

COULD RNA INTERFERENCE
BE INTRODUCED AS A
EFFECTIVE TOOL
FOR FIGHTING THE
FOOT AND MOUTH VIRUS?

BY
BETH RILEY

PASS WITH MERIT

RESEARCH PAPER
BASED ON
PATHOLOGY LECTURES
AT VET-MEDLINK 2009

ABSTRACT

Foot and mouth disease affects cloven hoofed animals and causes huge loss to the global agricultural industry every year. The foot and mouth virus spreads quickly and has huge genetic variation making vaccines aimed at activating antibodies difficult to produce, therefore the current measures in place are not sufficient to prevent an outbreak spreading widely and new strategies need to be developed. The newly discovered RNA interference (RNAi) pathway has thus far proven to be an effective antiviral measure however there are several issues regarding an RNAi based vaccine. The use of different vectors for the improved delivery of short interfering RNAs (siRNAs) and ways of stimulating the transfer of siRNAs between cells in the vaccinated animals are discussed. Two possible methods of prolonging protection are examined. Bio-security and moral issues need to be upheld throughout to ensure there is no unnecessary suffering or infection.

INTRODUCTION

Foot and mouth disease (FMD) is a disease that affects cloven hoofed animals (Chen *et al.*, 2006). This disease causes huge financial loss to the global agricultural industry each year through the destruction of stock due to infection or exposure to infection, the loss of productivity or product quality and trade embargo's on the products. It is estimated that the UK economy lost £2.4-£4.1bn in 2001 due to the FMD outbreak (BBC 2002). FMD is spread by a virus (FMDV), which is highly contagious (Pengyan *et al.*, 2008), can change its antigenic identity and has a high rate of mutation (Kahana *et al.*, 2004), making vaccination difficult. FMDV is from the genus *Aphovirus* of the family *Picornavidae*, it has 7 distinguishable serotypes and about 80 sub-types (Kahana *et al.*, 2004). The main types are; O, A, C, Asia1, SAT1, SAT2, and SAT3 (Chen *et al.*, 2006). FMDV can cause explosive epidemics as is illustrated in fig.1 which shows how many cases emerged daily during the 2001 UK epidemic. The potential to cause these epidemics is due to the low dose of FMDV needed for infection, the large amount of virus particles excreted, the multiple routes of transmission and a short incubation period (Chen *et al.*, 2006). The current measures in place may not be sufficient to prevent an outbreak spreading widely. This means that effective and fast acting antiviral strategies must be developed. The measures currently used worldwide to control a FMD outbreak are control of animal movement, slaughter and routine vaccination (Chen *et al.*, 2006). At present vaccines are based on an inactivated virus and are effective at eliminating the disease however there is a risk of the live virus escaping (Chen *et al.*, 2006). This was clearly highlighted in 2007 when the UK outbreak was probably caused by a leaking drain at a lab (BBC 2008). It has been demonstrated that a vaccine containing porcine alpha interferon, FMDV capsid and 3C proteinase coding regions gave immediate protection against viral challenge (Moraes *et al.*, 2003).

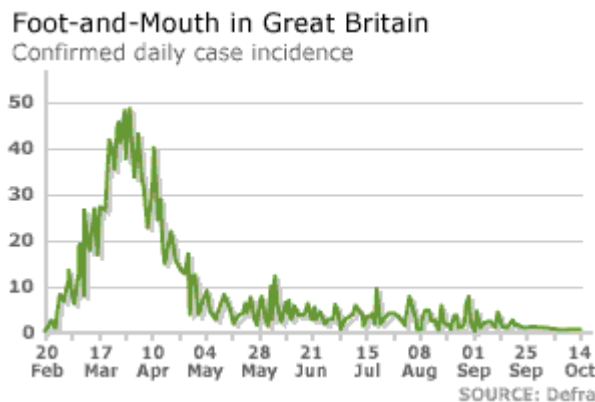


Fig 1 (BBC, (2002))

