Paper Reference(s)

6BI05/1

Edexcel GCE

Biology

Unit 5: Energy, Exercise & Coordination

Sample Assessment Material

Scientific Article for use with Question 8

Do not return the insert with the question paper.

Printer's Log. No.
N32919A

Turn over

W850/XXXX/57570 3/2/2

edexcel
advancing learning, changing lives

Epidemics

What was the Black Death?

The disease that spread like wildfire through Europe between 1347 and 1351 is still the most violent epidemic in recorded history. It killed at least a third of the population, more than 25 million people. Victims first suffered pain, fever and boils, then swollen lymph nodes and blotches on the skin. After that they vomited blood and died within three days. The survivors called it the Great Pestilence. Victorian scientists dubbed it the Black Death.

As far as most people are concerned, the Black Death was bubonic plague, *Yersinia pestis*, a flea-borne bacterial disease of rodents that jumped to humans. But two epidemiologists from Liverpool University say we've got it all wrong. In *Biology of Plagues*, a book released recently, they effectively demolish the bubonic plague theory. "If you look at how the Black Death spread," says Susan Scott, one of the authors, "one of the least likely diseases to have caused it is bubonic plague." If Scott and co-author Christopher Duncan are right, the world would do well to listen.

Whatever pathogen caused the Black Death appears to have ravaged Europe several times during the past two millennia, and it could resurface again. If we knew what it really was, we could prepare for it. "It's always important to re-evaluate these questions so we are not taken by surprise," says Steve Morse, an expert on emerging viral diseases at Columbia University in New York. Yet few experts in infectious diseases have even read the book, let alone taken its ideas seriously. *New Scientist* has, and it looks to us as though Scott and Duncan are on to something.

The idea that the Black Death was bubonic plague dates back to the late 19th century, when Alexandre Yersin, a French bacteriologist, unravelled the complex biology of bubonic plague. He noted that the disease shared a key feature with the Black Death: the bubo, a dark, painful, swollen lymph gland usually in the armpit or groin. Even though buboes also occur in other diseases, he decided the two were the same, even naming the bacterium pestis after the Great Pestilence.

But the theory is riddled with glaring flaws, say Scott and Duncan. First of all, bubonic plague is intimately associated with rodents and the fleas they carry. But the Black Death's pattern of spread doesn't fit a rat and flea-borne disease. It raced across the Alps and through northern Europe at temperatures too cold for fleas to hatch, and swept from Marseilles to Paris at four kilometres a day—far faster than a rat could travel. Moreover, the rats necessary to spread the disease simply were not there. The only rat in Europe in the Middle Ages was the black rat, *Rattus rattus*, which stays close to human habitation. Yet the Black Death jumped across great tracts of open country—up to 300 kilometres between towns in France—in only a few days with no intermediate outbreaks. "Iceland had no rats at all," notes Duncan, "but the Black Death was reported there too."

In contrast, bubonic plague spreads, as rats do, slowly and sporadically. In 1907, the British Plague Commission in India reported an outbreak that took six months to move 300 feet. After bubonic plague arrived in South Africa in 1899, it moved inland at just 20 kilometres a year, even with steam trains to help.

The disease that caused the Black Death stayed in Europe until 1666. During its 300-year reign, Scott and Duncan have found records of outbreaks that occurred somewhere in France virtually every year. Every few years, these outbreaks spawned epidemics that ravaged the rest of Europe. For *Yersinia* to do this, it would have to become established in a population of rodents that are resistant to the disease. It couldn't have been rats, because the plague bacterium kills them—along with all other European rodents. As a result, Europe, along with Australia and Antarctica, remain the only regions of the world where bubonic plague has never settled. So, once again, the Black Death behaved in a way plague simply cannot.

Nor is bubonic plague contagious enough to have been the Black Death. The Black Death killed at least a third of the population wherever it hit, sometimes more. But when bubonic plague hit India in the 19th century, fewer than 2 per cent of the people in affected towns died. And when plague invaded southern Africa, South America and the south-western US, it didn't trigger a massive epidemic.

The most obvious problem with the plague theory is that, unlike bubonic plague, the Black Death obviously spread directly from person to person. People in the thick of the epidemic recognised this, and Scott and Duncan proved they were right by tracing the anatomy of outbreaks, person by person, using English burial records from the 16th century. These records, which detail all deaths from the pestilence by order of Elizabeth I, clearly show the disease spreading from one person to their neighbours and relatives, separated by an incubation period of 20 to 30 days.

The details tally perfectly with a disease that kills about 37 days after infection. For the first 10 to 12 days, you weren't infectious. Then for 20 to 22 days, you were. You only knew you were infected when you fell ill, for the final five days or less—but by then you had been infecting people unknowingly for weeks. Europeans at the time clearly knew the disease had a long, infectious incubation period, because they rapidly imposed measures to isolate potential carriers. For example, they stopped anyone arriving on a ship from disembarking for 40 days, or quarantina in Italian—the origin of the word quarantine.

Telltale timing

Epidemiologists know that diseases with a long incubation time create outbreaks that last months. From 14th-century ecclesiastical records, Scott and Duncan estimate that outbreaks of the Black Death in a given town or diocese typically lasted 8 or 9 months. That, plus the delay between waves of cases, is the fingerprint of the disease across Europe over seasons and centuries, they say. The pair found exactly the same pattern in 17th-century outbreaks in Florence, Milan and a dozen towns across England, including London, Colchester, Newcastle, Manchester and Eyam in Derbyshire. In 1665, the inhabitants of Eyam selflessly confined themselves to the village. A third of them died, but they kept the disease from reaching other towns. This would not have worked if the carriers were rats.

