A brief history of vaccines and vaccination

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Summary
Human vaccinology, with its primary focus on the individual, seems far removed from veterinary medicine, with its concern for the health of the herd. Yet several episodes in the past (smallpox, fowl cholera, anthrax, swine erysipelas, rabies, tuberculosis, etc.) serve to illustrate the proximity between research on veterinary and human vaccines. In some cases the human vaccine was developed first, while in other cases it was the animal vaccine. The history of vaccinology clearly demonstrates the importance of these 'two medicines' working together. Foot and mouth disease (FMD) vaccines were among the first vaccines to be developed, beginning at the end of the 19th Century. Thanks to the discoveries of several researchers, including European researchers such as Vallée (French), Waldmann (German), Frenkel (Dutch) and Capstick (British), FMD vaccines began to be produced on an industrial scale from 1950 onwards, making possible vaccination of millions of animals in Europe and beyond. Vaccination strategies against FMD have always been dependent on the properties of the vaccines being used. At the beginning of the 21st Century FMD vaccines are designed in such a way that serological tests can differentiate infected from vaccinated animals, which has affected OIE regulations on international trade in animals and animal products. The history of vaccination against rinderpest, bovine contagious pleuropneumonia, and Marek's disease will also be dealt with.

Keywords
Bovine contagious pleuropneumonia – Foot and mouth disease – History of vaccinology – Human/veterinary medicine relationship – Marek's disease – Rinderpest – Vaccination – Vaccine – Veterinary vaccine.

The origins of veterinary vaccination: the human medicine viewpoint

In terms of its practices and concerns, human vaccinology, with its primary focus on the individual, seems far removed from veterinary medicine, with its concern for the health of the herd. Yet the history of vaccination provides evidence of close ties between what Dr Charles Mérieux affectionately called the 'two medicines'. It illustrates first of all their time-honoured collaboration, and it should be noted that the stock phrase in English, 'herd immunity', is directly derived from the veterinary concept of protecting the herd. After a century of totalitarianism in the name of general interest, people today are less inclined to accept measures that place the interests of society too far above those of the individual. In terms of vaccination, in recent years, the ideal of an individual vaccination 'à la carte' seems more in keeping with the demands of modern or even post-modern times. Yet, historically, vaccination has with some exceptions been predominantly a public health tool, aimed at populations rather than individuals.

What is a veterinary vaccine? A vaccine that a veterinarian applies to animals, be they companion animals, wild animals or herds of livestock. Yet the usefulness of veterinary vaccines extends beyond these limits since many of them also protect humans from

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anthropozoonoses, diseases common to humans and animals.

Veterinary vaccination differs *a priori* from human vaccination in terms of the ethical issues surrounding experimentation, and the importance, and even the priority, of economic considerations when it comes to animal health. There is also a major difference in the use of alternative solutions to the vaccination or treatment of sick livestock, such as mass culling, a strategy often employed in veterinary public health, despite the high cost and the shocking image it creates. No farmer can remain indifferent to having to have his livestock culled, especially if they are healthy. And even the wholesale destruction of mere battery chickens is not an operation to be treated lightly. Anyone who has tried to save a bird caught in an oil slick will find it hard to accept that in the 21st Century the only way of avoiding an epizootic is to destroy entire populations of poultry, cows or sheep, or even stray dogs.

Yet however unique it may be in many of its theoretical or practical aspects, veterinary or animal vaccination has a scientific history that is closely linked to that of human vaccination, for which it has served as a model, a tutor and a complement. This proximity and even interconnection illustrate how in many ways veterinary medicine offers a wealth of observations unmatched by human medicine, confined as it is to the anatomy and physiology of *Homo sapiens*. Not to mention that there are many human diseases where the reservoir is found in animals (rabies, for example), that the species barrier to infections is often crossed, and that many epizootic diseases prove to be potentially dangerous for humans, as in the case of avian influenza, all of which indicates the need for close collaboration in research (and decisions!).

Both human and veterinary medicine have certainly found a source of inspiration in the long tradition of empirical procedures where farmers have used fluids from sick animals to protect their herds. Several attempts at immunisation by inoculation were made for sheep pox, which is close to smallpox in humans, and bovine contagious pleuropneumonia. For the latter disease, the Belgian physician Willems brought this age-old practice into the scientific era when, from 1853, he inoculated animals at the base of the tail with a small amount of infective material. The tissues, and no longer just the 'humours', then came to be studied under the microscope and underwent all kinds of procedures to try to achieve a permanent, stable attenuation or neutralisation. The telling observations of animal farmers and veterinary practices thus provided the historical crucible for contemporary vaccinology.

The best example of the close relationship between human and animal vaccination, and certainly the best documented, is the history of successive vaccines against smallpox. Given the importance of the eradication of smallpox (proclaimed in 1979) as a success story, and the 'long and arduous hunting down of the disease', to paraphrase French historian Pierre Darmon, it seems appropriate to recall briefly this curious story that is in many ways indicative of the links between human and animal vaccination.

Vaccination against smallpox: an example of the historic links between human vaccine and animal vaccine

The inoculation of serous fluid under the skin is a procedure that has long been known as a way of protecting flocks against sheep pox. (The French language has a term which is used to refer specifically to inoculation with sheep pox, *clavélisation*, from the French word for the disease, *clavelée*.) In particular, there is documentary evidence of its use by nomadic herders in Africa, for example among the Tulani. There can be no doubt that this practice must have drawn attention to the possibility of acquiring protection from a serious disease by contracting a form of the disease that was attenuated to a greater or lesser extent. In earlier times people were closer to their animals, and animal farmers often had the reputation among neighbouring townsfolk of being healers. Yet it is difficult to know whether it was inoculation with sheep pox that led to the idea of human variolation or vice versa. It may seem more logical to favour the first hypothesis; however, even if inoculation against sheep pox was mentioned by explorers in Africa as long ago as the 16th Century, it is highly likely that human variolation was attempted in China or India even before then.

The history of the vaccine against smallpox, a human disease with no known animal reservoir, can be summed up as the replacement of inoculation with human smallpox (variolation) (Fig. 1) with inoculation with cowpox, a procedure invented by an English doctor, Edward Jenner (1749-1823). The use of cowpox is generally seen as a remarkable advance compared to variolation. The latter technique used only human material, serous matter from pustules and scabs taken from a subject with a mild form of the disease. It generally conferred solid immunity. However, the outcome was unpredictable and post-inoculation mortality was not inconsiderable.

In contrast, inoculation with cowpox, proposed by Jenner in 1798, seemed to be less dangerous and just as effective. Through a form of cross-immunity it provided humans with satisfactory protection, though probably less solid than that produced by the inoculation of smallpox. Indeed, during the 19th Century it proved necessary to revaccinate in order to reactivate the immunity since this tended to decline over the years. The need to revaccinate
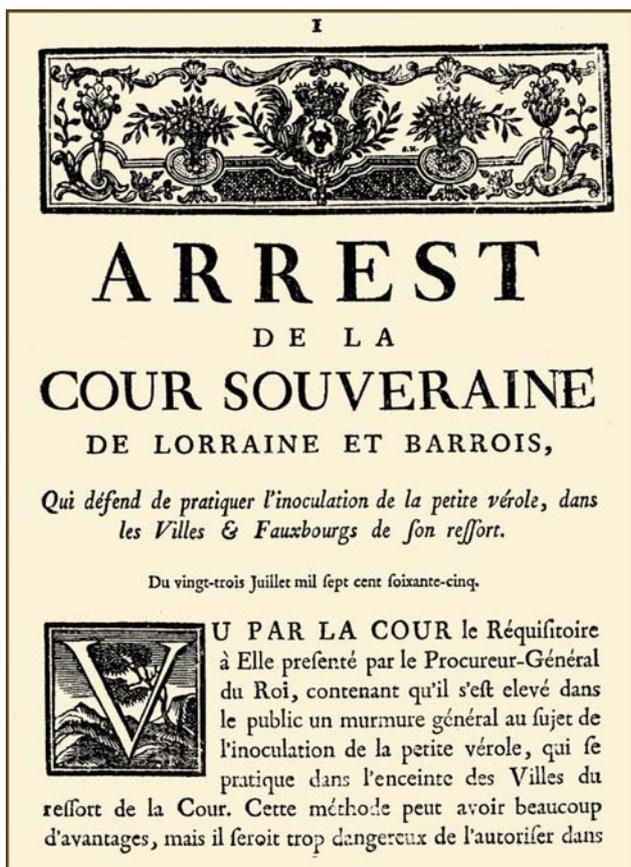
Fig. 1
Decision of the sovereign court of Lorraine and Barrois prohibiting smallpox inoculation (1765)
Source: Reproduced from *Mémoires des vaches et des bœufs* published by Equinoxe

Fig. 2
Historical treatise on the dangers of vaccinia by P. Chappon, 1803
Source: P.-P. Pastoret, personal collection

complicated the task of the health services and met with incomprehension on the part of the public, obliged to repeat a procedure that they had been led to believe was permanent.

