Foot-and-mouth disease: scientific problems and recent progress
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1. Key features of FMD

1.1. The disease: Foot-and-mouth disease virus (FMDV) causes an acute vesicular disease in cloven-hoofed animals, including cattle, pigs, sheep, goats, buffalo and various wildlife species. The onset of disease can be very rapid. In pigs and cattle, especially, this is accompanied by fever, painful blistering, lameness and loss of appetite. Secondary infections are not uncommon, particularly of the feet, causing chronic lameness and delayed healing and similarly mastitis may be a common sequel in dairy cattle. The acute phase of the disease lasts for approximately a week receding in the face of a mounting immune response of which the antibody response appears to be of particular importance as it is highly efficient in clearing virus from the blood stream. Mortality can occur in young animals, due to infection of the heart muscle causing circulatory failure. The loss of yield and the significant suffering coupled with the highly contagious nature of the disease are the ultimate reasons for controlling FMDV.

1.2. The virus: FMDV is one of the smallest of animal viruses. The name of the family to which FMDV belongs, the Picornaviridae, literally means small (pico) RNA (rna) virus (viridae). The family contains several members of medical importance, including those causing common colds (rhinoviruses), polio (poliomyelitis), and infectious hepatitis (hepatitis A). The particle (virion) consists of the genetic material, RNA, packed into a spherical protein shell approximately 1/40,000ths of a millimetre in diameter. The virus multiplies by invading a host cell and reprogramming it with its own genetic information to manufacture more virus. Picornaviruses are among the most elemental of parasites in that the genome – a single molecule of RNA – also serves as the messenger (mRNA), which redirects the cellular machinery to make viral protein. The FMDV RNA molecule carries the genetic information for 12 viral proteins, which have been given the names (we shall be referring to them later) L, 1A, 1B, 1C, 1D, 2A, 2B, 2C, 3A, 3B, 3C, and 3D. These proteins are "reeled off" in one long string, such that many of them also exist in combinations, like 3ABC. Viral proteins 1A, 1B, 1C, and 1D make up the protein shell of the virion, which also contains traces of non-structural proteins, some of which, such as 3D cannot be purified away. The other viral proteins listed above participate in replicatory and other functions within the host cell and are not part of the virion structure; hence their collective name, non-structural proteins, or NSPs.

1.3. Ability to contaminate the environment: FMDV multiplies in great abundance in superficial tissues, especially the skin of the mouth and feet. The blisters rupture, releasing vast numbers of infectious virus particles, which contaminate the environment in which the animals are kept. Virus is also excreted in relatively high levels in droplets and droplet nuclei (aerosols) in the breath and in addition, virus is excreted in faeces, urine, milk and semen, and may be shed before the onset of clinically apparent disease. The level of virus shedding is particularly high in pigs due to a very high level of replication of FMDV and pigs can excrete about 60-300 times as much virus in their breath as cattle or sheep.

1.4. Ability to infect: Tiny doses of infectious virus particles carried on the wind are sufficient to infect cattle and sheep, pigs being less susceptible to aerosol exposures. In addition, FMDV infects animals by mouth, or through cuts or abrasions in the skin or

mucosae and consequently animals with pre-existing lesions, such as pigs on concrete floors or sheep with foot-rot, are more susceptible to infection by that route. Oral infection (i.e via eating) requires a much bigger dose of virus than does respiratory infection (i.e. via breathing in). Pigs are more susceptible to oral infection than ruminants and in addition are more likely to be exposed this way, through consumption of uncooked, virus contaminated animal products present in some foods (i.e. swill).

1.5. FMDV's host range: The ability to infect both cattle, pigs, sheep and goats, exploits a strong host synergy that tends to promote the spread of infection over long distances. (i) The air exhaled by infected pigs is far more infectious than that of sheep or cattle, whereas cattle and sheep are much the most susceptible to infection by airborne virus. (ii) Pigs, being both omnivorous and more susceptible to infection by mouth than cattle or sheep, are at greater risk of contracting FMD from imported meat, and hence of initiating an epidemic in the first place. (iii) Sheep too can play an important part in spreading FMDV by harbouring acute infection without manifesting substantial clinical signs of disease. All three factors played a crucial part in initiating and spreading, often covertly, foot-and-mouth disease in the UK in 2001, particularly in the early part of the epidemic.

1.6. Infection is liable to become persistent in ruminants: An asymptomatic persistent infection is a common sequel following the exposure of ruminants to FMDV, producing so-called carriers. Carriers are historically defined as animals where virus persists beyond 28 days after the initial exposure. Vaccinated animals that are protected from clinical disease can still readily become carriers if exposed to sufficient levels of live virus. In carriers, the virus is maintained at the back of the throat in the oesophageal-pharyngeal region, but the amounts of virus present are very low, intermittently recoverable, and decline over time. The mechanisms whereby the virus is able to persist in the face of the host's immune response is not understood (Zhang et al., 2002). The longest recorded carriage of virus has been for 5 years in buffalo, more than 3 years in cattle, 9 months in sheep and 4 months in goats (Alexandersen et al., 2002b). There is a perceived risk that carrier animals could transmit disease to susceptible livestock, but this risk cannot be quantified. Despite several, mainly anecdotal, reports of carriers being responsible for field outbreaks of FMD, conclusive evidence of this has not been demonstrated under controlled, experimental conditions. The experimental studies that have been done, have by necessity included relatively few animals and few strains of the virus. Therefore, it can only be concluded that the risk is likely to be very small, but not necessarily zero as the presence of fully infectious virus in these animals under as yet undefined conditions could precipitate new outbreaks. The potential risk is sufficient to have had a major impact on international trade in livestock and their products and on the decision whether or not to use vaccines to assist in the control of an FMD outbreak. The lack of scientific consensus on the epidemiological significance of FMD carrier animals underlies the principle differences of opinion on methods of disease control. Although pigs do not become carriers after recovery from FMD, methods of serosurveillance to ensure that FMD virus is not circulating at a low level, especially in large vaccinated herds, would still be desirable.

1.7. There are distinct strains of FMDV: An animal immunised against one strain of FMDV may still be susceptible to another, just as humans may be susceptible to new strains of the common cold virus. On the basis of protection studies, seven distinct groups ("serotypes") of FMDV strains have been identified. Antibodies raised against a particular strain will bind that strain, but will not recognise strains belonging to another serotype. Within the FMDV serotypes, over 60 subtypes have been described, and new subtypes arise

continuously by mutation. Therefore, a number of vaccine strains for each serotype, particularly A and O, are required to cover the antigenic diversity and it is essential to monitor for the appearance of new strains internationally (one of the prime functions of the World Reference Laboratory, Annex 1, A1.1.1). The differences between strains can also be used to trace the likely origins of new outbreaks (Annex 1, A1.1.2.). To protect an animal against all the prevailing FMDVs in the world today would require a vaccine combining multiple, representative strains (in the large intensive dairy units of Saudi Arabia, the cattle are vaccinated every three months with up to ten different strains of FMD).

1.8. Rapidity of virus growth: FMDV is one of the fastest growing of all animal viruses. Intensively reared pigs may fall ill within one-two days of exposure. In the laboratory, FMDV can destroy a layer of cultured cells in less than two hours. The ability to infect and multiply with such extraordinary rapidity is a key factor that tends to confound any attempt to control outbreaks. In particular, it poses a severe challenge for a vaccine because protection requires the right antibodies to be available at the right time, and in sufficient amounts, to clear invading virus from the bloodstream. This accounts for two of the well-known limitations of FMDV vaccines: It takes several days for vaccination to take effect, and the resulting immunity tends to be relatively short-lived unless the animal is repeatedly vaccinated. In addition, even well vaccinated animals, in particular pigs, may be overwhelmed by the infection if exposed to large amounts of virus.

1.9. Instability of FMDV: The virus particle (virion) is inherently unstable (part of its strategy for rapid uncoating when it has entered a cell). Furthermore even intact virions lose infectivity at a detectable rate depending on the conditions. While this places a finite time limit on risks of infection from environmental contamination, it also limits the shelf life of vaccines, which are made from inactivated virus.

1.10. Immunity: Most of the studies of immunity to FMD have concentrated on antibodies, which are anti-viral proteins produced by certain types of white blood cells (B lymphocytes activated to become plasma cells). The FMD virus is found free in the blood and body fluids of infected animals where it can readily be neutralised by antibodies, which appear at around 5 days after the first clinical signs of disease. However, it is increasingly recognised that although they are easy to measure and a key player, antibodies are not the only important part of the immune response against this disease and moreover, it is obvious that antibodies are not capable of fully clearing the virus from carriers.

2. FMD Vaccination Issues

2.1. Current vaccines: All currently available FMD vaccines are based on cell culture derived preparations of whole virus, chemically inactivated and blended with suitable adjuvant(s), to potentiate the immune response to vaccination. Typically, FMD vaccines formulated with the adjuvants aluminium hydroxide and/or saponin provide protective immunity in cattle, sheep and goats, but are poor at conferring a similar response in pigs. However, mineral oil adjuvanted vaccines, developed for use in pigs, afford protection in all target species. FMD vaccines can be monovalent, i.e. formulated to contain one virus strain that is, antigenically, as closely related to the field virus as possible, or multivalent, including viruses of different strains and/or serotypes. There is a constant requirement to monitor contemporary outbreak strains of FMDV, to check the suitability of the available vaccines and to identify the appearance of antigenically novel strains (Annex 1, A1.1.3.).

2.2. Vaccine production and safety: FMD vaccines based on live FMDV must be produced under strict biocontainment, to exclude the risk of spreading virus from manufacturing plants. The European vaccine manufacturers are Merial, Intervet, ID-Lelystad and Bayer with manufacturing plants in Germany, France, the Netherlands and the UK (Merial at Pirbright). Large amounts of vaccine are also produced elsewhere, including South America, India, Russia, Iran, Turkey, Botswana, South Africa and Kenya. In the past 20 years, great advances have been made in the safety, efficacy and reliability of FMD vaccines available from appropriately licensed manufacturers. Recently, more consideration has been placed on the potential contamination of the final product with adventitious agents including transmissible spongiform encephalopathies. New batches of vaccine must be tested for safety and efficacy prior to use.

2.3. Vaccine efficacy: Efficacy testing of FMD vaccines is primarily concerned with checking their ability to prevent clinical signs of the disease in cattle. There is limited data relating to the vaccine's ability to inhibit local virus excretion and subsequent transmission to in-contact susceptible animals, or of the duration of protection following single administration, or the rate of protection under different challenge scenarios and use in other species such as sheep, goats and pigs. The International Vaccine Bank at IAH-Pirbright has done most of these types of studies. Using a high dose of a potent and antigenically appropriate vaccine will help to give the earliest onset of protection. Depending on their dose and on the severity of challenge, FMD vaccines may protect against disease within 4 to 5 days of vaccination (Barnett et al., 2002). There is no evidence that FMD vaccines induce a sterile immunity and clinically protected animals may still support FMDV replication after challenge, albeit at a reduced level, leading to some post-infection virus excretion and transmission. A proportion of ruminants (but not pigs) that are infected in this way can become virus carriers, in which live virus is found in the oropharynx at 28 days or more after infection. The possibility that vaccinated and subsequently challenged animals may be clinically protected but may nevertheless harbour live virus has important consequences for eradication strategies and regaining official virus-free status for international trade.