Despite the force of their argument, Scott and Duncan have yet to convince their colleagues. None of the experts that New Scientist spoke to had read their book, and a summary of its ideas provoked reactions that range from polite interest to outright dismissal. Some of Scott's colleagues, for example, have scoffed that "everyone knows the Black Death was bubonic plague".

"I doubt you can say plague was not involved in the Black Death, though there may have been other diseases too," says Elisabeth Carniel, a bubonic plague expert at the Pasteur Institute in Paris. "But I haven't had time to read the book." Carniel suggests that fleas could have spread the Black Death directly between people. Human fleas can keep it in their guts for a few weeks, leading to a delay in spread. But this would be unlikely to have happened the same way every time.

Moreover, people with enough *Yersinia* in their blood for a flea to pick it up are already very sick. They would only be able to pass their infection on in this way for a very short time—and whoever the flea bit would also sicken within a week, the incubation time of *Yersinia*. This does not fit the pattern documented by Scott and Duncan. Neither would an extra-virulent *Yersinia*, which would still depend on rats.

There have been several other ingenious attempts to save the *Yersinia* theory as inconsistencies have emerged. Many fall back on pneumonic plague, a variant form of *Yersinia* infection. This can occur in the later stages of bubonic plague, when the bacteria sometimes proliferate in the lungs and can be coughed out, and inhaled by people nearby. Untreated pneumonic plague is invariably fatal and can spread directly from person to person.

But not far, and not for long—plague only becomes pneumonic when the patient is practically at death's

door. “It is simply impossible that people sick enough to have developed the pneumonic form of the disease could have travelled far,” says Scott. Yet the Black Death typically jumped between towns in the time a healthy human took to travel. Also, pneumonic plague kills quickly—within six days, usually less. With such a short infectious period, local outbreaks of pneumonic plague end much sooner than 8 or 9 months, notes Scott. Rats and fleas can restart them, but then the disease is back to spreading slowly and sporadically like flea-borne diseases. Moreover, pneumonic plague lacks the one thing that links *Yersinia* to the Black Death: buboes.

If the Black Death wasn’t bubonic plague, then what was it? Possibly—and ominously—it may have been a virus. The evidence comes from a mutant protein on the surface of certain white blood cells. The protein, CCR5, normally acts as a receptor for the immune signalling molecules called chemokines, which help control inflammation. The AIDS virus and the poxvirus that causes myxomatosis in rabbits also use CCR5 as a docking port to enter and kill immune cells.

In 1998, a team led by Stephen O’Brien of the US National Cancer Institute analysed a mutant form of CCR5 that gives some protection against HIV. From its pattern of occurrence in the population, they think it arose in north-eastern Europe some 2000 years ago—and around 700 years ago, something happened to boost its incidence from 1 in 40,000 Europeans to 1 in 5. “It had to have been a breathtaking selective pressure to jack it up that high,” says O’Brien. The only plausible explanation, he thinks, is that the mutation helped its carriers survive the Black Death. In fact, say Scott and Duncan, Europeans did seem to grow more resistant to the disease between the 14th and 17th centuries.

Yersinia, too, enters and kills immune cells when it causes disease. But when O’Brien’s team pitted *Yersinia* against blood cells from people with and without the mutation, they found no dramatic difference. “The results were equivocal,” says O’Brien. “We don’t know if the mutation protected or not.” Further experiments are under way. Similar mutations occur elsewhere in the world, but at nowhere near the high frequency of the European mutant. This suggests that pathogens such as smallpox exerted some selective force, but nothing like whatever happened in Europe, says O’Brien.

The association between CCR5 and viruses suggests that the Black Death was a virus too. Its sudden emergence, and equally sudden disappearance after the Great Plague of London in 1666, also argue for a viral cause. Like the deadly flu of 1918, viruses can sometimes mutate into killers, and then disappear.

But what sort of virus was the Black Death? Scott and Duncan suggest a haemorrhagic filovirus such as *Ebola*, since the one consistent symptom was bleeding. In fact they think “haemorrhagic plague” would be a good new name for the disease.

They are not the first to blame *Ebola* for an ancient plague. Scientists and classicists in San Diego reported in 1996 that the symptoms of the plague of Athens around 430 BC, described by Thucydides, are remarkably similar to *Ebola*, including a distinctive retching or hiccupping. Apart from that, many of the symptoms of that plague—and one in Constantinople in AD 540—were similar to the Black Death.

Of course, the filoviruses we know about are relatively hard to catch, with an incubation period of a week or less, not three weeks or more. But there are other haemorrhagic viruses: Lassa fever in Africa is fairly contagious, and incubates for up to three weeks. Eurasian hantaviruses can incubate for up to 42 days, but are not usually directly contagious between people. Both can be as deadly as the Black Death.

Out of Africa

Perhaps we can narrow the search to Africa. Europeans first recorded the Black Death in Sicily in 1347. The Sicilians blamed it on Genoese galleys that arrived from Crimea just as the illness exploded. But the long incubation period means the infection must have arrived earlier. Scott suspects it initially came

from Africa, just a short hop away from Sicily. That continent is historically the home of more human pathogens than any other, and the people who lived through the epidemics that wracked Athens and Constantinople said their disease came from there. The epidemic in Constantinople, for instance, seems to have come via trade routes from the Central African interior. "And I'm sure that disease was the same as the Black Death," says O'Brien.

