REVIEW ARTICLE

Zoonoses

Proceedings of the third Liverpool Tropical School Bayer Symposium on Microbial Diseases held on 3 February 1996

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Introduction

Man has enjoyed a long and intimate relationship with the other animals. Some animals are taken into the home to provide companionship or to act as guards, others are reared to provide food, milk, clothing or for recreation. These interactions are most firmly in man's favour. In his interaction with wild animals man also usually triumphs but the animals are having notable successes. Rodents, such as the wild brown rat (*Rattus norvegicus*), have adapted very well to being parasitic upon man. For example, it is estimated that rats destroy 33 million tons of food worldwide each year [1]. Furthermore, over the period 1976–1979, 5.2% of UK premises inspected by environmental health inspectors were found to be infested by brown rats, but by 1993 the figure had risen to 7.7% [2]. The brown rat has been shown to carry and excrete various micro-organisms pathogenic for man including *Leptospira* spp., *Pseudomonas mallei*, *Coxiella burnetti* and hantavirus [1].

The concept that animals might be a source of infection for man is not new. Possibly the earliest reference is by Moses in the Bible (see ref. 3): 'The swine also because it divideth the hoof but cheweth not the cud shall be unclean to you, ye shall not eat of their flesh nor touch their dead carcasses, ye shall not eat anything that dieth of itself' (Deuteronomy Chapter 14 Verse 8). This is presumably a recognition that infections such as trichinosis, pig tapeworm (*Taenia solium*), anthrax, *Yersinia enterocolitica*, *Streptococcus suis*, *Brucella suis* or even Japanese B encephalitis might originate from pigs. On reading this text further it is also clear that godliness does not extend to neighbourliness (Verse 21).

Another zoonosis, rabies, has also been described in ancient literature. The Eshunni code of Mesopotamia (before 2300 BC) stated that the owner of a vicious dog that had bitten a man causing his death should be fined [4]. This, however, might not necessarily have been rabies but merely a badly behaved dog [5]. In 100 AD, Celsus recommended that 'Especially if the dog was rabid the virus must be drawn out with a cupping glass' [6]. In the 1880s great advances were made in understanding the pathogenesis of this zoonosis and in a classic series of experiments Pasteur was able to develop a vaccine which prevented rabies in a boy, Joseph Meister, who had been bitten by a rabid dog [7]. The term zoonosis was first coined by Virchow but its (almost) universal acceptance did not come until a joint Expert Committee of the WHO and FAO produced its deliberations [8]. In this, zoonoses were defined as 'Those diseases and infections naturally transmitted between vertebrate animals and man'.

It is estimated that there are over 200 different zoonoses transmitted from animals to man. However, it is difficult to estimate their overall impact on human health. For example, *Cryptosporidium parvum*, *Campylobacter* spp. and certain non-typhi salmonellae, which are arguably the most frequently occurring zoonoses in developed countries, may also be transmitted person-to-person.

In general, zoonoses may be transmitted by direct contact, by biting insects (within which the micro-organism replicates), by inhalation or by ingestion (Tables 1–4). With certain exceptions (e.g., rabies, orf, *Francisella tularensis*, *Y. pestis*) the reservoir host does not necessarily show signs of disease. Furthermore, a zoonosis may be transmitted to man from an animal that is not the definitive host but which acts as an amplifier (e.g., pigs are infected with Japanese B encephalitis virus by mosquitoes that have previously fed on migratory water birds) or a 'Trojan horse' (e.g.,

Table 1. Zoonoses spread by direct contact

Mode of spread	Organism	Disease	Reservoir host	Person-to-person
Throughcut, scratch abrasion or mucous membranes	Herpesvirus simiae	Encephalitis	Old world monkey	No
	Buffalopox virus	Buffalopox	Buffalo?	No
	Cowpox virus	Cowpox	Small rodents?	No
	Paravaccinia virus	Orf, Milker's nodes	Sheep, cattle	No
	Monkeypox virus	Monkeypox	Squirrel	Yes (limited)
	<i>Bacillus anthracis</i>	Cutaneous anthrax	Cattle, sheep, pigs	Yes
	<i>Bartonella henselae</i>	Cat scratch fever	Cats, dogs	No
	<i>Clostridium tetani</i>	Tetanus	Cattle, horses, sheep	No
	<i>Erysipelothrix rhusiopathiae</i>	Erysipelothrix	Pigs	No
	<i>Francisella tularensis</i>	Tularaemia	Wild mammals, domestic animals, birds	No
	<i>Leptospira</i> spp.	Hepatitis, meningitis	Rats, cattle, dogs	Rare
	<i>Mycobacterium leprae</i>	Leprosy	Armadillo	Yes
	<i>M. marinum</i>	Fish tank granuloma	Fish, reptiles	No
	<i>Pseudomonas mallei</i>	Glanders	Horses	No
	<i>Yersinia pestis</i> *	Plague	Rats	Yes
	Dermatophytes	Kerion, ringworm	Dogs, cats, pigs, monkeys, cattle	Yes
	(e.g. <i>Trichophyton simii</i> , <i>Microsporum canis</i>)			
	<i>Schistosoma</i> spp.	Bilharzia, schistosomiasis, Katayama fever	Many mammals, birds, snails	No
	<i>Sarcoptes</i> spp.	Sarcoptic mange	Pigs, dogs	No
	<i>Tunga penetrans</i>	Jiggers, tungiasis	Pigs	Yes
Through animal bites	Rabiesvirus	Rabies	Many species (dogs, foxes, bats, etc.)	No
	Hantavirus*	HFRS	Rats	No
	Tanapox virus	Tanapox	Monkey†	No
	<i>Capnocytophaga canimorsus</i>	Bite wound	Dogs	No
	<i>Pasteurella haemolytica</i>	Bite wound	Dogs, cats	No
	<i>P. multocida</i>	Bite wound	Dogs, cats	No
	<i>Spirillum minus</i>	Rat-bite fever	Rats	No
	<i>Streptobacillus moniliformis</i>	Rat-bite fever, Haverhill fever	Rats	No

*Not the most common mode of spread.

†Thought to be spread from monkey by biting insect which is not a biological vector.

HFRS, haemorrhagic fever with renal syndrome.

Table 2. Zoonoses acquired from arthropod bites

Agent	Arthropod	Animal reservoir	Disease
Togavirus*			
Chikungunya	<i>Aedes</i>	Monkey, rodent?	Febrile illness
Western equine	<i>Culex</i>	Birds, horses	Encephalitis
Flavivirus*			
Yellow fever	<i>Aedes</i>	Monkey, rodents?	Haemorrhagic fever (HF)
Dengue	<i>Aedes</i>	Monkey?	HF
Japanese encephalitis	<i>Culex</i> , <i>Anopheles</i>	Pigs, horses	Encephalitis
Louping ill	Tick (<i>Ixodes</i>)	Rodents	Febrile illness
Bunyavirus*			
Congo-Crimean HF	Tick (<i>Ixodes</i>)	Birds, rodents, sheep	HF
Rift Valley fever	Mosquito	Rodents?	Febrile illness
<i>Rickettsia tsutsugamuchi</i>	Trombiculid mite	Rodents	Scrub typhus
<i>R. prowazeki</i>	Louse	Flying squirrel	Endemic typhus
<i>R. typhi</i>	Flea	Rat	Murine typhus
<i>R. rickettsiae</i>	Tick	Dogs, birds	Spotted fever
<i>R. acari</i>	Mite	Mouse	Rickettsialpox
<i>Bartonella quintana</i>	Louse	Long-tailed vole?	Trench fever
<i>B. bacilliformis</i>	Sandfly (<i>Lutzomyia</i> spp.)	?	Oroya fever
<i>Ehrlichia chaffeensis</i>	Tick (<i>Dermacentor</i>)	Horses, ruminants	Ehrlichiosis (undifferentiated fever)
<i>Borrelia burgdorferi</i>	Tick (<i>Ixodes</i>)	Rodents, deer	Lyme disease
<i>Bor. recurrentis</i>	Louse	Rodents, armadillo, bats	Relapsing fever
<i>Bor. duttoni</i>	Tick (<i>Ornithodoros</i>)	Rodents, armadillo, bats	Relapsing fever
<i>Francisella tularensis</i>	Mosquito, tick, tabanids	Wild rodents	Tularaemia
<i>Babesia microti</i>	Tick (<i>Ixodes</i>)	Small rodents	Undifferentiated fever (<i>Babesiosis</i>)
<i>Bab. divergens</i>	Tick (<i>Ixodes</i>)	Cattle	Undifferentiated fever (<i>Babesiosis</i>)
<i>Leishmania</i> spp.	Sandfly	Dogs, foxes, cervines, rodents	Kala-azar, Oriental sore, Espundia
<i>Trypanosoma brucei</i>	Tsetse fly (<i>Glossina</i>)	Pigs, ungulates (e.g. bushback)	Sleeping sickness
<i>T. cruzi</i>	Reduviid bug (<i>Triatoma</i>)	Armadillo, opossum, domestic animals	Chagas disease

*Not a complete list.

Table 3. Zoonoses acquired by the respiratory route

Agent	Reservoir host	Disease
Ebola and Marburg viruses	Small rodents?	Haemorrhagic fever
Equine morbillivirus	Horses	Pneumonia, death
Hantavirus	Rodents, insectivores, bats	Haemorrhagic fever with renal syndrome Hantavirus pulmonary syndrome
Influenza A virus	Birds, pigs	Influenza (most often acquired person-to-person but new strains arise by recombination in birds)
Lassa, Junin, Machupo and Sabia viruses	Multimammate rat (Lassa), Vesper mouse	Haemorrhagic fever
Lymphocytic choriomeningitis virus	Mice	Aseptic meningitis
<i>Bacillus anthracis</i>	Cattle, sheep, pigs	Pneumonia, anthrax
<i>Bordetella bronchiseptica</i>	Pigs, dogs, cats	Pneumonia in immunocompromised patients
<i>Brucella</i> spp.	Sheep, goats, pigs	Brucellosis
<i>Chlamydia psittaci</i>	Birds, sheep	Pneumonia, abortion
<i>Coxiella burnetti</i>	Cattle	Q-fever, endocarditis
<i>Francisella tularensis</i>	Small mammals, horses	Tularaemia
<i>Mycobacterium avium</i>	Birds	Cervical adenitis, disseminated disease in AIDS
<i>M. bovis</i>	Cattle, badgers?	Tuberculosis
<i>Pseudomonas mallei</i>	Horses	Glanders
<i>Rhodococcus equi</i>	Horses	Pneumonia in AIDS
<i>Streptococcus suis</i>	Pigs	Meningitis, pneumonia
<i>Yersinia pestis</i>	Rats	Pneumonia, plague (most often person-to-person)
<i>Cryptococcus neoformans</i>	Pigeons, cats	Meningitis, pneumonia
<i>Histoplasma capsulatum</i>	Birds	Disseminated disease, pneumonia
<i>Pneumocystis carinii</i> *	Many animal species	Pneumonitis in immunocompromised patients

*Most evidence suggests it is species specific.

a pet dog infected by the bite of a rabid fox). It must also be remembered that zoonoses are not solely one-way traffic and that man may be a source of infection for animals (e.g., *C. jejuni* spread from owner to dog).

The risk of developing a zoonosis will, to a large extent, depend upon the frequency and intimacy of contact with an infected reservoir host. The risk of a particular pet dog or cat being a reservoir host for a zoonotic pathogen will also depend upon it having contact with infected dogs or cats. This results in a low overall risk of owners acquiring infection from their pampered pets. In contrast, veterinarians appear at greater risk. A survey of 1182 Illinois veterinarians revealed that 42.7% had experienced a zoonosis (the most frequent were bites and brucellosis!) and that those who were accident-prone were more likely to experience a zoonosis [9]. Another increasingly important risk for zoonoses is educational farm visits or holidays. It is estimated that over 4.7 million people visit farms each year in UK and that this might result in an increase in incidence of zoonoses [10]. This is exemplified by the recent report of an outbreak of cryptosporidiosis that affected 31 of 47 individuals who went on a week's educational visit to a farm [11].

Clearly it is impossible for one meeting to cover the vast spectrum of zoonoses. For this meeting, a group of scientists, veterinarians and physicians were assembled to address various aspects of zoonoses. The topics range from food-associated zoonoses, to hydatid disease, poxviruses to antimicrobial resistance and zoonotic protozoa to hantaviruses. The aim is to provide an up-to-date review of at least some current zoonotic problems.