2.4. Vaccine banks: FMD vaccine banks were initially composed of bulk reserves of conventional, formulated vaccine (i.e. ready to inject). However, the shelf-life of formulated vaccine was no more than 18 months, and constant replacement became expensive. In 1976, Denmark, which at that time stopped routine vaccination, established the principle of storing concentrated FMD virus antigen at ultra-low temperatures over liquid nitrogen for formulation into vaccine if required. This effectively extended the shelf life of the antigen indefinitely. In 1985, the International Vaccine Bank (IVB) was established at Pirbright by a consortium consisting of the UK, Australia, New Zealand, Finland, Ireland, Norway and Sweden. Malta later joined the IVB as an associate member in 1995. Since then, the European Union (EU) has established antigen banks (the EUVB), and a number of other countries, including EU Member States maintain their own national banks. Since vaccine banks do not make vaccine, they rely on operational vaccine plants to ensure the fast and high quality production of new FMD vaccines in sufficient amounts. Most currently available antigen reserves in Europe were made before new and more stringent rules on the safety of medicinal products were introduced. Consequently, they no longer meet the requirements for Market Authorisation for use, but instead rely on Article 8 of the European Directive 2001/82/EC (CEC, 2001a), which allows Member States to permit the release of an

unauthorised product in the event of a "serious disease epidemic", provided that no authorised product is available for the disease concerned, and provided that the Commission is informed of the detailed conditions of its application. The use of these antigens in a vaccinate-to-live strategy would be particularly problematic and therefore, they are being progressively replaced. In the establishment and supply of emergency vaccines, decisions on the quantity and potency of the product inevitably involve a compromise between the cost of purchase and the likely number of doses required. However, a minimum vaccine requirement might be based on the supply of the number of doses which could, in practice, be distributed and applied in the first week of vaccination, the expectation being that additional supplies could by then have been procured, either from other banks or from commercial sources. There would also be advantages in making formal reciprocal supply arrangements with other banks, and/or enlarging the size of such banks. It is now agreed that the IVB will not continue in its current form, and that the UK and others will withdraw and establish their own antigen reserves, directly from commercial vaccine producers, with or without new sharing arrangements. IVB Commissioners are currently considering future arrangements. In the meantime, the UK has obtained 500,000 doses of each of 7 strains of FMDV antigen, with such a commercial supplier, in addition to the several million doses of 01 Manisa it holds with a commercial supplier from the 2001 outbreak. The EU vaccine bank holds 500,000 to 5,000,000 doses of each of 13 strains of FMDV.

2.5. International disease status: The FMD status of a country is important for determining whether it can trade animals and animal products internationally. The world animal health organisation (office international des epizooties or OIE) in Paris produces an Animal Health Code that sets out the basis for determining the FMD status of a country or region and the trading rules that ought to apply (OIE, 2002). It also covers the steps that must be taken to recover disease-free status, following one or more outbreaks of FMD in previously FMD-free countries or regions. The chapter on FMD has been reviewed post 2001. A country with a previous disease-free status can get this back within three months of the last case if a stamping out policy has been used. If vaccination is used, but all of the vaccinated animals are slaughtered, then again a three month interval is required between the slaughter of the last vaccinated animal and the recovery of disease-free status. This was the policy adopted by the Netherlands in 2001. Before revision of the chapter, a twelve-month interval was required between the last vaccination and return of the disease-free status, if vaccinated animals were left alive (the so-called vaccinate-to-live policy). However, this interval has now been reduced to six months, so long as serosurveillance is also done using NSP tests to demonstrate absence of carrier animals. A set of guidelines on the performance of serosurveillance using NSP tests has been drafted by OIE, although there remain concerns that the necessary screening and confirmatory tests have not been fully validated in all of the target species and that a statistical sampling frame has not been established (Annex 1, A1.1.6.). In all of these considerations about recovery of disease-free status (and the so-called exit strategy from FMD), it should be borne in mind that notwithstanding the guidelines within the OIE Animal Health Code, some countries adopt a more stringent approach to reopening trade with countries that have suffered FMD outbreaks. For example, Japan does not yet recognise UK's FMD disease-free status 18 months after the last outbreak.

2.6. Marker vaccines: One of the principal objections to the use of emergency FMD vaccine is the subsequent difficulty in differentiating between animals which have been vaccinated and animals which have either recovered from infection or which have acquired sub-clinical

infection post vaccination. This is necessary after use of a vaccinate-to-live approach, in order to prove that FMD has been eradicated. Such a distinction can in principle be made by use of marker vaccines, which contain at least one immunogenic peptide or protein less than the corresponding field virus. This means that antibody to the protein that is absent from the marker vaccine will be a reliable indicator of infection with wild-type virus and this type of marker vaccine is consequently sometimes referred to as a 'negative' marker vaccine. 'Positive' marker vaccines, by contrast, would contain an additional immunogenic substance compared to the corresponding field virus and would enable vaccinated animals to be detected serologically, regardless of whether or not they had also been infected which are not currently available or required.

The concept of measuring antibodies to NSP as a means of distinguishing FMDV infected from FMD vaccinated animals is more than thirty years old. Tests for antibodies to the 3ABC NSP, which are particularly reliable in this regard have been under evaluation for the last ten years. Since NSP tests are not serotype-specific, they also have the advantage of being able to detect antibodies induced by all strains of FMDV. However, since not all infected animals become carriers, animals found seropositive in NSP tests may or may not actually be virus carriers. The use of NSP antibody tests is based on the fact that only replicating viruses produce NSPs in the host, whereas killed vaccines do not replicate and contain only the viral proteins that make up the virus coat i.e. the structural proteins. For this approach to be reliable, vaccines must be purified to remove all traces of NSP, which is now feasible, although it reduces the yield and increases the costs of production. A more serious difficulty is that the antibody response to the NSPs is less strong than that to the structural proteins of the FMD virus. Therefore, NSP tests are less sensitive than conventional serological approaches and, vaccinated animals exposed to infection may become asymptomatic carriers without producing any detectable antibody to 3ABC NSP. Further research on the duration of the NSP antibody response is also required. The NSP/marker vaccine approach to FMD has been most widely used in South America, but apart from significant differences in husbandry practices between the two continents, the application in South America is not quite the same as is being put forward for Europe. In South America, mass vaccination has been the mainstay rather than localised "emergency vaccination". Under these conditions, carriers are less of a threat, since the rest of the population is likely to be immune. Indeed, large scale NSP serology in South America has really been used to monitor the effectiveness of vaccination, rather than to specifically eliminate carriers. In South America, the consequences of carriers for international trade are instead mitigated by the export of deboned meat rather than live animals.

An alternative approach to the detection of subclinically infected carriers produced following vaccination and subsequent field challenge would be the use of reverse transcriptase–polymerase chain reaction (RT-PCR) tests to detect specific viral RNA in oropharyngeal samples (Annex 1, A1.1.8.). However, the sampling for this approach would be very laborious and the sensitivity of the test is still far lower than NSP serology, because of the fact that detectable virus shedding may be intermittent (Haas and Sorensen, 2002).

2.7. EU regulations: The new EU directive on the control of FMD in Member States makes clear that emergency vaccination should be available as one of the principle options to control future outbreaks, based on the use of marker vaccines and tests and a vaccinate-to-live policy. Difficulties arise in trying to reliably detect every carrier animal, when the tests available are not fully validated for all of the species concerned and/or lack sensitivity. Furthermore, confirmatory tests are not readily available. It is envisaged that emergency vaccination could

take place in small zones surrounding one or more outbreaks and that shortly afterwards restrictions would be lifted. However, if all carrier animals cannot be identified, then any remaining could pose a risk to the surrounding susceptible population, if the carriers are moved outside the original vaccination zone, or if susceptible animals are brought onto the farm where such carriers reside.

3. Challenges for science and recent progress

3.1 Diagnostics

Following the epidemic of 2001, there has been much interest in the development of tests for the earlier and more rapid confirmation of FMD. Much of this is a consequence of large-scale contiguous culling, which greatly increases the potential consequences of a misdiagnosis and increases the number of samples that need to be tested. A second factor in 2001 was the involvement of large numbers of sheep, in which clinical diagnosis is less reliable than in cattle and pigs (Hughes et al., 2002). The most talked about developments have been pen-side tests for FMD based on detection of FMD viral proteins (Reid et al., 2001; Annex 1, A1.1.4.) and the concept of taking real-time RT-PCR for FMD viral RNA detection (as described by Oleksiewicz et al., 2001), out of the laboratory and into the field (Callahan et al., 2002; Hearps et al., 2002). The former test is a chromatographic strip test (also known as a lateral flow device) similar to pregnancy diagnosis kits for women. It is very simple and quick, requires no sophisticated equipment and can literally be done pen-side in about 15 minutes. Its main disadvantage is that it is less sensitive than tests that can be carried out in laboratories and can only be used on cases that show vesicles, vesicular epithelium being needed for the test. By contrast, portable RT-PCR is very sensitive, but the test is more elaborate and less suited to field conditions. Robotic sample handling is also important to enable large-scale laboratory testing in an emergency during an epidemic (Reid et al., 2002 & 2003; Annex 1, A1.1.5.).

Another development of great potential significance is the use of gene chips to carry out miniaturised tests for multiple agents simultaneously (Annex 1, A1.1.4.). Once the method becomes simplified and more sensitive it will offer the potential to test for a wide range of pathogens in one go. This would mean that exotic viruses could be tested for at the same time as doing analyses for more common infections, improving the chances of early detection of an exotic disease incursion.

The other major area for FMD diagnostic test development is marker vaccine tests, which are covered separately.

3.2.Vaccinology

3.2.1. Novel vaccines: While existing, vaccines have been associated with notable success, there are a number of target areas for their potential improvement (see Table). New molecular approaches to FMD vaccination have been followed since the mid 1970s when proteins, protein fragments and viral subunits – principally isolated fragments of the viral coat protein- produced in bacteria, baculovirus, and transgenic plants have been investigated (Brown, 1999). Other avenues have since been pursued, including: synthetic peptide vaccines; the use of replicating vectors; genetically engineered attenuated strains; and DNA vaccines

(Grubman & Mason, 2002). Although some early promise has emerged from these experimental studies, only those based on adenovirus vectors expressing interferons have, as yet, progressed to the point at which they might offer advantages over existing, conventional vaccines (Chinsangaram et al., 2003). Genetic engineering has advanced to the stage that all manner of novel vaccines can be developed quite rapidly in many different laboratories. The real bottleneck is testing them out, which requires very expensive and ethically debatable, large animal challenge studies, which for FMD in the UK can only be done at IAH-Pirbright. One way of reducing our dependence on challenge studies would be to develop a better understanding of what immune responses equate with protection. At present the only correlate available is development of antibody, but it is increasingly realised that this is not enough and more research is clearly needed on the immune responses of farm animals, so that better correlates of protection can be developed.