One way to solve the puzzle could be to look for the pathogen's DNA in the plague pits of Europe. Didier Raoult and colleagues at the University of the Mediterranean in Marseilles examined three skeletons in a 14th-century mass grave in Montpellier last year. They searched the skeletons for fragments of DNA unique to several known pathogens—*Yersinia*, anthrax or typhus. They found one match: *Yersinia.* In their report they wrote: "We believe that we can end the controversy. Medieval Black Death was [bubonic] plague."

Not so fast, says Scott. Southern France probably had bubonic plague at that time, even if it wasn't the Black Death. Moreover, attempts by Alan Cooper, director of the Ancient Biomolecules Centre at Oxford University, and Raoult's team to replicate the results have so far failed, says Cooper. Similar attempts to find *Yersinia* DNA at mass graves in London, Copenhagen and another burial in southern France have also failed.

It's too early to conclude that the failure to find *Yersinia* DNA means the bacterium wasn't there, though. The art of retrieving ancient DNA is still in its infancy, Cooper warns. Pathogen DNA—especially that of fragile viruses—is extremely difficult to reliably identify in remains that are centuries old. "The pathogen decays along with its victim," he says. Scientists have had difficulty, for example, in retrieving the 1918 flu virus, even from bodies less than a century old and preserved by permafrost. And even if the technique for retrieving ancient DNA improves, you need to know what you're searching for. There is no way now to search for an unknown haemorrhagic virus.

But the possibility that the Black Death could strike again should give scientists the incentive to keep trying. The similarity of the catastrophes in Athens, Constantinople and medieval Europe suggests that whatever the pathogen is, it comes out of hiding every few centuries. And the last outbreak was its fastest and most murderous. What would it do in the modern world? Maybe we should find it, before it finds us.

(Source: From issue 2318 of *New Scientist* magazine, 24 November 2001, page 34)

How dangerous is Bird Flu?

In 1918, a woman boarded a subway train in New York to make a 45-minute trip. She must have felt healthy enough to travel, if perhaps a little achy and shivery. Yet a few minutes into the journey, she started having trouble breathing. Her condition rapidly deteriorated as her lungs filled with fluid. By the time the train reached its destination, she was dead.

Such was the ferocity of the 1918 flu. It killed some 40 million people worldwide. Most of the dead were otherwise healthy adults; not the very young, old or sick that flu usually kills. Disease experts' worst nightmare now is that the bird flu rampaging across Asia will mutate into a similarly deadly strain that could circle the globe in weeks.

If the nightmare does come true, what are our options? The good news is that we have the technology to defeat this killer. The template for mass-producing a vaccine is being made in British and American laboratories right now, something that would not have been technically possible only a year ago.

But it would still be a race against time to produce enough vaccine to immunise millions, and there are tough decisions ahead. Can we afford to take short-cuts with the usual safety tests? Will corporate interests get in the way of public health? And if the virus spreads so fast that there is not enough vaccine, or antiviral drugs, to go round, who will get them?

Every year 10 to 20 per cent of the world's population gets the flu. And because the influenza virus's two surface proteins are constantly undergoing small mutations, getting vaccinated or catching flu one year does not necessarily stop you catching it the next. Even so, flu is usually a mild disease because, despite its shifting disguises, our immune systems are more or less primed to recognise the main human flu viruses. The 1918 pandemic, and less lethal ones in 1957 and 1968, were triggered by big genetic changes when a flu virus incorporated surface protein genes from a strain that normally infects animals, and which human immune systems did not recognise.

Flu experts have long been warning of a repeat performance, largely because the huge, crowded chicken farms that have sprung up across Asia in the past decade are potent breeding grounds for a potentially devastating pandemic.

Bird flu normally lives harmlessly in ducks, which Asian farmers keep on paddies to eat pests. If duck flu passes to nearby chickens and is not immediately stamped out, it can readily mutate into a deadly and highly contagious virus that can spread like wildfire in poultry.

That is what has happened in east Asia, where at least eight countries, including China, are battling a particularly lethal strain of the H5N1 flu virus (the name refers to its surface proteins, haemagglutinin and neuraminidase). This was previously thought to infect only birds. But in 1997, six people died in Hong Kong when it somehow jumped to humans. So far it has done so only with difficulty - in the present outbreak, despite millions of sick birds, there have been only a few dozen confirmed cases in humans. And while human-to-human transmission has not been ruled out, if it is happening it seems that these infections do not spread any further.

The fear, however, is that H5N1 will change. The genetic material of the influenza virus is RNA, and RNA viruses are known to mutate frequently. A small change that makes it better at binding to human cells could make this flu much nastier.

H5N1 might also hybridise with a human flu virus. The flu virus genome consists of eight strands of RNA, each carrying one or two genes. When the virus infects a host cell, multiple copies of the eight strands are produced and new viruses self-assemble by taking one of each. If two virus strains infect the same cell at the same time, the progeny assemble from strands taken randomly from either of the original kinds. This means hybrids can emerge.

The worst-case scenario is a hybrid with genes from human flu that allow it to replicate and transmit easily in people, but with a gene for the bird flu surface protein, haemagglutinin, which is very different from the surface proteins on existing human flu. No human immune system would be prepared for that.

Right now in Asia conditions are ripe for such a hybrid to emerge. Thousands of workers are culling millions of sick birds, at the height of the region's human flu season. Few have protective clothing or masks. If just one worker is infected with bird flu while incubating human flu, he or she could become the breeding ground for a pandemic.

And it could be a monster. "To be bad, the virus needs new surface proteins, but it also needs attitude," says Earl Brown, a flu virologist at the University of Ottawa, Canada. And like many viruses that have recently jumped from animals to humans, H5N1 has attitude in spades. It has killed three quarters of known human cases in this outbreak. Medics believe that the H5N1 strain induces a massive overreaction by the human immune system. A hybrid version may or may not retain this talent but, ominously, the 1918 strain seems to have killed in the same way.