ZOONOSSES: A PERSONAL PERSPECTIVE ON THE JENNERIAN PRINCIPLE OF CROSS-PROTECTIVE IMMUNITY

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The organisers of this symposium have given me the privilege of discussing the zoonoses from a 'personal perspective' with reflections on my adventures with zoonoses in Africa including: anthrax in the hippopotamus and elephant; schistosomiasis in baboons; trypanosomiasis in bush buck; hydatid disease in dogs, jackals and hyaenas; trichinosis in bush pigs and large carnivores; bubonic plague in swamp rats; leishmaniasis and murine typhus in field rodents; tapeworms in hyaenas and wildebeest; and a whole host of other infectious diseases which if studied in detail would have proven to be zoonoses fitting the definition accepted by WHO and FAO as 'Those diseases and infections naturally transmitted between vertebrate animals and man' [8].

As a Medical Officer of Health in Uganda from 1950 to 1956 and specialist Parasitologist in Kenya from 1956 to 1963, I was expected to deal with zoonoses which presented as 'diseases' of man and not merely as 'infections'. In fact, I was sure that in the sea of pathogens in Africa, and elsewhere, it was the infections that produced no disease, or those that were only mildly pathogenic, that ensured man's survival through mechanisms of cross-protection. These mechanisms would include: (a) 'competitive exclusion', e.g., between non-pathogenic bacteria and pathogens and between different parasites, particularly

Table 4. Zoonoses acquired by ingestion

Agent	Reservoir host	Disease
Faecal-oral contamination		
<i>Campylobacter coli/jejuni</i>	Poultry	Diarrhoeal disease
<i>C. lari</i>	Seagulls, poultry	Diarrhoeal disease
<i>C. upsaliensis</i>	Dogs, cats	Diarrhoeal disease
<i>Clostridium botulinum</i>	Mammals, birds, fish	Botulism
<i>Cl. perfringens</i>	Pigs, cattle	Pig bel, food poisoning
Enterohaemorrhagic <i>Escherichia coli</i>	Cattle, pigs	Diarrhoeal disease, HUS
<i>Leptospira</i> spp.	Rats, cattle	Leptospirosis
<i>Listeria</i> spp.	Various domestic animals	Fever, meningitis, abortion
<i>Salmonella</i> spp. (non-typhi)	Large number of animal species	Diarrhoeal disease
<i>Yersinia enterocolitica</i>	Pigs	Diarrhoeal disease
<i>Balantidium coli</i>	Pigs	Diarrhoeal disease
<i>Cryptosporidium parvum</i>	Cattle, rats, many others	Diarrhoeal disease
<i>Cyclospora cayentanensis</i>	Moles? rodents?	Diarrhoeal disease
<i>Giardia lamblia</i>	Sheep, rodents, cattle?, dogs	Diarrhoeal disease
<i>Toxoplasma gondii</i>	Cats	Toxoplasmosis
<i>Ascaris suum</i>	Pigs	Usually no disease
<i>Capillaria philippinensis</i>	Monkeys	Chronic malabsorption
<i>Dracunculus medinensis</i>	Dogs, cats, cattle, cyclops	Guinea-worm
<i>Echinococcus granulosus</i>	Dogs	Hydatid disease
<i>Fasciola hepatica</i>	Sheep, snails	Hepatitis
<i>Fasciolopsis buski</i>	Pigs, snails	Malabsorption, obstruction
<i>Heterophyes heterophyes</i>	Cats, dogs, foxes, pigs	Usually asymptomatic but may spread to heart and brain
<i>Hymenolepis diminuta/nana</i>	Rat, dog, fleas	Usually no disease
<i>Oesophagostomum</i> spp.	Pigs	Colonic abscesses
<i>Parastrongylus costaricensis</i>	Slugs, rats	Eosinophilic granuloma
<i>Taenia solium</i>	Pig (ingestion of eggs excreted by humans)	Cysticercosis
<i>Toxocara canis</i>	Dogs	Visceral and ocular larva migrans
<i>Pentastomids</i>	Dogs, snakes	Halzoon (<i>Linguatula</i>), hepatitis (<i>Armillifer</i>)
Ingestion of meat (M), fish (F), shellfish (S), eggs (E), or dairy products (D)		
<i>Bacillus anthracis</i> (M)	Cattle, sheep, pigs	Intestinal anthrax
<i>Brucella</i> spp. (M,D)	Sheep, goats, cattle, pigs	Brucellosis
<i>Campylobacter jejuni</i> (M,D)	Poultry, cattle	Diarrhoeal disease
<i>Coxiella burnetti</i> (D)	Cattle	Q-fever, endocarditis
Enterohaemorrhagic <i>E. coli</i> (M,D)	Cattle, pigs	Diarrhoeal disease, HUS
<i>Salmonella</i> spp. (M,E,D)	Poultry, cattle, pigs, mice	Diarrhoeal disease
<i>Staphylococcus aureus</i>	Cattle (mastitis)	Food poisoning (vomiting)
<i>Streptococcus zooepidemicus</i> (M)	Cattle	Meningitis, pneumonia
<i>Vibrio parahaemolyticus</i> (S)	Filter feeders	Diarrhoeal disease
<i>Sarcocystis hominis</i> (M)	Cattle	Asymptomatic
<i>Toxoplasma gondii</i> (M)	Sheep, pigs	Toxoplasmosis
<i>Diphyllobothrium latum</i> (F)	Lake fish	Macrocytic anaemia (B ₁₂ deficiency)
<i>Gnathostoma spinigerum</i> (F)	Freshwater fish	Larva migrans
<i>Metorchis conjunctus</i> (F)	Freshwater fish, dogs	Fever, hepatitis
<i>Opisthorcis sinensis</i> (S)	Snails, fish	Obstructive jaundice
<i>Paragonimus westermani</i> (S)	Crabs, dogs, cats, fish	Pneumonitis
<i>Parastrongylus cantonensis</i> (S)	Prawns, rats	Eosinophilic meningitis
<i>Pseudoterranova decipiens</i> (F)	Sea fish	Anisakis
<i>Sparaganum mansonii</i> (M)	Frogs	Encephalitis
<i>Taenia saginata</i> (M)	Cattle	Beef tapeworm
<i>T. solium</i> (M)	Pigs	Pig tapeworm
<i>Trichinella spiralis</i> (M)	Pigs	Fever, myalgia, orbital oedema

HUS, haemolytic uraemic syndrome.

in the intestine; (b) cross-immunity between closely related organisms, e.g., cowpox and smallpox, non-pathogenic arboviruses and yellow fever, different species of animal trypanosomes and human sleeping sickness, yaws and syphilis and tuberculosis and leprosy; and (c) generalised cellular immunity such as the protective effect of toxoplasmosis against other protozoa, bacteria and even fungi. In the case of helminths, where the severity of the disease is usually proportional to the worm load, the cross-protection is usually incomplete, but by reducing the worm load or the number of pathogenic eggs in the tissues, as in schistosomiasis, the effect can be a marked 'ameliora-

tion' of the disease. It was by studying this phenomenon in areas where bovine and other animal schistosomes were prevalent and by experiments in rodents and primates that we suggested that the term 'zooprophylaxis' defined as 'the prevention or amelioration of disease in man as a result of previous exposure to infections of animal origin' should be used to designate this type of protective immunity [12]. At the time WHO had abandoned 'zooprophylaxis' in favour of 'deviation' to describe the phenomenon of animals being used to distract mosquitoes from biting man but other entomologists have persisted in using the term so that our much broader concept was never

generally accepted by microbiologists or epidemiologists. This was unfortunate because there was no label for this phenomenon and as a result the epidemiological significance has been greatly neglected.

In 1988, as a contribution to the hundredth anniversary of Virchow's promotion of the term zoonoses [13], I abandoned 'zooprophylaxis' in favour of the 'Jennerian principle of cross-protection', in recognition of Jenner's studies on cross-protection between cowpox and smallpox and also because Jenner was the first scientific zoonosologist. Although he never elaborated on the different diseases acquired from animals he said 'The deviation of man from the state in which he was placed by nature seems to have proven to him a prolific source of diseases. From the love of splendour, from the indulgence of luxury and from his fondness for amusement he has familiarised himself with a great number of animals which may not have been intended for his associates. The wolf disarmed of ferocity is now pillowed in the lady's lap. The cat, the little tiger of our island, whose natural home is the forest, is equally domesticated and caressed. The cow, the hog, the sheep and the horse are all for a variety of purposes brought under his care and dominion' [14]. This being the year of the bicentenary celebrations of Jenner's discovery I am using this opportunity to re-emphasise and redefine the Jennerian principle as follows in 'the prevention or amelioration of disease in man as a result of previous exposure to heterologous infections of animal or human origin'. This is not restricted to zoonoses but includes organisms depending on interhuman transmission, e.g., yaws and syphilis and human tuberculosis and leprosy. Some of the examples in Table 5 have been discussed elsewhere [3, 15]; here, the comments will be restricted to a few of those diseases where our observations in the field emphasised the importance of the Jennerian principle.

Smallpox and other pox viruses

As this 'personal perspective' is designed to promote the Jennerian principle mention must be made of smallpox – I must be one of the few remaining medical officers from Uganda who had to deal with an

extensive epidemic of smallpox which was controlled successfully by mass vaccination. This was in 1954 and it was thought to be *variola minor* but there were several congenital cases and the mortality was fairly high. There had been no cases in West Nile or in neighbouring Zaire for many years and there was nothing to indicate that it was an imported disease. I suspected that there was an animal reservoir. Monkeypox and Tanapox had not yet been described but cowpox and buffalopox were well known and Tanzanian *variola minor* was thought to be very close to the Iranian camel pox which had caused extensive epidemics. I still believe that we are premature in claiming total eradication of smallpox. The new generation of *Homo sapiens*, with no previous exposure to smallpox vaccination, is already showing an increased susceptibility to monkeypox and cowpox and new pox viruses such as Tanapox can suddenly infect man from their unknown reservoirs. Instead of devastating epidemics of previously unknown viruses like Ebola, with unknown reservoir hosts, we may encounter pox virus epidemics with the same virulence as smallpox. I was closely involved in the discovery of Tanapox in Kenya. It was quite frightening; whole villages in the delta of the Tana river were devastated with an acute febrile illness and on examination they all had a few umbilicated pox lesions similar to smallpox. Fortunately there were no deaths. We had no idea where the disease came from but we suspected monkeys and that transmission was due to the exceptional biting rate of *Mansonia* mosquitos. We sent specimens of the lesions to Professor Allan Downie in Liverpool and he described the virus and by self-inoculation and culture recognised that it was not related to smallpox but more closely related to myxomatosis virus [16]. It is fortunate that *Homo sapiens* is not related to the rabbit!

Yellow fever and other flaviviruses

Uganda was famous for the studies carried out in the virus research institute in Entebbe where the main emphasis was on yellow fever. It was here that the tree-top cycle between monkeys and the mosquito *Aedes africanus* was established. Subsequent sporadic transmission to man was by *Ae. simpsoni* and in some areas

Table 5. The Jennerian principle of cross-protective immunity*

Examples		
Cowpox	<i>versus</i>	Smallpox
Wesselsbrons virus	<i>versus</i>	Yellow fever
Yaws	<i>versus</i>	Syphilis
Bovine TB and BCG	<i>versus</i>	Leprosy and Buruli ulcer
Animal schistosomes	<i>versus</i>	Human schistosomiasis
Toxoplasma	<i>versus</i>	Intracellular fungi, bacteria and protozoa
Dog hookworm	<i>versus</i>	Human hookworm
Animal trypanosomiasis	<i>versus</i>	Human trypanosomiasis
Animal leishmaniasis	<i>versus</i>	Human leishmaniasis

* 'The prevention or amelioration of disease in man as a result of previous exposure to heterologous infections of animal or human origin'.

of West Africa interhuman transmission involved *Ae. aegyptii*. Many new viruses were identified including West Nile virus named after the district where I was Medical Officer of Health and it was here in Uganda where the Jennerian principle of cross-protection accounted for the almost complete absence of yellow fever in the human population. Viruses such as Wesselsbron, which was widespread in game animals, livestock and man, were shown by Henderson and his colleagues to give clear cross-protection in experimentally infected monkeys [17]. Other flaviviruses such as West Nile, Zika and dengue gave similar results and it was suggested that this cross-immunity prevented yellow fever from becoming established in monkeys and man in Asia, even though *Ae. aegyptii* was often the commonest man-biting mosquito [18]. Cross-protection between Murray valley fever and Japanese encephalitis is also thought to explain the absence of Japanese encephalitis in Australia [19].

