Publically funded research combats foot-and-mouth disease

Science & Technology Facilities Council

BBSRC bioscience for the future

Using the expertise in foot-and-mouth disease at The Pirbright Institute[a] and facilities for determining protein structures at Diamond Light Source[b] and its predecessor the Synchrotron Radiation Source (SRS)[c], researchers have developed a new vaccine against foot-and-mouth disease (FMD).

The new vaccine is synthetic and is not produced from live infectious viruses, making production much safer and cheaper. It also promises to be more effective than current commercially-available vaccines, because it has been designed to trigger an optimum immune response and engineered to be more stable. The improved stability has the benefit of removing the need for refrigeration of the vaccine, making it easier to transport and store.

A major international vaccine manufacturer is now working in partnership with the researchers to develop a marketable product. A commercial vaccine based on this research could improve the health of farm animals around the world, with resulting benefits for human wellbeing. Reducing the prevalence of FMD worldwide would also reduce the likelihood of a further outbreak in the UK.

"The preliminary results look very encouraging," says Dave Stuart[1], MRC Professor of Structural Biology at the University of Oxford, and Director of Life Sciences at Diamond. "We hope that the new vaccine will be able to get to market and really start to help animal health on a global scale."

Development of this vaccine is one of the latest breakthroughs to arise out of nearly a century of FMD research at The Pirbright Institute, with X-ray facilities at the Synchrotron Radiation Source (SRS) and Diamond playing a vital role in determining the structure of the FMD virus and subsequently producing a stable vaccine.

Work on the structure of the FMD vaccine was carried out by Stuart and his team at the University of Oxford, using the SRS and Diamond Light Source. Production and testing was performed by Dr Bryan Charleston at The Pirbright Institute and Professor Ian Jones at the University of Reading, and their teams. The vaccine was then analysed by the Oxford team to demonstrate it consisted of authentic copies of FMD viruses.

Together the three groups have developed a system for the production of the vaccine in commercially viable amounts. Funding from BBSRC, STFC, the Department for Environment, Food and Rural Affairs (Defra) and the Wellcome Trust, for both the research and the facilities used, made this breakthrough possible.

The foot-and-mouth problem

FMD is a highly infectious disease, caused by a virus that affects animals including cows, sheep and pigs[2]. It has a devastating effect on agricultural production as it reduces growth rates, fertility and milk production in affected animals.

The most recent UK outbreaks of FMD occurred in 1967, 2001 and 2007. During the worst of these, in 2001, the estimated cost to the UK economy was £8Bn[3].

Although FMD outbreaks are rare in most of Europe, the disease is regularly found in parts of South America, Central and Eastern Africa, and the Middle and Far East[4]. Three quarters of the world's population live in countries where FMD is endemic .

In the developing world, where animals are often

IMPACT SUMMARY

The new vaccine could reduce the incidence of FMD in endemic regions, where the annual cost of FMD is estimated at £4-13Bn[5], and help stabilise these countries' economies.

Use of the vaccine in endemic regions would reduce the likelihood of a major UK FMD outbreak. The estimated cost of the 2001 outbreak to the UK economy was £8Bn[3].

Unlike current vaccines, the new vaccine could allow vaccinated animals to be differentiated from diseased animals, protecting UK exports.

The new vaccine is produced from synthetic, non-infectious 'viruses' and can survive at up to 56˚C, making it safer to produce and easier to transport and store the estimated 2.35Bn doses of FMD vaccine administered worldwide each year[5].

A major international vaccine manufacturer is working in partnership with the researchers to develop a marketable product. The global market for FMD vaccines is expected to reach £330M by 2018[6].

Cow infected with foot-and-mouth disease.
Image: *The Pirbright Institute*

a. The Pirbright Institute is strategically funded by BBSRC. b. Diamond Light Source is funded by STFC and the Wellcome Trust. c. The SRS was funded by STFC when it closed in 2008, and formerly by predecessors to the current Research Councils.

relied on not only for food but also for draught power, such as for transport and cultivating land, FMD creates a food security issue and contributes to poverty and malnutrition[5].

"Foot-and-mouth disease is still one of the worst plagues of livestock around the globe," Stuart explains. "In large parts of the world, the disease is pretty much rampant; it's not under proper control. There's always the chance of the disease leaking from there into places like the UK which are normally disease-free."

Every year, an estimated 2.35Bn doses of FMD vaccine are administered in the world in an effort to control the disease[5]. The annual cost of FMD in endemic regions, in terms of visible production losses (due to death and reduced performance of animals) and vaccination, is estimated to be between £4Bn and £13Bn[5].