Table to show prospects for the development of improved FMD vaccines

Desired improvement	**Possible solution(s)**	**Likelihood of realisation**
Rapid onset of protection i.e. in less than 4 days	Stimulate non-specific immune defences, such as those based on interferons which have general anti-viral effects.	Principle recently demonstrated for FMD, using adenovirus vectors encoding interferon (Chinsangaram et al., 2003). This represents one of the most promising options for novel vaccine development. *Estimated time frame: at least 5 years.*
	Passive immunisation with antibodies to FMD.	Passive immunisation would require very large amounts of antibody. Donor animals would not easily produce sufficient quantities and their use is risky because of the possible presence of disease agents. Recombinant antibody technology is not yet proven. *Estimated time frame: at least 10 years.*
	Development of antiviral substances to block virus attachment.	Antiviral substances could include dummy receptors to divert FMD viruses from attaching to host cells. This is a purely theoretical consideration at present. *Estimated time frame: at least 10 years.*
Extended duration of immunity (ideally lifelong) preferably following a single application	Live vaccines usually give longer lasting immunity than killed ones, if safety concerns can be overcome.	Live attenuated FMD vaccines have had a poor safety record, but stable attenuation is possible using modern reverse genetic techniques. Previous work in this direction (Mason *et al*., 1997; McKenna *et al*., 1995; Chinsangaram et al., 1998b) does not seem to be being followed up very actively. The live virus vectors being most studied for delivery of FMDV genes are either human or porcine adenoviruses (Mayr *et al*., 2001; Moraes et al., 2002; Grubman & Mason, 2002). Safety concerns may require use of replication defective vectors, which may consequently have reduced vaccine potency. A new EU project will look at the use of porcine coronaviruses as vectors for delivery of FMDV genes (Annex 1, A1.4.2). *Estimated time frame: at least 10 years*
	A better understanding of memory mechanisms may enable immune modulators to be incorporated into a variety of different types of vaccines. The same effect may be achieved empirically by development of improved adjuvants.	A better understanding of the basis of immune memory in FMD and other viruses is a medium to long-term aim. *Estimated time frame: at least 5 years*

Sterile immunity to prevent acute and chronic viral shedding	A better understanding of the pathogenesis of FMD infection could help to understand why virus is not readily eliminated and why infection of immune animals is still possible. There are various options to try to develop vaccines which can be given orally and target the mucosal surfaces of the throat, for instance by using live virus vaccines, or vaccines coated to orally active microparticles.	*Estimated time frame: at least 10 years* Even animals recovered from FMD can frequently be re-infected. We currently lack a scientific basis for the development of vaccines to induce sterile immunity. The goal could possibly be achieved by inducing a potent local immune response at the site of primary virus replication – i.e. in the throat – especially in the early post-vaccinal phase, when immune responses to vaccination are likely to be strongest. Available novel vaccines are probably less effective in this respect than conventional killed vaccines given in high doses with powerful adjuvants.
Easier recognition of vaccinated animals that have become infected	Better purification of killed vaccines has already enabled them to be used as marker vaccines. A variety of subunit vaccines lacking defined viral epitopes or proteins can be produced which would offer better prospects for differentiating between antibodies to vaccination and to infection. Approaches include: peptide vaccines, recombinant protein vaccines, DNA vaccines, chimeric vaccines and live virus vectored vaccines. Positive markers for vaccination could also be incorporated. Peptide vaccines are perhaps the most ideal marker vaccines, since they elicit a very narrow spectrum of antibody	*Estimated time frame (new marker vaccines): 5-10 years* A general problem with many subunit vaccines is that they do not elicit as strong an immune response as live or killed whole virus vaccines. Genetically engineered FMD viral proteins do not seem to be good immunogens. Peptide vaccines so far described (Wang et al., 2002) do not mimic conformational B cell epitopes and stimulate a rather narrow spectrum of immunity, leading to concerns over whether viruses would be able to mutate and thereby evade the induced immunity. DNA vaccines are not yet potent enough when given in single doses and need more work on development of adjuvants. This is an active area of research in vaccinology (Beard etal., 1999; Chisangaram et al., 1998a) and is being addressed for FMDV as part of an EU project on DNA vaccination (Annex 1, A1.4.1.). Chimeric FMD vaccines were described several years ago and are about to be investigated for potential use as killed semi-conventional vaccines at IAH-Pirbright (A1.3.2.).

	response that can readily be distinguished from the broad spectrum response due to infection. Another approach to development of FMD marker vaccines has been to develop various systems that lead to the expression of viral capsids lacking viral RNA and most viral non-structural proteins. Similar tests can then be used for marker serology as are adopted for use with the current, purified killed vaccines. An alternative is to produce chimeric FMD viruses in which parts of the structural proteins are substituted by those from another FMDV serotype. For example a serotype O virus with a little bit of serotype A. Animals vaccinated with this can be distinguished from those infected with live serotype O virus, since only the vaccinated animals have anti-A antibodies and only infected animals have a full complement of anti-O antibodies.	
Safer vaccines that do not require large amounts of live FMDV to be grown up and	All types of recombinant vaccines that are not based on live FMDV offer this advantage.	*Estimated time frame: still at least 5 years off.*

then inactivated		
Thermostability (avoiding the current necessity for a cold chain)	Peptide vaccines have excellent stability and DNA vaccines should also be good in this respect. Some vectored vaccines may also be quite stable depending on the hardiness of the vector virus e.g. poxvirus vectors are very stable. Work done in collaboration with a vaccine manufacturer at IAH-Pirbright developed chemically-stabilised, recombinant empty capsids with good thermal stability	*Estimate time frame: at least five years.* A method of storing ready formulated vaccines in a stable frozen state for many years has been described (Barnett et al., 2002), but this vaccine still requires a cold chain once thawed for use.
More cross-protective between strains (conferring immunity against several, and ideally all, epidemiologically important serotypes and strains)	This will be difficult to achieve by means of induced antibody dependent immunity, since the viral protein targets of antibodies are highly variable. Currently, there is no clear scientific basis for developing a broad-spectrum vaccine other than by combining multiple antigens. A novel and practicable way of achieving this might be with peptide antigens. A possible solution is to elicit immunity to more conserved non-structural proteins of the virus.	*Estimated time frame: not in prospect, except by use of multivalent antigens.* Unfortunately no peptide-based vaccine effective in cattle has yet been described. Not yet clear that immune responses to NSPs are protective let alone cross-protective.
Can be administered topically (rather than	Orally active preparations or DNA vaccinations delivered by gene gun	*Estimated time frame: not in prospect.*

parenterally), to stimulate the primary sites of infection, through a novel application that allows simultaneous immunisation of large numbers of animals with minimal effort, time and risk to animals and handlers	would fulfill this requirement.	Negotiations underway over use of gene gun in IAH DNA vaccine trials.

3.2.2. Marker vaccine tests: Various studies have defined which non-structural proteins (NSP) elicit the most reliable antibody response following infection, and a variety of recombinant NSP have been expressed for use as serological antigens (Mackay et al., 1998b). Peptide antigens have also been developed and blocking tests have been designed using monoclonal antibodies. In unvaccinated, but virus-challenged animals, the NSP tests are less sensitive at detecting infection than tests for anti-structural protein antibodies (Mackay et al., 1998a,b), and the duration that NSP antibodies remain detectable is rather poorly documented.

The 2000 edition of the OIE Diagnostic Manual lists a screening and confirmatory test for NSP serology (OIE, 2000). The methods described have been widely used in South America, to monitor virus circulation amongst vaccinated populations of cattle. The screening test is an indirect ELISA detecting antibody binding to bacterially-expressed 3ABC protein. The confirmatory test is a form of western blot to measure the binding of antibodies to a range of NSP. The availability of a confirmatory test is important because once very large numbers of animals are tested, then even relatively specific tests will start to throw up false positive results, and therefore all positive results need confirmation by an independent method. An ELISA based on similar principles to the South American test was developed in Europe at IAH-Pirbright and Brescia, and has been recently turned into a commercially available test kit (Schalch et al., 2002). A North American commercial company is also developing a peptide based assay (Liu et al., 2002). Their test has three components. Sera are screened for antibodies to a bound peptide representing a fragment of the NSP 3B. A proportion of false positive results are excluded by checking that the reaction can be blocked by soluble 3B. A final confirmation is achieved by testing for antibodies to a second peptide representing part of NSP 3A. A Danish 'in-house' laboratory test has also been described that tests for the ability of antibodies to bind to a baculovirus expressed 3ABC protein and to thereby displace an anti-3ABC monoclonal antibody (Haas and Sorensen, 2002).

The various NSP tests are at different stages of validation. Several seem to be quite specific for infection regardless of vaccination status. However, most of the available data is for cattle and there is rather little information on how the tests would perform looking for carriers amongst vaccinated sheep. There is also, critically, a lack of data concerning the ability of these tests to identify vaccinated and subsequently infected animals. The lack of data is because

(1) vaccination-and-challenge experiments for FMD are very expensive and animals are not generally kept for long periods after protection has been assessed and

(2) field testing rarely provides sufficient certainty as to the true status of animals that are assessed.

An important variable is likely to be the potency of the vaccine used as well as the specific challenge conditions. It is known that higher potency vaccines are more effective at reducing virus replication following challenge, but it is yet to be determined whether this diminishes the likelihood of subsequent virus persistence, or affects the NSP antibody response. The nature of the viral exposure, including dose and route of exposure, may also be significant. This is being studied in a research project underway at IAH-Pirbright (Annex 1, A1.1.7).

Preliminary results suggest that some NSP tests are more sensitive than others (Haas and Sorensen, 2002). A working group set up by OIE to examine the available data, concluded that North American and European NSP tests had sensitivities of 80-90%, i.e. if 100 carriers are tested, 10-20 might be missed. Their specificity is rather high and may well be around 99.5%, but this still means that every time a herd of 200 cattle are tested, then on average there will be one false positive result.

It might be expected that killed vaccines would elicit a different type of immune response to that induced by infection with a live virus. Some studies have attempted to separate vaccinated and infected animals on the basis of the classes of antibody produced systemically and in various body fluids (Salt et al., 1996). This is a promising approach worthy of further study (Amadori et al., 2000; Annex 1, 1.1.6.). In addition, different cell mediated immune responses are to be expected and this could be exploited by future tests (A1.3.3.).