AIDS

When I was in Uganda HIV had not yet been discovered but the West Nile District was regarded as the epicentre of the chronic form of Kaposi's sarcoma and also Burkitt's lymphoma and we saw occasional cases that were indistinguishable clinically from AIDS. No serious studies have been carried out to see if there is an animal reservoir of AIDS in East Africa but in West Africa it is now known that HIV-like viruses are present in green monkeys, mangabeys and chimpanzees (simian immunodeficiency virus: SIV) and that HIV2 almost certainly originated as a zoonosis (see review in Laurie Garrett's *The Coming Plague* [20]). Is it possible that cross-protection affects the distribution of AIDS? Some of these HIV-like variants may be non-pathogenic in man in Africa and this may account for some of the cases of prolonged HIV positivity without AIDS and also for the long-term maintenance of low levels of transmission such as occurs in some rural areas in Zaire. This is an exciting area of research; the tools are available but the study is neglected because of a lack of field workers.

Yaws and syphilis

In 1950, when I arrived in the West Nile district of Uganda, yaws was universal. Every year >20 000 men were recruited to work on the sugar plantations around Lake Victoria – they came back with a high prevalence of gonorrhoea but never syphilis. With the support of WHO and UNICEF, who supplied free penicillin, we practically eradicated yaws from West Nile between 1951 and 1954. A few years later syphilis started to appear. This phenomenon was observed all over Africa and the protective effect of yaws was clearly documented by Hackett in the Pacific islands and re-emphasised by Hackett and Guthet in summarising the WHO eradication campaign [21]. Similar protective immunity against venereal syphilis occurs with the

non-venereal forms and yet neither yaws nor any of the lowly pathogenic treponemas has ever been developed as a vaccine. Perhaps the moralists regarded this as an invitation to promiscuity!

Bovine tuberculosis, BCG, leprosy and Buruli ulcer

My brief as MOH in West Nile was to organise a leprosy control programme because it was in this district that Ross Innes had found almost the highest prevalence of leprosy in the world with >10% of the 400 000 people infected [22], confirming Lucetius who in his 'Natura Rerum' says 'High up the Nile midst Egypt's Central plain springs the dread leprosy and there alone'. Innes's figures turned out to be grossly exaggerated due to the enthusiasm of the local chiefs who collected cases of leprosy from very wide areas and included them in Ross Innes's samples. It was not difficult to get accurate figures because the whole population was registered for the 6-monthly sleeping sickness inspections. We examined >20 000 people in 19 samples representing all the different tribes and geographical areas with the dramatic result of a clear division of very low prevalence in the densely populated cattle owning areas of the highland and a high prevalence in the lowlands where game and tsetse prevented cattle husbandry. The highland areas were also the source of labour for the down-country sugar estates and it was in the highlands that we saw a good deal of tuberculosis. The conclusion was that the low prevalence of leprosy in the highlands and similar parts of Africa was due to cross-protective immunity from bovine and human tuberculosis [23]. The same seemed to be true of many parts of the world, with leprosy disappearing where tuberculosis subsequently increased in prevalence [24]. Here we have an excellent example of the Jennerian principle of cross-protective immunity which was first demonstrated by Fernandez who used BCG to protect family contacts of leprosy patients in Brazil [25]. As a result of our studies in West Nile and those of Kinnear Brown elsewhere in Uganda, BCG vaccination was used extensively to control leprosy in Uganda with very good results. The same is true in many parts of Africa where it is now admitted by WHO that BCG is more effective in the control of leprosy than in the control of tuberculosis. The cross-protection seen with mycobacteria was also clearly demonstrated in other areas of Uganda where BCG vaccination was shown to be very effective in controlling *Mycobacterium ulcerans* which caused Buruli ulcer and was a major problem in refugees [26].

Leprosy is undoubtedly a zoonosis according to the WHO/FAO definition; monkeys and chimpanzee have been found naturally infected in West Africa [27] and there has been natural transmission of leprosy to man from wild armadillos in Texas in the USA [28]. What is now required is a detailed search for leprosy in many other species of animals using more effective

methods of diagnosis such as the PCR technique, which detected numerous human carriers of leprosy in Indonesia [29].

Animal trypanosomiasis and human sleeping sickness

In Uganda, between 1902 and 1906, Sir David Bruce and his fellow members of the Royal Society Commission discovered that sleeping sickness was caused by *Trypanosoma gambiense* and was transmitted by the riverine and lacustrine tsetse fly, *Glossina palpalis* (later in Uganda shown to be *G. fuscipes*). At least 300 000 people died of sleeping sickness and control involved the resettlement inland of the people from the islands in Lake Victoria and from the lakeshore. One of the islands was Buvuma, where >43 000 people of a total population of 56 000 died. At that time there was almost complete deforestation, very little game and the crops, mainly bananas, were grown on terraces. In the 1940s the people were allowed to return to the island but by now it was covered with tropical rain forest with lots of game animals, particularly bush buck and bush pig, and it was not long before there was a new outbreak of sleeping sickness. This time it was caused by *T. rhodesiense* which was known to cause sporadic infections in savannah areas. Circumstantial evidence strongly suggested that this was a zoonosis with game animals as reservoir hosts. In 1950 I was sent to Buvuma to (a) find the vectors by catching tsetse and isolating trypanosomes by getting the flies to feed on rats and (b) to control the epidemic by blood slide survey and treatment of positive cases with Antrypol: (b) was easy, the unco-operative people became very co-operative when I shot a hippopotamus and exchanged a kilo of meat per blood slide! On the other hand, proving which of the tsetse flies was the vector was not easy because although they were infected with trypanosomes resembling *T. rhodesiense* there was no way of distinguishing them from *T. brucei* – the non-pathogenic, non-infective trypanosome which was prevalent in many parts of Africa in game animals and cattle. The only way to distinguish these trypanosomes was to put living trypanosomes from the rats into human volunteers or into patients with general paralysis of the insane. (This end stage of cerebral syphilis could be cured by hyperpyrexia combined with arsenicals but Ugandan patients could not be made hyperpyrexia with malaria as in Europe and the USA because of their immunity.) As it turned out the vector proved to be *G. fuscipes*.

This contradicted Bruce and the accepted dogma of the textbooks that this was the vector of *T. gambiense* and not *T. rhodesiense* so my work on sleeping sickness was discontinued and I was posted to West Nile! Later in Kenya in 1957–1958, I was again involved in studying 'Rhodesiense' sleeping sickness on Utonga ridge on the shore of Lake Victoria. In neighbouring Uganda, the vector was *G. fuscipes* but

in the uninhabited peninsula, fishermen became infected and the vector was shown to be *G. pallidipes*. However, the reservoir host had still not been identified despite enormous efforts in Tanzania where >600 volunteers were infected with *T. brucei* (?*T. rhodesiense*) from game animals. No-one became infected and they were all riding around on bicycles (the fees for participating in the experiment). I strongly suspected that the absence of human sleeping sickness in most of the local people and in the volunteers was due to the principle of cross-protection, because Africans living in close proximity to game and tsetse are constantly bombarded with different antigenic variants of *T. brucei* and other species of trypanosome. No-one had either the courage or imagination of Jenner to challenge any of the volunteers to see if they were resistant to *T. rhodesiense*. Despite antigenic variation there is now evidence of resistance to re-infection in *T. gambiense* infections [30]. A further outcome of our studies in Kenya was the first demonstration of an animal reservoir following the inoculation of volunteers with trypanosomes originating from a bush buck on Utonga Ridge. In the very brief and inaccurate account of this investigation in the *British Medical Journal* [31], Heisch and his colleagues say 'The bush buck strain was inoculated into the forearm of Mr C. A. W. Guggisberg, mammalogist on our staff. On the ninth day the arm was very swollen. Fluid from the blister contained about three typanosomes per oil immersion field. At 11 pm the temperature rose to 106.9°F (41.6°C). At 11.45 am he was given his first injection of Antrypol. Response to treatment was dramatic and recovery uneventful'.

The authors, who were not present, failed to give credit to the field and laboratory staff and they made no mention of the problem we had of keeping the hyperpyrexial Guggisberg alive. He fell unconscious in my laboratory at 11 am, not 11 pm as reported, and I had to put him in the cold room with my *Trichinella*-infected hyaenas whilst I searched for some Antrypol. Guggisberg made a good recovery but due to my 'interference' I was again prevented from working on the trypanosomes. Fortunately I moved to Turkana to study the equally fascinating subject of hydatid disease; another zoonosis where the Jennerian principle of cross-protective immunity may explain the peculiar incidence and distribution of the disease in Kenya and elsewhere.

Conclusion

With all the recent advances in immunology and molecular biology we can now study the interactions of pathogenic and non-pathogenic organisms in man and demonstrate the fundamental significance of the Jennerian principle of cross-protective immunity in terms of man's survival. It is my hope that the 200th anniversary of Jenner's experiments on smallpox will

emphasise the much wider significance of his studies in terms of most of the infectious diseases of man and animals.

FOOD- AND MILK-BORNE ZOOTIC INFECTIONS

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Food animals can be infected with or colonised by bacteria which are potentially pathogenic for man. Principal amongst such organisms are campylobacters, salmonellae and pathogenic serotypes of *Escherichia coli*, and this article will concentrate on these bacteria. Zoonotic infections most often occur as a consequence of product contamination during either slaughter or food production. It is also possible for people to become infected as the result of direct or indirect contact with infected animals. Such a route would seem to be important in the current upsurge in the UK of infections caused by *Salmonella typhimurium* DT104 [32, 33]. However, infections of this sort are outside the scope of this review.

Campylobacter infections

The epidemiological pattern of human campylobacter infections in the UK is typical of the picture seen in most developed countries, with *Campylobacter* spp. being more frequently isolated from infected stools than other bacterial pathogens. At present, 15 species of *Campylobacter* have been identified and eight are considered to be of clinical importance (Table 6). Of these, *C. jejuni* is the most important and, in the UK, this species is responsible for c. 95% of human infections. Almost all of the remaining 5% are caused by *C. coli*, although *C. upsaliensis* is becoming increasingly recognised. There has generally been a year-on-year increase in campylobacter infections since it was first identified as a human pathogen in the late 1970s (Fig. 1) although the number of confirmed cases

Fig. 1. *Campylobacter* (◆), and *salmonella* (■) infections in England and Wales 1981–1995.

in England and Wales in 1995 showed a small decrease (Fig. 1).

Most campylobacter infections are sporadic and between 1988 and 1992 only 10 general outbreaks of infection were reported. *C. jejuni* and *C. coli* can be isolated from the intestinal tract of a range of domesticated and wild animals and environments. Their ubiquity means that various vehicles have been identified with infection. However, of these, contaminated chicken meat and unpasteurised cows' milk are the most important [34, 35].

Campylobacter and poultry

The production of chicken meat is highly efficient and birds are reared under the broiler system where they

Table 6. *Campylobacter* species of clinical or potential clinical importance

Species	Frequency of importance	Common illnesses	Uncommon illnesses
<i>C. jejuni</i> <i>C. coli</i>	Common	Diarrhoea	Bacteraemia, appendicitis, reactive arthritis, Guillain-Barré syndrome
<i>C. fetus</i> subsp. <i>fetus</i>	Common	Bacteraemia Diarrhoea	Septic abortion, septic arthritis, abscesses, meningitis, peritonitis, salpingitis
<i>C. upsaliensis</i>	Uncommon	Diarrhoea	Bacteraemia
<i>C. lari</i>	Uncommon	Diarrhoea	Bacteraemia
<i>C. butzleri</i>	Unknown	Diarrhoea	Bacteraemia, appendicitis
<i>C. hyointestinalis</i>	Unknown	Diarrhoea Proctitis	...
<i>C. sputorum</i>	Unknown	Abscesses	...
<i>C. concisus</i>	Unknown	Periodontal disease	Foot ulcer, diarrhoea

are housed intensively in controlled conditions with food and water available *ad libitum*. Chickens generally become campylobacter-positive during the first 2–3 weeks of life [36] and remain colonised until slaughter at 6–7 weeks [36]. Poultry slaughter is intensive and modern production lines can process >100 birds/minute. Cross-contamination is common and the prevalence of campylobacter-positive carcasses usually exceeds that of colonised live birds. Studies in the UK have demonstrated that chicken carcasses can be heavily contaminated with campylobacters on their surface [37] and in the abdominal cavity and this can lead to extensive cross-contamination in the kitchen [38]. However, of possible greater public health importance is the fact that *C. jejuni* can be isolated from muscle tissue of processed chicken carcasses [39, 40]. The importance of undercooked chicken in the epidemiology of human campylobacter infections may be evidence of the significance of muscle contamination [41].

