

Tried and tested: From smallpox to measles

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'Anyone in the Roman Empire who had smallpox and measles could always still moan that he might get the plague' This proposition in the thesis of Frederik Rijkels [1] seems to me an appropriate opening to an inaugural lecture entitled 'Tried and tested – From smallpox to measles', in which the author takes you from our earliest history and that of infectious diseases and vaccination through to the challenges facing the vaccinology of today and in the future.

The history of infectious diseases: Tried and tested

In modern industrial society our dependence on other animal species is often considerably underestimated. In the current urbanised societies we largely live separate from agricultural activities and, as a result, hardly ever come in contact with farm animals any more. The average city dweller generally comes across a cow, pig or chicken in the refrigerated section of the supermarket, where humans encounter animals as a vacuum-packed piece of meat that serves as part of the evening meal. Indeed, it is highly likely that the supermarket visitor is even unaware of the origin of that food. The book by Joanna Swabe, 'The Burden of Beasts' [2] deals with the dependence of modern-day humans on other species and describes the serious consequences of this. If we go back in time: it was about 10,000 years ago that our ancestors took the first hesitant steps towards domestication, i.e. towards transforming wild animals into domestic animals, and keeping livestock. This led to a fundamental change in human-animal relationships, a change in the hunter-prey link, a shift of attention from the dead to the living animal and its offspring.

As the American historian William McNeill demonstrated in his book 'Plagues and Peoples' [3], the new intimacy between humans and animals resulting from domestication has a considerable impact on our health. Through the closeness with animals, humans were for the first time brought in contact with pathogenic animal microorganisms. Domestication created conditions where infectious agents could be transmitted from animals to humans, leading to diseases called zoonoses. It has been found that many epidemics, which ravaged the human race – and still do – such as tuberculosis, have their origins in animal diseases. Infectious diseases can only survive in fairly big communities; measles, smallpox, tuberculosis and whooping cough are linked to what we call civilisation, with settlements and adjacent areas, and contacts between them. Studies have shown that measles continues to occur only when there is a constant pool of at least 7000 susceptible persons available. It is interesting that the oldest known urban civilisation in Mesopotamia consisted of about that number of people. We must assume that infectious diseases began their advance approximately 3000 BC. Microbes can have a great impact on the stability of human societies. For instance, infectious diseases wiped the Inca Empire from the map, ravaged Europe during the Middle Ages and still wreak havoc in developing countries [2] [3] [4] [5].

The history of vaccination - smallpox

We find the first observation of immunity against infectious diseases in the description of the great plague of Athens by the historian Thucydides in 430 BC. He found that people who had survived the disease (including himself) could care for the sick without fear of falling victim to it again [6] [7]. This was the basis for the concept of giving a healthy person a mild form of smallpox, in order to provide a life-long immunity. In ancient China, around the year 1000, a form of immunisation was practiced using dried material from scabs of human smallpox cases being inhaled or blown

into the nose.



Figure 1. Ancient Oriental print illustrating the technique of variolation. Dried material from scabs of human smallpox is blown into the nose of an unaffected person with a small blowpipe. The word 'variolation' comes from the Latin word 'variola' for human smallpox.

This technique was called 'variolation', from 'variola', the name for smallpox, and was widely used in the Middle East. Natural smallpox first presents as small blisters, which turn into ulcerated pustules, often leaving permanent scars - the familiar pockmarked appearance. In those days, the primary purpose of variolation was to prevent pockmarks and to preserve female beauty, not to save lives. How variolation eventually reached Europe can be traced back to trade between Turkey and a country called Arcassia. The Arcassian people were poor, but blessed with beautiful women, who were unfortunately the main commodity for the Arcassian economy; many were sold to the Sultan of Turkey. [VetScite Editors' remark: this is a copy from <http://trevor.butler.edu/~jshellha/323/History.html>; the historic validity of this statement has not been verified]. Variolation eventually became popular in Great Britain through Lady Mary Wortley Montagu (Figure 2a), the wife of the British Ambassador to Turkey. She had survived smallpox, was introduced to variolation in Constantinople and had her son inoculated in 1718.



LADY MARY WORTLEY MONTAGU IN TURKISH DRESS

Figure 2a. Variolation was popularised in England by Lady Mary Wortley Montagu, who had her son inoculated against smallpox in Constantinople in 1718.