The OIE health code is being redrafted to accommodate the principle of using NSP tests, post vaccination, in order to help regain FMD free status. It has been frequently stated that because of the risk of false negative results, NSP testing for this purpose should be used on a herd basis rather than for individual certification. However, the definition of what constitutes a herd needs to be clarified. Another area of uncertainty is over how many vaccinated animals are likely to become infected in a herd that undergoes virus challenge after vaccination. This will clearly be affected by many variables, but the likely range and most probable outcomes are needed in order to estimate sampling rates for assured detection of a given prevalence of infection.

3.3.Epidemiology

From the questions that have been raised after the UK 2001 FMD epidemic over control methods and the role that biomathematical modelling played in determining these (especially the contiguous cull), there is a clear need to characterise in detail the dynamics of FMDV infection/replication in target animals. This should ultimately improve our understanding of the epidemiology of FMD, lead to an increased accuracy in risk predictions and thus facilitate efficient control efforts. As FMD affects a number of species and many different strains of the virus exists, each having slightly different biological properties, this is in itself an immense effort as it makes generalisation from single experiments or single outbreaks difficult or impossible. Consequently, a better understanding of the underlying mechanisms as well as accurate quantitative data is needed in order to develop or optimise mathematical models to predict the risk of entry of FMD into the UK and to assist decision making and direct control activities should outbreaks occur in the future. Essential for the development of such models are the key determinants of the spread and maintenance of FMDV in livestock populations in a quantitative format. These quantitative data should be generated for FMDV strains of varying virulence and in populations that are fully susceptible to infection or immune following vaccination and kept under varying contact structures resembling intensive and extensive farming (Annex 1, A1.1.7.; A1.1.9.; A1.3.5.). The opportunity to generate these data has been greatly enhanced by the development of methods for exposing animals to FMDV by natural routes and for tracking the infectious processes in animals by "real-time" quantitative PCR technologies (Alexandersen & Donaldson, 2002; Alexandersen et al., 2002a,c).

Such studies should investigate the dynamic, molecular and immune characteristics of FMDV transmission and persistence in livestock and address differences observed in replication, excretion and persistence in different species and with different virus strains to improve epidemiological understanding and, furthermore, to assess the risk of transmission from carrier animals. The studies should include detailed characterisation of the parameters of airborne spread (Donaldson and Alexandersen, 2002) as well as spread by other routes. It is envisioned that the studies should provide a framework for generating data that are vital for accurate modelling but which are currently not available unless as rather inaccurate average calculations that are inappropriate to use for accurate assessment of the impact of various control options.

Additional studies should also be initiated to strengthen biological and molecular characterisation of outbreak strains and to contribute towards a cohesive framework for solid advice and for simulation modelling of FMD spread and control. Furthermore, it would provide a strengthened “link” between basic studies and more applied studies on diagnosis, disease control and biomathematical modelling. Ideally, studies should be conducted as collaborations between animal disease specialists and experts in various aspects of biomathemathical modelling, including airborne spread.

Studies of infectivity, pathogenesis, transmission, and vaccination, in the target species are essential for determining these data and this in turn requires the availability of biosecure experimental facilities of which the current capacity is limited and if the proposed topics are to be comprehensively investigated additional facilities will be required.

3.4. Basic/strategic sciences:

There is now a general recognition, as exemplified by the the report of the Royal Society committee of inquiry, that the basic science base needs to be strengthened as an integral part of any national strategy for research on infectious animal diseases. This applies particulary to the more intractable problems posed by FMDV, e.g. of vaccinology. There are many gaps in our knowledge, for example how the virus selects its hosts and its preferred sites of replication within these hosts. We have very little idea how the virus manages to do what it does so much faster than most viruses. These are issues of practical importance in making the virus so difficult to control.

The field of livestock immunology is in its infancy compared with that of humans and mice, and yet the carrier state has to be seen as a failure of the ruminant immune system. The immune system of mammals is very complex. It involves the responses of individual cells to detect and attack foreign agents, followed by the secretion of immunologically active substances (cytokines) to activate neighbouring cells and to recruit various arms of the immune system (including antibody production and killer cells that attack virus infected cells). Much of this knowledge has been gained in the last 10-15 years and is yet to be applied to some animal diseases including, to a large extent, FMD. Differences between the immune systems of farm animals and those studied by medical immunologists mean that it is not just a matter of technology transfer to achieve this. Such knowledge is vital for the design of new vaccines. For example, it is now clear that early events in infection have a profound effect on which parts of the immune system are armed, and if one wants to have protection earlier than 5 days after vaccination it is no use looking to stimulate antibody production. Similarly, if one wants a longer duration of immunity to FMD vaccination, one needs to better understand why memory cells are either not sufficiently stimulated or maintained following vaccination with the currently available vaccines (Annex 1, A1.2.4.).

The interactions between this most “simple” of viruses and its host are actually very complex at the molecular level. We know a little about how FMDV subverts and commandeers the synthetic machinery of the cell but we are only beginning to study how the virus completely re-organises the cell internally, quickly turning it into a production line for new virions. Basic replicatory mechanisms are also poorly understood in vivo. . Our view of research needs in this area were presented to BBSRC Council and DEFRA SD (then MAFF CSG) in the spring of 2001 in a paper entitled “FMDV persistence: from genotype to phenotype, proposal for multidisciplinary research” in which we set out a comprehensive plan for studying the host-virus interactions (Annex 1, A1.2.1-3.).

By its very nature the practical gains from basic research are liable to be serendipidous while strategic research is focused on distant goals. A breakthrough could arise from work on a related virus, and then put to use though subsequent strategic and applied research on FMDV. For all these reasons, it is impossible to review the basic FMDV virology/immunology in a document of this kind, but we can point to the followingrecent advances that might prove insightful for the design of future control strategies. (i) A lot of progress has been made in identifying cell surface molecules (receptors) that FMDV selectively binds in order to initiate infection. The properties of the key receptor, an epithelial protein called αvβ6, suggest that it could be very important in determining which tissues FMDV infects, and hence what kind of disease it causes (Jackson et al, 2000). (ii) Another likely determinant of tissue tropism,

without which FMDV will not replicate efficiently, has been identified as ITAF-45. What makes this interesting is that it is found only in rapidly dividing cells (Pilipenko et al, 2000). (iii) The FMDV strain that has been causing severe disease in Taiwanese pigs is unusual in being unable to replicate in bovine cells. The defect was found to be associated with the loss of part of the NSP called 3A (Mason et al, 2001). (iv) The same workers identified a small region located within the RNA genome, shaped like a hairpin, which acts a signal enabling that genome to be copied into daughter strands (Mason et al, 2002). We have since shown that this stretch of RNA doesn't only function for the genome containing it, but can "help" others to be replicated as well (Tiley et al, 2003).

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Annex 1

A1. Synopsis of FMD work at IAH

A1.1. Defra funded projects

A1.1.1. Surveillance contract
There is an annually renewable contract for the provision of national and international reference services for FMD (including subsidising the World Reference Laboratory for FMD). This underpins the diagnostic capability of the laboratory and the provision of surveillance and consultancy. Activities: contingency planning, test validation, quality accreditation, consultancy to UK and international disease control agencies, international surveillance and test harmonisation. A substantial increase in funding has been requested for the year 2003/4.

A1.1.2. Tracing the origins and spread of FMD
A three-year research project carrying out genetic characterisation of FMD isolates from around the world to monitor the emergence and evolution of new strains and to help understand factors involved in the spread of the disease. A new version of the project begins in 2003/4.

A1.1.3. Vaccine selection
A three-year research project to develop improved methods for rapidly matching field isolates to the most appropriate vaccine strains. This includes development and characterisation of monoclonal antibody typing panels and studies to predict antigenicity from genetic sequence data. A new version of the project begins in 2003/4.

A1.1.4. Novel detection methods
A three-year research project to develop improved methods for rapid detection and diagnosis of FMD virus. This includes development of penside tests, microarrays and improved cell culture systems. A new version of the project begins in 2003/4.

A1.1.5. RT-PCR detection methods
A three-year research project to improve RT-PCR methods for rapid and sensitive detection and diagnosis of FMD virus, including differential diagnosis of other viruses causing vesicular disease. This includes development and validation of automated, high throughput testing and in process control systems. There is a study to evaluate the use of RT-PCR for detection of FMD virus in milk. This project is about to enter its second year.

A1.1.6. FMD sampling strategies
A three-year research project to develop improved tests for differentiation of infected animals from ones that have been merely vaccinated. This project aims to obtain data on the kinetics of antibody development in vaccinated and subsequently challenged animals. As well as evaluating currently available NSP serological methods, the study aims to develop and evaluate alternative assays, including penside tests for use in the field and tests for mucosal antibody responses, which may be used to confirm the results of NSP serology. This project commenced in October 2002.

A1.1.7. Use of currently available vaccines

A three-year research project to evaluate the consequences of challenging cattle at different times after administration of conventional vaccines that have been given at different dosages. This study will provide information on how quickly vaccines protect against contact challenge and on how much virus replicates in vaccinated and subsequently challenged animals. This is of great importance in planning and modelling vaccination strategies. Furthermore, the animal work provides the samples underpinning much of the diagnostic development work described above. The project began in November 2002.

A1.1.8. Identification of animals persistently infected with FMDV

A three-year research project to identify persistently infected cells in carrier cattle, to identify factors which reduce persistence of virus in carrier cattle and to develop suitable RT-PCR methods for identifying carrier cattle. A new more comprehensive version of the project begins in 2003/2004. This new project will also focus on the carrier state but will study both cattle and sheep and will also include studies of the pathogenesis and spread of acute disease.

A1.1.9. Airborne infection and pathogenesis of foot-and-mouth disease

A three-year research project with the objectives: (i) to determine the minimum infectious dose for pigs of two strains of FMD virus, delivered as natural aerosols, in order to expand the capability of the computer Rimpuff model to analyse and predict the risk of airborne spread of FMD virus; and (ii) to identify the target sites and cells in the respiratory tract of pigs infected by airborne FMD virus and also those from where the virus is subsequently excreted. The project ends in June 2003. A new proposal aimed at providing needed experimental data and improved simulation models for prediction of airborne spread is currently being written in collaboration with the UK Met Office and will be submitted shortly.

A1.1.10 Molecular aspects of host specificity and adaptation in FMDV

04/1999-06/2002. This project was to study virus interactions with the cell surface to understand how FMD viruses select the host cells they infect, and how the host cell can, in turn, drive change in the virus. The purpose was to enable (a) epidemiologically useful predictions of the host range and pathogenicity of FMDV strains on the basis of sequence data, and (b) vaccine manufacturers to produce more effective FMDV vaccines which avoid cell culture-induced antigenic changes.