Control at slaughter is rarely successful and there is general agreement that on-farm measures are more likely to succeed. Investigations in the UK have demonstrated that the principal source of campylobacters in broiler flocks is the environment around the broiler house [36]. Biosecurity measures such as boot dipping in strong disinfectant or boot changing before staff enter the broiler house have been shown to prevent the colonisation of chickens [36]. These measures may be difficult to maintain over a long period and other approaches are being explored.

Past work in Exeter revealed that young, campylobacter-free chickens have a caecal microflora which is capable of inhibiting *C. jejuni* *in vitro* [42]. It would also seem that the campylobacter status of a flock is linked closely to the *in-vitro* inhibition. Once this can no longer be demonstrated birds rapidly become colonised [42]. Current research is aimed at producing cultures, derived from campylobacter-free caeca, which could be given to chickens to better enable them to resist campylobacter infection.

Campylobacter and milk

Dairy cows are frequently campylobacter-positive and appear to acquire the organism by the consumption of contaminated water [43]. Some strains of *C. jejuni* can cause mastitis in dairy cows and such infections have led to milkborne outbreaks [44]. More usually, however, milk becomes campylobacter-positive as a consequence of faecal contamination during milking [43]. Although levels of contamination are generally low (<1 cell/ml) studies in Exeter showed that some milk samples contained levels of *C. jejuni* sufficient to cause infection [43]. The examination of samples of raw milk on sale to the public in Devon revealed that 6% were campylobacter-positive [45]. Milkborne campylobacter-

iosis can be controlled by pasteurisation as the bacteria are heat-sensitive [46].

Zoonotic salmonellosis

There are >2200 different *Salmonella* serotypes and the great majority are believed to be capable of causing human infection. In practice, the epidemiology is dominated by a few serotypes, with Enteritidis and Typhimurium being most important. In the mid-to-late 1980s, Western Europe, including the UK, saw a marked increase in the incidence of human salmonellosis (Fig. 1) almost exclusively caused by Enteritidis [47]. Phage type 4 (PT4) was most important and the pandemic of human infection caused by this bacterium and related phage types is a consequence of their invasive nature in chickens. In broilers (meat birds), infected flocks often show raised mortality and stunting with seriously ill birds exhibiting pericarditis and perihepatitis [48]. PT4 readily causes septicaemia in chickens which leads to the bacterium being lodged in small blood vessels at slaughter and the resultant contamination of muscle tissue [39]. The identification that cooked take-away chicken is an important vehicle of PT4 infection may be a consequence of this [49].

The behaviour of Enteritidis in laying hens is very different. Although the organism is highly invasive and can be isolated readily from reproductive and other tissues [50, 51] there are no overt signs of illness, with infected birds laying and eating as normal. Infection of reproductive tissue will lead to contamination of egg contents and studies on infected commercial flocks have demonstrated contamination rates of *c.* one egg per 1000 (Table 7). The most important site of infection in the bird with regard to contamination of egg contents is the upper oviduct [50]. Infection at this site means that, in contaminated egg contents, Enteritidis will be either on the outside of the yolk membrane or in the albumen immediately surrounding it. In fresh, intact eggs, contamination levels are low (<20 cells/egg) and the bacterium is unable to grow because albumen is an iron-restricted environment, until it can invade the yolk which is rich in iron [52]. The yolk membrane will only permit the movement of salmonellae once storage-related increases in permeability have taken place [52].

In eggs stored at a constant 20°C, yolk invasion is possible after 3–4 weeks [52]. In eggs stored at 30°C, yolks will become salmonella-positive after 6–10 days [53]. Yolk membrane breakdown is also accelerated by storage at fluctuating temperatures or under high humidity [53]. The safety of eggs can be much improved by storage at low temperature, particularly in the home [54]. Control of the public health problems associated with Enteritidis infection in chickens is being mediated through the European

Table 7. Prevalence of *Salmonella* serotype Enteritidis* in eggs originating from naturally infected poultry laying flocks

Flock type	Number of flocks	Number of eggs examined	Number (average %) of contents positive
Commercial layer, free range	9	2412	24(1.0)
Commercial layer, battery	8	2489	10(0.4)
Layer-breeder	1	1120	1 (0.1)
Broiler-breeder	2	1558	10(0.6)
Experimental†	2	1119	11(1.0)
Total	22	8698	56 (0.6)

*All isolates, except one which was PT33 and another which was PT4a, were Enteritidis PT4.

†Two small flocks of naturally infected laying hens were caged individually with each egg laid by each hen examined over a 3-month period.

Union Zoonoses Directive which requires the regular testing of poultry breeding flocks [55]. Those found to be positive for Enteritidis are slaughtered.

There has been a recent marked rise in human infection caused by Typhimurium DT104 [32, 33] and 3837 cases were recorded in England and Wales in 1995 (P. Wall, personal communication). Similar trends have been seen in food animals, particularly cattle [56]. The great majority of isolates are resistant to a wide range of antibiotics and infection with these strains resulted in higher rates of admission to hospital and mortality than with many other salmonellae [32]. Infection has been associated with a range of vehicles, including pork sausages from a restaurant or take-away, chicken from similar outlets, chicken from a local butcher and consumption of a particular brand of meat paste [32]. The epidemiology of Typhimurium DT104 in cattle is not yet understood but is being investigated [56]. Studies in South West England on 79 infected cattle herds in 1995 have indicated that animals can become infected following consumption of sewage-contaminated water or pasture. Initial investigations in Exeter suggest that strain DT104 is better able to survive in water or on surfaces than other salmonellae.

Verocytotoxic Escherichia coli

Many serotypes of *E. coli* are capable of producing toxins that damage cultured African green monkey kidney cells (Vero cells). Such bacteria are classed as verocytotoxic [57]. The most important serotype is *E. coli* O157:H7 and infections caused by this bacterium have shown a marked upward trend although, in comparison with salmonellae and campylobacter, infection rates are relatively low [57], but this bacterium can cause haemorrhagic colitis. Haemolytic uraemic syndrome (HUS), a type of kidney failure, is an important complication of haemorrhagic colitis. Children are most at risk and in some outbreaks up to 30% of infected people have later developed HUS. The condition is now the commonest cause of renal failure in children in the UK [57].

The primary reservoir of *E. coli* O157:H7 seems to be cattle [58, 59], although the bacterium has been isolated, on occasions, from other food animals. A variety of foods, ranging from raw potatoes to turkey roll, have been implicated in outbreaks, but milk and beef products are most important [57]. Milk may be contaminated as a consequence of poor milking hygiene and beef carcasses become contaminated during slaughter. *E. coli* O157:H7 has been isolated from the faeces of both beef and dairy cattle and from both young and adult animals [57–59]. The epidemiology of the bacterium in cattle is not yet fully understood. Current data suggest that infection in cattle is transitory and there is cycling of the bacterium within herds, particularly if they are housed [60].

Control measures for *E. coli* O157:H7 in animal production have not yet been identified and the control of food- and milk-borne infection rests principally with the pasteurisation of milk and the proper cooking of comminuted beef products [61]. *E. coli* O157:H7 appears to be more acid tolerant than other serotypes of *E. coli* and this may enable cells to survive gastric acidity, which facilitates transfer to the lower intestinal tract.

HANTAVIRUS INFECTIONS

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There have been several reviews of hantavirus and hantavirus infections recently [62–65], so this text will concentrate on recent developments and zoonotic aspects of the virus.

The virus

Hantavirus is a genus within the family *Bunyaviridae*. Unlike all the other genera it is not transmitted from its

Fig. 2. A negative stain electron micrograph of Puumala virus (bar = 50 nm). (From: *A Colour Atlas of Medical Microbiology*: Mosby Wolfe, UK).

reservoir host *via* an insect vector. Hantavirus (Fig. 2) is a spherical enveloped virus (100–120 nm in diameter) with a single-stranded negative sense RNA genome of 4.5 MDa. The genome consists of three segments: large (L = 6500 nucleotides), medium (M = 3700 nucleotides) and small (S = 1800 nucleotides). The L segment encodes a virion-associated RNA-dependent RNA polymerase [66]. The M segment encodes two glycosylated envelope glycoproteins (G1 and G2) and the S segment encodes a protein [67] that forms the helical nucleocapsid. G1 and G2 are glycosylated envelope glycoproteins that have haemagglutinating activity and are therefore presumed to mediate attachment of the virus to its host cell. The receptor for the virus is unknown but monoclonal antibodies that inhibit haemagglutination do not necessarily inhibit infectivity, suggesting receptors other than those expressed on erythrocytes [67]. Viral entry is presumably by membrane fusion [68] and appears to be mediated by the G2 glycoprotein. Recently, a single base change that resulted in a substitution of serine for glycine at amino acid position 1124 of the G2 glycoprotein has been associated with increased virulence of Hantaan virus for suckling mice [69]. Replication follows production of mRNA by the viral RNA polymerase. The virus is assembled at the smooth intracellular membranes of, in particular, the

Golgi apparatus. As it does not possess a matrix (or M) protein, its assembly must be orchestrated in a manner different to other negative strand RNA viruses such as influenza or measles viruses [67]. The virions bud into the Golgi apparatus and are transported to the cell surface where they are released by exocytosis. Hantaviruses produce persistent and usually asymptomatic infection in their natural mammalian hosts (usually rodents). Infection is normally transmitted to man by inhalation of dried excreta (urine or faeces) of the reservoir host. Hantavirus is subdivided into at least 18 (Table 8) different subtypes based on animal reservoir, human disease pattern, antigenic properties and more recently on RNA sequence data (principally M and S segments). The nucleocapsid protein carries antigens (or has a gene sequence) that are common to all hantaviruses, and the G1 and G2 gene sequences show variability that can distinguish between subtypes.

The application of PCR technology to the study of hantaviruses has not only led to rapid diagnostic methods but to an explosion in the description of 'new' hantavirus subtypes. Hantaan-like viruses persistently infecting fieldmice (*Apodemus flavicollis*) and causing haemorrhagic fever with renal syndrome (HFRS) have been described from the Balkan region. These include Dobrava [70], Porogia [71] and

Table 8. Hantavirus subtypes

Subtype	Animal reservoir	Human disease	Geographic distribution
Hantaan	<i>Apodemus agrarius</i> (field mouse)	HFRS (severe)	Japan, Korea, East China, Russia
Belgrade	<i>A. flavicollis</i> ?	HFRS (severe) + HPS	Balkans
Dobrava	<i>A. flavicollis</i>	HFRS (severe)	Balkans
Porogia	<i>A. flavicollis</i>	HFRS (severe)	Balkans
Bat	<i>Rhinolophus ferrum-equinum</i> (bat)	Unknown	Korea
Seoul	<i>Rattus norvegicus</i> (rat)	HFRS (moderate)	Worldwide
Puumala	<i>Clethrionomys glareolus</i> (bank vole)	HFRS (mild) (Nephropathia epidemica + HPS)	North-west Europe
Puumala-like	<i>C. rufocanus</i>	Not yet known	Japan
Leaky	<i>Mus musculus</i> (mouse)	Unknown	USA
Prospect Hill	<i>Microtus pennsylvanicus</i> (vole)	Unknown	USA
Sin Nombre	<i>Peromyscus maniculatus</i> (deer mouse)	HPS	USA
Shelter Island	<i>P. leucopus</i> (white footed mouse)	HPS	USA
Bayou	<i>Oryzomys palustris</i> (rice rat)	HPS	USA
Black Creek Canal	<i>Sigmodon hispidus</i> (cotton rat)	HPS	USA
El Moro Canyon	<i>Reithrodontomys megalotis</i> (harvest mouse)	Unknown	USA
Rio Segundo	<i>R. mexicanus</i> (harvest mouse)	Unknown	USA
Rio Mamore	<i>Oligoryzomys microtis</i>	Unknown	Bolivia
Tula	<i>Microtus arvalis</i> (vole)	Unknown	North-west Europe
Thottapalyam	<i>Sorex araneus</i> (shrew)	Unknown	India
Thailand	<i>Bandicota indica</i> (bandicoot)	Unknown	Thailand

HFRS, haemorrhagic fever with renal syndrome; HPS, hantavirus pulmonary syndrome.

Belgrade [72]. In addition to HFRS, Belgrade also appears to cause hantavirus pulmonary syndrome (HPS) [72]. Two 'new' Hantaan-like viruses have been isolated from the lungs of bats (*Rhinolophus ferrum-equinum* and *Eptesicus serotinus*) but their role in human disease is unknown [73].