RNA interference (Ribonucleic acid interference (RNAi)) is a pathway by which double stranded RNA (dsRNA) is fragmented by the enzyme Dicer, these short strands then activate the RNA Induced Silencing Complex (RISC) which opens up the dsRNA and copies its fingerprint. The RISC then destroys all RNA containing the same sequence. This pathway is a vitally important immunological

response to viruses in plants and invertebrates (see Nobel (2006) for more information) but it does not occur in higher animals as an antiviral measure. Instead they use an interferon based inflammatory response that shuts down all protein production when any long dsRNA enters the cells. However the RNAi pathway is still present and is involved in silencing the cells own genes (Pengyan *et al.*, 2008). This leads to the possibility of using short strands of dsRNA or short hairpin RNAs (shRNA) to activate the RNAi pathway. This may allow cells to fight viruses and other dsRNA related conditions such as cancer. This pathway becomes ineffective if the sequence on the virus mutates, Pengyan *et al.* (2008) chose siRNAs relating to an area of the virus which was the same in all the FMDV serotypes recorded in an international database GenBank. The RNAi pathway is thus a vitally important area of research in medicine and veterinary medicine today.

This paper will address some of the problems facing this area of research, including delivery of the siRNAs to the cells and how to prolong protection. It will also discuss some of the moral issues surrounding the research, the problems with some of the current research and possible drawbacks of a vaccine based on this technology.

DISCUSSION

The delivery of the siRNAs remains one of the greatest problems in developing an RNAi based vaccine. Currently adenovirus has been one of the favoured vectors, and although it does seem to be reasonably effective there are problems (Chen *et al.*, 2006). It appears that the FMDV and adenovirus target different areas of the body therefore the protection is not full. Chen *et al.*, (2006) showed this when they examined several organs from some of their experimental animals, they found that the majority of the adenovirus was in the liver while the majority of the FMDV was in the epithelium of the foot. This is a major problem as a vaccine will be far more efficient if delivered to those areas with greatest viral load; therefore further investigation of this is needed. To do this several viruses that are known to affect similar areas of the body to FMDV should be isolated. These should then be modified to allow them to enter the body without causing harm. Groups of all of the main commercial animals that suffer from FMD should then be treated with each virus. The organs of these animals should then be examined to assess which target the same areas as FMD most effectively. A final vaccine may need to use more than one vector to give the most effective protection in all species. In fact the best solution may be to use a modified FMDV as a vector as it will target the same areas as the active virus. Another drawback of the adenovirus vector is that it appears that a gene within it inhibits the RNAi effect; therefore further modification is needed to delete the relevant genes (Chen *et al.*, 2008). This may become an issue with the FMDV although at present there is no evidence of an RNAi inhibiting gene, there is a possibility of mutations occurring that could cause the inhibition of RNAi, this may make a vaccine more expensive to produce. Therefore any suitable virus identified using the process above should be further tested to investigate whether there are any inhibitory effects. The final vector should both target the correct areas of the body and be easy to modify.

The adenovirus, one of the preferred vectors (Chen *et al.*, 2006), has many serotypes and causes a variety of complaints from a mild cold to gastroenteritis (CDC 2005). The modification of these viruses to carry the FMD siRNAs and to make the virus itself harmless may inadvertently create a far more dangerous virus. Rigorous testing will be necessary, as with all new vaccines, to ensure the safety of all species.

An effective vaccine must provide protection for every cell. It is important to note is that siRNAs are incredibly powerful thus only a small amount must enter each cell to give protection compared to a vaccine based on the technologies of antisense or ribozyme gene targeting (Elbashir *et al.*, 2001). This

may make a vaccine cheaper to produce. The RNAi effect has been shown to spread between cells in plants (Ding *et al.*, 2004), and in the worm *C. elegans*, but the mechanism is still unclear. There is also some evidence to suggest that this occurs in mammals however there is no evidence that one infected tissue can trigger a systematic response (Chen *et al.*, 2006). This is important as if siRNAs can be stimulated to spread through tissues the effectiveness of the vaccine may increase.