A1.1.11 Cellular receptors of foot-and-mouth disease virus: their role in tropism during persistent and acute disease

07/2002-06/2005. The objectives of this ongoing project are to: (i) complete the task of identifying which (integrin) receptors serve as receptors for field strains of FMDV in cell culture, (ii) correlate sites of virus replication in susceptible animals with integrin expression, and (iii) investigate the cell-signalling role of integrin(s) in regulating acute and persistent infection. These studies will open up new anti-viral strategies for blockading the viral receptor in the animal host, and/or for depressing its expression or activation state. A useful spin-off will be improved cell lines for growing/detecting field strains of FMDV.

A1.2. BBSRC funded FMD work at IAH

A1.2.1. The survival strategy of persistent foot-and-mouth disease virus in cattle

A three-year response-mode grant from the BBSRC funds a cross-divisional collaboration at Pirbright to understand the cellular and molecular basis of FMDV persistence. The project began in July 2001 and is investigating whether the genome of the virus isolated from sites of persistence has changed. This tests the hypothesis that the virus may become attenuated during the development of persistence. The project will also determine whether non structural proteins encoded by FMDV have the ability to inhibit MHC class 1 antigen presentation and offer an immune evasion strategy to the persistent virus. A three year link grant with Dr Martin Ryan at the University of St Andrews started in 2001 and is investigating the effects of FMDV non structural proteins on membrane trafficking in cells. This will provide molecular insight into how the virus modulates the transport of immunomodulatory proteins in infected cells.

A1.2.2. Molecular aspects of integrin specificity of foot-and-mouth disease virus

03/2002-02/2005. The narrow range, and properties, of the integrin receptor(s) used by FMDV suggest that these cell surface molecules play a profoundly important role in determining tissue tropism, and hence disease pathogenesis, and mechanism of spread. This study aims to dissect, using mutants viruses altered in the integrin-binding loop, the molecular basis of integrin recognition.

A1.2.3. Viral replication and host cell damage

FMDV infection results in a rapid change in the pattern of protein synthesis within the cells. There is a dramatic reduction in the production of cellular proteins and a rapid rise in the synthesis of virus-encoded proteins. This raises two questions: a) how is the inhibition of host cell protein synthesis achieved? and b) how does the production of viral protein occur under these conditions? It has been shown that virus-encoded proteins induce the specific breakdown of key cellular factors that are involved in the recognition of the cellular mRNAs (which are translated to make proteins). These particular factors are not required for the recognition of the viral RNA due to the presence of a specific RNA element, termed an IRES. The mechanism by which FMDV proteins modify these host translation factors is being examined and the structure and function of the FMDV IRES is also being studied, both are potential targets for anti-viral agents.

A1.2.4. FMD proposals are in preparation for submission to the recent BBSRC initiative on Control of Viral Diseases in Livestock.

A1.3. PhD studies on FMD at IAH

A1.3.1. FMD epidemiology in an endemically infected country

A PhD thesis has just been completed by a veterinarian working at IAH-Pirbright and in the Cameroon in Africa. The project was a collaboration between Liverpool University (Professor Kenton Morgan) and IAH-Pirbright. It has investigated the cross-sectional prevalence of infection and related this to husbandry practices, carried out analyses of the viruses isolated and evaluated NSP serology.

A1.3.2. Chimeric FMD vaccines

This IAH studentship commences in October 2003 and is a collaboration with Plum Island. The student will be registered with Professor Brownlie at the Royal Veterinary College in London. The aim is to evaluate the protective effect and immune responses elicited by killed FMD vaccines that are formulated like conventional ones, but are derived from genetically engineered mixtures of different FMDV serotypes. Since these recombinant viruses are already available, it is anticipated that this could be a rapid means of developing FMD vaccines with a similar efficacy to conventional killed vaccines, but with improved marker qualities. The experimental animals will be provided by the EU project A4.2.

A1.3.3. Immune responses to FMD virus and vaccines

This is collaboration with the National Veterinary Research and Quarantine Service in Korea and will be funded by the Korean Government. It is due to start in September 2003. It has two components. Researchers in Korea will be developing new FMD vaccines based on peptides and DNA, whilst a PhD student funded at Pirbright will look at differences in the mucosal and cell mediated responses of cattle vaccinated and/or infected with FMD virus. The experimental animals will be provided by the defra project described under A1.1.5. and A1.1.6.

A1.3.4. Defining FMD virus epitopes

This is collaboration with Professor Ian Jones at Reading University. It is due to start in October and aims to define the conformational epitopes against which most antibodies to FMD virus seem to be directed. Recombinant technology will be used to design random libraries of epitopes some of which will mimic those found on the FMD virus. These epitopes may subsequently be of value in development of diagnostic assays and vaccines.

A1.3.5. Quantitative modelling of the viral load in pigs infected with foot-and-mouth disease virus

This IAH studentship started in October 2001 and involves collaboration with Mark Woolhouse at University of Edinburgh. The experimental parts of the project are determining the viral load in blood and various tissues at different times after infection. The other part of the project is to develop a mathematical model describing the viral load under different conditions. If successful, the model should provide an increased understanding of the kinetics of viral loads in single animals, which, together with additional experimental data on transmission, should result in the development of an improved epidemiological model for predicting the spread of FMD.

A1.3.6. Quantitation of cytokine mRNA in animals acutely or persistently infected with foot-and-mouth disease virus

This IAH studentship started in October 2001 and the objectives are to investigate the mRNA concentrations of selected cytokines during infection with FMD virus and to determine whether they correlate with the stage of virus replication and disease progression and the development or not of persistent infection.

A1.3.7. Molecular pathogenesis of foot-and-mouth disease (FMD)

This BBSRC studentship started in October 2002 and will continue and expand ongoing studies on the molecular pathogenesis of FMD in pigs and ruminants. Both the acute stage, as well as the persistent state (ruminants only) of the infection are of concern. The studies will focus on new molecular techniques such as real-time RT-PCR, in situ hybridization and laser dissection microscopy (LDM). The data obtained will further our understanding of this

important disease and will possibly pave the way for a knowledge-based development of vaccines capable of preventing persistent infection.

A1.3.8. Endocytosis of foot-and-mouth disease virus
10/2001-09/2004. Two striking characteristics of FMDV are its extreme sensitivity to acid and the rapidity with which it infects cells. FMDV enters the host cell attached to its receptor, trapped inside compartments called endosomes. These become progressively more acidic until the virus falls apart and injects its infectious RNA into the interior (cytosol) of the cell. Many viruses infect cells in this way, but we suspect that FMDV is unusual in entering through early endosomes rather than, the more acid, late endosomes. The aim of this project is to test this theory by means of a microscopical examination of cells undergoing infection. The project entails the use of mutant forms of both the virus and of the receptor.

A1.3.9. Dissecting the molecular interactions for FMDV within the host cell
10/2003-09/2005. This project will use RNA interference to target cellular, rather than virus, genes (targeting of viral genes is proposed as part of an application to the BBSRC's new initiative on Control of Viral Diseases of Livestock). The method will be used to explore the requirement for specific cellular proteins during key steps of FMDV replication. Initial targets will be those cellular proteins known to be involved in virus uptake mechanisms.

A1.3.10. FMD virus interactions with host cell proteins
The specific interactions of cellular proteins with the FMDV RNA element that is required for viral protein synthesis, (termed an internal ribosome entry site, IRES), is being analysed. Such interactions may determine which cells (and tissues) the virus can replicate in, this can be a major determinant of the outcome of a virus infection. For example, vaccine strains of poliovirus are modified within the IRES and these changes determine that the vaccine induces protection rather than disease. We want to know which cellular proteins bind to the FMDV IRES and how these modify its activity.

Two different FMDV proteins (L and 3C) modify cellular translation factors that are required for the production of cellular proteins. The sites of these modifications are being determined and the effect of these modifications on the activity of these cellular proteins is being analysed.

The replication of FMDV RNA occurs very rapidly within FMDV infected cells. We are attempting to identify the regions of the viral RNA that are required for RNA production, to determine which cellular and viral proteins interact with these regions and to determine the role of such proteins in RNA replication.

A1.4. EU projects on FMD involving IAH

A1.4.1. Optimizing DNA based vaccination against FMDV in sheep and pigs.
The specific objectives are to produce and to compare several new DNA vaccines and several vaccination protocols, including adjuvants, route of administration, heterologous prime-boost regimen, and their ability to induce early and protective immune responses in target species. The partners are: INRA, France (P1, coordinator), IAH-Pirbright (P2), Ploufragan, France (P3), CISA-INIA, Spain (P4). The project began in September 2002.

A1.4.2. Novel coronavirus vector-based vaccine for prevention of FMD.

Novel coronavirus vector-based vaccine for prevention of foot-and-mouth disease. The objective is to use a recently developed, biosafe transmissible gastroenteritis virus (TGEV)-based vector (BAC-TGEVFL) to induce protective immunity against foot-and-mouth disease virus (FMDV) and against TGEV. The vector system should evoke both a systemic and a mucosal immune response. The response of the vaccine will be distinguished from the response to FMDV infection by the absence of specific anti-FMDV 3AB antibodies, the absence of 3a3b and S gene specificity of TGEV. No infectious FMDV can be generated by the constructs. Humoral and cellular immune responses, tissue-distribution of antigens and antibodies, mucosal immunity, protection against challenge with FMDV, and virus transmission after challenge will be investigated in swine. The final goal is to develop a new general strategy for preparation of marked, safe and effective anti-FMD vaccines that would prevent the establishment of the carrier state in ruminants. The partners are: CISA-INIA, Spain (P1, coordinator), IAH-Pirbright (subcontractor to P1), BFAV-Riems, Germany (P2), Fort Dodge, Spain (P3), ID-Lelysstad, The Netherlands (P4), IVI Mittelhausern, Switzerland (P5). The project began in September 2002.

A1.4.3. Improvement of FMD control by ethically acceptable methods, based on scientifically validated assays and new knowledge on FMD vaccines.
This is a proposal for a four-year project incorporating some new vaccine and test developments as well as applied research to validate existing tests and vaccines. It should make a significant contribution to implementing the desired policy shift towards use of vaccination in Europe. The partners are: CODA Brussels, Belgium (P1, coordinator), IAH-Pirbright (P2), CIDC-Lelystad, Netherlands (P3), DVI Copenhagen, Denmark (P3), BFAV Greifswald, Germany (P4), CISA-INIA Madrid, Spain (P5), IZSLER Brescia, Italy (P6), SAP Ankara, Turkey (P7).

A1.4.4. Improved FMD diagnosis
This is a concerted action (i.e. a coordination project and not a research project) currently in its fifth and final year. It involves the main laboratories working on diagnostic tests in Europe and provides a forum to discuss the development and validation of tests. For example, it has organised interlaboratory comparative testing and pulled together available validation data on FMD NSP tests. The partners are largely the same as in A4.2., except Turkey is not involved.