Few variants of Seoul virus have been described, but comparisons of the M segment single large open reading frames (ORF) of SR-11 (the prototype strain) with the Biken (B1) strain showed 97% homology at the nucleotide level and 98% and 99% homology for the deduced amino acids of G1 and G2, respectively [74]. However, there was significant heterogeneity in the non-coding part of the M segment. Recently a Puumala-like virus has been isolated from *Clethrionomys rufocanus* in Japan [75]. Again, no human disease has yet been described.

In 1993 a hantavirus (Sin Nombre) was shown to be the cause of HPS [76]. Since then there has been an explosion of 'new' related hantaviruses. These include Shelter Island [77], Bayou [78], Black Creek Canal [79] and El Moro Canyon [80]. The rodent host for Sin Nombre is the deer mouse (*Peromyscus maniculatus*); for Shelter Island, the white-footed mouse (*P. leucopus*); for Bayou, the rice rat (*Oryzomys palustris*); for Black Creek Canal, the cotton rat (*Sigmodon hispidus*) and for El Moro Canyon, the western harvest mouse (*Reithrodontomys megalotis*). There is also evidence of genetic re-assortments occurring naturally in HPS-associated hantaviruses [81]. Finally, in north-west Europe the common vole (*Microtus arvalis*) has been found to excrete Tula virus, a hantavirus that is related to Puumala, Prospect Hill and Sin Nombre viruses [82].

The reservoir

In addition to the rodent hosts listed in Table 1 hantaviruses have been detected in or isolated from *C. rustilus*, *A. sylvaticus* and *Microtus agrostis* in European Russia [83] and *Calomys musculinus* (the vesper mouse) in Argentina [84]. There is also evidence that hantavirus infection is not limited to rodents. Infection has been demonstrated in insectivora such as *Suncus murinus* [85] and *Crocidura russula* (the common shrew) [86], in bats [73], in feral and domestic cats [87, 88], and even birds [89]. Whether these species play a role in transmission of infection to man is not clear, although possession of cats is a risk factor for HFRS in China [90]. In serological surveys hantavirus infections have been detected in most parts of the world [91], although different subtypes appear to be geographically limited. The exception to this appears to be Seoul virus, which is found in *Rattus* spp. throughout the world but especially in harbour and urban areas. The effect of hantavirus infection on the reservoir host is minimal to non-existent, despite the fact that virus is present in many tissues throughout the body. It appears that once infected the rodent host will continue to excrete virus for its lifetime. Hantavirus is transmitted to man when either man enters the reservoir hosts' ecological niche (for example in the Korean War) or the reservoir host enters man's niche (at times of rodent population explosion or shortage of food). Infection is transmitted to man by inhalation of dried rodent faeces, urine or saliva and has also been transmitted by bite. Rare cases of infection have been associated with handling rat plasmacytoma cells in the laboratory [92]. The fact that the reservoir host species do not develop disease when infected with hantavirus makes development of animal models difficult. In-

traperitoneal or intracerebral inoculation of suckling mice with Hantaan virus produces fatal infection [93], but this does not replicate the human disease. Recently infection of macaques with Puumala virus has been shown to provide a model which reproduces human disease [94].

Human disease

Infection in man falls into two major manifestations: one with haemorrhage and renal failure (HFRS) and one presenting with acute respiratory distress (HPS). HFRS can be severe (due to Hantaan and Hantaan-like viruses), moderate with some evidence of hepatitis (Seoul) or mild (Puumala). The most severe form (e.g., Korean haemorrhagic fever) progresses through five distinct phases – influenzal, hypotensive, oliguric, diuretic and convalescent. Most deaths occur in the oliguric phase but the high mortality (10–20%) seen in the Korean War has been decreased to 5% with appropriate management. In some instances, for example with Belgrade virus, pneumonic signs also occur [72]. Moderate HFRS due to Seoul may progress through phases but most often they are blurred. Liver involvement occurs more often with Seoul but the mortality rate is low (*c.* 1%). Puumala causes nephropathia epidemica (NE) in northwestern Europe. This is usually a mild disease with only 1 in 14 to 1 in 20 of those infected developing clinical disease. The clinical features of NE include high fever, abdominal or back pain (or both), headache, nausea and vomiting. There is usually proteinuria, microscopic haematuria and elevation of serum creatinine, reflecting the occurrence of acute tubular and interstitial nephritis. Up to a third of patients will develop haemorrhagic manifestations following thrombocytopenia but virtually all recover. NE in children is very similar to that in adults [95]. Finally there have been recent reports of infection with Puumala in Germany which had only pulmonary features [96].

Previously, a strong association between Seoul virus infection and chronic renal failure was demonstrated in Baltimore [97]. However, subsequent studies have not demonstrated such an association for Puumala in the Netherlands or Sweden [86, 98] nor for Seoul or Sin Nombre viruses in Pima Indians [99].

The first cases of HPS were described in May 1993 in south-western USA [100], although there is evidence of a fatal infection occurring 10 years earlier in Kansas [101]. Most patients have an influenza-like prodromal phase lasting 3–4 days followed by a rapid progression to tachypnoea, tachycardia, hypotension, extensive pulmonary oedema, hypoxia and metabolic acidosis [100, 102, 103]. The mortality rate is high (50–70%) and mild disease is extremely rare [103]. The features that most clearly distinguish HPS from pneumococcal pneumonia, adult respiratory disease syndrome and influenza on admission are dizziness,

nausea and vomiting and absence of cough; and in the laboratory, thrombocytopenia, low serum bicarbonate and elevated haematocrit [102]. HPS with Sin Nombre-related viruses has been described only from the Americas. Most infections are in North America and Canada [62] but some have been reported from Brazil [104].

Despite the high prevalence of persistent infection in reservoir hosts, infection in man is not common. In China and European Russia there is an annual incidence of 30–168/100 000 population [91]. In Sweden over a 4-yearly cycle the incidence of NE is 1.3–20/100 000 [105]; these changes corresponded to changes in bank vole population numbers. In the USA the incidence of HPS is estimated at 0.02/100 000 population. Serosurveys in the Netherlands (25) and Northern Ireland [106] have indicated that 0.9% of those tested have anti-Puumala and 2% have anti-Seoul antibodies, respectively. The degree and method of exposure to the rodent reservoir host is probably the major determinant for developing disease [107, 108]. In this respect it is noteworthy that hantavirus (presumably Seoul) antibodies were detected in *R. norvegicus* on four of 11 farms sampled in England and Wales and that, overall, 4% of rats were seropositive [1].

Treatment and prevention

For severe HPS or HFRS the management must include early admission to an intensive care unit where it is important to monitor blood and tissue oxygenation, cardiac output, central blood pressure and intracranial pressure. Ribavirin has been administered intravenously on a named patient basis in HPS with some benefit. A placebo-controlled trial of intravenous ribavirin in HFRS demonstrated benefit [109]. Other adjunctive therapies remain speculative except that corticosteroids have also been shown to be of benefit in HFRS [110].

Despite the absence of evidence for person-to-person transmission of hantavirus it must be remembered that tissue, blood and other body fluids from patients with HFRS or HPS might be infective. Universal precautions should be taken to avoid inoculation or mucosal contamination and if there is a likelihood of splashes or aerosolisation, respiratory and eye protection should be used. All hantaviruses are currently classified at category 3 containment.

To a large extent, prevention of hantavirus infection involves prevention of contact between man and excreta of the reservoir hosts [62]. Transmission does appear to require close contact. For example, in an outbreak of Puumala infection in US troops on manoeuvre in Germany, infection was most often associated with seeing rodents or using hay for bedding [108]. It is noteworthy that no such infections occurred over the same period in the local civilian

population. Infection also appears to be an occupational hazard. A survey of European mammalogists revealed that 11% had antibodies to Puumala [111], a significantly higher proportion than that (0.7%) seen in Dutch blood donors [86]. In the Dutch survey [86], 2.2% of laboratory workers, 4% of foresters and 5.8% of trappers were also seropositive.

Inactivated Hantaan virus vaccines have been used in Korea in high risk groups since 1990 [112]. Unfortunately this has as yet not resulted in a decrease in numbers of cases [73].

POXVIRUS ZONNOSES

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Now that smallpox has been eradicated the remaining poxvirus infections of humans are, with the exception of molluscum contagiosum, acquired directly or indirectly from animal sources (Table 9). Representatives of three poxvirus genera are involved and the lack of an antigenic relationship between members of different genera and the characteristic morphology of parapox virions aids laboratory diagnosis [113, 114]. The infections range from the relatively trivial occupational hazard posed by parapox infections which occur world-wide, to relatively rare but serious infections such as monkeypox which is geographically restricted (Table 9). Prevention of human infection, where possible, is largely dependent on a better understanding of the relationship between indicator hosts, including man, and reservoir hosts.

Parapoxvirus infections

Parapoxviruses cause world-wide infection in cattle (paravaccinia, pseudocowpox, ring sore) and sheep and goats (orf, contagious pustular dermatitis, scabby mouth). The viruses are very closely related if not identical, and human infection is designated according to the animal source if known; an alternative term for human parapox infection acquired from cattle is milker's nodes. Current UK practice is for human parapox infections to be recorded as orf/paravaccinia, whatever the source.

Human infection is an occupational hazard of farm, abattoir and veterinary workers [115, 116]. Rather more human cases are acquired from sheep than from cattle (Table 10), and this probably represents differences in farming practice. Reports of human infection are higher in spring and autumn, which coincides with lambing and calving seasons.

Human infection usually results in a single, granulomatous, relatively painless lesion which heals uneventfully if sometimes slowly [115, 116]. Many patients do not seek medical help, some diagnosed cases go unreported and the true incidence of human infection is unknown (Table 10). A limited special survey in one rural general practice in which cases were actively sought detected a relatively high incidence (Baxby and Welton, unpublished observations, Table 10). Lesions may occasionally be multiple and 'giant' lesions sometimes occur [117] which may be misdiagnosed as malignancy [115]. Attention paid to the occurrence of erythema multiforme in those cases seen at referral [115] overestimates its incidence because so many routine cases go unreported.

Table 9. Zoonotic poxvirus infections

Virus (genus)	Distribution	Sources of human infection*	Comment
Orf/Paravaccinia (Parapoxvirus†)	World-wide	Sheep (R), goats (R), cattle (R)	Relatively painless granulomatous skin lesions; occupational hazard of those in contact with reservoir hosts
Monkeypox (Orthopoxvirus)	W. Africa especially Zaire	Squirrels (R), (monkeys)	Severe generalised infection; >400 cases since 1970; c. 90% in children with c. 11% mortality; limited case-to-case spread
Cowpox (Orthopoxvirus)	Europe including some states of former USSR	Cats (cows), (rodents, R)	Painful haemorrhagic ulcer; relatively more common in young girls
Buffalopox (Orthopoxvirus)	India	Buffalo (R?)	Occupational hazard; painful skin lesion; skin lesion virus is subspecies of vaccinia
Tanapox (Yatapoxvirus)	Zaire, Kenya	Monkeys (R)	Nodular skin lesions usually singular, with pyrexia

*R, reservoir hosts; (), less important known sources. With all infections indirect spread *via* fomites is probably important.

†Morphologically distinct.

Table 10. Human parapoxvirus infections in England and Wales, 1978–1995*

Period†	Number of human cases	Source		
		Ovine	Bovine	Slaughterhouse
1978–1986 total	412	135	26	31
Year mean	46	15	3	5
Range	39–61	9–25	1–5	3–8
1990–1995 total	86	26	4	1
Year mean	15	4.3	0.7	—
Range	11–25	2–9	0–1	—
‡1981 special survey	12	10	2	0

*Source: Communicable Disease Surveillance Centre and unpublished data.

†Reported cases dropped significantly during 1987–1989, and from 1990 CDSC records were simplified.

‡An active prospective survey in one rural general practice covering a sheep farming area. Data not supplied to CDSC (D Baxby, J Welton, unpublished data).

The immune response is poor and re-infections occur [116, 118]. It is estimated that most people exposed will be infected at least once [116], although any prudent steps available should be taken to prevent infection, and in particular to prevent transmission to contacts. Debility in animals is caused by interference with feeding of lambs and calves, rather than by the infection itself.