In 1721 the first 'clinical trial' was carried out in England, where six prisoners, who had been sentenced to death, were variolated and rewarded with their freedom when they survived variolation and the subsequent exposure to smallpox sufferers. So - the clinical trial went well. However, variolation had important drawbacks: the person inoculated with smallpox material represented a source of infection for others, and 2 to 3% of the variolated people died.



Figure 2b. Illustration of the famous first vaccination experiment. In 1796 Edward Jenner infected eight-year-old James

Phipps with fluid from a lesion on the hand of Sarah Nelmes, who had caught cowpox. The word 'vaccination' comes from the Latin word 'vaccinia' for cowpox. This was a successful experiment, because the boy was subsequently protected against a human smallpox infection. Years later Louis Pasteur proposed that all inoculations intended to protect against infectious diseases should be called vaccinations, in honour of Jenner.

Where are you going, my pretty maid?
I'm going a-milking, sir, she said
May I go with you, my pretty maid?
You're kindly welcome, sir, she said
What is your father, my pretty maid?
My father is a farmer, sir, she said
What is your fortune, my pretty maid?
My face is my fortune, sir, she said

This 18th century verse is a reminder of Edward Jenner (Figure 2b), a country doctor from Berkeley, England.

Jenner provided an enormous break-through in the control of infectious diseases. He had observed that milk-maids from the surrounding area had beautiful, perfect skin compared to the pock-marked village-dwellers. He discovered pock-like scars on the hands of farm girls and farm labourers because, during milking, they had been in contact with the udders of cattle infected with cowpox. In 1796 Jenner carried out his famous experiment: he infected the eight-year-old James Phipps with fluid from a lesion on the hand of Sarah Nelmes, who had caught cowpox. Two months later, Jenner wrote to a colleague: "The boy has since been infected with smallpox and, just as I predicted, that did not have any consequences." There was a violent reaction from religious factions; the inoculation of humans with disease material from animals, God's lowly creatures, was said to be unacceptable. The opposition even went so far that cartoons appeared showing people who had acquired cows' heads and hooves as a result of vaccination (Figure 3). Jenner fought for 25 years to convince the medical establishment of the benefits of his new technique – as compared to variolation. The British Parliament did not ban variolation until 1840, and in 1853 vaccination became compulsory. The term comes from the Latin word for cow – vacca – and cowpox, vaccinia. It was not until 1803 that the term was used for the first time. Years later Louis Pasteur proposed that all inoculations aimed at protecting against infectious diseases should be called vaccinations – in Jenner's honour [6] [7].



Figure 3. Cartoon on smallpox vaccination from 1802 entitled "The Cow Pock or the Wonderful Effects of the New Inoculation". It shows Edward Jenner and persons who are developing cow-like projections as a result of allowing themselves to be inoculated with an 'animal disease'



Figure 4. Vaccination became popular, as this poem from 1860 shows [8]. Mrs. Diklijf talking to her son Gerrit: "Gerrit if you carry on telling lies, you will surely get smallpox! And it will serve you right, said Mrs. Diklijf to her son. "Oh, really" thinks Gerrit. 'Not to worry. My cheeks are still smooth and red; my mother's quite wrong to think that, and anyway... I've been vaccinated!'

Incidentally, it is very likely that Edward Jenner used the results of a study by the Dutchman Geert Reinders [Figure 5], nicknamed 'the Inoculator', as the basis for developing his cowpox inoculation. As a cattle merchant Geert Reinders had become involved in the - not particularly successful - experiments of the noted Professor Petrus Camper to find a remedy for the devastating rinderpest disease. On his own initiative and often at the expense of his own livestock, Reinders showed great persistence in carrying out follow-up experiments with the objective to control rinderpest [9].



Figure 5. Engraving of Geert Reinders, who was successful in controlling rinderpest. He was born in Bedum on 19 April 1737 and died in Bellingeweer on 4 February 1815. This is the picture “of a man who, in a farmer’s smock, was a charitable angel for all of society, blessed with Franklin’s spirit but not given to hovering on high, he drove rinderpest from the cattle-shed.”