An effective vaccine would need to bring both instant and prolonged protection to prevent the spread of an outbreak of FMD. The current FMD vaccine can only give protection after about seven days post inoculation (Chinsangaram *et al.*, 2003). Moraes' group's (2003) vaccine using a combination of approaches was immediately effective but protection lasted less than 5 days. This is obviously a major drawback because protection needs to be both instant and long lasting if it is to be totally effective at preventing an outbreak. The vaccine should last for a greater length of time than the incubation period (twelve days (Chen *et al.*, 2006)). Multiple inoculations would achieve this, however this will be time consuming and expensive; therefore a longer acting vaccine needs to be developed. A way of doing this may be by using an implant which releases doses of the vaccine every 4 days for a certain length of time this could mean that animals are protected until the risk of epidemic is over. The volume of drug however may be too great and the cost prohibitive. Another way of increasing the length of vaccine may be to give two simultaneous injections one in a fast acting medium and other in a slow release medium.

There are several moral issues surrounding this research. There is the risk of the live or modified virus being inadvertently released from laboratories or test farms. Although bio-security is important for all research involving viruses and the development of vaccines, it is particularly emotive where FMD is concerned due to the scale of the 2001 UK outbreak (BBC 2002), and the accidental release from a laboratory complex producing FMD vaccines in 2007 (BBC 2008). It is the duty of all research projects dealing with the FMDV or any other viruses to operate under the strictest bio-security. Another moral issue is that any investigations into FMD vaccines require the deliberate infection of animals. This may cause suffering if they are left to be observed and not euthanized when they show signs of disease. This occurred in the study described by Moraes *et al.*, (2003). These authors carried out their work under guide lines set by the "Institutional Animal Use and Care Committee" in a USA Department of Agriculture facility, but during their earlier investigations animals with pre-existing rashes were used, which may have compromised their health and welfare. Therefore the rigour and clarity of these guidelines, may be compromised. It must also be remembered that not all countries have regulations on animal experimentation. To offer consistency, and prevent unnecessary suffering, international legislation could be introduced, with strict guidelines on the use of animals in research. The suffering of experimental animals should be weighed against the long term benefit of a vaccine both in terms of animal welfare and loss to the agriculture industry worldwide.

Thorough and detailed investigations must be carried out to ensure that any vaccine is effective and safe. Some of the current papers are flawed; Chen *et al.*, (2006) had an animal that was infected after probable cross contamination as it had passed the maximum incubation period of 12 days with no infection. This shows that the bio-security during this project was poor and raises doubts about the reliability of the results. Careful analysis and investigation is vital to ensure that results are accurate and that those results that seem unpromising at first are due to the experimental treatments and not other factors. This is clearly illustrated by Moraes *et al.*, (2003), two animals died in this investigation which on necropsy prove to be due to two different conditions unrelated to FMD. To prevent this, more thorough veterinary tests should be made on test animals prior to an investigation ensuring that there are no underlying problems. Moraes *et al.*, (2003) discuss how in an earlier experiment results had been poor but goes on to say that these animals all had skin rashes prior to the investigation. These animals

should not have been included in the experimental groups. The fact that the vaccine was not effective in animals with pre-existing conditions is an important issue, as in the event of an outbreak emergency vaccination will need to cover all animals. Research is needed to ensure a vaccine provides protection for all animals including those with compromised immune systems. Both these papers were published in reputable peer reviewed journals so the above drawbacks were not deemed great enough to compromise the results, but need to be considered when comparing papers with contradictory results.

Once an effective RNAi vaccine has been developed there may still be barriers to its introduction as an effective tool for the control of a FMD outbreak. One important factor to consider when deciding whether to vaccinate livestock in an emergency situation is that current EU regulations state that the country in question must then wait 6 months before it is designated free of the disease when a 'vaccination to live' policy is in place (DEFRA 2008). If a 'vaccination to slaughter' policy is adopted, or a slaughter only approach is used it is only 3 months before the country is designated as free of FMD (DEFRA 2008). All vaccinated animals must have their products sold cooked in the period prior to the country being declared free of the disease (DEFRA 2008). These regulations are based on a vaccine that is not an RNAi based one and the policy may change for RNAi based vaccines if they are deemed to be safe. Careful consideration needs to be made of the economic implications of a change in vaccination policy. Loss of FMD free status for six months following vaccination may cost the economy less in the long term than loss of exports for only three months following a policy of slaughter. However this may be a politically unacceptable decision to take. If vaccination brings an outbreak under control quickly the total time that the country will be deemed to have the disease may be less than when an outbreak spreads widely as it did in 2001, cases were still arising 6 months after infection (see fig 1).