At the beginning of the 19th Century, Jenner's vaccination procedure rapidly spread around the world (Fig. 2), supported by governments favourable to a measure that could reduce the devastating effects of epidemics on their populations. The President of the United States of America (USA); the Tsar of Russia; the King of Sweden; the Emperor of France, Napoleon I; and the Pasha of Egypt, Ali Mohammed, to mention but a few, were greatly enthusiastic about the vaccine and actively promulgated it, in some cases, as with Napoleon I in 1812, going as far as to make it compulsory in the army, and even in society as a whole. When it came to putting these plans into action, however, it was of course quite a different story.

Yet it was not long before vaccination with animal vaccine underwent changes. In fact the use of lymph of animal origin that was subsequently 'humanised' soon became
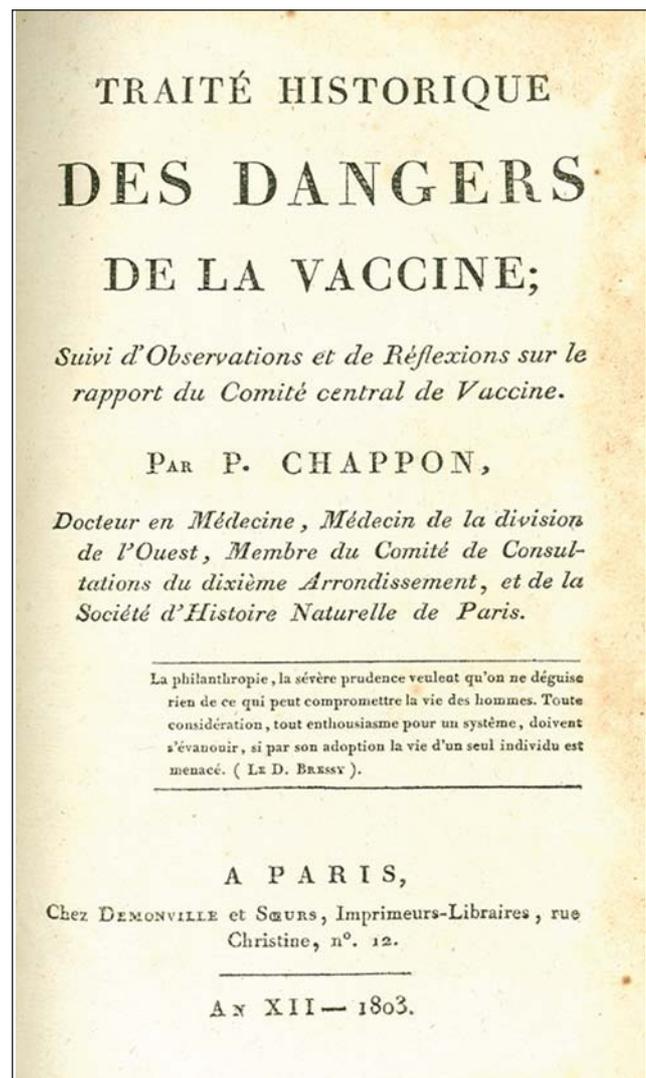
established: the original vaccine, derived from a cow, was first propagated from arm to arm, usually in children, who were used as vaccinifers. The method raised numerous problems. The lymph eventually lost its potency and produced hardly any pustules. Parents were also reluctant to have their offspring used as a reservoir for producing vaccine. Lastly, because repeated samples were taken from the same pustules they were soon emptied of smallpox virus, either because the pustules dried up or because they became superinfected and they then produced a fluid of dubious content.

During the latter half of the 19th Century, it seemed more natural and more practical to go back to the original source, namely cows or indeed calves, which were the only means of obtaining an authentic cowpox vaccine and ensuring an abundant and readily available supply of

lymph. This had to be organised in a completely different way. Breeding centres had to be established for animal vaccinifers, and animals had to be transported from village to village by road or rail or else the vaccinal lymph itself had to be transported, kept in an appropriate medium to conserve it and protect it from superinfection. Various excipients such as liquid paraffin, lanolin and glycerine were tested, with glycerine eventually being preferred.

As well as causing specific organisational problems, the transition from a vaccine of human origin to a vaccine of animal origin met with socio-cultural problems. In India, for example, where smallpox was a terrible scourge for the densely populated continent, variolation was an ancient tradition dating back to at least the 17th Century. Throughout the 19th Century, the British colonial administration went to great lengths to develop the vaccine but had to contend with the reluctance of Hindus. They found the use of sacred animals for this purpose abhorrent, and the presence of a fatty excipient led them to suspect the use of animal fat prohibited by culture. Furthermore, the highest castes commonly practised smallpox inoculation which, as in England in the 18th Century, was accompanied by a set of dietary measures and isolation of inoculated subjects that were considered quite satisfactory. Compared to the results of these inoculations, the variations in efficacy of inoculation with vaccine lymph were sometimes far from convincing. For a long time, the British administration steered a delicate course by using vaccination only for mass campaigns among the lower social classes. Vaccination, as a mark of solidarity against contagion, was thus confronted by the imperviousness of the caste barriers within Indian society (10).

Yet, in the case of smallpox vaccine, can one legitimately call it an animal vaccine? Right from the start, when the Jennerian procedure was first disseminated, it became difficult to determine the exact origin of the vaccine being used. In England, the practice of vaccination in hospitals formerly used for smallpox inoculation (promoted by physicians such as Pearson and Woodville) took place without the subjects being isolated, and was accompanied by a hybridisation of strains.

Moreover, in countries that were in favour of vaccination it seemed preferable to identify and use local cases of cowpox rather than having to rely on a supply from abroad. Yet in many countries in Africa (e.g. Egypt in the 1830s) or Asia (e.g. Indochina after the founding of the Pasteur Institute in Saigon in 1891), spontaneous cowpox could not be found. An alternative solution that was tried in India was to inject cows with human smallpox in the hope of obtaining an unlimited supply of attenuated material. Small institutes, in Bombay, for instance, bred calves and produced stocks of lymph for distribution to villages. The lymph was injected into subjects and then transferred from arm to arm. Children were targeted first because of their lower susceptibility, and served as 'guinea pigs' to standardise the vaccine fluid (according to the number and appearance of the pustules produced), before its use in adults. Parallel controls to test for innocuousness were sometimes performed using donkeys or rabbits.

In the history of smallpox vaccination, it is therefore very difficult to distinguish between the paths of these two fluids, one human and one animal. We can do no more than speculate about the origins of the strains that we have today. It seems likely that Jenner's original strain has been irremediably lost. Three types of virus are commonly distinguished, according to the type of cell lesions in the culture media (embryonated egg or allantoic membrane): 'historic' cowpox virus (thought to be closely related to the strain used by Jenner), vaccinia virus, and 'classic' smallpox virus. However, it seems likely that what we have today are in fact intermediate strains. Virologists are currently discussing a possible link between the smallpox vaccine and an equine virus that no longer exists in the wild (4).

We have therefore eradicated smallpox before fully elucidating the origin and behaviour of poxviruses and their vaccines throughout history. The development of a new vaccine, free from the dangers of its predecessor, to protect against any future use in bioterrorism, will probably not help us to learn more about the past (5).

The vaccine against smallpox, despite its many particularities, served as the inspiration for the development of vaccination against other diseases and as a springboard for the Pasteurian programme sometimes summed up as '*une maladie, un vaccin*' ('for each disease, a vaccine').

Veterinary vaccines and human vaccines during the Pasteurian era

In tracing the origin of modern vaccines one is inevitably confronted by the legend of Louis Pasteur (1822-1895) (Fig. 3), which presents a picture of a man of genius who knew no precursor other than himself. Yet the variety and eclecticism of Louis Pasteur's research, which ranges from the scientific basis of vinification to diseases of silkworms and human diseases, suggest that he relied more heavily on the results of his contemporaries than is generally realised. In fact, by tracing the path of his scientific research, it is easy to identify those who made his work possible and whose names were obliterated by his glory. At each stage in his career, Louis Pasteur kept himself very well informed of the scientific output of his time, even if he sometimes omitted to cite his sources (22). He obtained information from veterinary practitioners and specialists, agronomists, surgeons, farmers and herdsmen. Of these, the veterinarians and livestock farmers played a predominant role.

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Fig. 3
Portrait of Louis Pasteur (1822-1895) in 1865
Source: Reproduced with kind permission of Mérial
(provided by Philippe Dubourget)

In 1881, on the basis of his preliminary research, Louis Pasteur called for an extensive programme of prophylaxis against all diseases potentially of infectious origin. In an emotional speech to the French Academy of Science in the same year, he introduced the term '*virus-vaccin*' (synonymous with attenuated microbe), which he subsequently shortened to '*vaccin*':

'*Nous possédons maintenant des virus vaccins. Ces vaccins peuvent protéger contre la mort sans être eux-mêmes mortels*' (33).

(I.e. 'We now have virus vaccines. These vaccines can protect against death, without being lethal themselves.')

This was in spite of the fact that at the time he still only had two available candidate vaccines, both of which were veterinary vaccines, one against fowl cholera and the other against anthrax.

Veterinarians were traditionally very involved in trying to find ways of preventing the diseases that were decimating herds of livestock. The discovery of microbes under the microscope, the demonstration of their pathogenicity and,

particularly, their culture in the laboratory paved the way for the development of new preventive techniques, while at the same time providing the animal models needed for experiments in human medicine.