The challenge of vaccination

Vaccination of animals with FMD presents a range of problems. One issue is that there are seven types (serotypes) of the FMD virus, and vaccination against any one of these does not offer any protection against the others. Also, present vaccines only protect against the disease for a matter of months.

A further problem with currently available vaccines is that they are produced from a live but inactivated virus. Consequently, they can only be produced in elaborate, highly contained facilities to prevent the virus escaping into the environment and causing disease. This makes vaccine production expensive, and raises safety issues. As a result of these production problems, there is a shortage of the vaccine.

"In places like India, they've got a very good programme of vaccination, but it is challenging to produce sufficient quantities using current technology," says Stuart.

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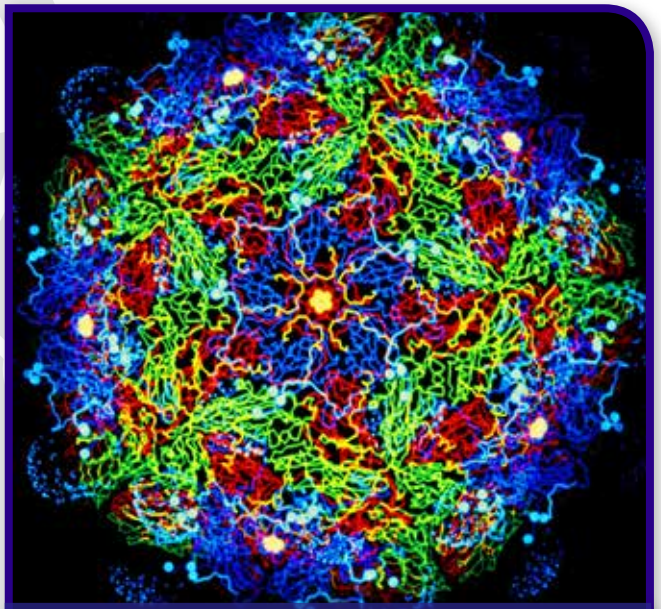


Structure of foot-and-mouth disease virus. Image: *The Pirbright Institute*

Another difficulty in vaccinating animals against FMD is that the vaccine is highly unstable and must be kept refrigerated between 4 and 6°C until it is used, making it complicated to transport and store, and limiting its use in parts of the developing world.

The new vaccine

If it becomes commercially available, the new FMD vaccine could solve many of these problems. It is synthetic and not based on live viruses, making its production safer. This also means high containment facilities are not required for growing or handling the virus, lowering production costs.

The new vaccine is also more stable than currently available vaccines and can survive at up to 56°C. This makes transportation and storage much easier and facilitates its use in developing countries. Because of its stability, the vaccine should also produce an optimum immune response in vaccinated animals, making it more effective.

These factors mean the new vaccine could be produced safely and cheaply on a large scale and used throughout the world.

"I think it would completely transform the way the disease is contained," says Stuart, "and if the disease load around the world was significantly reduced, the chance of an outbreak in this country would be significantly reduced."

This would also deliver economic benefits for vaccine manufacturers. The global market for FMD vaccines in 2013 was estimated to be worth £260M, and it is expected to reach £330M by 2018[6].

The new vaccine has another advantage: because it is synthetic, it can be distinguished from live viruses, so a test could be developed to differentiate vaccinated from diseased animals. This is not possible with current FMD vaccines. As a result, the new vaccine has the potential to be an invaluable new weapon in the fight to eradicate FMD and has clear advantages over current technology as a possible option to help control the disease should another outbreak occur in the UK.

Stuart explains, "What you need to be able to do is to very reliably discriminate between an animal that has been vaccinated and one that has been infected, so you can say 'There is no chance that any of our animals have been infected.' If there is any doubt over that, then you lose the ability to export your meat and that's clearly economically disastrous."

Tests of the new vaccine have so far shown that it is as effective as current vaccines against one of the seven types of the virus[7]. The researchers are now producing and testing vaccines against other types. A major international vaccine manufacturer is working in partnership with the researchers to develop the vaccine into a commercial product.

Unravelling the virus structure

The first stage in developing the new FMD vaccine was to know the structure of the virus.

In the 1980s, Stuart studied the structure of the FMD virus in collaboration with Professor Fred Brown, a highly respected virologist at the Wellcome Foundation laboratories on the Pirbright site. Brown held the belief, which was highly unusual at the time, that knowing the structure of a virus was the key to designing better vaccines against it. At that point, virtually no virus structures were known.