So what can we do? The World Health Organization is trying to get protective gear, antiviral drugs and vaccines against ordinary flu to people culling sick birds in Asia, to prevent simultaneous infection with human and bird flu. If the pandemic does start, antiviral drugs may minimise the effects and spread of the disease - provided manufacturers can produce enough in time.

But the best way to stop infectious disease has historically been mass immunisation. Manufacturers make standard flu vaccine by injecting an innocuous strain of flu into chicken embryos in eggs along with the disease-causing strain they want to protect against. The two strains jumble their genes, and some of the progeny get the two surface proteins from the disease strain and the rest of their genes from the harmless virus. This strain is selected and multiplied in millions more chicken eggs, then purified, killed and sold as vaccine.

But this won't work with this strain of bird flu. It is lethal to chickens, so injecting the virus into eggs would simply kill the embryos.

If an H5N1 pandemic had emerged back in 1997, we wouldn't have had a lot of options at this point. But the sense that 1997 was a near miss galvanised virologists into finding another way of producing a vaccine.

Designer viruses

Less than a year ago, they cracked it. Teams at St Jude Children's Research Hospital in Memphis, Tennessee, and the National Institute of Biological Standards and Control in the UK managed to create designer flu viruses without using chicken embryos. The technique they used is called "reverse genetics". They inserted genes from the bird and human viruses into plasmids, which are small circles of DNA that can be incorporated into cultured human or monkey cells. The cells then churned out viruses containing the chosen genes. Crucially, the mutation in the surface protein gene that makes it lethal to chickens had been snipped out, so these "seed viruses" could be multiplied up in eggs as normal.

The designer virus grows well in eggs, and in initial tests it elicited a strong immune response in ferrets and chickens. A similar vaccine made from another strain of bird flu, H9, got a good response in humans.

Now those labs, and the Centers for Disease Control and Prevention in Atlanta, Georgia, are brewing a similar vaccine virus carrying the H5 and N1 genes from the bird flu strain in Vietnam. The labs will hand the seed virus over to the WHO this month for further safety testing in ferrets and chickens, says Klaus Stöhr, head of the WHO's influenza programme. In April or May, vaccine manufacturers and

national research agencies will test the killed virus for safety in humans, and will make sure it triggers antibody production.

By July the manufacturers could be ready to start mass-producing the vaccine, using chicken eggs. At that point, their factories will already be occupied making regular human flu vaccine for the northern hemisphere winter of 2004-2005. “But if we see rapid spread of H5N1 among people,” says Stöhr, “I will ask them to switch to pandemic vaccine.” It will just be a matter of injecting each new batch of eggs that come in every week or two with the pandemic virus instead of the regular vaccine.

How fast could they make it? The companies are equipped to produce only enough flu vaccine for current demand. Global capacity is some 125 million doses per month of a one-virus vaccine. Production cannot be scaled up quickly, even if the producers commandeered more eggs from the food industry, as the processing capacity can’t be easily increased. “You can’t just put more eggs in the front door and get more vaccine out the back,” says Bram Palache of Belgian-based Solvay, a major producer.

The four biggest manufacturers have long been planning to switch to making flu vaccine in cultured cells instead of eggs, a system that could respond better to emergency surges in demand. But no one is quite ready. Solvay’s new plant needs another year of testing. The biggest manufacturer, Aventis, has only just signed a deal to start developing a human cell-based flu vaccine. Baxter’s new Czech factory is due to start production next year but could be pressed into service this year in an emergency, says Otfried Kistner, the company’s head of virology. Chiron’s new plant in Germany is also nearly ready but still requires regulatory clearance. “In an emergency, as the pandemic would be, we could grow a vaccine generated by reverse genetics in the facility,” says Rino Rappuoli of Chiron. If things get bad, governments may have to cut corners to clear plants to operate fast.

There are other potential hitches. Several firms hold patents on technologies involved. The overall patent holder on reverse genetics, a US biotech firm called MedImmune based in Gaithersburg, Maryland, says it will charge only modest fees for using the technology to make pandemic vaccine, as long as no one else profiteers. It is not yet clear whether other patent holders will be so accommodating.

And Chiron can’t even work with the seed virus in Europe for lack of a laboratory licensed to handle genetically modified organisms under European law. It is trying to build one, quickly. But if people start dying, concerns about patent rights and GMOs may well have to be postponed.

On the bright side, recent research suggests the pandemic vaccine may require less killed virus per dose than ordinary flu vaccines. Pandemic vaccine contains only one strain of flu, while ordinary flu vaccine has three. So it needs only a third as much virus, effectively trebling production capacity. But recent research suggests that if immune-stimulating chemicals called adjuvants are added as well, the amount of virus needed per dose might be further cut to half, or even a sixth as much, says Tony Colgate of Chiron - although people might need two doses, three weeks apart to be protected.

“It will be up to the WHO to decide how to distribute the vaccine,” says Colgate, who heads the industry taskforce on pandemic flu. “We hope it will be equitable.” If human cases are confined at first to limited areas, it may be possible to contain the virus with some form of local vaccination. But nearly all vaccine is made in Europe and North America. Would those countries be happy to send their limited supplies to Asia? “We hope there might be some sort of international registration of this vaccine,” says Colgate. “Our big concern as producers is that our governments might nationalise it.”