Not all French veterinarians were immediately won over to the microbian theory of diseases, no doubt because, with their experience of working in the field, they were aware of the multitude of factors that could be involved in triggering diseases and were suspicious of the notion of a single cause. While the veterinary school in Lyons led by Jean-Baptiste Chauveau (1825-1917) subscribed to the new ideas, Henri Bouley (1814-1885), then director of the prestigious veterinary school in Maisons-Alfort, near Paris, long remained attached to the doctrine of spontaneous generation, which he defended in his publication *Recueil de médecine vétérinaire*. In 1877, however, probably under the influence of a group of young teachers working with Edmond Nocard (1850-1903), Henri Bouley did a complete about-turn and from then on conducted a regular correspondence with Louis Pasteur on all aspects of 'vaccination', both human and animal (31). The use of the word 'vaccine' as the generic term to designate all existing and future vaccines, and not just the Jennerian vaccine, came into use in the international scientific community around 1880 before being included in the French dictionary.

What were the explanations for virulent microbes becoming attenuated while retaining their protective effect and maintaining the stability of attenuation? Nowadays, we attribute these changes in virulence to genetic mutations that occur spontaneously and are then selected by changes in the synthetic media used in the laboratory. The approach adopted by the contemporaries of Louis Pasteur was above all empirical, even if they were only too eager to theorise on the basis of their initial successes.

Fowl cholera

In 1876, the French veterinarian Henri Toussaint (1847-1890) cultured a causal bacterium of fowl cholera in neutralised urine, described two years later by Perroncito (and subsequently known as *Pasteurella avicida* or *gallicida*, and now as *P. multocida*). Were the cultures of the organism that causes fowl cholera accidentally left on a laboratory bench by one of Pasteur's assistants during the holidays, as the legend goes? Was it a chance discovery that the cultures which had become acidic due to aging had acquired attenuated virulence? Whatever the case may be, the hen survived inoculation with the 'forgotten' cultures and even became resistant to a subsequent, virulent inoculation. It was in fact an empirical trial to attenuate the culture by re-seeding the medium at longer intervals devised by Emile Roux with the help of a system of continuous oxygenation to accelerate the aging process.

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Anthrax

Whereas fowl cholera was not known to occur in humans and was rather more an academic exercise in exploring artificial immunisation at Pasteur's laboratory, anthrax was a constant source of concern for farmers faced with the seriousness of the outbreaks that affected herds grazing in the so-called *champs maudits* ('cursed fields') and with the risk of inadvertently inoculating themselves with the fatal black pustule while handling carcasses.

The team working with Louis Pasteur endeavoured to attenuate the bacteria in the laboratory by comparing or cumulating different methods borrowed from one another. In England, in 1878, John Burdet-Sanderson and William Greenfield, by re-seeding the culture at 35°C succeeded in attenuating the virulence of the strain without affecting its immunising potential. In 1880, Henry Toussaint proposed that if animals were vaccinated with blood heated at 55°C they could then survive an otherwise lethal inoculation. He successfully immunised five ewes using this technique.

Applying the laboratory method in the field was to prove decisive. In 1881, Louis Pasteur undertook his still famous trial at the farm in Pouilly-le-Fort, near Paris. In the presence of an extensive public consisting of farmers and veterinarians, he compared the behaviour of vaccinated and unvaccinated sheep. Initially, his vaccine had consisted of a culture attenuated simply by heating. However, Pasteur's disciples persuaded him to take the precaution of using an attenuated culture also containing an antiseptic known to inhibit the formation of spores (this was 'the secret of Pouilly-le-Fort'), and in so doing saved the day. The carefully staged-managed experimental trial ended in triumph, with the death of the unvaccinated animals. This success was the prelude to the Pasteurian vaccine being distributed to livestock-producing areas in the world affected by anthrax. Even though the years that followed were not without controversy, since the results of vaccinations sometimes proved difficult to interpret regarding the 'natural' immunity of some herds, this date marked a decisive turning point in the history of the fight against animal diseases.

Swine erysipelas

Although Louis Pasteur relied heavily on professors of French veterinary schools, he also mobilised the network of livestock farmers and veterinarians in the provinces. In 1881, at the invitation of a modest veterinarian from Bollène (a village in the south of France), Louis Pasteur conducted research into an attenuated vaccine against swine erysipelas, a disease caused by a bacillus that had recently been discovered by Louis Thuillier. This attenuated vaccine was lapinised, in other words attenuated by serial passages through rabbits.

The observation of an increase in virulence when a disease is passed from one individual to another during an epidemic is common to both physicians and veterinarians. In contrast, the notion of *in vivo* attenuation of virulence when germs affecting one species are passed through another species is an empirical observation of long date made by veterinarians. It proved to be a fruitful source of research for the Pasteurian school.

Rabies

In 1879, Louis Pasteur, left fowl cholera, anthrax and swine erysipelas to one side to concentrate on this rare, but invariably fatal disease: *'Si la rage pouvait être attribuée à l'action d'un organisme microscopique, il ne serait peut-être pas au-dessus des ressources naturelles de la science de trouver le moyen d'atténuer l'action du virus de la terrifiante maladie, pour la faire servir ensuite et en préserver d'abord les chiens et ensuite l'homme'* (34). (I.e. 'If rabies could be attributed to the action of a microscopic organism, it would perhaps no longer be beyond the natural resources of science to find a means of attenuating the action of the virus of this fearful disease, and thereafter put it to use, first to protect dogs and then to protect humans.')

It was with the vaccine against rabies, the cornerstone of Pasteurian science, that collaboration with veterinarians was to prove most crucial. It involved a human vaccine against an animal disease. Humans only become infected as an unfortunate accident and do not play a role in maintaining the natural cycle of rabies, because once the disease has developed in a human patient it is virtually never transmitted to others. Today, contrary to the hopes of Louis Pasteur, rabies has still not been eradicated, and is unlikely to be so in the near future since its animal reservoir is not restricted to domestic carnivores but now includes wild animals, such as foxes, among which Lyssaviruses, a group of viruses that includes rabies, are known to circulate. The reservoir also includes other wildlife species that are currently being identified, such as the many species of bats.

At the time of Louis Pasteur, veterinarians alone had the necessary expertise to study rabies. It was the veterinarians who monitored the disease in towns and the countryside, looking for evidence of rabies lesions during the post-mortem examination of dogs suspected of biting humans. They also provided dogs from the animal pound for use in experiments. It was the veterinarian Pierre-Victor Galtier (1846-1908), a pupil of Chauveau at the Lyons veterinary school (France), who showed rabies to be an affection of the nervous system, with a variable incubation period. In 1879, he suggested that laboratory dogs could be replaced by rabbits, which develop a paralytic form of the disease with a faster course than in dogs, thus making them more manageable. Moreover, after studying rabies immunity in

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sheep injected with blood from a rabid dog, he put forward the idea of a 'preventive treatment, undertaken before the onset of lesions of the nerve centres', which amounted to a treatment for the disease!

In 1881 and 1882, Louis Pasteur and his pupils Charles Chamberland, Emile Roux and Louis Thuillier entered the fray and modified Galtier's technique by inoculating nervous tissue from a rabid animal directly into the brain after trephination. By successive passages in dogs, they obtained a virus of maximum virulence coupled with a fixed incubation period of around 10 days. They then needed to attenuate the virulence of the causal microbe and measure the degree of attenuation indirectly by passages through rabbits. The chosen attenuation procedure was invented by Emile Roux. It consisted of suspending the spinal cord of a rabid rabbit in a flask, in a warm dry atmosphere, to achieve slow desiccation. Using animals as a live propagating medium, Pasteur and his team succeeded in producing 'attenuated viruses of different strengths', in short a standardised range of viruses, the weakest of which could be used to prepare a vaccine. Inoculating dogs with a sequence of spinal cords of increasing virulence rendered them resistant to inoculation with medulla of absolute virulence (42). The dog could then without danger be exposed to a street virus. This was the protocol that Louis Pasteur successfully applied to the young Joseph Meister on 6 July 1885 even though the experiments on dogs were still in progress (the dogs had not yet been subjected to the final test with an infective bite) and two trials had not been wholly conclusive.

Throughout the experiments with rabies, there was constant collaboration between veterinarians and physicians, even if Louis Pasteur is the name that tends to remain in the collective memory. The clinical know-how of veterinarians proved very important during all stages of rabies research, which they followed closely before returning to their main concern, namely animal rabies, the cornerstone of all programmes aimed at eliminating rabies in humans. The same vaccine was for a long time used to protect humans and animals, until genetically engineered oral vaccines were developed which could be distributed in baits by plane or helicopter as a means of immunising foxes.

The success of antirabies vaccination led to the founding of the Pasteur Institute, which to this day still conducts internationally recognised research. Edmond Nocard, who became Director of the veterinary school in Maisons-Alfort (France), worked tirelessly with Pasteur and his group, first at the laboratory in the rue d'Ulm in Paris and then at the Pasteur Institute. His presence was considered indispensable on the French mission to Egypt in 1883 during the cholera epidemic in Alexandria, which was to cost Louis Thuillier his life. On the well-known group photo at the Institute library, it is certainly no accident that Edmont Nocard is seated to the right of Pasteur, with Emile Roux on the Master's left (Fig. 4).