A1.4.5. Foot and Mouth Disease: the molecular basis of tissue tropism and persistence
This project will study how the virus enters and replicates in epithelial cells ‘in vivo’ and will exploit new technologies such as laser microdissection (LMD) and manipulation of recombinant viruses to cross the bridge between molecular virology and veterinary pathology. LMD allows individual cells and microanatomical features to be dissected from histochemical preparations and assayed for gene expression. LMD will be used to gain quantitative estimates of FMDV genomes produced in specific epithelial cells during acute and persistent infection. These genomes will be sequenced to follow changes that may occur during the transition to persistent infection. FMDV uses the integrin family as receptors for cell entry. Mutant viruses will be constructed to identify crucial amino acids that determine the specificity of FMDV for specific integrins. LMD will be used to gain a quantitative estimate of specific integrin expression at sites of FMDV infection and persistence. The ability of integrins expressed by monocytic cells to bind FMDV and traffic the virus to sites of persistence ‘in vivo’ will also be determined, as will the effects of FMDV on monocytic cell function. The project funds a collaboration between IAH, Pirbrght, the Institute of Virology and Immunoprophylaxis in Mittelhausern and INIA at Valdeomos.

A1.4.6. International Research and Surveillance Co-ordination

A new proposal has recently been submitted to the EC under the FP6 "ERA-NET SCHEME" - "support for the co-ordination activities". The title is “Responding to the global demand for FMD R&D through coordinated programme development of European reference laboratories and international organisations.” The co-ordinator is from Brussels and the other partners are IAH, The Canadian National center for Foreign Animal Disease Control, the European Union’s FMD Commsiiion of FAO, OIE, a professional "co-ordinations" group from Brussels and a UK international development group.

A1.5. Other collaborative work

A meeting will take place at Pirbright at the end of June 2003 between senior scientists from IAH, the Australian Animal Health Laboratory in Geelong , the Plum Island Animal Disease Center in USA and the Canadian National Center for Faoreign Animal Diseases. It is planned to discuss a concerted strategy for collaborative research towards improved vaccines and diagnostics for accelerated global FMD control.

Several collaborative studies on FMD are in train or under discussion involving commercial vaccine companies and IAH.

How FMDV recognises its receptor: As part of a wider research programme to understand how FMDV selects its cellular receptor, we are collaborating with the Wellcome Trust Centre for Human Genetics, Oxford, to determine the structure of a virus-integrin complex. By combining cryo-electron microscopy (using the new Phillips microscope at Pirbright) with 3D image reconstruction, it is possible to visualise such structures (we have previously done this with virus-antibody complexes) in sufficient detail to enable the interacting surfaces to be modelled at atomic resolution from the known crystal structures of the components.

Annex 2

A2. FMD research outside IAH

A2.1. Europe

IAH has by far the greatest critical mass of scientists working on FMD and is unusual in having strength in both applied and basic aspects of research. Many other European groups have small teams mainly dedicated to providing national reference laboratory functions. Few have the ability to carry out experimental infection of large animals with FMD. The institutes with significant FMD programmes are: Valdeolmos in Spain which has a strong reputation in FMD viral evolution, structure, immunology and vaccine development (Baranowski et al., 2001); Lelystad in the Netherlands (which was formerly the Community Co-ordinating Institute for the European FMD Vaccine Bank and where there is a vaccine plant), Tuebingen in Germany (recently relocated to the Isle of Reims), Mittelhausern in Switzerland; Lindholm Island in Denmark and Brescia in Italy. FMD vaccine manufacture is carried on in Russia, but research is not very active in Eastern Europe.

A2.2. Middle East and Australasia

Some applied research is carried out in Turkey, India, Taiwan and Korea, but notable publications are few. More research is likely from China in coming years. Japan does not have a particularly strong FMD research programme. Australia is hampered by not being able to work with FMD virus, despite having excellent containment facilities at Geelong (AAHL). The Geelong laboratory has an interest in the development of both diagnostics and vaccines and has worked with vector vaccines and DNA vaccines for the control of other exotic virus diseases. The Australian government is very active in promoting FMD control measures in SE Asia.

A2.3. Africa

The only institute of standing in FMD research is at Ondestepoort in South Africa. They have been involved in many studies to investigate the epidemiology of FMD in Africa in domestic and wildlife species.

A2.4. North America

Until recently, the only laboratory working with live FMD was at Plum Island in USA. They have been the most active group in recent years working on FMD vaccines and have produced the most promising vaccine candidates, including live-attenuated, chimeric, DNA, peptide and adenovirus-vectored vaccines (Grubman and Mason, 2002). They also do important work on factors affecting the host tropism and virulence of FMD viruses, including identification of viral receptors and use of reverse genetics to directly study the genetic basis of phenotypic changes. The laboratory is to come under the control of Homelands Security and is likely to be increasingly concerned with countering the threat of bioterrorism. There are a number of private companies working on FMD diagnostic kits including pen-side antigen, antibody and nucleic acid detection. The Canadians have recently opened a high containment laboratory in Winnipeg and hope to expand their FMD group.

A2.5. South America

FMD control is a high priority for S America. The foremost laboratory is PANAFTOSA in Brazil, which has a co-ordinating and surveillance role for the subcontinent as a whole. They have pioneered the use of NSP tests and the application of killed vaccines in the field. They are less strong on basic research.

Annex 3

A3. Notable recent publications

A3.1. Reviews

Special issue of OIE Revue Scientifique et Technique. 2002. Vol 21 (3). **Foot-and-mouth disease: facing the new dilemnas.** Editor G Thompson. Covers many aspects. In particular, see Grubman MJ, Mason PW. 2002. **Prospects, including time-frames, for improved foot and mouth disease vaccines.** Rev Sci Tech. 21(3):589-600. An excellent and up-to-date review of vaccine developments.

Special Issue of Comparative Immunology Microbiology & Infectious Diseases. **Foot-and-Mouth Disease.** Editor B Toma. Covers many aspects including vaccination and the role of OIE. CIMID 25: 279-280.

EU Scientific Committee on Animal Health and Animal Welfare report on **"Diagnostic techniques and vaccines for Foot-and-Mouth Disease, Classical Swine Fever, Avian Influenza and some other important OIE List A Diseases".** Covers most issues relating to diagnostics and vaccinaology for FMD.
http://europa.eu.int/comm/food/fs/sc/scah/out93_en.pdf.

A3.2. Research reports

A3.2.1Vaccines

Chinsangaram J, Moraes MP, Koster M, Grubman MJ. 2003. **Novel viral disease control strategy: adenovirus expressing alpha interferon rapidly protects swine from foot-and-mouth disease.** J Virol. 77(2):1621-5.
Abstract: We have previously shown that replication of foot-and-mouth disease virus (FMDV) is highly sensitive to alpha/beta interferon (IFN-/ß). In the present study, we constructed recombinant, replication-defective human adenovirus type 5 vectors containing either porcine IFN- or IFN-ß (Ad5-pIFN or Ad5-pIFNß). We demonstrated that cells infected with these viruses express high levels of biologically active IFN. Swine inoculated with 109 PFU of a control Ad5 virus lacking the IFN gene and challenged 24 h later with FMDV developed typical signs of foot-and-mouth disease (FMD), including fever, vesicular lesions, and viremia. In contrast, swine inoculated with 109 PFU of Ad5-pIFN were completely protected when challenged 24 h later with FMDV. These animals showed no clinical signs of FMD and no viremia and did not develop antibodies against viral nonstructural proteins, suggesting that complete protection from infection was achieved.

Wang CY, Chang TY, Walfield AM, Ye J, Shen M, Chen SP, Li MC, Lin YL, Jong MH, Yang PC, Chyr N, Kramer E, Brown F. 2002. **Effective synthetic peptide vaccine for foot-and-mouth disease in swine.** Vaccine. 20(19-20):2603-10.

Abstract: We have designed a peptide-based vaccine for foot-and-mouth disease (FMD) effective in swine. The peptide immunogen has a G-H loop domain from the VP1 capsid protein of foot-and-mouth disease virus (FMDV) and a novel promiscuous T helper (Th) site for broad immunogenicity in multiple species. The G-H loop VP1 site was optimised for cross-reactivity to FMDV by the inclusion into the peptide of cyclic constraint and adjoining sequences. The incorporation of consensus residues into the hypervariable positions of the VP1 site provided for broad immunogenicity. The vaccine protected 20 out of 21 immunised pigs from infectious challenge by FMDV O1 Taiwan using peptide doses as low as 12.5 microg, and a mild adjuvant that caused no lesions. A safe chemically-defined product would have considerable advantages for vaccination against FMD.

Wong HT, Cheng SC, Sin FW, Chan EW, Sheng ZT, Xie Y. 2002. **A DNA vaccine against foot-and-mouth disease elicits an immune response in swine which is enhanced by co-administration with interleukin-2.** Vaccine 20(21-22):2641-7.

Abstract: A plasmid DNA vaccine candidate (pCEIS) encoding two foot-and-mouth disease virus (FMDV) VP1 epitopes (amino acid residues 141-160 and 200-213) has been demonstrated to have the ability to elicit both FMDV-specific T cell proliferation and neutralizing antibody against FMD in swine. In this study, the efficiency of the pCEIS DNA vaccine when administrated by intramuscularly injection in swine was confirmed, and the immunogenicity of the pCEIS vaccine candidate was found to be enhanced through co-administration with a newly constructed plasmid (pIL2S) encoding the swine interleukin-2 (IL-2) cDNA. The expression of the pIL2S plasmid was driven by a CMV promotor provided by a pcDNA3.1 vector. Swine IL-2 cDNA was cloned by RT-PCR from swine spleen cells. The pIL2S plasmid was expressed in COS-7 cells after 24 and 96h of transfection in vitro. In an animal trial, results from T cell proliferation assay indicated that the stimulation index (SI) in response to stimulation of FMDV proteins in the swine groups injected with pCEIS plus pIL2S (SI ranging from 9.9 to 15.5) were significantly higher than that with pCEIS alone (SI ranging from 3.3 to 6.6). However, there was no significant difference in FMDV-neutralizing antibody level detected in these two swine groups. Mouse protection tests (MPTs) showed that the blood sera from immunized swine injected with either pCEIS alone or pCEIS plus pIL2S were able to protect suckling mice from FMDV challenge, with protection levels ranging from 10(1) to 10(2) lethal dose 50 (LD(50)) M. In a direct FMDV challenge, all swines immunized with either pCEIS plus pIL2S or with pCEIS alone were challenged with 50LD(50)S (50 x lethal dosage in swine) of FMDV. The animals were fully protected (100%) from the FMD viral challenge. These results suggest that co-administration of the plasmids, pCEIS and pIL2S, enhances of the immunogenicity of the pCEIS DNA vaccine candidate, and both intramuscular injection of pCEIS alone and co-administration of the vaccine candidate with pIL2S can protect the swine from direct FMD challenge.