As most viruses are too small to see with a standard microscope, the virus structure could only be determined using protein crystallography: shining an intense beam of X-rays through a crystal made up of the viruses and using the pattern produced by the emitted X-rays to work out the structure of the viruses in the crystal.

The world's first dedicated X-ray source for protein crystallography had recently been created at the Synchrotron Radiation Source (SRS) based at the Daresbury Laboratory in Cheshire. A synchrotron light source, such as the SRS and Diamond, is a facility that produces radiation, in this case X-rays, by accelerating tiny particles to almost the speed of light.

The SRS was funded at the time by the Science and Engineering Research Council (SERC) and gave the researchers ready access to state-of-the-art facilities without leaving the UK.

"Because of the facilities at the SRS, UK researchers had the leading edge in protein crystallography, and in particular in virus structure determination," says John Helliwell, Emeritus Professor at The University of Manchester, who carried out the first protein crystallography studies at the SRS[8,9].

In 1989, using the SRS, Stuart and his colleagues determined the structure of the FMD virus[10].

Developing the vaccine

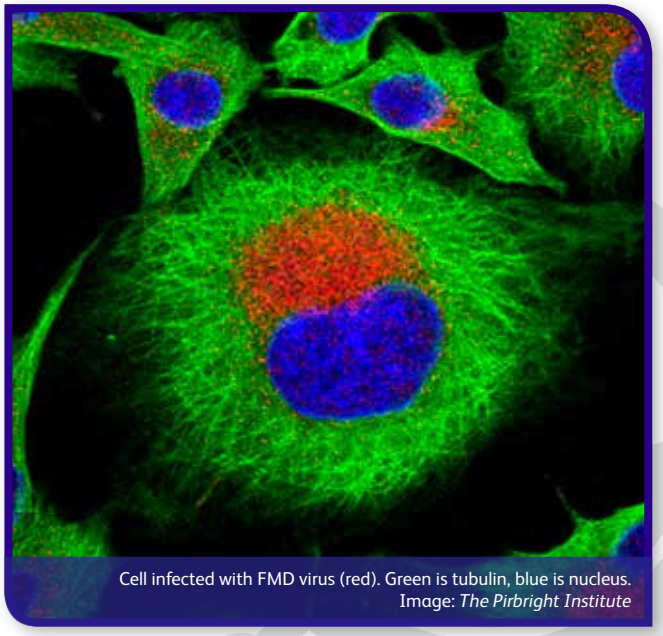
Following the 2001 UK outbreak of FMD, a report by the Royal Society recommended research into a new vaccine. The work began with BBSRC Combating Viral Diseases of Livestock funding to understand the immune response to the FMD virus and virus transmission in more detail. Out of this knowledge came the vaccine development programme, which was led by Dr Bryan Charleston, Head of the Livestock Viral Diseases Programme at The Pirbright Institute[11].

The first step was the ability to make a synthetic copy of the virus[12], which Charleston's research team achieved in collaboration with Ian Jones[13], Professor of Virology at the University of Reading, and his team. Charleston and Jones then worked with Stuart and his team at the University of Oxford to engineer the structural changes required to enhance the stability of the synthetic 'virus' and enable commercial development. The work was funded through grants from BBSRC, Defra and the Wellcome Trust.

Aerial view of Diamond Light Source. Image: *Diamond Light Source.*

Using his expertise in genetically engineering insect cells to produce proteins, Jones was able to produce a synthetic replica of the FMD virus' protein outer shell (the capsid)[12]. These empty shells are effective as a vaccine because it is the outer shell of a virus that is recognised by the body's immune system and causes it to mount a defence.

Not only are these shells safe, because they are not infectious, they can also be made more stable than the live viruses used in currently available vaccines, because radical changes can be made to the synthetic shells that would kill a live virus. This increased stability makes the new vaccine more effective.



Cell infected with FMD virus (red). Green is tubulin, blue is nucleus.
Image: *The Pirbright Institute*

"The shells are like a football," Charleston explains. "If they are intact, they will drive an immune response that will protect the animal against disease. If the shells break down into their individual components, then that doesn't stimulate a protective immune response. So physical stability is key to producing a useful vaccine."

Live viruses are intrinsically unstable, which is why vaccines based on these viruses require refrigeration, and still degrade over time despite this. Since the new vaccine is synthetic, in that it contains no live virus, it can be altered so that it retains high stability even without refrigeration.