Fig. 4
Louis Pasteur with his team in 1894
Back row from left to right: Eugène Viala, Paul Reboud, Marcel Mérieux, Auguste Chaillou, Amédée Borrel, Louis Marmier, Auguste Marie, Andrien Veillon, Ernest Fernbach, Auguste Fernbach. Front row from left to right: Albert Calmette, Louis Martin, Emile Roux, Louis Pasteur, Edmond Nocard, Henri Pottevin, Félix Mesnil
Source: Reproduced with kind permission of Mérial (provided by Philippe Dubourget)

Sera was used for preventive or curative purposes in both human and veterinary medicine from the late 19th Century onwards. Serum therapy for children suffering from diphtheria was introduced in Germany and France in 1894, by Emil von Behring and Emile Roux, respectively. Serum therapy for anthrax was used by Sclavo and Marchoux in 1895. Seroprotection of cattle against foot and mouth disease (FMD) was attempted by Friedrich Löffler (1852-1915) in 1897 and applied on a large scale in Denmark. The sera proved to be of variable efficacy and many were abandoned. Some were later used in association with vaccines in the belief that they rendered the vaccines more effective.

At the beginning of the 20th Century, a new vaccine developed by the Pasteur school bore witness to the constant intermingling of the history of human and veterinary vaccines, but this time it was for a disease that was very different from the earlier ones, namely, tuberculosis.

Bovine and human tuberculosis, the same fight?

Vaccination against tuberculosis is still based on the historic vaccine of Calmette and Guérin whose initials it bears (BCG vaccine [bilious bacillus vaccine of Calmette and Guerin], the fruit of collaboration between a physician and a veterinarian.

In 1882, Robert Koch (1843-1910) described the tubercle bacillus responsible for tuberculosis in humans. Tubercular infection was also well known in cattle. However, Theobald Smith in the USA drew attention to differences between the bovine and human bacilli: their chemical characteristics and differences in virulence in experimental animals. This marked the beginning of the controversy over the role of bovine tuberculosis in human tuberculosis, notably through the ingestion of milk, and vice versa. Animal tuberculosis like human tuberculosis often went undetected, due to the frequently insidious nature of the disease and its chronic course, complicating the task of epidemiologists at that time. However, the analogies between the two diseases resulted in virtually parallel lines of research.

Initially, vaccinating cattle with human bacilli, considered to be less adapted to animals and less virulent, appeared to be simpler, and perhaps more urgent. Koch suggested inoculating a calf with human tubercle bacilli treated with phenol. Working on behalf of the firm Hoechst, Emil von Behring prepared a bovo-vaccine based on desiccated human bacilli reduced to a powder. At around the same time, a physician in Berlin named Friedmann suggested using a tuberculosis bacillus in humans that was not

thought pathogenic since it came from an animal of a distant species, a turtle. This vaccine, which was reputedly both preventive and curative, was extremely popular for a number of years.

In France, the veterinarians Vallée and Rossignol (the son of the veterinarian who had organised Pasteur's anti-anthrax vaccine trials at Pouilly-le-Fort) carried out trials in cattle in 1904. The results were equivocal, the protection afforded being relatively short-lived and not consistent. A quarter of the animals were not protected and contracted active tuberculosis. Gaston Calmette, at the Pasteur Institute in Lille, was particularly interested in these observations, which suggested that in some cases an abortive infection resulted in immunisation against further contamination, acting like an attenuated vaccine, and which he believed constituted a protective infection.

In 1897, Albert Calmette and Camille Guérin, a veterinary pupil of Nocard, began working together. A bovine bacillus, isolated by Nocard in a sample taken from the udder of a tuberculous cow, was cultured by passages through glycerinated bile potato medium, eventually resulting in an attenuated form. The tubercular bacillus has a fatty capsule which makes it difficult to blend. The idea of using bovine bile in the culture medium most likely came from the veterinarian Vallée, who had used delipidated bacilli in his vaccination trials: at that time, ideas were readily passed from team to team. The bacillus, from 1908 to 1921, was subsequently transformed by serial passages (230 passages) without regaining virulence in susceptible animals. The vaccine was called 'BCG' (which stands for '*vaccin bilié de Calmette et Guérin*'). In 1921, amid concerns at the upsurge in tuberculosis after the First World War, two experiments took place that, today, with the benefit of hindsight, are striking in their parallelism. Together with his co-workers, Henri Vallée, newly appointed director of the veterinary school in Maisons-Alfort near Paris, experimented with the BCG vaccine at a farm near Fécamp in Normandy. They tested the vaccine under different conditions, such as adding powdered pumice to the attenuated bacilli inserted under the skin and using intravenous injection. The trials were not judged to be entirely conclusive. The cattle did not acquire 100% protection even though every precaution had been taken during the experiment. It had taken place in a model farm with the best possible conditions of hygiene, far removed from conditions existing elsewhere in the country at that time.

Also in France in 1921, the first clinical trial of BCG took place, involving a newborn child in a family with a history of tuberculosis. The paediatrician, Weill-Hallé, administered several doses of BCG with a spoon. Faced with the prospect of almost inevitable contamination, the

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well-off parents had preferred to try an unknown vaccine rather than have to send the child away from home (9).

During the years that followed, scientific research was marked by a constant cross-over between human and bovine tuberculosis. For his part, Calmette demonstrated the reduced mortality from tuberculosis in children vaccinated with his vaccine after a follow up of several years, and the expansion of human BCG provided an argument in favour of bovine vaccination. Conversely, while BCG gave results that were far from satisfactory in herds of cattle, it showed no tendency to regain virulence and reassured the medical profession regarding the genetic stability of the strain for use in human medicine.

In 1928, an international veterinary commission, comprising Italy, the Netherlands, Austria, Poland, France and Germany, recommended extending the use of BCG in cattle. In 1929 over a hundred vaccinated children died in the town of Lübeck, Germany, which led to intense discussions over the safety of BCG. The official verdict attributed the deaths to the accidental contamination of the vaccine with a virulent strain, but, due to a lack of genetic knowledge on the subject, questions remained as to a possible reversion to virulence. The court case in Lübeck probably resulted in the world being divided into two camps over the use of BCG in clinical medicine, both for humans and for animals. To this day, some countries such as the USA have never used BCG, even though it is included in the UNICEF Extended Vaccination Programme for children throughout the world. The use of the BCG vaccine was not included in French legislation for the protection of bovines against tuberculosis, voted in 1933, and remained at the discretion of farmers.

Veterinary use of BCG continued after the Second World War but gradually declined, having to compete with the systematic slaughter of tuberculous cattle (known as Bang's method, after the name of a Danish veterinarian), and despite the cost of such a measure for governments and especially for farmers. The argument in favour of the latter method was not only that vaccination gave uneven results but that tuberculin tests could not differentiate between an allergic reaction indicating previous sensitisation to the bacillus and actual infection with tuberculosis. France, in the face of increasing isolation and problems with exporting meat from vaccinated animals, eventually stopped using the vaccine in 1954.

Up to the present day, BCG vaccination in humans has continued to plough a lone furrow. It has not yet been superseded by a genetically engineered vaccine, though several teams are actively engaged in research, notably at the Pasteur Institute in Paris. Due to its innocuousness, as clearly demonstrated during a century of use in humans,

BCG has also been thought of as a possible vector, through the use of genetic engineering, of vaccine antigens to prevent diseases other than tuberculosis.

Adjuvants

Another famous example of the fruitful exchange between human and animal medicine, concerns the discovery of adjuvants of immunity by Gaston Ramon (1886-1963) (Fig. 5), a veterinarian at the Pasteur Institute who became one of the first Directors General of the World Organisation for Animal Health (OIE) (then known as the Office International des Epizooties), following its creation in Paris in 1924.