It wouldn’t be the first time. In 1976, swine flu was expected to become pandemic, though in the end that never materialised. The American government’s response was to prohibit export of US-made vaccine until US needs were filled, causing panic in erstwhile importers such as Canada.

We’re not yet at that desperate stage. “It’s very important we remain calm about worst-case scenarios,” said Mike Ryan, the WHO’s head of epidemic response, at a bird flu summit in Rome earlier this month.

But that does not mean the nightmare can be ruled out. “This could be the most serious influenza threat since 1918,” says D. A. Henderson of Johns Hopkins University in Baltimore, who led the smallpox eradication drive.

There are some tough choices ahead. What if we start vaccinating people, only to see the pandemic fizzle out, leaving companies to face lawsuits because regulations were overlooked in the rush? Should authorities bend the rules to get vaccine on the market faster - even though there will never be enough for all? Should someone start churning out industrial quantities of antiviral drugs, never mind the patents?

Officials like Ryan and Stöhr are doing their best to reassure the public that the pandemic hasn’t happened yet, and that there are things we can do. If this turns into another near miss, we will, if nothing else, be better prepared when the next flu pandemic does strike. But at meetings and in conversations with scientists, the fear in people’s eyes and voices is palpable: this could be the big one.

(Source: From issue 2436 of *New Scientist* magazine, 28 February 2004, page 36)

Will there ever be a malaria vaccine?

In the 1970s, the World Health Organization (WHO) set about the global eradication of smallpox. The production of an effective vaccine allowed the WHO eventually to declare the elimination of the disease. Scientists hope to emulate this success as they strive to eliminate one of today's biggest killers — malaria.

Malaria is a disease caused by the parasitic protozoon *Plasmodium*. Four species cause disease in humans, the most devastating being *Plasmodium falciparum*. Malaria is now endemic only within tropical regions. Over a million people die every year from malaria, mostly children under 5 years old. Malaria causes economic problems for poorer nations and places tremendous strains on healthcare systems.

The need for a malaria vaccine

The malarial parasite is transmitted in the saliva of a feeding female *Anopheles* mosquito. The parasite passes through stages in both the mosquito and human hosts. Attempts to control the disease have therefore been two-pronged, attacking both the mosquito and the parasite.

Early efforts to kill the mosquito vector used DDT insecticides. This approach failed due to the emergence of DDT-resistant populations of mosquitoes resulting from natural selection. The process was repeated with other compounds and the mosquitoes are now resistant to most insecticides.

Natural selection has also been responsible for setbacks in developing effective drugs to kill the malarial parasite. Strains resistant to chloroquine — the cheapest antimalarial drug — emerged in the 1960s and multidrug-resistant strains are now widespread. More effective drugs have been developed but these are expensive. A cost-effective method of control for malaria is needed — a vaccine. This should be one that will be long-lasting and affordable to both governments and to individual patients.

It has proved much harder to produce a vaccine for malaria than for many other diseases. Polio vaccine, for example, contains a weakened (attenuated) version of the polio virus which nevertheless stimulates appropriate immune responses. The malarial parasite, however, is large and complicated, so it cannot be used as a whole organism in this way. We need to find those parts of the parasite that are responsible for stimulating a long-lasting and strong immune attack and use only those to generate a vaccine. We could perhaps produce synthetic or recombinant forms of the antigens that stimulate the optimum responses and stick them together to make a subunit vaccine.

***Plasmodium*: master of disguise!**

This is not as straightforward as it sounds. One difficulty is that the parasite's life cycle involves a number of different stages, during which the parasite's antigens are continually changing. As these antigens are recognised by the host's immune system and initiate responses, the parasite changes and gains new antigens. The antibodies produced against antigens from an earlier form no longer work. As there are thousands of potential antigens, the immune system lags behind the parasite's development. The parasite's antigens also alter. This antigenic variation is just one of the ways *Plasmodium* evades the immune response

So is a vaccine for malaria a realistic prospect? Unlike other diseases, such as chickenpox, we know that complete immunity to malaria cannot be acquired after suffering just one bout. However, it is also known that people living in areas where malaria is endemic gain a degree of immunity from each infection, gradually building up a low-level immunity. For some adults, an attack of malaria may be relatively insignificant due to the many *Plasmodium* antigens encountered previously. Frequent exposure to parasites means that this natural immunity is boosted regularly. On the other hand, children, who have not had time to develop their immunity, are at risk of more serious disease. This observation suggests

that vaccination may be possible. The responses that protect adults could theoretically be promoted in a vaccine that could be given to children.

The structural changes throughout the parasite's life cycle mean that the mechanisms the host uses against it differ accordingly. We would need a number of different antigens from several life cycle stages for inclusion into one 'multistage' vaccine. This would lead to an attack on the parasite from all angles. However, this is a long-term goal. Vaccine attempts to date fall into three categories, each aiming to enhance a particular part of the host's immune response.

Pre-erythrocytic vaccines

The pre-erythrocytic stage of the parasite has a sporozoite stage, when the injected parasites are free in the blood, and a subsequent stage in the liver cells. During the first stage, antibodies against sporozoite surface antigens are produced, which prevent the parasite from entering the liver cells. In the liver stage, cytotoxic T cells are activated, which directly kill the infected liver cells. An 'ideal' pre-erythrocytic vaccine would prevent all parasites from reaching the blood (the stage associated with the fatal symptoms of malaria).

The first candidate to emerge was a synthetic vaccine called Spf66, which contained several antigens from the sporozoite and liver stages of *Plasmodium falciparum*. It reached clinical trials and first results were promising, but later trials showed disappointing effectiveness and testing was stopped.

