenic in their natural host species and lethal in their new host. Myxoma virus in American cottontail rabbits became deadly and epidemic in European rabbits (Fenner 1988); a mild herpesvirus infection of African elephants kills Asian elephants and possibly vice versa (Richman *et al*. 1999); and herpesvirus B of rhesus monkeys causes lethal encephalopathy in humans (Palmer *et al*. 1998). SIVsm appears harmless in sooty mangabey monkeys yet causes AIDS in humans. These examples are a further reason not to be complacent about xenotransplantation; what might be a harmless commensal in the source animal may cause disease on crossing the species barrier.

On the other hand, changing hosts and serial transmission often diminishes pathogenicity. Pasteur's success in immunizing against rabies and anthrax was based on this principle (Brock 1975; Porter 1997). Similarly, the vaccine strains of poliovirus types 1, 2 and 3 were attenuated through serial passage, and yellow fever virus and measles became attenuated through passage in eggs. We need to gain a greater understanding of virulence determinants in microbes, the ways they evade or delay immunity, and how immune responses differ from host to host (Nowak & Bangham 1996). There still seems to be a yawning gulf of communication and intellectual debate between those who model infectious disease epidemiology using the term virulence, and those who analyse the molecular biology and immunology of infection, using the term pathogenesis.

8. LOOKING TO THE FUTURE

With all the new infections that humans have acquired from animals, we might wonder why infectious disease has not steadily accumulated rather than diminished during the past century. We do not clearly know why epidemic diseases waxed and waned, e.g. tuberculosis increasing in the 18th and 19th centuries, and polio in the 20th century. However, increasing freedom from infection has more to do with sanitation and immunization than with antibiotics. Eradication of smallpox was possible because variola virus has no animal reservoir and has no asymptomatic or persistent infection in people. Other candidate viruses for eradication for the same reasons are polio, measles and mumps viruses.

One cannot predict the future of zoonoses except that there will be further surprises. I have indicated a potential problem in xenotransplantation. Three other foreseeable possibilities of cross-species infection deserve brief mention.

(a) *Biological weapons*

The red, black and pale horses of the apocalypse—war, famine and pestilence—ride well together, as I mentioned in reference to smallpox, typhus and plague. Sir Jeffrey Amherst and his pox-infested blankets notwithstanding, deliberate germ warfare has not found much practical use in past conflicts. There should, nevertheless, be considerable concern that rogue nations or terrorist groups could exploit microbiological weapons in the future. These weapons may include human pathogens such as variola virus (Berche 2001; O'Toole 1999), but it would appear that more development has been placed in animal microbes that can kill humans (Alibek & Handelman 1999). The stockpiling of anthrax, Q fever, tularaemia, plague packaged in fleas, and other pathogens, has less danger of rebounding on the aggressor when delivered by long-range missiles. Bacteria and viruses have been genetically engineered with the aim of producing attenuated versions. But a recent attempt to do just that for ectromelia virus (mousepox), by the insertion of the host gene encoding interleukin 4, actually increased its virulence by disrupting cell mediated immunity (Jackson *et al*. 2001). Molecular microbiology, like nuclear energy, can be exploited to promote human health, or to generate lethal weapons.

(b) *Immunosuppressed populations*

It is well known that immunosuppressed individuals are more susceptible to animal infections that are common in the environment but seldom cause serious disease in immunocompetent people. When the proportion of immunosuppressed individuals in a population remained very low, the chance of transmission of a pathogen from one to another was even smaller. Inherited immunodeficiency, medically immunosuppressed transplant patients and neutropenic cancer patients on chemotherapy are at high risk of infection as individuals but are not a great risk to others.

With AIDS the situation has changed. HIV infected patients have already become a high load reservoir of human pathogens such as tuberculosis, which can then be transmitted to others who are not at risk of HIV itself. The HIV pandemic may also hamper campaigns for disease eradication because normally acute infections or live vaccines (e.g. polio) may become persistent in immune deficiency (Weiss 2001*b*). So what might be the risk of HIV-infected persons acting as incubators of microbes that previously relied solely on animal reservoirs? Alphaviruses of birds, enteric infections of farm animals, *Mycobacterium avium*, canine *Toxoplasma*, *Borrelia* and numerous other microbes not normally transmissible between humans are known to present as opportunistic infections in AIDS (El-Helou *et al*. 1997; Zuckerman *et al*. 2000). They now have a vast population of immunodeficient hosts in which to play Darwinian selection. Where 10% or more of a community are HIV-infected, direct transmission between immunodeficient individuals becomes plausible. I can envisage a number of microbes that are poorly adapted for human infection evolving to become well adjusted, first to the immunodeficient host and eventually to immunocompetent humans (Weiss 2001*b*). These could include non-obligate parasites from the environment, e.g. non-tuberculous *Mycobacteria*, *Legionnella* and *Aspergillus* (Shafer & Sierra 1992; Squier

et al. 2000) as well as microbes and macroparasites from animal sources. This issue of the potential community impact of a massive immunodeficient reservoir merits the attention of those who model infectious disease dynamics and evolution.

(c) *Human-to-animal transmission*

This lecture has been presented from an unashamedly human point of view. Yet the exchange of microbes can pass both ways. New World primates were invaded by yellow fever virus from humans after *Aedes aegypti* mosquitoes established themselves in the Americas. Subsequently, the virus became transmissible through mosquito species indigenous to the New World tropical forest. Mankind has also been the agency whereby related but distinct animal species can exchange microbes, as already mentioned for animals in captivity such as African and Asian elephants with herpesviruses (Richman *et al*. 1999) and African and Asian monkeys with SIV. Ross McPhee considers that the demise of mammoths and other large animals of the Pleistocene era might have occurred through epidemics transferred from humans or the animals such as dogs that accompanied human migration (Wong 2001).

Today, a danger may be posed to the great apes, chimpanzees and gorillas in Africa, and the orang-utan in South-East Asia. Their survival increasingly depends on sanctuaries and national parks that attract visits from primatologists, ethologists and 'ecotourists'. Humans could be the reservoir menace from whom these animals acquire, say, measles, influenza or the common cold. Let us hope the apes do not follow the same fate as naive human populations. 'Wherever the European has trod, death seems to pursue the aboriginal' (Darwin 1836).

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