Gaston Ramon developed an anti-tetanus vaccine in 1924 (38), consisting of the tetanus toxin treated with formaldehyde and heat, which he called 'anatoxin' (i.e. toxoid). This discovery was to prove a model for many subsequent applications. He also proposed that the efficacy of this 'anatoxin' could be enhanced by using, in addition
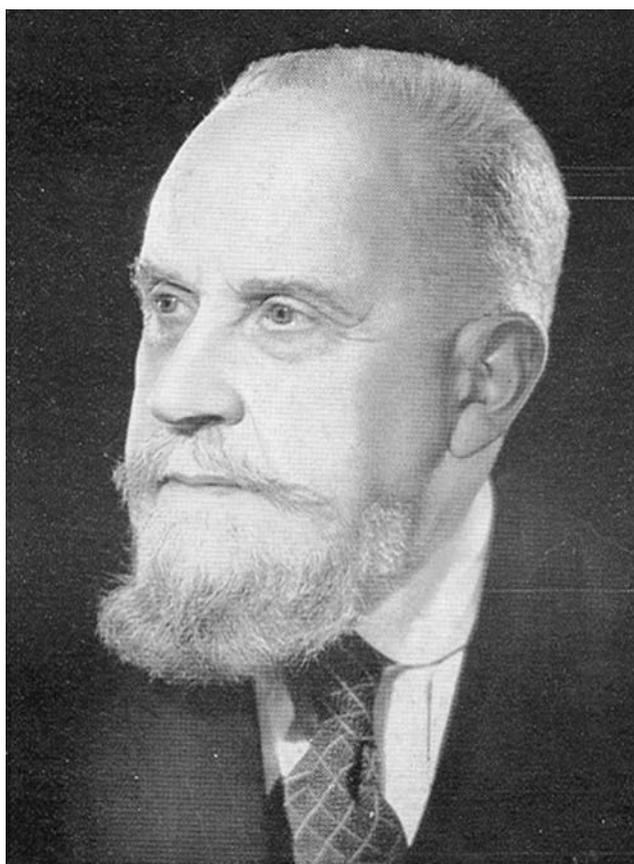

Fig. 5
Gaston Ramon (1886-1963)
Ramon was a veterinarian at the Pasteur Institute and also held the position of Director General of the World Organisation for Animal Health (OIE) from 1949 to 1959
Source: OIE

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to the specific antigens, substances known as adjuvants of immunity, such as aluminium hydroxide, thereby creating the first adjuvanted vaccine. Gaston Ramon had reached this conclusion after observing differences in the effectiveness of the various immunisation protocols he had been using in horses in order to produce anti-diphtheria and anti-tetanus immune sera, an activity he was in charge of at the Pasteur Institute annex in Marnes-la-Coquette.

The use of these toxoids in association with aluminium hydroxide in a suitably adapted vaccination programme, helped to prevent the dreaded occurrence of the form of infantile diphtheria still known today as '*croup*', which had long been a scourge across rural areas of Europe, and tetanus, a disease that in those days often proved fatal when even the most superficial of wounds became infected with the bacillus. During the Second World War, the disease took a heavy toll among soldiers wounded during battles fought over tetanigenic terrain. With hindsight, it would appear unjust that this fundamental advance in the prevention of toxin infections has not brought its discoverer more universal recognition.

The independent history of veterinary vaccinology

Rinderpest

Rinderpest is one of the great historic plagues that have ravaged human livestock for centuries. In Europe, rinderpest was the major plague of cattle up to the end of the 19th Century, when it was eliminated. At about the same time rinderpest was introduced with devastating effects in Africa where it decimated the cattle and buffalo populations *(Syncerus caffer)*, along with those of other susceptible domestic ruminants and many wildlife species (3, 35).

It is remarkable that rinderpest was eliminated from Europe by the end of the 19th Century by the simple application of sanitary measures, before the nature of the infectious agent was known. In fact, the ability to control rinderpest effectively was often considered to be a measure of the quality of a country's veterinary services. When rinderpest was reintroduced in Belgium in 1920, it was again eliminated purely by sanitary measures within seven months and without spread to neighbouring countries. The history of medical prophylaxis (vaccination) against rinderpest illustrates the evolution of medical thinking (24). The Italian Lancisi (1654-1720) wrote very lucidly: 'if such a dreadful disease were to threaten our cattle, I would be in favour of destroying all sick or suspect animals, rather than allowing the contagion to increase, simply to gain time in the hope of achieving the honour of

discovering a specific remedy, which is often a fruitless quest…'. His contemporaries did not necessarily share this view and Ramazzini was convinced that rinderpest, being a disease similar to smallpox, could be controlled by homologous inoculation. This lead to a whole series of unsuccessful inoculation trials. Some clearly stated that inoculation should only be recommended for areas already contaminated, otherwise there was a risk of spreading the disease further. Among all the inoculation trials against rinderpest carried out during that period, it is worth mentioning the work of Geert Reinders (1737-1815) in the Netherlands. He was a farmer in the Province of Groningen and a self-taught man. During his experiments Reinders noticed that calves from recovered cows were resistant to infection. This was most probably the first recognition of the phenomenon of maternally-derived immunity, since, according to him, this resistance was not of hereditary origin, depending solely on the immunity of the dam. He also noticed that the transferred protection gradually disappeared, leaving the calves just as susceptible as those from dams who had not had the disease. He also took advantage of this temporary resistance to inoculate calves with minimal risk and realised that he increased his chances of successful inoculation by repeating the procedure at different ages, because in some of the calves the first inoculation would not 'take' (i.e. have the desired effect).

Nevertheless, in the end it became obvious that inoculation was not a valid solution for rinderpest control. Not only were the losses after inoculation too high but, more importantly, the procedure perpetuated the circulation of the causative agent in the cattle population. At least all these experiments proved that smallpox was not unique in being preventable by inoculation and that the procedure, when successful, provided lifelong protection.

After Jenner's discovery of vaccination against smallpox in 1796, and due to the suspected analogy between the two diseases, there were trials to vaccinate cattle against rinderpest using the smallpox vaccine. This practice was passionately supported in England during the epizootics of 1865 to 1867 (18); finally, one of the main advocates of this practice, a Dr Murchison (1830-1879), wrote to *The Times* (30 January 1866) saying that: 'the analogies between smallpox and rinderpest were so obvious that it was logical to try to vaccinate cattle against rinderpest; but it is becoming also obvious that, despite all the trials, there are nowadays sufficient evidence that vaccination does not confer a continuous protection against rinderpest'. In fact, Henri Bouley demonstrated the total lack of cross-protection between rinderpest, smallpox and vaccinia in 1865. For this purpose he sent eight cows to England, where the rinderpest epizootics were raging. These cows, which had already been used in France to produce the anti-smallpox vaccine, all contracted rinderpest.

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Later on, Robert Koch, working in South Africa, suggested that cattle could be protected by subcutaneous injection of blood and bile from an infected animal. This highly dangerous method was soon replaced by the use of immune serum and later by a mixture of immune serum and virulent virus. Subsequently, the technique was improved by serial passages of the bovine virus through goats, which enabled Edwards to produce a caprinised vaccine in India in the 1920s. Trials with inactivated vaccines also took place. Finally, the successful isolation of the virus in cell culture (37) led to the *in vitro* development of an attenuated strain and from this the production of a safe and highly effective vaccine.

The Plowright tissue culture vaccine has been used with great success over the past forty years to vaccinate against rinderpest and has been the major reason behind the success of the global campaigns to eradicate the disease. We may justifiably hope that rinderpest will follow smallpox into oblivion, as only the second great plague to be eliminated on earth.

Bovine contagious pleuropneumonia

Another important disease of cattle, bovine contagious pleuropneumonia (CBPP), also played a key role in the history of veterinary vaccinology.

The disease was a real scourge in Europe during the 19th Century, reaching Belgium in 1828, the Netherlands in 1833, and the United Kingdom (UK) in 1841 (28). Louis Willems, a Belgian physician, tried to use inoculation to prevent the disease (45). Willems was the son of a distiller in Hasselt; his father had a huge fattening cowshed where he held cows coming from many different herds, which provided the ideal conditions for the transmission of a contagious disease such as bovine pleuropneumonia. Willems chose to inoculate cattle at the tail, provoking large abscesses (45); the animals presented general clinical signs but not the typical signs of the disease (pleuropneumonia) and became protected when exposed again.

It must be noted that a procedure similar to that proposed by Willems was empirically developed in Western Africa, where cattle were vaccinated with virulent pleuropneumonia tissues; vaccination sometimes provoked exostosis leading to a horny protrusion on the nasal bone. The skulls of such vaccinated animals even led to false identification of a new species named *Bos triceros* (11, 12, 21).

Bovine contagious pleuropneumonia also led to the discovery of *Mycoplasma* by Nocard and his colleagues in 1898 (32); for a long time *Mycoplasma* were called pleuropneumonia-like organisms (PPLO), a term which

was applied to the group of microorganisms similar to *Mycoplasma mycoides*, the cause of pleuropneumonia in cattle.

Foot and mouth disease

The protection of herds against the consequences of FMD has been a concern for cattle breeders for centuries, probably since antiquity. Vaccination is a recent development (between the two World Wars) in the history of farm animal breeding, and was preceded by various alternative measures, all of them oriented to protect the herd from losses induced by the threatened disease.

The oldest known strategy used by cattle breeders in the distant past to confer active protection on their herd was to practise 'aphtisation' as soon as the first case of FMD was observed in the herd or in the neighbourhood. The simultaneous inoculation of all animals in the herd by rubbing muzzle or lips with virulent saliva taken from the lesions of FMD-ill animals conferred a very early, strong and long lasting post-infectious immunity. The clinical disease triggered was comparable to the spontaneous disease, but nevertheless, there were some positive aspects like the brevity of the clinical signs, the synchronisation of infection in the full herd, the absence of aggravation of virulence by passages and finally the aim of the operation, the immunity (monovalent) conferred for several years. This kind of general method for 'prevention' is well documented as having been carried out in both animal species and humans (small pox) for centuries in Asia, Africa and Europe (12, 25, 30).