The leading malaria vaccine currently is the pre-erythrocytic RTS, S -ASO2. This vaccine is based on a recombinant form of a protein located on the surface of sporozoites. This antigen was found to stimulate good antibody and T cell-mediated responses. The active part of the vaccine has been attached to an antigen of the hepatitis B virus. This boosts the immune response as well as providing antibody protection against hepatitis. Trials on children in Kenya and on infants in Mozambique have been promising.

A group in Oxford has developed a new type of vaccine that targets the parasite within the cells of the liver. DNA sequences coding for segments of immunogenic parasite antigens are inserted into a viral vector, modified to eliminate its virulence. After injection, the virus travels to the liver, transcribes the DNA into proteins and exposes them to the immune system on the surface of virally infected liver cells. These vaccines have a promising future and have undergone trials. When two vaccinations were given sequentially in different viral vectors — first to prime the immune system and second to boost it — the resulting T cell responses protected the recipient against disease.

Blood stage vaccines

Attempts to produce a blood-stage vaccine have so far concentrated either on inhibiting the invasion of parasites into erythrocytes, or preventing complicated malaria associated with *P. falciparum*. Complicated malaria occurs when parasite proteins protrude from the infected erythrocyte's surface and adhere to blood vessel walls, or to other infected erythrocytes. The cells clump together in small vessels, potentially obstructing the blood flow and causing vital organs to fail. Attempts to develop a vaccine based upon such a surface antigen have proved difficult, as it has one of the fastest antigenic variation rates known.

Most of the work done on blood stage vaccines has concentrated on preventing invasion of the erythrocyte. One possible antigen is a merozoite surface protein (MSP-1). Adults with some immunity to malaria produce antibodies that prevent parasite invasion by attaching to one part of this antigen. These antibodies therefore reduce the numbers of parasites able to enter the replication cycle. Blood stage vaccines are still early in their development, but some promising ones are entering the clinical trial stage.

Transmission-blocking (sexual stage) vaccines

These vaccines aim to prevent the passing of the parasite from person to person by attacking the sexual stages which lead to the formation of infective sporozoites. If humans are immunised with antigens expressed by the parasite while in its mosquito stage, antibodies will be produced against them. This is important as these antibodies can prevent the fertilisation of the parasite inside the mosquito. These vaccines will reduce the number of parasites in circulation in a locality, but as they do not work in the liver and blood stages, they do not alleviate symptoms. Therefore, a sexual stage component may only be useful as a method of transmission control when combined with preerythrocytic/blood stage components. However, in countries where malaria is endemic, acquired immunity is maintained by the boosting effect of regular parasite exposure. If this were to be eliminated, the naturally acquired immunity would eventually be lost.

Financial hurdles to vaccine development

The major problem for the development of malarial vaccines is that they are not commercially attractive. Pharmaceutical companies concentrate on products for use in wealthy countries to ensure a return on their investment, although vaccines used by travellers to endemic areas have found some commercial interest. Public funding is needed and happily there has recently been a huge boost in the charity money dedicated to malaria vaccine research, most notably by Microsoft millionaire Bill Gates.

The WHO has launched a campaign to halve deaths due to malaria by 2010 — a tough target considering the timescale involved in developing any vaccines, let alone the intricate form needed for malaria. In the meantime, the work goes on, highlighting and investigating various potential candidates with the help of the recently sequenced *Plasmodium falciparum* genome. This was a breakthrough for malaria drug and vaccine research, because it will allow us to search for weak spots in the parasite's genetic make-up that we might be able to exploit. For example, if we can identify a gene coding for a protein essential for parasite survival, but not essential for humans, we can target that protein. The sequenced genome has also opened up many more possible antigens for vaccine development. The key is in learning more about the immunological interaction between the host and parasite and identifying potentially useful antigens. Following this, rigorous human testing must be done to ensure safety and immunogenicity of potential antigens, all of which takes time and money. However, despite the problems, the future looks promising for the development of a malaria vaccine. Its mass production, distribution and administration, however, will remain a serious problem without a large injection of funds from wealthy countries.

(Source: From *Biological Sciences Review* Vol 19, No 1 Sept 2006)

HIV in South Africa

South Africa is currently experiencing one of the most severe HIV epidemics in the world. By the end of 2005, there were five and a half million people living with HIV in South Africa, and almost 1,000 AIDS deaths occurring every day, according to UNAIDS estimates. A survey published in 2004 found that South Africans spent more time at funerals than they did having their hair cut, shopping or having barbecues. It also found that more than twice as many people had been to a funeral in the past month than had been to a wedding.

A number of factors have been blamed for the rapid rise in HIV prevalence in South Africa, and debate has raged about whether the Government's response to the epidemic has been sufficient.

While richer countries began to use combinations of antiretroviral drugs (ARVs) to effectively treat HIV in 1996, this treatment was for a long time only available to a small minority of South Africans who could afford to pay for private healthcare.

The Treatment Action Campaign (TAC) – an organisation led by Zackie Achmat, who would later become a Nobel Peace Prize nominee for his campaigning – was started in 1998 with the aim of putting pressure on the Government to increase public access to ARVs. Achmat, himself HIV positive, publicised the situation by refusing to take ARVs until they were available to all South Africans. He argued that the cost of providing treatment and preventive education was ultimately less expensive than the economic impact of an unchecked AIDS epidemic.

In March 2003, the TAC laid culpable homicide charges against the Health Minister and her trade and industry colleague, claiming that the pair was responsible for the deaths of 600 HIV-positive people a day in South Africa who had no access to ARV drugs. By this time many poorer African countries were already implementing public treatment programmes, including Uganda, Nigeria and Zambia. South Africa's neighbour Botswana had started providing ARVs in early 2002.