Orthopoxvirus infections

Monkeypox. Monkeypox virus was so named because it was first isolated from captive Asiatic monkeys. However, human cases have only been detected in west and central Africa, particularly Zaire. Serological surveys of wildlife have found little or no evidence of simian monkeypox, although antibody to monkeypox virus has been found in 25% of two species of African squirrels [119], which are believed to be the reservoir hosts [119, 120]. Surveillance activities during the later stages of the smallpox eradication campaign, particularly in Zaire, provided detailed information on >400 cases of human monkeypox and their contacts [120–122]. Clinically the disease resembles smallpox but with more obvious lymphadenopathy particularly involving the submandibular, cervical and inguinal regions. In a survey of 338 cases seen during 1981–1986 in Zaire [121], 291 (86%) were in children under 10 years old and of these only 12 (4%) had previously been vaccinated against smallpox. In all, 33 deaths occurred among the 338 cases (10%) but all the deaths were of unvaccinated children <8 years old, and in the 0–4 age group mortality was 15% [121].

Despite the severity and mortality of endemic monkeypox, control *via* routine smallpox vaccination is not recommended, although it is necessary for laboratory workers who study the virus [123]. Secondary attack and case-to-case transmission rates are low and rarely occur for more than two or three generations [122, 124]. These can be further reduced by early recognition and isolation of suspect cases. The threat of initial infection can be reduced by education in the use of animals and land so that

human activity and cultivated land are separated from the arboreal reservoir by an appropriate buffer zone [119, 120]. Studies of smallpox and monkeypox viruses have shown their separate identities [125], and there is no reason to suppose that the virulence of human monkeypox will increase.

Cowpox. Human cowpox is a relatively rare zoonosis which occurs sporadically in the UK and across Europe and some western states of the former USSR. Attention was first drawn to cowpox by Edward Jenner, the bicentenary of whose vaccination was celebrated in 1996. His analysis of ‘true’ cowpox which protected against smallpox, and ‘spurious’ cowpox which did not, were important early contributions to the comparative study of zoonoses long before the nature and diversity of microbial pathogens were known [126].

However, the term cowpox, like monkeypox, is a misnomer. Although occasional human infections are acquired from cattle, most human patients have no contact with cows, and bovine infection is too rare for the virus to be maintained in cattle [127] (Table 11). Of the 24 cases with a known source surveyed since 1969 only three were acquired from cattle [114], and the last human case acquired from cattle in the UK occurred in 1976 [128]. The virus circulates in wild rodents; in the UK these are field and bank voles and woodmice [129] (Table 11). However, the most commonly detected victim and source of human

Table 11. Prevalence of antibody to cowpox virus in British animals

Species	Number tested	Number (%) positive
Field vole	16	8 (50)
Bank vole	63	25 (40)
Wood mouse	106	17 (16)
House mouse	13	1 (8)
Cattle	1076	7 (0.7)
Domestic cat	138	1 (0.25)
Rat	65	0 (<1.5*)
Badger	31	0 (<3.0)
Squirrel	39	0 (<2.5)
Deer	27	0 (<3.7)

*Figure given in percentage represented by one positive specimen out of the number sampled.

cowpox is the domestic cat (Table 12). The cat is not a reservoir host but acts as a major link between the reservoir and humans [114, 130]. Although the case numbers are too low for detailed analysis, a higher proportion of patients are young females, which is perhaps consistent with a tendency for such individuals to play with and care for cats [114] (Table 12). Of 54 cases surveyed since 1969, 25 were in patients <18 years old, 16 of them female [114].

In most cases (80%) of human cowpox [114] there is one lesion, usually on the hand (46%) or face (35%). It presents as a painful haemorrhagic lesion, sometimes in its later stages mistaken for anthrax [114, 131]. Lymphadenitis and general symptoms such as pyrexia or a 'flu-like' illness are common and 30% of patients are admitted to hospital. Most cases recover in 6–8 weeks but severe cases may take up to 12 weeks. Occasionally severe generalised infection and death have been reported in immunodeficient individuals [114, 132], but no cases have been reported in HIV-infected individuals in whom such severe infections might be expected to occur.

The key to the clinical diagnosis of human cowpox is the painful nature of the lesion and a properly taken history which takes account of feline contact, and the fact that most cases occur in late summer to early autumn [114, 130]. Given the restricted host range and geographical distribution of poxviruses with a similar morphology, electron microscopy can be used to provide a rapid diagnosis of cowpox and eliminate, or where appropriate provide an alternative diagnosis of, parapox or herpesvirus infections [114]. Feline cowpox is a generalised infection but with a low mortality [130]. The virus is probably not very infectious for man, and no case of human cowpox has occurred in anyone caring for a sick cat after the feline case has been diagnosed and simple precautions taken [114, 130].

Buffalopox. Buffalopox virus, closely-related to vaccinia virus [133] and now regarded as a subspecies of it [134], circulates in India and causes localised painful vesicular lesions in those in contact with infected

animals [135, 136]. Infection is an occupational hazard but it is not known whether other, perhaps wildlife, reservoirs are involved in its epidemiology.

Tanapox

Tanapox virus is the term given to a distinct poxvirus, isolated from patients with localised skin lesions in the Tana river valley of Kenya in 1962 [16]. Since then the virus has been detected in other countries of central Africa, particularly Zaire, where details of 264 laboratory-confirmed human cases were recorded in 1979–1986 during monkeypox and post-smallpox eradication surveillance activities [137]. Most patients (80%) have one lesion, usually on the exposed areas, although often the face is spared. The nodular lesions are relatively large (>10 mm) and often break down to form ulcers. There is usually a short prodrome with fever, head- and back-ache, and lymphadenopathy when the lesions develop [16, 137].

Tanapox is a zoonosis acquired from monkeys, but relatively little is known about its epidemiology. Epidemics have occurred due to crowding of people and animal species during floods [16] but man-to-man spread is extremely rare. It has been suggested [16] that indirect transmission may involve arthropod vectors, a suggestion consistent with the distribution of lesions in human cases.

Conclusions

Now that smallpox has been eradicated human poxvirus infections are perhaps of interest rather than importance. Although orf may cause economic problems for communities dependent on sheep or goat farming [116], animal poxvirus infections are relatively unimportant [138]. Diagnosis of individual human cases can be improved by knowledge of the geographical distribution and host range of the viruses (Table 9) and the use of electron microscopy to exclude herpesviruses and recognise the characteristic morphology of parapoxviruses. Further information about the reservoir hosts and transmission of monkeypox, cowpox, buffalopox and tanapox would significantly

Table 12. Sources of human cowpox 1985–1993*

Number (%) of cases according to age (years) and sex											
	Total	0-6		7-12		13-18		>18		Total	
Source		M	F	M	F	M	F	M	F	M	F
Unknown	12 (34)	0	1	1	2	3	1	2	2	6 (38)	6 (32)
Feline	18 (51)	0	2	1	4	1	1	5	4	7 (44)	11 (58)
Occupational	3 (9)	0	0	0	0	0	0	2	1	2 (12)	1 (5)
Rodent?†	2 (6)	0	0	0	1	0	1	0	0	1 (3)	1 (5)
Bovine	0	0	0	0	0	0	0	0	0	0	0
Subtotals	35	0	3	2	7	4	3	9	7	16 (46)	19 (54)
Total	35	3		9		7		16		35	

*re-arranged from data in ref. [114].

†Epidemiological evidence only, no laboratory studies on suspect animals.

increase our understanding of the infections and their management. However, in the UK, financial restraints may restrict investigation of rare infections such as human cowpox [114, 139]. Consequently, future cases may go undiagnosed and trends in epidemiology unexplored.

ZOONOTIC PROTOZOA

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This short review focuses on the three most important zoonotic protozoal infections in the UK – toxoplasmosis, cryptosporidiosis and giardiasis (although there is still debate about the degree to which giardiasis is zoonotic) and will briefly highlight some of the recent developments in understanding of the biology and transmission of the causal organisms. The number of diagnosed cases reported to the Communicable Diseases Surveillance Centre of the PHLS during the last 5 years is shown in Table 13. Whilst these are undoubtedly underestimates, they reveal no trends in incidence over that period, with giardiasis and cryptosporidiosis being the most frequently occurring endoparasitic infections in Britain.

Although reported cases of toxoplasmosis are much fewer, this is arguably the most serious infection, as disease can follow either primary infection in pregnancy, leading to fetal abnormality, or recrudescent chronic infection provoked by immunosuppression. Toxoplasmosis is also an important veterinary problem, as a major cause of ovine abortion [140]. The causal agent, the apicomplexan protozoan *Toxoplasma gondii* (Fig. 3) has a complex life-cycle in which systemic infection and asexual multiplication can occur in any warm-blooded animal but enteric infection involving sexual reproduction occurs only in *Felidae* – which for practical purposes in Britain means only in the cat. As the only animal that excretes infective forms into the environment (as oocysts), the cat is pivotal in the epidemiology of toxoplasmosis. Transmission from all other hosts can

only be by either ingestion of their tissues (carnivorism or cannibalism) or by transplacental infection. The flow of infection of *Toxoplasma* is indicated in Fig. 4.

The frequency of human congenital infection in the UK is low but there is an increasing antibody prevalence with age to a rate of c. 40% [141], emphasising that the vast majority of human infections are acquired post-natally. These post-natal infections in man are all derived either directly or indirectly (*via* meat animals) from the cat. The situation is similar in meat animals in which the rate of congenital transmission is very low but seroprevalence rates in sheep and pigs are high [142], due to post-natally acquired infection – which in the case of herbivores, like sheep, is entirely contracted from ingestion of oocysts. Whilst some of these infections undoubtedly result from contamination of stored feed (hay and grain), many occur at pasture. Recent epidemiology studies in Liverpool, involving seromonitoring sheep flocks throughout the year, have shown seroconversion, indicative of infection, occurring throughout the summer when sheep consumed only grass at pasture, (Owen and Trees, unpublished observations). These data not only have significance in determining prophylactic strategies in sheep, but indicate the degree of environmental contamination produced by cats. There are some 6–7 million cats in the UK and the prevalence of infection in them based on antibody is variously reported to be between 16 and 48% [141, 143–145]. Whilst antibody prevalence tends to be higher in stray or farm cats, it is still high in domestic cats. As domestic cats are increasingly fed manufactured feed, the major source of infection to cats is likely to be from birds and small rodents caught while hunting. Even well-fed domestic cats hunt and a study of pet cats reported 14 known kills/year/cat in one English village [146, 147]. Toxoplasma antibody prevalence in small rodents is variable and has been reported to be between 0 and 20% [148, 149], whilst 8% of British starlings have been found to be infected [150]. It has been assumed that the rodent reservoir of infection for cats is maintained by congenital infection, but this has not been investigated for small wild rodents until recently. By use of the polymerase chain reaction (PCR) with B1 gene primers [151] to detect infection in tissues, in addition to antibody assay, congenital infection has been found to occur commonly in both *Apodemus* and *Mus*, even when dams are chronically infected. Intriguingly, antibody assay is very unreliable in the detection of congenital infection in *Mus* in such circumstances (Owen and Trees, unpublished observations).

Although almost all human infections are ultimately derived from cats, the proportion of human infections contracted by ingestion of oocysts compared with tissue cysts in meat is unknown. As regards meat-producing animals, cattle are rather insusceptible to infection, antibody prevalences are low and it is

Table 13. Protozoal infections reported to the PHLS Communicable Disease Surveillance Centre: laboratory reports – all identifications England and Wales 1991–1995

Infection	Number of reports in				
	1991	1992	1993*	1994*	1995*
Toxoplasmosis	636	580	454	627	322
Giardiasis	6299	6898	6227	6009	6186
Cryptosporidiosis	5165	5211	4819	4424	5705

*Provisional.

Fig. 3. Thin section electron micrograph of *Toxoplasma gondii* growing in cultured epithelial cells. The cells show the apical complex (arrow) which is characteristic of the Apicomplexa (bar = 1 μ m).

difficult to recover viable parasites [152]. Thus beef is a low-risk meat. Sheep and pigs are both very susceptible to toxoplasma infection; antibody prevalences and rates of recovery of viable parasites are relatively high [152] and thus both species are potentially important sources of human infection. The relative importance of pork or mutton may depend on the eating habits of populations.

Some of the outstanding epidemiological questions on toxoplasmosis may be addressed by modern methods of molecular characterisation. Initial examination of

genotypic heterogeneity by examination of phenotypic variation in isoenzyme electrophoretic mobility has revealed relatively limited heterogeneity in *Toxoplasma* isolates [153] and DNA analysis of strains from both man and animals showed a single clonal lineage for all isolates examined which were acutely virulent in mice [154]. More recently, examination of 106 isolates from human and animal infections in Europe and America, by restriction fragment length polymorphisms (RFLP) on PCR amplification products from six different loci, has revealed that all the isolates can be grouped into just three clonal lineages which display correlations with biological and pathological characteristics [155]. Most human cases were associated with Type II strains, which were significantly more common in AIDS patients with toxoplasmosis than in animals, whilst Type III strains were more common in animals. As most human isolates are from clinical cases, these data reveal more about the pathogenicity of clonal types than their transmissibility or infectivity, but similar studies of asymptomatic as well as symptomatic infections in man and animals may reveal greater insights into the epidemiology of this infection.