The next step just before vaccine use, was the injection of therapeutic immune serums for preventing or curing FMD symptoms in cattle. Friedrich Löffler, the co-discoverer of the filterable nature of the FMD agent (1897), pioneered this new preventive means to protect herds, which was then further developed by many other researchers (12). After the First World War, the production of cattle immune serum was organised at industrial level in many European countries, for example, records show that nearly 13,000 cattle were treated in one year in France and that 112,000 litres of immune serum were used in nine years in Denmark in the 1920s (25). It is interesting to note that several authors promoted the use of immune serum associated with aphtisation to minimise the consequences of the inoculated disease (25).

The history of vaccination as a whole is very interlinked with the history of FMD vaccines, the progress in industrial vaccine technology offering new opportunities to modify the opinion on vaccination or on the way to use it. For this reason, the history of the development of FMD vaccines will be presented here in some detail.

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History of foot and mouth disease vaccine technology

The pioneers

The first published attempt at using a protective FMD vaccine was that of French researchers Vallée, Carré and Rinjard in 1926 (43). Since 1922 they had been testing the action of formaldehyde on different agents of infectious diseases. In 1925 they published an article on the first vaccine, which was made from ground mucosal FMD lesions in saline buffer that had been filtered and inactivated at 20°C for 4 to 7 days with formaldehyde at 0.5%. The protection given was irregular but when present was reported as good for the standards of that time.

In 1932 in Denmark, Schmidt completed the laboratory process by the simultaneous use of aluminium hydroxide gel, a compound that had been used with formaldehyde in the domain of tetanus and diphtheria toxins by Ramon since 1924 at the Pasteur Institute in Paris.

Semi-industrial production of FMD vaccine began after the technique was further improved by the team of Professor D. Waldmann (44), working at the German Institute of Riems Island in the Baltic Sea. In 1937, they published a paper in which they highlighted the beneficial role of certain key factors, i.e. ensuring a pH>9 during the inactivation process, using a lower concentration of formaldehyde (0.05%), and maintaining the material at a higher temperature (25°C) for 48 hours. Thus, the first modern technology for turning FMD viruses into antigens for vaccines was born, and it was used with almost no modification for 50 years up until the 1970s, when attempts were made in industrial production to use other inactivants like glycidaldehyde, or aziridines.

Live attenuated vaccines against FMD have not succeed, even if there has been some semi-industrial production of such vaccines at certain times (reported by Kemron in Israel in eggs, Gribanov and Onufriev in the USSR in baby rabbits and Villegas in Venezuela in eggs).

Industrial development

Once the difficult process of turning the virulent FMD viruses into safe antigens was mastered, the second difficulty to solve was to obtain enough virus material for vaccine production.

Once again, it was the Riems Island research team which found a solution to this problem by developing an original technology for harvesting larger quantities of virulent material, which has been known ever since as the Waldmann's method. This method was used in Europe until the 1950s and it was still being used in South America in the 1970s. To encourage the standardisation of this method worldwide, the OIE organised an International Meeting in Bern in 1947 (25). According to

the method, the virulent material is obtained from infected cattle which are kept in a restricted stable, inoculated at the same time at several points in the tongue, and slaughtered when the tongue lesions are at their worst. All tongues are isolated and scraped to collect lymph and epithelial lesions. The carcasses are kept in the fridge for lactic maturation for 48 hours before rejoining the commercial circuits for fresh meat. The virulent tongue lesions are ground in saline buffer, centrifuged, then diluted before the inactivation step. At the earliest stage in the development of the method, one cattle dose of monovalent vaccine was a volume of 60 ml and each cattle tongue allowed for the preparation of 40 to 50 commercial cattle doses. One disadvantage of the Waldmann's method was the necessity to use FMD-free cattle to develop large lesions after inoculation. So, as the use of vaccination progressed across the country, fewer susceptible animals were available for the production of vaccine.

The second breakthrough in FMD vaccine production was made by Professor Frenkel, a Dutch scientist from the Amsterdam Veterinary Institute. Taking advantage of the work of Maitland on tissues maintained in a special medium, Prof. Frenkel had the brilliant idea of collecting epithelia fragments taken from the tongues of healthy cattle immediately after slaughter in normal abattoirs. Maintained for 48 hours or more in an appropriate medium at 37°C under oxygen bubbling, the small pieces of epithelia (the surface areas of which were equivalent to that of a hand) were infected with a virulent seed virus. The virus multiplied in the epithelial cells and at the end of the culture time virus was present both in the epithelia and in the maintenance medium. The process was presented as experimental at the OIE meeting in Bern in 1947, but industrial development started in 1950. The concept was revolutionary for this time because the source of raw materials (tongues) was without limit in normal abattoirs, the vaccination status of the animal had no effect on virus multiplication, and the yield of FMD virus harvested per animal was 100 times more than in the Waldmann's method (400 commercial doses). In Chile, in 1951, Espinet discovered that saponins could be used as an effective adjuvant in the aluminium hydroxide gel (19), which led to the first modern vaccine available for vaccination campaigns. To meet the demand, 500 l culture tanks were used for vaccine production which induced economy of scale and made each vaccine batch bigger and each vaccine dose cheaper. And the cherry on the cake was that the vaccine prepared with bovine homologous material did not induce allergic reactions in repeated vaccination campaigns, an issue which subsequently became a huge problem with the vaccines obtained using heterologous hamster cells for virus growth.

The third major technical step in the progression of FMD vaccine production was the use of cells, first in monolayer, then in suspension, to satisfy the huge demand for millions

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of litres of vaccine for vaccination campaigns in development in Europe or in South America. Cells in monolayer were used on an industrial scale mainly in Italy. At first, the cells used were primary or secondary kidney cells (from calves, piglets, lambs) taken from abattoirs. Subsequently, the advantages of a clean cell line like the baby hamster kidney cell line (BHK 21) isolated by Macpherson and Stocker became apparent. But soon, the capacity of plants to produce vaccine using cell monolayer culture in roller bottles was exceeded by demand; additionally, the harvest of thousands of bottles was not without risk of bacterial contamination. Consequently, the culture of cells in suspension became the method of choice for manufacturing huge volumes of vaccines.

The credit for the development of the BHK 21 cell line in suspension must go to Capstick and Telling, who carried out their work at the Pirbright Laboratory in the UK in 1962. The main advantage of this new technology was that everything could now be done in a completly closed circuit: cell growth, the infection of cells with sterile seed virus, the clarification in line of the virus harvest, its inactivation, concentration and formulation with adjuvant, and, finally, the filling of vaccine vials. At a time in the 1970s when FMD outbreaks were rare after successful mass vaccinations, virus escapes from manufacturing plants were seen as scandalous; consequently, the new process which was safely contained in a closed circuit, itself located in an appropriate containment unit, was the beginning of real biosecurity. The unique but huge disadvantage of this process was the presence of allergens from cell culture in the vaccine and the allergic reactions this provoked during regular vaccination campaigns. It took a decade to fine-tune purification steps so that a potent, non-allergenic vaccine could be produced in huge volumes without impairing the virus yield (1).

After the search for new adjuvants for potent FMD vaccines for pigs by McKercher and his group at Plum Island in the USA after 1965, it became obvious in the early 1970s, that oil-adjuvanted vaccines for cattle could have a promising future in regions such as South America where cattle breeding was extensive. In that region the oil-adjuvanted vaccines were well accepted both from an immunological and a political point of view, because they offered a new approach to rectify the errors of the past in disease control. Oil-adjuvanted vaccines administered by intra-muscular route protected cattle under a great variety of breeding conditions and appeared to provide longer-lasting immunity than the previous aqueous vaccines (41). Injected by vaccinators in planned programmes, oil-adjuvanted vaccines proved to be more efficient than classical vaccines bought by cattle breeders to comply with legislation but rarely injected. In fact, the great successes observed in FMD control in infected areas of South America are essentially due to the intensive use of oil-adjuvanted vaccines of good quality.

Scientific discoveries

It had been well known since the paper by Moosbrugger in 1948 (29) that after inactivation with formaldehyde FMD vaccines could remain virulent for a few days after their date of manufacture. The kinetic studies of inactivation in the 1950's confirmed that formaldehyde was not an inactivant of the first order. In 1959, Brown and Crick (15) explored the properties of a new family of inactivants: the aziridines, which were first used in the vaccine industry in 1971 by Pay *et al.* (36). But the breakthrough came in 1973 from Bahnemann (2), working for PANAFTOSA (Pan American Foot-and-Mouth Disease Center) in Rio de Janeiro, who demonstrated that in a very simple chemical reaction, an aziridine, the cyclised ethylene-imine, can be synthesised by vaccine manufacturer using a halo-ethylamine, most often the 2-bromo-ethylamine, just before the inactivation process starts. The method was immediately adopted worldwide and often repeated for biosecurity reasons in a double inactivation step. Of the hundreds of billions of vaccine doses that have been tested worldwide for safety since the introduction of this method, not one has been reported to be virulent.