The Government eventually approved plans to provide public access to the drugs in November 2003, in the form of the Operational Plan for Comprehensive Care and Treatment for People Living With HIV and AIDS. This followed years of debate in South Africa about the cost of implementing such a scheme and the effectiveness of antiretroviral drugs; the Government had frequently argued that an increase in access to antiretroviral treatment was not necessarily the best way to stop the AIDS epidemic, and that other treatment options needed to be considered. The Government's change in attitude towards ARVs was partly a result of a court battle in which GlaxoSmithKline and other pharmaceutical companies agreed to allow low-cost generic versions of their drugs to be produced in South Africa. This made South Africa one of the first African countries to produce its own AIDS drugs.

While the decision to start an ARV program was widely commended, many have since expressed dismay at the slow pace at which treatment is being made available. Although the Government's 2003 plan aimed to have 381,177 people on Government-funded ARVs by 2005-2006, only 85,000 people in the public sector were receiving treatment by September 2005. UNAIDS estimated that at least 79% of South Africans who needed ARVs were not receiving them at the end of 2005. In April 2006, a representative of the TAC said on the subject of access to ARVs:

"It is improving – slowly. It's also patchy. Some places, like Khayelitsha, are doing well. In many parts of the country, the rollout is pitiful, such as Limpopo and Mpumalanga provinces. These areas are less urban and less wealthy."

There has been some tension in South Africa between the methods used by different medical practices to treat HIV. Around 80% of people living in African countries consult traditional African healers and use traditional African remedies, even if they use conventional medicines as well, and some of these traditional methods of treatment are potentially harmful to people living with HIV. For instance, some

people (such as the Health Minister, Manto Tshabalala-Msimang) claim that African potato boosts the immune system and thereby helps to fight off AIDS. Yet a recent study shows that people taking ARVs should not eat African potato, because it lowers the level of antiretroviral chemicals in the body and increases the likelihood of HIV developing resistance to the drugs.

At the same time, some traditional medicines and practices have been shown to be beneficial in the treatment of HIV. Traditional healers are treated with respect in South African society, and in 2004 the Traditional Health Practitioners Bill was passed to formally recognise and regulate their legitimacy. Many such practitioners recognise the benefits of ARVs, and counsel people living with HIV to continue with antiretroviral treatment. The TAC argues that traditional healers have an important role to play in the treatment of HIV.

In 2000, the Department of Health announced plans to provide two prevention of mother-to-child transmission (PMTCT) sites in each province of South Africa. There was still, however, discontent about the lack of antiretroviral drugs available to pregnant women with HIV.

The following year, the TAC took the Government to court, seeking an order to make nevirapine (an antiretroviral drug proven to be effective and economical in reducing the transmission of HIV from mothers to their babies) available in all state hospitals and clinics. Many health care professionals had become frustrated by the Government's lack of progress in supplying the drug, which, the Government argued, was due to questions about its toxicity. Doctors had started applying to non-governmental organisations (NGOs) for grants to pay for nevirapine, and in some cases used their own money to buy the drug. Official policy stated that the doctors were forbidden to provide nevirapine, and those who did so risked being disciplined or sacked.

Later that year, the High Court ruled against the Government, ordering that nevirapine be made available to all pregnant women with HIV. A subsequent Government appeal was overturned, but they continued to display reluctance about distributing the drug, and even threatened to revoke its approval in 2003 unless the company that produced it (Boehringer Ingelheim) could provide additional data proving that it was safe. The Department of Health has continued to question its safety, in spite of the consensus medical opinion.

Pregnant women with HIV can now access nevirapine at most hospitals, health centres and clinics in the country. This is a huge progression from the original eighteen PMTCT sites set up in 2000. The Government estimates that in 2004, 78.7% of pregnant women who were HIV positive received nevirapine. However, the Government has been accused of failing to monitor the programme accurately, and there are concerns that many pregnant women are still unable to access the drug. More recently, a 2006 UNAIDS global report has stated that only 14.6% of pregnant women in South Africa are receiving the drug - the discrepancy between this figure and the Government's has yet to be explained. It is likely that the UNAIDS statistic is a more accurate reflection of what is really happening.

'Denialism' and misinformation in South Africa

Many people argue that the response to HIV/AIDS in South Africa has been hampered by 'AIDS denialism', a minority scientific movement that refutes the orthodox idea that HIV causes AIDS. Some leading figures in South Africa have flirted with this school of thought, much to the dismay of AIDS activists. President Mbeki has consistently refused to acknowledge that HIV is the cause of AIDS; he argues that HIV is just one factor among many that might contribute to deaths resulting from immunodeficiency, alongside others such as poverty and poor nutrition.

"Does HIV Cause AIDS? Can a virus cause a syndrome? How? It can't, because a syndrome is a group of diseases resulting from acquired immune deficiency. Indeed, HIV contributes, but other things contribute as well."

Although Mbeki has never declared outright that he rejects the link between HIV and AIDS, he has continually inferred as much through statements such as this. He has also failed to publicly state that he believes HIV to be the cause of AIDS.

While international scientific consensus holds that antiretroviral medication is an effective treatment for HIV, Mbeki has claimed that it is harmful and unsafe. Drug companies, he argues, have exaggerated the importance of ARV treatment in order to further their profits.

In 2000 Mbeki included a number of 'AIDS dissidents', such as the controversial American scientist Peter Duesberg, in a committee set up to advise the Government on tackling the AIDS crisis. In the same year, hundreds of delegates walked out of the International AIDS Conference in Durban in protest after Mbeki reiterated his view that HIV is not wholly responsible for AIDS.