In 1774, he found that cows, which had recovered from rinderpest gave birth to calves that were immune to the disease for the first months of their life. He actually discovered the phenomenon known as ‘maternal immunity’ in current terminology. He also observed that calves infected

during this period by inoculation with virulent rinderpest material generally showed only mild disease signs. Thereafter the animals appeared to be protected. By repeating the inoculation a few times at intervals (currently termed 'booster injections'), he was able to increase the protection. In a letter of 1774 to the regent Willem V, Geert Reinders wrote that he had made an important discovery. The results were then published as a letter to the professors Petrus Camper and Wynoldus Munniks, who translated it into English and sent it to the Royal Society in London. It is highly likely that Edward Jenner saw the results of this study and used them to develop his cowpox inoculation of humans. Petrus Camper publicised the work abroad through articles in which he did not mention Geert Reinders, to make it appear as if he had made the discovery. This may be the reason why the merits of Geert Reinders regarding immunology have not been sufficiently appreciated. Over the last years this may have improved.

At the time of Geert Reinders, rinderpest had haunted the Netherlands. In 1713, an epidemic broke out in Groningen, which lasted until 1721. Within a year, the Province of Holland had lost two-third of its cattle, and later the disease became endemic. Animals were inoculated according to the 'Reinders method' from 1774 onwards, with favourable results. In the ten subsequent years, mortality in the inoculated cows dropped from 71% to 6%.

The history of vaccination - measles

About 30 years after Lady Mary Wortley Montagu had her son variolated, a Scottish doctor, Francis Home, tried to mimic the method by inducing a mild form of measles in children. The process consisted of injecting blood from a measles patient into a healthy person. This was successful in 10 out of 12 persons. The experiment unequivocally demonstrated the presence of measles in blood, 100 years before the first animal virus was described. Just like smallpox, measles was, and still is a disease of the large town [4]. It is likely that the people who, thousands of years ago, populated Mesopotamia on the Tigris and Euphrates river banks were the first to suffer from measles. How measles virus entered the human species is not known. The source was perhaps rinderpest virus in cattle that eventually 'jumped species' and infected their herders and subsequently the people in urban settlements. The similarities between measles virus on the one hand and the canine distemper and rinderpest viruses on the other hand cast suspicion on these two viruses regarding the origin of measles virus. This is another example of an infectious disease, which may have been acquired from animals and had established itself in humans. Definitive proof is difficult, because contemporary descriptions are vague, and measles was difficult to distinguish from smallpox. Both were seen as a single entity, as in the expression 'pockmarked and measly'. This continued until the 17th century, when the Englishman Thomas Sydenham documented measles as a separate disease. It was not until 1911 that the cause of the disease was identified as a virus.

At the end of the 19th century it was discovered how to culture bacteria outside the body, mainly as a result of the work of Robert Koch and Louis Pasteur. It became possible to isolate the causative bacteria in pure cultures, which allowed their manipulation and characterization. For example, the possibility of culturing bacteria in vitro was instrumental in the discovery of antibiotics. In addition, toxins could be produced and inactivated, and also antibodies to toxins and vaccines could be produced in this way. Bubonic plague, cholera, typhoid fever, diphtheria and sepsis could largely be prevented by means of the products of laboratory research. However, viruses require living cells in order to multiply. The main obstacle to getting viral infections such as polio and measles under control was the long time it took before animal cells could be cultured outside the body. In the successful development of a vaccine against measles, John F. Enders was guided by the 1746 finding of variolation described above. Enders took a throat swab from a young man, David Edmonston, who suffered from acute measles, and isolated the virus in cell cultures. He then selected viruses with a low disease potential, which still offered protection against the original, virulent virus. In 1961, Enders and his colleagues reported that measles virus infection could be prevented by vaccination [4].

Emerging, re-emerging infectious diseases and zoonoses

In present-day circumstances, new diseases and epidemics no longer arise as a result of

contacts with previously isolated populations and continents [3]. Now other events are required, such as mutations of known pathogens that acquire new properties, or new pathogens which make the transition from animal hosts to humans. Even in this situation, the closer together people and animals live, the greater the chance of such 'species jumps'. Partly because of the outbreaks of AIDS, SARS, BSE, Marburg, Ebola, Q-fever and avian flu, infectious diseases are once again the focus of concern. International travel and trade contribute to the introduction or re-emergence and wider spread of infectious diseases. Factors relating to the pathogens proper play a part here, as do factors that are the result of human activity (such as deforestation, intensive livestock farming or ecological changes). Most of the recently introduced infectious diseases are zoonoses [10]. The increasing intensive contact between humans and animals is one of the reasons why zoonoses represent a growing risk to public health.