Later, in the middle of the 1990s, in laboratories involved in FMD research, new studies shed light on the role of FMD virus non-structural proteins (NSPs) in the immune response and on their potential use for diagnosis (20). These findings were a revolution, as a vaccine that did not contain NSPs could be used in vaccination programmes without hampering the serological diagnosis of virus infected/carrier animals. That was the wish of all the vaccine manufacturers, who were being blamed because their products were hiding potential infection behind the protection conferred by vaccination. The DIVA system (Differentiating Infected from Vaccinated Animals) was finally applicable to FMD vaccination. That was a real change, with many consequences for the image and use of FMD vaccination.

The purification of antigens became a double necessity for manufacturers using BHK cells, firstly to remove the heterologous proteins of cell culture origin because of their allergenic role and secondly to remove the FMD virus NSPs of virus culture origin for their interference with the new serological method of diagnosis. Technical discoveries like chromatography or the use of poly-ethylene-glycols and high polymers of oxide of ethylene helped to solve this industrial challenge, without affecting the potency of FMD vaccines.

A beneficial consequence of the high purification process of the FMD antigens was the high degree of concentration of antigens, from 250 to 1,000 times (1); moreover, these concentrated antigens could be frozen and stored in vaccine banks as strategic reserves for emergency vaccinations. The possibility of obtaining on request, in just a few days, several million doses for emergency

vaccination brought a big change in vaccination strategies. Vaccine producers were also able to create their own banks to enable them to respond, within a very short time, to any request for vaccine for the formulation of multivalent vaccines anywhere in the world (27).

Regulation

After the publication of reports by Beck and Strohmaier (6) of repeated outbreaks of FMD in Germany, the sources of which were vaccines with residual virulence and virus escapes from vaccine plants, the European and the international communities reacted. They promulgated various regulations concerning biosecurity, good manufacturing practice, and marketing authorisations. More recently, to prevent transmissible spongiform encephalopathies, an EU directive has been introduced on the control of the origin of biological raw materials. Export controls on FMD products and equipment are also in force to prevent dual-use. Verification of compliance is carried out by national or international inspectors at vaccine plants.

Nowadays, in Europe and South America, FMD vaccines are the most inspected and controlled vaccines of all veterinary vaccines. When free of FMDV NSPs they are very useful tools for controlling the disease in endemic areas. Vaccines that do not contain NSPs mean that serological surveys can be used to differentiate vaccinated from infected animals, so this type of vaccine is also useful during outbreaks in previously FMD-free countries when stamping out is not sufficient to stop the disease progression.

History of foot and mouth disease vaccination

This brief summary of the various ways in which vaccination has been used over the years provides an overview of the different phases of the history FMD control in many regions of the world. It is clear that the history of FMD vaccination strategies is closely linked with the evolution of vaccine technology in general. Joubert (25) describes four distinct phases in the history of FMD vaccination and it will be useful to include them here:

a) The initial phase is characterised by the absence of national or regional level health plans and by limited funds for controlling the disease. Very often this is accompanied by ignorance amongst rural populations, vaccines of questionable quality, and an unreliable cold chain. This situation is observed in countries or regions after serious political conflicts, as seen in Europe in the past or in other continents presently. The result is the use of FMD vaccines on an individual basis by some farmers, vaccine being obtained by purchase or donation. The vaccination strategy is absent and vaccination takes place in scattered areas of the country. Sanitary measures are not applied. This method of conducting FMD vaccination is inefficient

and surprisingly costly because there is no return on investment.

b) The second phase is characterised by a better awareness of the benefit gained in controlling the disease and vaccination is always the first option. The strategy is generally limited by the lack of funds; consequently the vaccine is used where considered useful, i.e. around outbreaks, following a ring or a zone vaccination strategy. Vaccine batch control is often inadequate due to absence of structure, expertise and funds. The required sanitary measures are known, but are rarely in force due to a lack of funds or of qualified personnel. In such countries, FMD vaccination is always initiated after the appearance of viruses and always takes time to stop virus progression. The consequence of this is a failure to protect national livestock from the disease. Additionally, new FMDV isolates demonstrate the constant genomic evolution of current virus(es) after selection through the 'filter' of partially immunised or convalescent animals. There is no return on the investment made in controlling FMD. Many developing countries are still in this phase of their history of FMD vaccination.

c) The third phase is characterised by a true national willingness to control and eradicate the disease. Often a National Commission for FMD Control is created for centralising information and directives. With more money allocated or/and collected from farmers or from commercial meat circuits, the control of FMD proves to be rapidly effective if farmers are educated and convinced. The 'winning trilogy' for full FMD control using vaccination is the following:

– the national programme should be enshrined in law and 100% of vaccine batches should be controlled, with failing batches destroyed and not only refused;

– throughout the country (or zones to be controlled), vaccination should be compulsory at the same period(s) of the year, with more than 90% coverage of the designated species for each campaign, and vaccination should be carried out by registered personnel, not farmers. Vaccinated large ruminants should be individually identified and recorded;

– farmer's associations should be encouraged and regularly informed and educated. During outbreaks, fair compensation for elimination of animals should be given to encourage breeders to declare suspicion of disease and respect the sanitary legal measures in force.

When the French National Vaccination Programme (promulgated on 23 August 1961) was extended to include almost 100% of the (individually identified) French cattle population in 1962, and small ruminant populations along borders in 1972, the number of outbreaks fell dramatically in a short period of time (Fig. 6). The same causes induced

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the same effects when Germany started its National Programme in 1966 (Fig. 7).

Such extensive national vaccination strategies were successfully used in all European countries between 1960 and 1992, up until the EU decided to ban FMD vaccination, immediately followed by other European countries. A similar vaccination strategy is currently being employed at regional level in South America to enable countries in this region to obtain the status of a country that is 'FMD free with vaccination', a status created by the OIE in the 1990s and described in the OIE *Terrestrial Animal Health Code* (47). In such recognised countries, the use of FMD vaccines that do not contain NSPs is of prime importance: it allows for conclusive serological surveys on the absence of FMD virus circulation, facilitates the export of cattle products, and can enable countries to achieve 'Free of FMD without vaccination' status more quickly.

d) The fourth phase is the end of the long process of vaccination or is adopted right away by countries geographically protected from FMD threat. This phase is characterised by the absence of vaccination in livestock, the existence of national/regional antigen reserves for emergency vaccination and the implementation of very strict sanitary prophylaxis, i.e. sanitary controls at borders and inside national territory. This phase could be recognised by the OIE as 'Free of FMD without vaccination' (47). Of course, any strategic reserves for emergency vaccination (27) should be made from purified antigens without detectable levels of NSPs; using this type of vaccine will take advantage of the new OIE rules concerning the use of serological testing (46) for re-gaining 'FMD-free without vaccination' status in six months.

This fourth phase, including storage of strategic reserves, was reached some years ago by European continental countries after 25 to 27 years of medical prophylaxis as described in phase 3, but it was adopted straight away by the UK, Norway, the USA, Canada, Australia and New Zealand due to their favourable geographical situation.

Other veterinary contributions

A major step in the development of vaccines took place in the USA, when Salmon and Smith (1886) (40) demonstrated that pigeons could be protected against infection with a hog *Salmonella* (at the time called 'hog cholera virus') by inoculation with a heat-killed preparation of a culture of the organism. This method of vaccination proved to be widely applicable for bacterial infections, and by the end of the 19th Century killed vaccines had been developed for typhoid, plague and cholera in humans and for several bacterial diseases of animals.

The discovery by Roux and Yersin (1888) (39) of a soluble product in a culture of diphtheria bacilli (diphtheria toxin) that could produce all the symptoms of diphtheria in experimental animals, and the subsequent demonstration by von Behring and Kitasato (1890) (8) of antitoxic potency in sera of animals which had recovered after inoculation with such toxins, were of major significance in the development of both immunology and vaccinology. Initially, toxins were used for vaccination by preparing balanced toxin-antitoxin mixtures (7) but, while success could be achieved in experimental animals, it was not a practicable procedure for use in the field. However, the production of toxoids by formalin inactivation of toxins by Glenny and Hopkins in 1923 (23) and independently by Ramon in 1924 (Fig. 8) (38) added another tool to vaccinology, one which could combat bacteria whose virulence was mediated through toxins, e.g. those causing
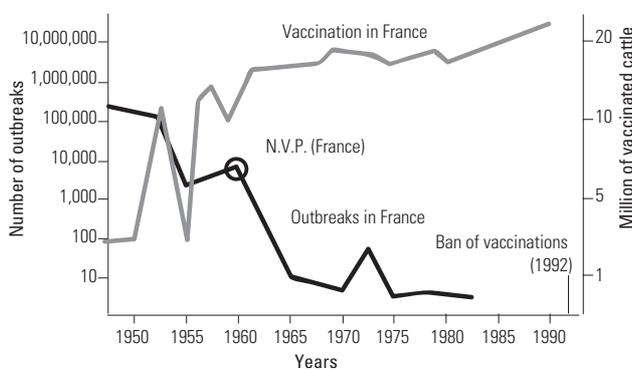
Fig. 6
The effect of the National Vaccination Programme (NVP) (which covered 100% of the cattle population) on reported outbreaks in France between 1962 and the ban of vaccination in 1992
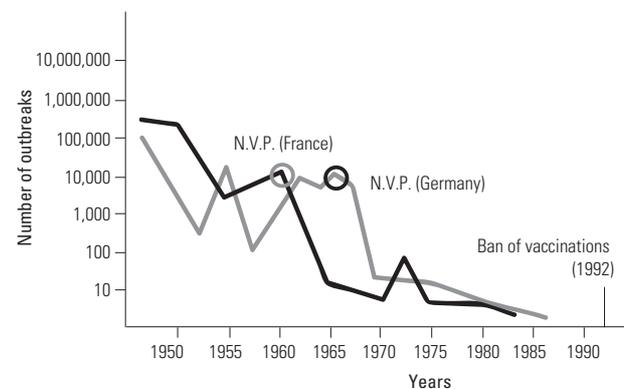
Fig. 7
The comparative effects of National Vaccination Programmes (NVP) in France and Germany

Fig. 8
**Gaston Ramon at the World Organisation for Animal Health
(OIE) in 1959**
Source: OIE

diphtheria and tetanus in humans, and tetanus and a range of other clostridial diseases in animals.