The logistics of emergency vaccination must also be considered. In 2007, the UK would have logistically been able to begin vaccinating within 5 days (DEFRA 2008). In 2001 however, the outbreak had reached Tyne and Wear from Essex within 4 days and there were almost 100 cases within a week (BBC 2002). If emergency vaccination is to be used as an effective measure to fight the disease an effective system needs to be introduced. This is likely to be difficult as factors such as the location of the outbreak, the shelf life of the vaccine, the cost of the vaccine and how quickly it can be produced must be taken into account. The current vaccine can only be produced after the strain has been identified; this means that countries cannot stockpile the vaccine. However if an RNAi vaccine is developed using a similar process as Pengyan *et al.* (2008) then all strains will be covered, making stockpiling possible. This may be expensive for individual countries; vaccine may not be used before it expires. A possible solution is that countries share resources, several countries could share a stockpile of the vaccine that is centrally located, jointly funded and can be deployed within hours to quickly prevent the spread of disease. The spread to Tyne and Wear from Essex may have occurred before clinical signs had occurred in the Essex stock therefore using animal movement information to aid the tracing of contact made by infected animals is vital to help stop the spread of disease. Once contact has been ascertained testing can take place identifying cases prior to clinical signs appearing. These tests should also be made within all surrounding farms as these are the most at risk due to contact between stock and infected surfaces.

Animals vaccinated with the current vaccine can become long term carriers if they come in to contact with the FMDV (Moraes *et al.*, 2003), this may also be the case with an RNAi vaccine and should be investigated. Animals vaccinated with the current vaccine are hard to distinguish from infected animals using standard tests (Moraes *et al.*, 2003). However Moraes *et al.*, (2003) produced a vaccine which included both RNAi and interferon technologies that when tested meant that vaccinated and infected animals were clearly distinguishable. Some of the current papers for example Chen *et al.*, (2006) only

looked at clinical signs and antibodies in their test animals, further investigation should have been done to find whether these animals had become carriers. This is important as it will make importing and exporting animals easier and could reduce the time taken to regain FMD free status as discussed above. If a problem does occur with the final vaccine, as it might if an FMDV vector was used (as suggested above), a form of labelling could be introduced on the vector. This could be a protein that is of a size that when is clearly distinguishable from either FMDV or animal proteins when run on a protein gel; this test could take less than 2 days to be completed thus only slightly delaying any export or import.

In the past when vaccines have been introduced for the prevention of epidemics the take up has been poor, for example when the bluetongue vaccine was introduced in Wales only 26% of the doses available were initially used (Enticott). This is unlikely to happen with a FMD vaccine. Bluetongue was a new disease to Britain and farmers did not have firsthand experience of it, whereas the horrors of FMD will be far more familiar to farmers as they have seen two outbreaks in the last decade alone. To prevent a poor uptake of vaccine compulsory ring vaccination should be introduced, this would mean that all stock in an area around a case would be vaccinated to prevent the spread of disease. It is important to avoid misinformation about vaccination, after the introduction of the bluetongue vaccine many farmers believed that it would affect the fertility of their stock which contributed to poor uptake of vaccine (Enticott). Any vaccine must be thoroughly investigated prior to introduction; this means that farmers can be given sound evidence that there are no such problems. Early signs indicate that siRNA should not adversely affect the cells that have been treated as Kahana *et al.*, (2004) found that there was no effect on the messenger RNA of treated cells.