There are a few striking examples to illustrate this view. As a result of climate change, pathogens are moving with their arthropod vectors from warmer regions, and their spread is made easier by greater human mobility (e.g. Dengue virus, yellow fever virus, Lyme's disease, Chikungunya virus and malaria). Factory farming also results in a higher risk of zoonoses (e.g. avian influenza). Companion animals also play a part in the spread of infectious diseases such as cat scratch disease and rabies. The increasing use of antibiotics in livestock farming, in household pets as well as in humans has contributed to the emergence of multi-resistant microorganisms. As a result, certain bacterial diseases can only be effectively controlled with vaccines (e.g. tuberculosis, *Staphylococcus* and *Streptococcus A* infections). Finally, in a time of biological weapons, plague and smallpox are no longer diseases of the past, and epidemics after their use constitute a real threat. By definition, 'emerging infections' occur unexpectedly. For their optimum control, the important success factors are not only political concern and coordination, but also efficient communication and exchange of knowledge between medical and veterinary professionals. As in the immune system, only good communication produces effective responses.

Immunity (cells with an experience)

Geert Reinders and Jenner did not know they were actually exploiting a property of the immune system - it can react more quickly to microorganisms it had seen earlier. The immune system is in fact 'tried and tested', which literally means that it has acquired experience, has developed memory and had overcome adversities. With its help, the body is able to create a defensive response to the building blocks of pathogens: the antigens. Certain white blood cells - the T and B lymphocytes - play a leading part here. For example, when pathogenic bacteria enter the body, they are immediately grabbed by a 'police force', the antigen-presenting cells (APCs; Figure 6). These are constantly keeping an eye on the body, owing to the fact that they can move with the blood stream. Employing special antennae such as the recently discovered Toll-like receptors, APCs recognise widely occurring molecular patterns that are present on various invading pathogens (Figure 7).

Upon activation, the APCs migrate to the nearest draining lymph node, which forms part in a complex network of lymph vessels distributed throughout the body (e.g. groin, armpit, or throat). After absorbing and disintegrating antigens into manageable chunks (termed antigen processing), fragments of the pathogen are routed to presentation sites (the MHC molecules) on the surface of APCs. Training places are located in the lymph nodes, a kind of barracks, where armies of soldiers of simple white blood cells, the T and B cells are lodged. The APC alerts the 'general' or helper T cell, which determines whether it is necessary to take action. If the answer is positive, B lymphocytes are activated to produce large quantities of specific antibodies, which will eventually destroy the invading bacteria.



Figure 6. The cartoon illustrates how an antibacterial antigen-specific immune response is generated. After a microorganism invades the body (the hooligans in the diagram, represent an invading bacterium *Neisseria meningitidis*), this can be detected by various antennae or sensors (e.g. Toll-like receptors) and captured by dendritic cells (DCs, the 'policemen'). After absorption of the antigen, processing and formation of peptides-MHC complexes, the DCs migrate to the T and B cell areas of the lymph nodes. After specific recognition of peptide-MHC complexes, clonal expansion of the peptide-specific CD4+ T helper cells (or 'generals') occurs. Specialised CD4+ T helper cells migrate to the B cell follicles and there stimulate the development and division of specific plasma cells. Antigen-specific plasma cells ('bombers') produce antibodies ('bombs') which are able to eliminate extracellular bacteria, in this case *Neisseria meningitidis*. (© Eric Reits, with changes by Claire Boog and Wilma Witkamp)

In the case of intracellular pathogens like viruses, a different sort of white blood cells, the cytotoxic T lymphocytes or 'killer T cells' launch the attack. They can recognise the infected cell because again chunks of viral antigens are displayed on its surface. After recognition by means of 'sniffer structures' or T cell receptors, the T cells can kill the diseased cell, and the virus loses the chance to spread. After a successful defence, the immune system is able to remember: the communication between T and B lymphocytes is stored in a cellular memory, and the body will react more quickly in the event of a subsequent contact with the same pathogen. This is, in a nutshell, an explanation of specific immunity and also the guiding principle in vaccination.