Vaccination against Marek's disease in poultry is considered to be the first example of widespread use of a vaccine to effectively control a naturally occurring cancer agent. Although Marek's disease vaccine was primarily developed for protecting chickens, its importance extends beyond the field of animal health and it has contributed to our understanding of related human diseases and fundamental biology (17). Within the last decade there has been a dramatic change in the method of delivery of Marek's disease vaccines in commercial broiler chickens. Previously, they were administered at hatching by the subcutaneous route. Today, most major commercial hatcheries use the *in ovo* delivery system. With this system, live vaccine viruses are administered to embryonated eggs before hatching. Injection in ovo is given at the time eggs are transferred from the incubator to the hatchery, usually around embryonation day 18. Automated, multiple head injectors deliver a precise quantity of vaccine simultaneously to an entire tray of eggs (16). This simultaneous inoculation of large numbers of eggs saves on the labour costs associated with injecting individual chicks after hatching. There is no apparent adverse effect from *in ovo* injection on either the hatchability of the eggs or the long-term performance of the chickens.

A recent development in veterinary medicine is the extension of vaccination to wildlife. Through the systematic vaccination of the European wildlife reservoir, the red fox (*Vulpes vulpes*), terrestrial rabies was eliminated from most of Western European countries (14). Wild boars (*Sus scrofa*) are presently vaccinated to eliminate classical swine fever *Pestivirus* from this wildlife reservoir (26); even vaccination of wildfowl against highly pathogenic avian

influenza virus, or vaccination of African buffaloes (*Syncerus caffer*) against foot and mouth disease are envisaged.

Conclusion

These few episodes in the past illustrate the close relationship between veterinary and human vaccines that still holds true today, and a whole book could be written on the subject. Nowadays, as in the past, when there are both human and animal forms of a disease, sometimes it is the human vaccine that arrives first and sometimes the animal vaccine. Whichever comes first serves as a guide for the other. An area where advances in veterinary vaccines are particularly well developed is in parasitic diseases. For instance, although a human vaccine against human schistosomiasis is still not available, there is a satisfactory vaccine against bovine schistosomiasis, even though the parasite involved is very similar to *Schistosoma mansoni*. There is also a vaccine against bovine lungworm, based on irradiated larvae. We are still awaiting one or more of the promised vaccines against malaria, whereas a vaccine against canine babesiosis is already on the market.

Where there is a risk of epizootic diseases passing to humans as a result of a reassortment involving different strains, as in the case of avian influenza, physicians see the animal vaccine as the first line of defence in avoiding a possible pandemic. The very latest human vaccine against rotaviruses, the result of a cross between an avian strain and an attenuated bovine strain, is a reminder of what the history of vaccination has revealed: the movement of pathogens between species can pose a very real threat but can also be exploited for prophylactic purposes.

Another line of convergence between human and veterinary vaccines has arisen in recent years. In the legislation to ensure greater reliability and safety of vaccines, we see the extent to which veterinary vaccines are now controlled at all stages of trials before being licensed, in a way that does not fundamentally differ from the situation with human medicine. This tendency to converge merely confirms the historic vocation of these 'two medicines' to work together. Nowhere is this more apparent than in the history of vaccinology, to which so many veterinarians have contributed.

Foot and mouth disease, which was among the first diseases recorded centuries ago in literature, has always mobilised shepherds, farmers, veterinarians and government authorities to find a means to minimise the consequences of the disease for livestock. Vaccination against the disease appeared possible between the two World Wars, but not until the 1950s, when vaccines began to be produced on an industrial scale, did it become

feasible to make vaccine available in large quantities for mass vaccination strategies. Vaccination has been a successful alternative to the more risky practices of aphtisation and serotherapy.

From the First World War up to the middle of the 1950s, FMD was observed throughout most of the European continent in the form of widespread epizootics occurring at intervals of approximately six years (13). This situation is over in Europe thanks to vaccination, but is nowadays still prevailing in some parts of the Asian and African continents. The history of FMD eradication in Europe, culminating in the ban on vaccination in 1992, should be an encouragement and a model for countries were FMD remains a scourge that impairs the development of the dairy and meat industry at a time when the Livestock Revolution has already started.

Une brève histoire des vaccins et de la vaccination

M. Lombard, P.-P. Pastoret & A.-M. Moulin

Résumé

La vaccinologie humaine, qui s'intéresse d'abord à l'individu, semble très éloignée de la médecine vétérinaire dont l'objet principal est la santé du troupeau. Pourtant, nombre d'épisodes du passé (variole, choléra aviaire, fièvre charbonneuse, rouget du porc, rage, tuberculose, etc.) illustrent la proximité de la recherche sur les vaccins à usage vétérinaire et humain. Dans certains cas, le vaccin humain fut le premier à être développé, dans d'autres ce fut le vaccin à usage vétérinaire. L'histoire de la vaccinologie révèle l'importance de la collaboration entre ces « deux médecines ». Les vaccins contre la fièvre aphteuse ont été parmi les premiers à être mis au point, dès la fin du XIXᵉ siècle. Grâce aux découvertes de plusieurs chercheurs, notamment européens, tels que Vallée (un Français), Waldmann (un Allemand), Frenkel (un Néerlandais) et Capstick (un Britannique), la production à échelle industrielle des vaccins contre la fièvre aphteuse a démarré dans les années 1950, permettant de vacciner des millions d'animaux, en Europe et ailleurs. Les stratégies de vaccination contre la fièvre aphteuse ont toujours été tributaires des propriétés des vaccins utilisés. En ce début de XXIᵉ siècle, les vaccins contre la fièvre aphteuse sont conçus de telle sorte que les tests sérologiques sont désormais capables de différencier les animaux infectés des animaux vaccinés, ce qui a modifié les prescriptions de l'OIE en matière d'échanges internationaux d'animaux et de produits d'origine animale. Les auteurs abordent également l'histoire de la vaccination contre la peste bovine, la péripneumonie contagieuse bovine et la maladie de Marek.

Mots-clés

Fièvre aphteuse – Histoire de la vaccinologie – Maladie de Marek – Péripneumonie contagieuse bovine – Peste bovine – Relation entre les médecines humaine et vétérinaire – Vaccin – Vaccin vétérinaire – Vaccination.

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Una breve historia de las vacunas y la vacunación

M. Lombard, P.-P. Pastoret & A.-M. Moulin

Resumen

La vacunología humana, centrada sobre todo en el individuo, parece muy alejada de la medicina veterinaria, que se ocupa esencialmente de la salud de los rebaños. Sin embargo, en el pasado ha habido numerosos episodios (de viruela, cólera aviar, carbunco bacteridiano, erisipela porcina, rabia o tuberculosis, por ejemplo) que han puesto de relieve los estrechos vínculos que existen entre la investigación sobre las vacunas humanas y la dedicada a las vacunas veterinarias. En algunos casos la vacuna humana ha precedido a la animal, mientras que en otros ha ocurrido lo contrario. La historia de la vacunología demuestra a las claras la importancia de que estas 'dos medicinas' trabajen conjuntamente. Las vacunas contra la fiebre aftosa se cuentan entre las primeras que empezaron a fabricarse, desde finales del siglo XIX. Gracias a los descubrimientos de una serie de investigadores, entre ellos varios europeos como Vallée (francés), Waldmann (alemán), Frenkel (neerlandés) y Capstick (británico), a partir de 1950 se empezaron a fabricar a escala industrial, cosa que sirvió para vacunar a millones de animales tanto en Europa como en otras regiones. Las estrategias de vacunación contra la fiebre aftosa han dependido siempre de las propiedades de la vacuna empleada. Hoy en día, en los albores del siglo XXI, las vacunas están concebidas de tal manera que una prueba serológica permite distinguir entre un animal infectado y uno vacunado, hecho que ha influido en los reglamentos de la OIE sobre el comercio internacional de animales y productos de origen animal. Los autores también abordan la historia de la vacunación contra la peste bovina, la perineumonía contagiosa bovina y la enfermedad de Marek.

Palabras clave

Enfermedad de Marek – Fiebre aftosa – Historia de la vacunología – Perineumonía contagiosa bovina – Peste bovina – Relación entre la medicina humana y la veterinaria – Vacuna – Vacuna veterinaria – Vacunación.

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