CONCLUSION

There are potentially many advantages of using an RNAi based vaccine as part of an anti-FMD strategy. Many areas of this work are exciting and offer promise but more research is needed before such a vaccine can be introduced. One area where further work needs to be concentrated is the finding of an effective vector or combination of vectors for delivery of the vaccine, these vectors must target the same tissues as FMDV as well as having no inhibitory effects. Another important area of investigation is whether when the RNAi mechanism comes into play in one cell there is a systematic response; it may be possible to find a way of stimulating the spread of siRNAs to increase the effectiveness of a vaccine. The development of a test to distinguish the vaccinated from infected animals is also vital as this will help the vaccine be accepted by governments. Implants and a double injection may be options to ensure long lasting protection but further work is needed to examine the effectiveness and feasibility of these methods. Care must be taken while this work is carried out; investigations need to be thorough to ensure a safe effective vaccine is developed and that vaccinated animals do not become carriers of FMD. All work should be carried out under the highest bio-security to ensure both reliable results and to protect the agriculture industry. Investigators should also ensure that they carry out their work ethically.

The most important tool to fight FMD is government strategy and awareness within the industry. A strategy using compulsory ring vaccination to prevent spread of the disease, testing of stock to identify the disease before clinical signs have appeared, the tracing of infected animals contact with other stock, and slaughter in combination is the best way to prevent an outbreak spreading. The agricultural industry needs to be aware of disease control at all times ensuring that all animals are healthy; this needs to be done on the farm, at the market and in the abattoir.

In conclusion RNAi offers great promise as a potential FMD vaccine but research is in its early stages with several significant problems to overcome.

REFERENCES

- BBC (2008) Labs escape outbreak prosecution, <http://news.bbc.co.uk/1/hi/uk/7424887.stm>
- BBC (2002) Foot-and-mouth: The key stats, <http://news.bbc.co.uk/1/hi/uk/1334466.stm>
- BBC, (2001), FMD report: Outbreak's economic impact, <http://news.bbc.co.uk/1/hi/uk/1515327.stm>
- CDC, (2005), Adenoviruses, <http://www.cdc.gov/ncidod/dvrd/revb/respiratory/eadfeat.htm>
- Chen, W., Liu M., Jiau Y., Yan W., Wei X., Chen J., Fei L., Liu Y., Zuo W., Yang F., Lu Y., Zheng Z., (2006), Adenovirus-Mediated RNA Interference against Foot-and-Mouth Disease Virus Infection both In Vitro and In Vivo, *Journal of Virology* April 2006, Vol. 80, No 7., 3559-3566
- DEFRA, (2008), FMD: Disease control and vaccination, <http://www.defra.gov.uk/foodfarm/farmanimal/diseases/atoz/fmd/control/index.htm>
- Elbashir, S. M., Harborth J., Lendeckel W., Yalcin A., Weber K., Tuschli T., (2001), Duplexes of 21-nucleotide RNAs mediate RNA interference in cultured mammalian cells, *Nature*, Vol. 411, 494-498, May 2001
- Enticott, G., *Bluetongue is coming! But what should we do about it?*, http://www.brass.cf.ac.uk/uploads/Bluetongue_is_coming.pdf
- Kahana, R., Kuznetova L., Rogel A., Shemesh M., Hai D., Yadin H., Stram Y., (2004), Inhibition of foot-and-mouth disease virus replication by small interfering RNA, *Journal of general Virology* (2004), 84, 3213-3217
- Moraes, M.P., Chinsangaram J., Brum M.C.S., Grubman M.J., (2003), Immediate protection of swine from foot-and-mouth disease: a combination of adenoviruses expressing interferon alpha and a foot-and-mouth disease virus subunit vaccine, *Vaccine* 22 (2003), 268-297.
- Nobel, (2006), The Nobel Prize in Physiology or Medicine for 2006, http://nobelprize.org/nobel_prizes/medicine/laureates/2006/info_en.pdf
- Pengyan, W., Yan R., Zhiro G., Chuangfu C., (2008), Inhibition of foot-and-mouth disease virus replication in vitro and in vivo by small interfering RNA, *Virology Journal* July 2008 5:86