The ability of the researchers to increase the stability of the vaccine was completely reliant on two world-class Research Council-funded facilities: The Pirbright Institute and Diamond Light Source, the UK's national synchrotron, which was built to replace the SRS.

To improve the stability of the protein shells used in the vaccine, Stuart and his colleagues studied the structure of FMD viruses with differing degrees of stability, using protein crystallography, at Diamond. They used this information about the virus structure to produce a computer programme that could predict appropriate changes to make to a shell to stabilise it.

In the laboratories at The Pirbright Institute, Charleston and his colleagues made the changes calculated using the computer programme, then tested the shells' stability to confirm that it had indeed improved. Once this was established, Stuart and colleagues examined the shells at Diamond to see if they resembled the more stable versions of the virus, as expected.

It would not have been possible to produce a stable vaccine in this way without the use of Diamond to visualise the structure of the virus shells.

"Given that within the virus there are 1000 different residues in the structural proteins that you could change, and you could change any one of those residues into 20 other residues, if you didn't know the structure of the virus, it would be impossible to stumble upon what you should do to stabilise it," explains Stuart.

"The particles are so small you can't see them with a normal microscope – you probably get a billion or so on a pinhead – and yet, using Diamond, we can see them at the atomic level, which is amazing."

This work also relied on the long history of FMD research at The Pirbright Institute, dating back to the 1920s. Pirbright has been the World Reference Laboratory for FMD since 1958 and has played a central role in global efforts to fight the disease over the years, including developing diagnostic tests and technology to determine the origins of outbreaks. As a result, Pirbright is a world-leading source of FMD expertise and provides world-class facilities to study the disease.

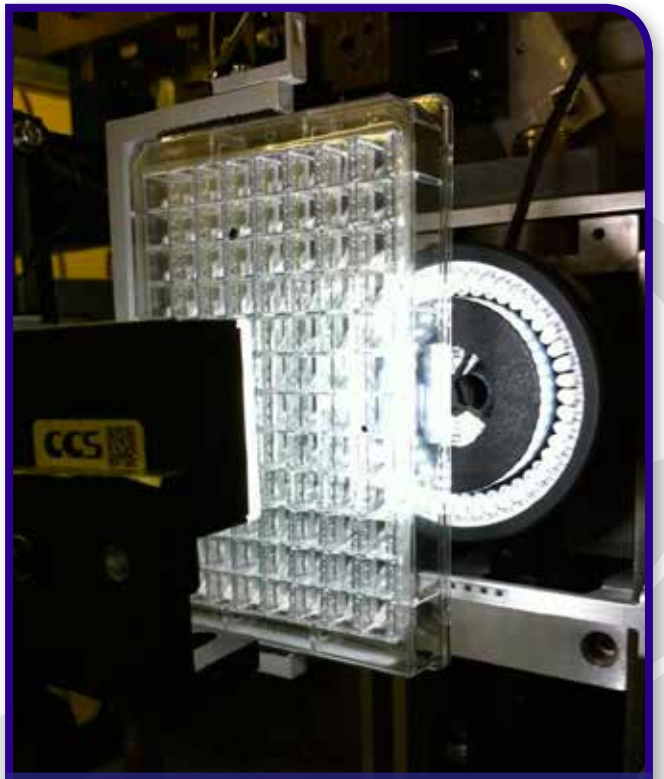
It is due to the history of FMD research at The Pirbright Institute (formerly the Institute for Animal Health) and protein crystallography at the SRS and later at Diamond that development of this vaccine in the UK was possible.

"I think we have got great added value from these facilities," says Charleston. "The facilities are there and available, and we have sought funding from additional sources to carry out this work in them. Clearly we would not have received that funding if we didn't have access to these facilities."

The future

The researchers are continuing to work on the new FMD vaccine, with funding from Defra and the Wellcome Trust, to produce vaccines against the more common and less stable of the seven types of the FMD virus. At the same time, they are working with a commercial partner to produce a marketable product. Whilst this is still several years away the team believes that if it reaches the market, the vaccine will have a dramatic effect on animal health around the world.

The benefits of this research may also extend beyond preventing FMD. The researchers are now collaborating with scientists at the University of Leeds, the National Institute for Biological Standards and Control (NIBSC) and Harvard University, to see whether the same approach can be used to produce a synthetic vaccine against the virus that causes polio. This is a highly infectious disease in humans that can result in paralysis. A synthetic vaccine against polio could contribute to worldwide efforts to eradicate the disease.



Scrutinising the vaccine on Diamond's microfocus beamline, I24.
Image: *Oxford University*

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