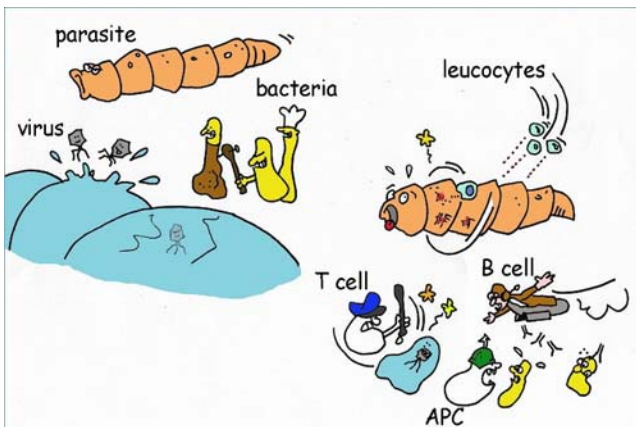


Figure 7. The cartoon illustrates the threat to the body from various pathogens (viruses, bacteria, parasites, worms) and the various types of immune responses. The illustration shows leucocytes, which are killing worms, B cells ('bombers') which form specific antibodies that destroy bacteria, and cytotoxic T cells ('police with truncheons'), which destroy virus-infected cells. (© Eric Reits)

The future of vaccinology

Smallpox was the first disease to be tackled in a modern way, and variolation became the flagship of the enlightened way of thinking; it testified to greater confidence in human understanding rather than in divine providence. Smallpox has been eradicated through a worldwide WHO vaccination programme and was the first – and hitherto only – disease that has vanished from the planet through human intervention. All traditional, successful vaccines up to

now have been developed according to the Pasteur principle: isolation, inactivation or attenuation of infectivity and injection. This led to the development of killed and live attenuated vaccines and vaccines containing fragments of pathogens ('subunit' vaccines). Traditional vaccine development has been successful against pathogens that hardly change their antigens over time [11]. For example, vaccines against diphtheria and tetanus are targeted at 'poisonous' substances of the bacteria, the toxins, and these have not changed much over the last century. The same applies to the measles and to rubella viruses. However, the development of new and better vaccines against more complex and/or more rapidly changing pathogens such as influenza, tuberculosis, malaria, the AIDS virus, HIV requires more fundamental knowledge - not only of the pathogen but also of the workings of the immune system. Many unanswered questions still linger in vaccinology: what precisely is the basis for a solid immune response? How can long-term 'immunological memory' be achieved? How can immunity be manipulated in the desired direction (humoral, cellular)? In short: rational vaccine design instead of the historic 'trial and error' approach. www.niv-vaccin.nl

The vaccine world has changed over the last decades. Vaccine design has become increasingly complex, quality requirements are now stricter, and vaccine development has become very expensive. As a result of concentration and conglomeration, only a few big players now dominate the vaccine market. Industry R&D cannot provide all the answers to an increasing number of vaccine dilemmas. Vaccines which have insufficient market prospects, but are necessary from a public health perspective, are not developed or are unavailable e.g. for target groups in new EU member states and for developing countries. The industry increasingly focuses on new combination vaccines covered by exclusive patents, where big financial margins can be achieved. The result is a vulnerable vaccine market. Furthermore, the industry gives little thought to improving existing vaccines (such as pertussis products) or reducing the number of required vaccinations.

In the light of the threat from 'emerging diseases', most of which are due to infections that cross species barriers from animals to man, it is important to combine the knowledge from medical and veterinary microbiology and immunology. This is also the objective of the "One Health Initiative - a movement to forge co-equal, all inclusive collaborations between physicians, veterinarians, and other scientific-health related disciplines, (which) has been endorsed by various major medical organizations and health agencies, including the American Veterinary Medical Association, the American Medical Association, the American Society of Tropical Medicine and Hygiene, the American Society for Microbiology and the Centers for Disease Control and Prevention (CDC). Additionally, more than 400 prominent scientists, physicians and veterinarians worldwide have endorsed the initiative." <http://www.onehealthinitiative.com/>

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