Fig. 4. The flow of *Toxoplasma gondii* infection. Dotted line indicates less frequent route of transmission.

Although related to *Toxoplasma* (as an apicomplexan protozoan), *Cryptosporidium* – the causal organism of

cryptosporidiosis – has a much simpler life-cycle. The parasite multiplies asexually (Fig. 5) and then sexually in the gut, resulting in the excretion of resistant oocysts into the environment. Transmission is by the faecal-oral route, without the intervention of any intermediate hosts. However, the host range is wide. Since its first description early this century, there have been changing views with respect to the host specificity and number of species of *Cryptosporidium*. Suffice to say that Tyzzer was correct in his original descriptions of two mammalian species, one (*C. muris*) infecting the stomach, and one (*C. parvum*) the intestine [156, 157]. The currently accepted list of species is shown in Table 14 [158]. Whilst mammalian species do not usually infect birds and *vice versa*, there is a lack of host specificity within mammals. Most cryptosporidial infections and disease in man and other mammals is associated with the enteric species, *C. parvum*, although there is one report of infection in an AIDS patient, with *C. baileyi* [159].

In man cryptosporidiosis is important as a life-threatening infection in AIDS patients, although not uncommonly it also causes enteritis in immunocompetent people [158]. In a children's hospital study in Liverpool, cryptosporidiosis was the diagnosed cause of 7.2% of all cases of diarrhoea, almost all of which

Table 14. *Cryptosporidium* species

Species	Host	Site
<i>C. parvum</i>	Mammals	Intestine
<i>C. muris</i>	Mammals	Stomach
<i>C. meleagridis</i>	Birds	Intestine
<i>C. baileyi</i>	Birds	Bursa, cloaca, trachea
<i>C. serpentis</i>	Reptiles	Stomach
<i>C. nasorum</i>	Fish	Intestines/stomach

involved non-immunocompromised individuals [160]. In addition to man, *C. parvum* infects a wide range of farm and companion animals [158]. In the UK, infection has been described in diarrhoeic cats [161] and in the faeces of 8.1% of non-diarrhoeic cats presented for various reasons at Glasgow University veterinary clinic [162], but *Cryptosporidium* appears to be much less frequently detected in dogs. In calves, cryptosporidiosis is a major cause of neonatal diarrhoea [163] and probably all calves undergo an infection with a transient period of high oocyst excretion. Adult cows have been found excreting oocysts and it is also common in lambs. Recent studies have also indicated that wild rodents in Britain often excrete oocysts [164, 165]. Therefore, there is a large variety of sources of environmental contamination. Whilst some evidence indicates human-to-human

Fig. 5. Thin section electron micrograph of *Cryptosporidium parvum* infecting human duodenal enterocytes (bar = 2 μ m).

infection, other human cases have been associated with zoonotic sources, through farm visits, caring for ruminant neonates or laboratory handling of bovine isolates [158]. Most importantly, a number of major outbreaks have been associated with water supplies presumed to have been contaminated by waste of animal or human origin [158, 166]. Water treatment systems do not remove cryptosporidial oocysts because of their small size and resistance to chemical treatment [166], thus control of cryptosporidial infection in water supplies has now become a major issue in the water industry.

In spite of the large number of human cases which, by association, have been linked to animal sources of infection, recent techniques of molecular analysis using different approaches have all indicated differences between human and animal isolates. Thus bovine and human isolates could be distinguished by RFLP analysis of repetitive DNA sequences [167]. By random amplified polymorphic DNA (RAPD) analysis, 25 isolates were shown to fall into two major groupings – one containing all of nine animal isolates and the other containing 12 of 14 human isolates [168]. Of the other two human isolates, one formed a distinct subgroup, whilst the second resembled the animal isolates. A fourth subgroup was formed by two reptilian isolates of *C. serpentis*. In another study with

isoenzyme gel electrophoresis, human and animal isolates were uniformly different from one another but homogeneous within the respective sources of origin, with one exception [169]. Thus molecular typing techniques suggest a high degree of compartmentalisation of cryptosporidial infections, although it will be necessary to examine many more isolates before firm conclusions can be drawn. In this respect, RAPD analysis of faecal preparations will be valuable as it can be performed in asymptomatic as well as symptomatic infections, without the need to amplify oocyst numbers by passage [168].

Giardia spp. are biologically not closely related to *Cryptosporidium*, but there are many similarities in their epidemiology. Trophozoites (Fig. 6) inhabit the intestinal lumen and are transmitted by the faecal-oral route when resistant cysts are excreted. Moreover, there has been a similar degree of uncertainty about the speciation and host specificity of *Giardia*. Five species are currently recognised based on morphological and electrokaryotypic characteristics, but many more have been described based on apparent host specificity [170, 171]. However, human giardia have infected a number of animals [172] and a human volunteer was recently infected with a giardia of rodent origin [173]. There has been much debate about the zoonotic potential of *Giardia* spp., a debate

Fig. 6. Thin section electron micrograph of a trophozoite of *Giardia intestinalis* showing the attachment organelle (arrow) (bar = 2 μ m).

which is still unresolved, and which centres partly on the extent to which animals might merely be reservoirs of human-derived infections [174, 175]. *Giardia* infections occur commonly in many animals but just how commonly has only recently been recognised. In the UK, 12–35% of non-diarrhoeic dogs and cats have been found to be excreting *Giardia* cysts [176–179]. Even more surprising is the extent of infection in large animals. In the UK, 69% of a group of growing lambs excreted giardia cysts over a 9-week period [180]. Elsewhere in the world, infection occurs at high prevalence in cattle [181]. Whilst the zoonotic potential of bovine infections remains unclear, biological similarities between ruminant and human giardia infections have been reported [182]. Because many human giardia infections are associated with water-borne infection [183], there is the clear possibility of farm animal waste contaminating water supplies, analogous to the situation with *Cryptosporidium*. In this case, as with *Cryptosporidium*, molecular characterisation may help to clarify the extent of zoonotic infection. There has been a considerable amount of analysis of *Giardia* isolates by isoenzyme electrophoresis, karyotyping and DNA polymorphisms [170, 171] and the current situation is complex. It appears that diversity in *Giardia* is more extensive than in *Toxoplasma* or *Cryptosporidium*, with a large number of zymodemes [170], although isolates may be grouped into two major assemblages [171]. However, there appears to be no clear association of genetic type with host, and heterogeneity within human isolates is as great as between human and animal isolates, and some animal isolates are indistinguishable from human isolates. This is interpreted as evidence for zoonotic transmission [170].

The three infections considered here vary from the clearly zoonotic (*Toxoplasma*) through the occasionally, perhaps frequently, zoonotic (*Cryptosporidium*) to the arguably zoonotic (*Giardia*). They present fascinating problems for the biologist, physician and veterinarian and there is still much to be understood about their epidemiology. Molecular typing techniques will undoubtedly shed new light on the relationship between human and animal infections, but a true understanding of the risk to man from animal infections will require a consideration, not only of genotypic similarities, but also of basic biological and sociological factors that affect exposure to infection.

HYDATID DISEASE

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Hydatid disease is caused by the development of a very large cyst, the larval stage of a very small cestode worm, belonging to the genus *Echinococcus*. The worm is believed to have originated in wolves with the larval

stage in reindeers, which illustrates the fact that the lifecycle of all *Echinococcus* species involves a predator and a prey. Man becomes infected by ingesting the eggs of the worm passed by the predator, and the parasite develops in man in the same way that it does in the prey.

Species of *Echinococcus*

Four species of *Echinococcus* are recognised, the adult worm occurring in the small intestine of a wide range of predators. The species are *E. granulosus*, *E. multilocularis*, *E. oligarthrus* and *E. vogeli*. *E. granulosus* is found throughout the world; the distribution of the other species is more restricted, although there are many parts of the world where more than one species occurs. Human infections with all four species have been described but cystic hydatid disease caused by *E. granulosus* and alveolar hydatid disease caused by *E. multilocularis* are by far the most important. *E. granulosus* is the only species found naturally in the UK. The cysts are found in many organs but the liver and lungs are most commonly infected.

Strains of *E. granulosus*

One of the important features of *E. granulosus* is that it occurs as a number of strains, which have been described as subspecies and may even be separated into different species [184]. The original recognition that different strains existed and were important in the epidemiology of hydatid disease in man was based on the sheep and horse strains in Britain, which were distinguished by morphological characteristics like hook size and by iso-enzyme patterns. Seven well-recognised strains have now been identified in various parts of the world [184]. The horse-dog strain is found throughout the British Isles, including Eire but the sheep-dog strain is much more restricted, being found mainly in the west, especially in Wales and the Western Isles of Scotland. As hydatid disease does not occur in Eire, it has been known for many years that the strains possess different infectivity for man and this has been borne out by an increasing number of studies in many parts of the world. It is now believed that the horse, the camel and the pig strains are of low or even no infectivity to man whereas the sheep, cattle and reindeer strains are infective to man.

Modern typing by molecular techniques can rapidly and reliably identify different strains [185, 186]. The application of these techniques to the typing of material from human cases has shown that all except one isolate was the sheep strain, the one exception being in a child in Holland, which was the cattle strain, although the absence of material for study from northern Canada is believed to be responsible for the absence of reindeer strains, which are clearly implicated on epidemiological grounds [187]. The importance of different strains possessing differing

infectivity for man in the epidemiology and control of hydatid disease is obvious. It should be mentioned that although the strains are identified according to the major intermediate host, other host species may also become infected, although the development and particularly the fertility of the parasite, may be impaired. Thus, the sheep strain does develop in camels, pigs and cattle and may occur in the same geographical area as the homologous strain [187]. As the strains have recently been shown to be discrete and not interbreeding, it has been suggested that they should be recognised as distinct species [184, 187].

Infection of Canidae with the sheep strain in the UK

In the UK, the sheep-dog strain is responsible for human hydatid disease and, therefore, it is possible to concentrate on sheep and dogs alone in formulating a control programme. Studies of the prevalence of infection show that up to 25% of farm dogs are infected in mid-Wales, 10% in the Western Isles of Scotland but none in the Eastern counties of England. These results were obtained by the tedious and unpleasant method of purging the dogs with arecoline and examining the purge for adult worms, which tends to under-estimate the prevalence. Examination of faeces for worm copro-antigen is now preferred [188]. Although foxes have also been found to harbour adult *E. granulosus* in the wild, the number of worms has been much smaller than those found in dogs [189] and experimental infections have demonstrated that foxes are much less susceptible than dogs to the development of gravid infections [190]. It seems highly probable, therefore, that once infection is eradicated from dogs, and particularly farm dogs, the rate of infection will fall in sheep and, incidentally, in foxes [191].

Epidemiology of human infection

The epidemiology of hydatid disease in man in the UK has been studied by several workers [192–194]. In one study based on the serology carried out at the PHLS Hydatid Reference Laboratory for England and Wales in the Liverpool School of Tropical Medicine over 2 years during 1981–1983, 160 cases of surgically confirmed hydatid disease were identified. Eighty-four were new cases and 31 of these were normally resident in England and Wales. A detailed study of the indigenous cases, both old and new, indicated that 46% resided in Wales or Herefordshire and at least 25% more probably acquired the disease in Wales. Eighty-nine percent had close contact with dogs, three-quarters of these with sheepdogs and 38% worked, lived or were brought up on farms. Forty-one percent fed raw meat or allowed their dogs to scavenge sheep carcasses [193]. The Welsh county of Powys was identified as the major focus of infection, which led to the introduction of the official control scheme there.

Treatment of dogs and man

Fortunately, there are very effective anthelmintics against the adult worms in the dog, praziquantel (Droncit, Bayer) being the most efficient. This drug is available as tablets or as an injection and it destroys immature and mature tapeworms in the small intestine. In addition to *E. granulosus*, there are a number of other tapeworms in dogs in the UK, several of which are responsible for serious disease in the intermediate hosts and for the condemnation of offal at meat inspection. This gives an additional encouragement to farmers to treat their dogs regularly. In order to ensure that mature infections of *E. granulosus* do not develop in dogs and thus prevent eggs being passed which may infect man and sheep, it is necessary to repeat treatment every 6 weeks, which forms one feature of the majority of present-day control schemes, including the Powys scheme.