Cox SJ, Aggarwal N, Statham RJ, Barnett PV. **Longevity of antibody and cytokine responses following vaccination with high potency emergency FMD vaccines.** Vaccine. 21(13-14):1336-47.

Abstract: The ability of high potency emergency foot-and-mouth disease (FMD) vaccines to promote sustainable immune responses in sheep and pigs following a single application was examined. All vaccine formulations induced a rapid seroconversion in both species, as expected, which was maintained at near peak titres for up to 6 months in sheep and 7 months in pigs. The Montanide ISA 206 formulation gave the best results in sheep. Vaccinated pigs challenged with homologous FMDV were protected from disease at 7 months post vaccination. Systemic levels of cytokines IL-6, IL-8, and in some pigs IL-12, increased following vaccination and were often maintained at an increased level for the duration of the trials. These initial results suggest that high potency vaccines may promote longer lasting immunity than the conventional lower potency vaccines in ruminants and a comparable response in pigs. Results indicate that in an outbreak situation, should emergency vaccination be done with these high potency vaccines, protection should be conferred for a long enough period for the outbreak to be brought under control without the need to revaccinate. Given the increased interval for re-vaccination the use of high potency vaccines for routine prophylactic campaigns could provide a more cost-effective and efficient means of maintaining herd immunity and is an area thus worthy of further examination.

A3.2.2 Diagnostics

Reid SM, Ferris NP, Hutchings GH, Zhang Z, Belsham GJ, Alexandersen S. 2002. **Detection of all seven serotypes of foot-and-mouth disease virus by real-time, fluorogenic reverse transcription polymerase chain reaction assay.** J Virol Methods 105(1):67-80

Abstract: A fluorogenic RT-PCR (5'-nuclease probe-based) assay using a primer/probe set designed from the internal ribosomal entry site region of the virus genome was developed for the specific detection of all seven serotypes of foot-and-mouth disease (FMD) virus in epithelial suspensions and cell culture virus preparations. The reverse transcription polymerase chain reaction (RT-PCR) specifically detected FMD virus in sample submissions from the UK 2001 FMD outbreak with greater sensitivity than our conventional RT-PCR procedure and our routine diagnostic procedures of ELISA and virus isolation in cell culture. The fluorogenic RT-PCR provides relatively fast results, enables a quantitative assessment to be made of virus amounts and can handle more samples and/or replicates of samples in a single assay than the conventional RT-PCR procedure. Therefore it is seen as a valuable tool to complement the routine diagnostic procedures for FMD virus diagnosis.

Reid, S. M., Grierson, S. S., Ferris, N. P., Hutchings, G. H., and Alexandersen, S. 2003. **Evaluation of automated RT-PCR to accelerate the laboratory diagnosis of foot-and-mouth disease virus**. J.Virol.Methods, 107, 129-139.

Abstract: Automated fluorogenic (5' nuclease probe-based) reverse transcription polymerase chain reaction (RT-PCR) procedures were evaluated for the diagnosis of foot-and-mouth disease (FMD) using suspensions of vesicular epithelium, heparinised or clotted blood, milk and oesophageal-pharyngeal fluid ('probang') samples from the United Kingdom (UK) 2001 epidemic and on sera from animals experimentally infected with the outbreak serotype O FMD virus strain. A MagNA Pure LC was initially programmed to automate the nucleic acid extraction and RT procedures with the PCR amplification carried out manually by fluorogenic assay in a GeneAmp(R) 5700 Sequence Detection System. This allowed 32 samples to be tested by one person in a typical working day or 64 samples by two people within 10-12 h. The PCR amplification was later automated and a protocol developed for one person to complete a single test incorporating 96 RT-PCR results within 2 working days or for two people to do the same thing in around 12 h. The RT-PCR results were directly compared with those obtained by the routine diagnostic tests of ELISA and virus isolation in cell culture. The results on blood, probang and milk samples were in broad agreement between the three procedures but specific RT-PCR protocols for such material have to be fully optimised as perhaps have the positive-negative acceptance criteria. However, the automated RT-PCR achieved definitive diagnostic results (positive or negative) on supernatant fluids from first passage inoculated cell cultures and its sensitivity was greater than ELISA on suspensions of vesicular epithelium (ES) and at least equivalent to that of virus isolation in cell culture. The combined tests of ELISA, virus isolation in cell culture and RT-PCR might, therefore, only be required for confirmation of a first outbreak of FMD in a previously FMD-free country. Should a prolonged outbreak subsequently occur, then either ELISA plus RT-PCR or else RT-PCR alone could be used as the laboratory diagnostic tool(s). Either approach would eliminate the requirement for sample passage in cell culture and considerably advance the issue of laboratory diagnostic test results.

Callahan JD, Brown F, Osorio FA, Sur JH, Kramer E, Long GW, Lubroth J, Ellis SJ, Shoulars KS, Gaffney KL, Rock DL, Nelson WM. 2002. **Use of a portable real-time reverse transcriptase-polymerase chain reaction assay for rapid detection of foot-and-mouth disease virus.** J Am Vet Med Assoc. 220(11):1636-42.

Abstract: OBJECTIVE: To evaluate a portable real-time reverse transcriptase-polymerase chain reaction (RT-PCR) assay designed to detect all 7 viral serotypes of foot-and-mouth disease virus (FMDV). DESIGN: Laboratory and animal studies. STUDY POPULATION: Viruses grown in tissue culture and animals experimentally infected with FMDV. PROCEDURE: 1 steer, pig, and sheep were infected with serotype O FMDV. Twenty-four hours later, animals were placed in separate rooms that contained 4 FMDV-free, healthy animals of the same species. Oral and nasal swab specimens, oropharyngeal specimens obtained with a probang, and blood samples were obtained at frequent intervals, and animals were observed for fever and clinical signs of foot-a nd-mouth disease (FMD). Samples from animals and tissue cultures were assayed for infectious virus and viral RNA. RESULTS: The assay detected viral RNA representing all 7 FMDV serotypes grown in tissue culture but did not amplify a panel of selected viruses that included those that cause vesicular diseases similar to FMD; thus, the assay had a specificity of 100%, depending on the panel selected. The assay also met or exceeded sensitivity of viral culture on samples from experimentally infected animals. In many instances, the assay detected viral RNA in the mouth and nose 24 to 96 hours befor e the onset of clinical disease. CONCLUSIONS AND CLINICAL RELEVANCE: The assay reagents are produced in a vitrified form, which permits storage and transportation at ambient temperatures. The test can be performed in 2 hours or less on a portable instrument, thus providing a rapid, portable, sensitive, and specific method for detection of FMDV.

Hearps, A., Zhang, Z., and Alexandersen, S. 2002. **Evaluation of the portable Cepheid SmartCycler real-time PCR machine for the rapid diagnosis of foot-and-mouth disease**. Vet.Rec., 150, 625-628.
Abstract: The ability of the portable Cepheid SmartCycler real-time PCR machine to detect foot-and-mouth disease (FMD) virus sensitively and accurately was evaluated by comparing the results of the analyses of nasal swab and serum samples from experimentally infected animals with those obtained from the real-time PCR assay currently in use in the laboratory. The results indicated that the ability of the machine to detect viral RNA is greatly affected by the PCR reagents used for the assay. When it was used with PCR beads it was unable to detect weakly positive samples, but when TaqMan core reagents were used for the assay, its sensitivity was significantly increased. The machine could be used for the laboratory-based detection of FMD; however, as with all assays, significant optimisation of assay conditions as well as solid validation of the technique is required

Reid SM, Ferris NP, Bruning A, Hutchings GH, Kowalska Z, Akerblom L. 2001. **Development of a rapid chromatographic strip test for the pen-side detection of foot-and-mouth disease virus antigen.** J Virol Methods 96(2):189-202 .
Abstract: Foot-and-mouth disease (FMD) is the most contagious animal virus disease of cloven-hoofed livestock and requires reliable and accurate diagnosis for the implementation of measures to control effectively its spread. Routine diagnosis of FMD is carried out at the OIE/FAO World Reference Laboratory for Foot-and-Mouth Disease (WRL for FMD), Pirbright by the combined use of ELISA and virus isolation in cell culture supplemented by reverse transcription polymerase chain reaction (RT-PCR) methods. These techniques require skilled personnel and dedicated laboratory facilities which are expensive. The development of a rapid and simple test for the detection of FMD virus antigen using Clearview chromatographic strip test technology for field application is described. This device detected FMD viral antigen in nasal swabs, epithelial suspensions and probangs from clinical samples submitted from the field, from animals infected experimentally and in supernatant fluids resulting from their passage in cell culture. The test system was more sensitive than ELISA for the diagnosis of all seven serotypes of FMD virus in the epithelial suspensions and nasal swabs and had equivalent sensitivity to the ELISA for the detection of contemporary virus strains in cell culture supernatant fluids. The study demonstrated the potential for this device to confirm a clinical diagnosis at the site of a suspected FMD outbreak, thereby offering the possibility of implementing control procedures more rapidly. Such pen-side diagnosis would have particular benefits in FMD emergencies, relevance to FMD control programmes which operate in endemic regions of the world such as South East Asia and for increasing disease awareness in other areas where efforts to control disease may be difficult. In each circumstance the availability of a pen-side device for diagnosis would reduce the necessity for sending routine diagnostic samples to an FMD laboratory and thereby reduce the delay in diagnosis, which can in some areas be considerable.

Wang CY, Chang TY, Walfield AM, Ye J, Shen M, Zhang ML, Lubroth J, Chen SP, Li MC, Lin YL, Jong MH, Yang PC, Chyr N, Kramer E, Brown F. 2001. **Synthetic peptide-based vaccine and diagnostic system for effective control of FMD.** Biologicals 29(3-4):221-8.
Abstract: We have designed synthetic peptides corresponding to two different regions of the genome of foot-and-mouth disease virus (FMDV) that are effective as (a) a vaccine or (b) a diagnostic reagent which differentiates convalescent from vaccinated animals, respectively. The peptide vaccine is based on a sequence from the prominent G-H loop of VP1, one of the four capsid proteins. The sequence was optimized by the inclusion of a cyclic constraint and adjoining sequences, and broader immunogenicity was obtained by the incorporation of consensus residues at hypervariable positions. The peptide also included a promiscuous T-helper epitope for effective immunogenicity in outbred populations of large animals.The diagnostic reagent, a peptide based on non-structural (NS) protein 3B, is used in immuno-assays for the detection of antibodies. Antibodies to this NS protein are present in the sera of infected animals but not in the sera of vaccinated animals. The VP1 peptide can be used in complementary immuno-assays for confirmation of NS test results and to monitor for vaccination. This system for differential diagnosis is important to establish the disease-free status of a country.