In October 2000, Mbeki stated that he would withdraw from the public debate about whether HIV causes AIDS, after admitting that his stance had created confusion amongst the public. Since making this statement he has largely avoided the issue of what causes AIDS, but has repeatedly suggested that the impact of AIDS in South Africa may have been overstated.

In 2002 the Cabinet issued a statement on their latest AIDS campaign, declaring:

"In conducting this campaign, Government's starting point is based on the premise that HIV causes AIDS".

While this remains the official stance of the Government, there is evidence that certain politicians continue to question scientific consensus on AIDS. President Mbeki has repeatedly stressed the importance of a good diet in halting the progression of AIDS, as has the Health Minister Manto Tshabalala-Msimang, who famously urges people to eat lots of beetroot and garlic to fight off the illness. While it is true that a good diet is an important part of treatment, it is certainly no substitute for antiretroviral medication, as she has suggested. Her stance has angered many, including the revered South African cleric Desmond Tutu:

"We are playing with the lives of people, with the lives of mothers who would not have died if they had had drugs. If people want garlic and potatoes let them have them, but let's not play games. Stop all this discussion about garlic."

The Health Minister has also voiced support for the Dr Rath Health Foundation, an organisation that promotes vitamin supplements as a substitute for ARV drugs. The foundation has previously published adverts in South Africa claiming that antiretroviral drugs are toxic and cause AIDS. In August 2005, The Advertising Standards Authority ruled that such statements were a threat to public health, and that the organisation would not be allowed to make such claims in future adverts. Manto Tshabalala-Msimang later stated in newspapers that:

"No reason exists to criticise Rath, his treatments and his foundation"

The Dr Rath Health Foundation continues to promote its ineffective vitamin treatment in South Africa despite widespread international condemnation. The organisation has been banned from almost all other countries in which it has tried to operate. The TAC, which recently won a court case to prevent the Rath Foundation from wrongly labelling them 'a front for the pharmaceutical industry', has strongly criticised the Government for failing to condemn the organisation.

The Health Minister continues to make statements that play down the importance of ARVs, and it is likely that the attitude towards the drugs taken by her and other politicians has been central to the slow rate of progress in providing access to treatment. Amongst the scientific community there is little doubt about the benefits of ARVs; a recent study in South Africa reported that 93% of HIV positive people

surveyed were alive after one year of treatment.

Alongside AIDS denialism and misinformation about AIDS treatment, false beliefs about how HIV can be transmitted are also a concern. In April 2006, on trial for the alleged rape of a HIV positive woman, South Africa's former Deputy-President Jacob Zuma was found not-guilty but confessed that he had had consensual sex with the woman despite being aware that she was HIV positive. He stated his belief that HIV was not easily transmitted from women to men, and that he had showered after sex in the belief that this would minimise his chances of contracting HIV. There was widespread dismay amongst the AIDS prevention community that a politician (particularly one who had once been head of the National AIDS Council) could display such ignorance, and a fear that his statement would cause confusion amongst the public, undermining years of AIDS prevention campaigns. The National AIDS Helpline was subsequently inundated by callers querying the validity of his statement.

Many people believe that the widely publicised views of politicians such as Mbeki, Tshabalala-Msimang and Zuma have added to the climate of misinformation that surrounds the problem of AIDS in South Africa. Zackie Achmat, leader of the TAC, argues that the real hindrance to antiretroviral drug provision in the country is not lack of funding, but the attitude of the Government.

How Many South Africans Have HIV?

Two recent studies have attempted the difficult task of estimating the number of HIV infected people in South Africa. Each used a different method for collecting data and looked at different groups of the population.

The South African Department of Health Study, 2005

Based on its sample of 16,510 women attending 399 antenatal clinics across all nine provinces, the South African Department of Health Study estimates that 30.2% of pregnant women were living with HIV in 2005.

Estimated HIV infection among antenatal clinic attendees, by age

Age group /years	Proportion of population infected / %					
	2000	2001	2002	2003	2004	2005
Under 20	16.1	15.4	14.8	15.8	16.1	15.9
20-24	29.1	28.4	29.1	30.3	30.8	30.6
25-29	30.6	31.4	34.5	35.4	38.5	39.5
30-34	23.3	25.6	29.5	30.9	34.4	36.4
35-39	15.8	19.3	19.8	23.4	24.5	28.0
Over 39	11.0	9.8	17.2	15.8	17.5	19.8

The South African National HIV Survey, 2005

The National HIV Survey is a "household" survey. This involves sampling a proportional cross-section of society, including a large number of people from each geographical, racial and other social group. The researchers take great pains to try to make the sample as generalised as possible, and the findings are later adjusted to correct for likely over- or under-representation of individual groups (according to census data).

The survey's fieldworkers visited 12,581 households across South Africa, of which 10,584 (84%) took part in the survey. Of the 24,236 people within these households who were eligible to take part, 23,275 (96%) agreed to be interviewed and 15,851 (65%) agreed to take an HIV test. This means that only 55% of eligible people were tested.

Estimated HIV infection among South Africans, by age

Age group /years	Proportion of population infected / %	
	Male	Female
Under 20	3.5	5.3
20-24	6.0	23.9
25-29	12.1	33.3
30-34	23.3	26.0
35-39	23.3	19.3
Over 39	12.1	4.9

(Source: From http://www.avert.org)

AVERT is an international HIV and AIDS charity based in the UK, with the aim of AVERTing HIV and AIDS worldwide.

BLANK PAGE

BLANK PAGE

BLANK PAGE