The treatment of human hydatid disease generally involves surgery but there are now a number of drugs which may contribute to the management of patients. As cysts in naturally infected sheep are very similar to those in man, this model may be used for chemotherapeutic trials. Naturally infected sheep from Powys were used to examine the effect of albendazole on cysts [195], which had been detected in the lungs of live animals by radiography. The viability of the cysts was established before treatment by thoracotomy and needle puncture. No viable protoscoleces were found after a course of treatment at a dose of 10 mg/kg body weight/day for 6 weeks whereas treated controls which had only received the thoracotomy and needle puncture still had viable cysts. Profound changes in cyst morphology were seen macroscopically and microscopically, and electron microscopy showed that the germinal membrane was destroyed by the treatment. Promising results were reported with albendazole in 32 patients, some of whom had been treated previously with mebendazole [196] and there have been many other studies with these drugs since. Whilst the results have been variable, albendazole is undoubtedly of value either for treatment alone or prior to surgery, in causing shrinkage of the cyst and by reducing the danger of secondary cyst development during surgery. A comparative study of albendazole and mebendazole indicated some of the factors which influence drug efficacy [197]. The localisation of the cyst (brain 80% efficacy, liver and lungs 60%, bone 30%), their size (small 80%, large 8%) and number (best response when many) affected the efficacy of both drugs and albendazole was more effective than mebendazole (65% *cf.* 44%). Overall, 54% of all the treated cysts completely disappeared. Recent initial trials have suggested that these results may be improved by combination therapy with albendazole and cimetidine [198], or albendazole and praziquantel [199] or by the incorporation of albendazole in liposomes [200]. There seems hope that these devel-

opments may reduce the need for surgery, particularly of hydatid disease of organs like the brain, where surgery is more hazardous. Guidelines for treatment schedules are published by the World Health Organization.

Control programmes

However, there is no doubt that with hydatid disease, prevention is much better than cure, and there have been a number of extremely successful control programmes in many parts of the world. The disease was eradicated from Iceland in 1950 and the incidence of disease reduced to virtually nil in Tasmania, New Zealand and Cyprus, before the advent of effective anthelmintics, largely by education, the banning of home slaughter of sheep or stray dog control. Control programmes are also in varying degrees of progress in other countries including Argentina, Chile, Uruguay and mid-Wales. It is important that each scheme should be 'tailor-made' for each ecological or epidemiological area, as not only do the important hosts differ between countries but agricultural and cultural practices markedly influence the natural history of the host-parasite relationship. A list of the key features of control programmes is given in Table 15 and the measures which are being used in the Powys scheme are outlined in Table 16 (Walters, personal communication). It is likely that new methods of control will be devised as our knowledge of the worm and the reaction of its hosts to infection increases and it may be possible, for instance, that vaccination of dogs or intermediate hosts, including man, will play a part in disease control in the future [201]. It should be remembered, of course, that the hydatid cyst is remarkably adept at evading host responses, as it may remain in man and other intermediate hosts for many years [202, 203].

Table 15. Main features of hydatid control schemes

1.	Health education
2.	Stray dog control
3.	Dog registration
4.	Regular anthelmintic treatment of dogs
5.	Prevention of scavenging
6.	Centralised slaughterhouses
7.	Disposal of slaughterhouse waste
8.	Identification of origin of infected carcasses
9.	Voluntary or legislation?

Table 16. Features of control scheme in Powys, Wales

1.	Health education <i>via</i> schools, shows, farmers meetings, etc.
2.	Voluntary anthelmintic treatment of dogs every 6 weeks; praziquantel is used as 'Droncit' or 'Drontal'
3.	Surveillance of dogs by coproantigen and by monitoring sheep
There appears to have been a reduction in the incidence of human cases:	
1974–1983 43 cases (4.3 per annum)	
1984–1989 19 cases (3.1 per annum)	
1990–1995 7 cases (1.4 per annum)	
'Gid' (coenuriasis) in sheep has also been reduced	
The scheme is to be extended to Gwent	

DEVELOPMENT OF ANTIBIOTIC RESISTANCE: A VET'S TALE

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Modern livestock production relies on the use of antimicrobial substances, not only for the treatment of infectious diseases but also for the prevention of disease and the promotion of growth.

Growth promoters

Since the first description of the growth enhancing properties of some antimicrobial agents, there have been many papers confirming their efficacy and economic value for livestock production, although their mode of action is still not understood. Because of concerns about an increasing prevalence of multi-resistant *Salmonella* serotype typhimurium [204], the agricultural use of antimicrobial agents was reviewed by a committee, chaired by Professor M. Swann [205]. They recommended that growth-promoting antimicrobial agents should have little application to human therapy, will not impair the efficacy of therapeutic drugs through the development of resistance, be of economic value and not be absorbed from the gut to produce residues.

In the UK, the permitted growth promoters fall into four categories: inhibitors of cell synthesis, inhibitors of protein synthesis, inhibitors of DNA synthesis and modulators of cation permeability. The maximum permitted levels are low and usually range from 20 to 50 ppm; most have a gram-positive spectrum of activity and usually require a relatively short withdrawal period prior to slaughter. In the European Union, the use of tetracyclines and penicillins is not permitted, although both are used in the USA.

Some growth promoters have been found to increase the excretion of enteric pathogens, especially salmonellae. However, before being licensed growth promoters must now be tested for their effect on the shedding of pathogens. Another concern was that their use may give rise to the development of antimicrobial resistance. Recently vancomycin-resistant enterococci have been detected in farm animals in the UK, Denmark and Germany [206, 207] and it has been suggested that the use of the glycopeptide, avoparcin, for growth promotion may be associated with this resistance. However, in the USA the use of avoparcin is not permitted but the prevalence of vancomycin-resistant enterococci is increasing.

The prophylactic use of antimicrobial agents

Antimicrobial agents may be used prophylactically to prevent the introduction of a pathogenic bacterium into

a previously uninfected herd or to prevent and minimise the spread of pathogens within a herd. The high incidence of new intramammary infections during the dry period of lactating cows is widely recognised and many of these infections can be prevented by the use of antimicrobial agents. This so-called 'dry cow therapy' was developed to shorten the duration of existing intramammary infections and to prevent further re-infection of the non-lactating udder. Robinson and others [208] found that even if the bacteriological examination of milk samples was routinely carried out to select and to treat infected quarters, the udder health of the herd would deteriorate rapidly unless all uninfected quarters also received prophylactic therapy.

During an epidemiological study of Typhimurium infection of calves, nine of 11 farms studied gave antimicrobial agents to the calves on their arrival to prevent disease. Despite this antimicrobial prophylaxis, salmonellae spread rapidly, although few animals showed disease [209].

Therapeutic use of antimicrobial agents

The benefits arising from the therapeutic use of antimicrobial agents in animal production are: animal welfare, carcass quality and economics. On welfare grounds alone there is a constant need to treat sick animals to mitigate against the effects of infection. However, it is widely believed that the benefits derived from the advent of antimicrobial agents in combating bacterial diseases in man and other animals have been largely offset by their indiscriminate use. Numerous publications have documented the emergence of antimicrobial-resistant bacteria.

Antimicrobial resistance

Salmonellas. In Great Britain, studies of antimicrobial resistance in Typhimurium isolated from man and other animals, particularly calves, showed that prior to 1961–62, 2% of the isolates were resistant but this proportion increased to 61% in 1964–65 when phage type 29 became common and constituted 73.2% of bovine isolates and 22% of human isolates [204]. Subsequently the incidence of phage type 29 declined and most salmonellas isolated from animals in England and Wales were sensitive to antimicrobial agents until 1977 [210]. During 1977, the prevalence of the genetically related, multi-resistant phage types of Typhimurium 204, 193 and 204C increased, especially in cattle. Phage type 204C became the predominant Typhimurium isolated from calves and some isolates were resistant to seven antimicrobial agents [211]. The incidence of strain DT204C has declined but it has been replaced by another multi-resistant type, DT104 (Fig. 7), which differs because the resistance genes are carried on the chromosome rather than on plasmids [212].

In contrast, Dublin – which is a frequent isolate from cattle – has remained sensitive during the period 1971–1995 to all the antimicrobial agents used for testing, as have most of the other *Salmonella* serotypes from farm animals (Table 17).

The introduction of new antimicrobial agents into veterinary practice may provide opportunities to study the development and epidemiology of antimicrobial resistance. The aminoglycoside antimicrobial apramycin was licensed for use in the UK in 1980 and resistance was detected in salmonellas in 1982 [213]. During the period 1982–1990 the incidence of

Fig. 7. Incidence of *Salmonella* serotype Typhimurium phage types DT204C □ and DT104 ■ and percentage of antibiotic-resistant Typhimurium PT104.

Table 17. Resistance in salmonellas isolated from animals and their environment in England and Wales during 1994 and 1989

Year	Organism	Number of isolates tested	Percentage of isolates sensitive to all 16 antimicrobial agents	Percentage of isolates resistant to antimicrobial agents									
				T	N	A	Fr	Tm	C	S	Su	Apr	NA
1994													
	Dublin	647	98.5	0.3	—	0.3	—	—	1.1	0.8	0.8	—	—
	Typhimurium	2339	15.2	81.6	1.2	74.1	0.4	12.4	70.7	75.7	82.1	0.7	0.7
	Other <i>Salmonella</i> serotypes	6227	67.1	11.4	1.6	4.3	2.4	11.2	1.8	9.8	19.8	1.4	8.0
1989													
	Dublin	731	98.6	—	—	0.1	—	0.1	—	1.0	0.7	—	—
	Typhimurium	2151	47.0	49.5	2.9	32.3	0.5	28.2	22.8	21.9	46.0	4.8	—
	Other <i>Salmonella</i> serotypes	6608	77.6	13.8	1.4	4.6	2.0	4.2	0.9	5.2	15.9	0.1	0.3

T, Chlorotetracycline (10 µg); N, neomycin (10 µg); A, ampicillin (10 µg); Fr, furazolidone (15 µg); Tm, sulphamethoxazole/trimethoprim (25 µg); C, chloramphenicol (10 µg); S, streptomycin (25 µg); Su, Sulphonamide (500 µg); Apr, apramycin (15 µg); NA, nalidixic acid (30 µg).

apramycin resistance increased from 0.6 to 4% but has subsequently declined to 0.7% in 1995.

The quinolone antimicrobial agent nalidixic acid was added to the surveillance programme in 1988 and resistance in salmonellas was detected in 1989 [214]. These isolates were also resistant to the fluoroquinolone, enrofloxacin, and although this resistance was detected in poultry isolates, some cattle isolates of Typhimurium now show resistance to fluoroquinolones (Table 17).

Escherichia coli. This organism has been the subject of numerous studies and many factors may affect its ecology and also its antimicrobial resistance [215]. Recent studies of clinical isolates from farm animals suggest that the level of antimicrobial resistance has increased [216] although most isolates of *E. coli* O157 from cattle remain sensitive.

Staphylococcus aureus. Francis and Carroll found that 59.7% of *S. aureus* strains were penicillin resistant and produced β -lactamases but resistance to other antimicrobial agents was uncommon [217]. Similar findings have been reported from other European countries [218, 219].

Other species of *Staphylococcus* from the skin of animals frequently show multiple antibiotic resistance and the presence of multi-resistant *S. hyicus* on a pig farm was associated with the use of antibiotic-containing feedstuffs [220].

Conclusions

Most of the studies of antimicrobial resistance in micro-organisms from food animals have been on pathogens and there have been only a few studies on commensal bacteria. Studies in the abattoir have

detected direct and indirect carcass contamination by multi-resistant *E. coli* which were demonstrated to pass down the food chain [221].

The enzyme AAC(3)IV which mediates apramycin resistance was originally detected in bacteria from farm animals, and the first report of its occurrence in human isolates (Typhimurium DT204C) was by Threlfall *et al.* [222]. Subsequent studies have reported the clinical isolation of AAC(3)IV-producing enterobacteria from man in a number of European studies [223–227] indicating the possible zoonotic spread of bacterial resistance genes. To what extent antimicrobial resistance genes spread from farm animals to man is unknown and the subject of debate. The most recent investigation concluded that: 'Years of expert review testify to the difficulty of coming to any generally accepted conclusions about the effects of long-term, low-level feeding of antibiotics to food-producing animals and the appearance of antibiotic-resistant bacteria in humans' [228].

In view of these uncertainties it is advisable that antimicrobials are used prudently in agriculture and with due regard to the possibility of compromising human therapy.

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