Chung WB, Sorensen KJ, Liao PC, Yang PC, Jong MH. 2002. **Differentiation of foot-and-mouth disease virus-infected from vaccinated pigs by enzyme-linked immunosorbent assay using nonstructural protein 3AB as the antigen and application to an eradication program.** J Clin Microbiol 40(8):2843-8.

Abstract: Baculovirus-expressed foot-and-mouth disease virus (FMDV) nonstructural protein 3AB was used as the antigen in an enzyme-linked immunosorbent assay. This assay allowed the differentiation of vaccinated from infected pigs. Serial studies were performed using sera collected from pigs in the field. Positive reactions were found in sera from fattening pigs and sows 16 weeks and 3.5 years postoutbreak, respectively. There was, however, no positive reaction in sows with at least 10 vaccinations. Maternally derived antibodies to the 3AB antigen persisted in piglets up to 13 weeks of age. A high correlation coefficient (r = 0.93) was found between the test results from sow sera and those from their offspring. Therefore, piglet serum was a good substitute for sow serum to monitor the infection status of the dam. The application of this assay to serological surveillance in an FMD eradication program in Taiwan showed that the positive reactors steadily decreased over time in both finishers and sows, indicating that the pig population risk of infection by FMDV has decreased.

A3.2.3 Epidemiology and carriers

Gibbens JC, Wilesmith JW. 2002. **Temporal and geographical distribution of cases of foot-and-mouth disease during the early weeks of the 2001 epidemic in Great Britain**. Vet Rec 2002 Oct 5;151(14):407-12.

Abstract: Estimates of the likely dates of infection of the early cases of the 2001 foot-and-mouth disease (FMD) epidemic indicate that at least 57 premises in 16 counties in Great Britain were infected before the first case was disclosed. Nationwide animal movement controls were imposed within three days of the first case being confirmed on February 20, when FMD was only known to be in two counties, and these controls limited its geographical spread. After the first few cases were confirmed, new cases were rapidly discovered, and the epidemic curve for the daily number of confirmed cases peaked five weeks later, 11 days later than the peak of the curve based on the estimated dates of infection. In the peak week, both curves showed an average daily number of 43 new cases. The estimated dates of infection are believed to be relatively unbiased for the early cases, for which they were derived from a known contact with infection. However, for the later cases they were estimated mainly from the age of the clinical signs of the disease, and were biased by species and other factors, a bias which would probably have made the estimated dates later than was in fact the case.

Ferguson, N. M., Donnelly, C. A., and Anderson, R. M. 2001. **The foot-and-mouth epidemic in Great Britain: pattern of spread and impact of interventions**. Science, 292, 1155-1160.

Abstract: We present an analysis of the current foot-and-mouth disease epidemic in Great Britain over the first 2 months of the spread of the virus. The net transmission potential of the pathogen and the increasing impact of control measures are estimated over the course of the epidemic to date. These results are used to parameterize a mathematical model of disease transmission that captures the differing spatial contact patterns between farms before and after the imposition of movement restrictions. The model is used to make predictions of future incidence and to simulate the impact of additional control strategies. Hastening the slaughter of animals with suspected infection is predicted to slow the epidemic, but more drastic action, such as "ring" culling or vaccination around infection foci, is necessary for more rapid control. Culling is predicted to be more effective than vaccination.

Keeling, M. J., Woolhouse, M. E., Shaw, D. J., Matthews, L., Chase-Topping, M., Haydon, D. T., Cornell, S. J., Kappey, J., Wilesmith, J., and Grenfell, B. T. 2001. **Dynamics of the 2001 UK foot and mouth epidemic: stochastic dispersal in a heterogeneous landscape**. Science, 294, 813-817.

Abstract: Foot-and-mouth is one of the world's most economically important livestock diseases. We developed an individual farm-based stochastic model of the current UK epidemic. The fine grain of the epidemiological data reveals the infection dynamics at an unusually high spatiotemporal resolution. We show that the spatial distribution, size, and species composition of farms all influence the observed pattern and regional variability of outbreaks. The other key dynamical component is long- tailed stochastic dispersal of infection, combining frequent local movements with occasional long jumps. We assess the history and possible duration of the epidemic, the performance of control strategies, and general implications for disease dynamics in space and time.

Morris, R. S., Wilesmith, J. W., Stern, M. W., Sanson, R. L., and Stevenson, M. A. 2001. **Predictive spatial modelling of alternative control strategies for the foot-and-mouth disease epidemic in Great Britain, 2001**. Vet.Rec., 149, 137-144.
Abstract: A spatial simulation model of foot-and-mouth disease was used in March and early April 2001 to evaluate alternative control policies for the 2001 epidemic in Great Britain. Control policies were those in operation from March 20, 2001, and comprised a ban on all animal movements from February 23, 2001, and a stamping-out policy. Each simulation commenced with the known population of infected farms on April 10, 2001, and ran for 200 days. For the control policy which best approximated that actually implemented from late March, the model predicted an epidemic of approximately 1800 to 1900 affected farms, and estimated that the epidemic would be eradicated between July and October 2001, with a low probability of continuing beyond October 2001. This policy included the slaughter-out of infected farms within 24 hours, slaughter of about 1.3 of the surrounding farms per infected farm within a further 48 hours, and minimal interfarm movements of susceptible animals. Delays in the slaughter of animals on infected farms beyond 24 hours after diagnosis slightly increased the epidemic size, and failure to achieve pre-emptive slaughter on an adequate number of at-risk farms substantially increased the expected size of the epidemic. Vaccination of up to three of the most outbreak-dense areas carried out in conjunction with the adopted control policy reduced the predicted size of the epidemic by less than 100 farms. Vaccination of buffer zones (designed to apply available vaccine and manpower as effectively as possible) carried out in place of the adopted control policy allowed the disease to spread out of control, producing an epidemic involving over 6000 farms by October 2001, with no prospect of immediate eradication.

Alexandersen, S. and Donaldson, A. I. 2002. **Further studies to quantify the dose of natural aerosols of foot-and- mouth disease virus for pigs**. Epidemiol.Infect., 128, 313-323.
Abstract: Foot-and-mouth disease virus (FMDV) can be spread by a variety of mechanisms, including wind. Simulation models, developed to predict the risk of airborne spread, have played an important part in decision making in some outbreaks. The amount of airborne virus excreted as well as the minimal infectious dose (MID) of FMDV for different species are important determinants of airborne spread. The objective of this study was to obtain data for the O1 Lausanne, O SKR 2000 and O UKG 2001 strains of FMDV to enhance the capability of such models. Pigs were exposed to naturally generated aerosols of the three strains using an experimental design which delivered high doses of the two strains O1 Lausanne and O SKR 2000 over a short period, or of the O UKG 2001 strain over an extended period. The average excretion of the O1 Lausanne strain was 10(6.4) TCID50 per pig per hour. The excretion of the O SKR 2000 strain averaged 10(5.8) and the O UKG 2001 strain 10(6.1) TCID per pig per 24 h. The results show that the previous estimate of 'above' 800 TCID50 as the MID50 for the O1 Lausanne strain is a considerable under-estimate and that the real dose may be as high as 6000 TCID50. A dose of around 650 TCID50 of the O SKR 2000 strain failed to infect any pigs. Thus, the aerosol MID50 for pigs for this isolate is at least 1000 TCID50 and likely to be as high or higher than the O1 Lausanne strain. The exposure of pairs of recipient pigs kept physically separated from donor pigs in a series of rooms to aerosol exposure doses of the O UKG 2001 strain of around 50 TCID50 per min for 24-48 h failed to infect any of eight pigs. Thus, the present experiment confirms our previous findings that pigs, compared to cattle and sheep, are relatively resistant to infection with airborne FMDV.

Alexandersen, S., Zhang, Z., Reid, S. M., Hutchings, G. H., and Donaldson, A. I. 2002. **Quantities of infectious virus and viral RNA recovered from sheep and cattle experimentally infected with foot-and-mouth disease virus O UK 2001**. J.Gen.Virol., 83, 1915-1923.
Abstract: The profiles of virus production and excretion for sheep experimentally infected with the UK 2001 strain of foot-and-mouth disease (FMD) virus by inoculation and by direct and intensive contact have been established. Virus replicated rapidly in the inoculated sheep from which a peak infectivity of airborne virus of 104.3 tissue culture infectious doses 50% (TCID50) per sheep per 24 hours was recovered. Around 24 hours later contact-infected sheep excreted airborne virus maximally. Similar amounts of airborne virus were recovered from cattle.
The excretion of virus by the sheep under these conditions fell into three phases. Firstly, a highly infectious period of around 7 to 8 days. Secondly, a period of 1 to 3 days soon afterwards when trace amounts of viral RNA were recovered in nasal and rectal swabs. Thirdly, at 4 weeks after exposure the demonstration, by tests on oesophageal-pharyngeal samples, that 50% of the sheep were carriers. The implications of the results and the variable role that sheep may play in the epidemiology of FMD are discussed.

Alexandersen, S., Kitching, R. P., Mansley, L. M., and Donaldson, A. I. 2003**. Clinical and laboratory investigations of five outbreaks of foot-and-mouth disease during the 2001 epidemic in the United Kingdom**. Vet.Rec., 152, 489-496.
Abstract: Clinical and laboratory investigations of five outbreaks of foot-and-mouth disease (FMD) were made during the early stages of the 2001 epidemic in the UK. The first outbreak, confirmed on February 20, was at an abattoir in Essex which specialised in the processing of culled sows and boars. On February 23, the disease was confirmed at a pig farm in Northumberland which held cull sows and boars fed on waste food; the findings indicated that it was the first of the five premises to be infected. The disease had probably been present since early February, and it was the most likely origin of the epidemic. The other premises investigated were a waste food-fed cull sow/boar pig unit in Essex, approximately 30 km from the abattoir, which was probably infected at the same time or before the abattoir, a sheep and cattle farm approximately 6 km from the Northumberland pig farm, which was probably infected by airborne virus from it in the period immediately before February 13, and a sheep and cattle farm in Devon which had clinical disease from February 20 and was probably infected by sheep transported from Northumberland on February 13 which arrived on February 15

Gloster, J., Champion, H. J., Sorensen, J. H., Mikkelsen, T., Ryall, D. B., Astrup, P., Alexandersen, S., and Donaldson, A. I. 2003. **Airborne transmission of foot-and-mouth disease virus from Burnside Farm, Heddon-on-the-Wall, Northumberland, during the 2001 epidemic in the United Kingdom**. Vet.Rec., 152, 525-533.
Abstract: The results of a detailed assessment of the atmospheric conditions when foot-and-mouth disease (FMD) virus was released from Burnside Farm, Heddon-on-the-Wall, Northumberland at the start of the 2001 epidemic in the UK are consistent with the hypothesis that the disease was spread to seven of the 12 farms in the immediate vicinity of the source by airborne virus, and airborne infection could not be ruled out for three other premises; the remaining two premises were unlikely to have been infected by airborne virus. The distances involved ranged from less than 1 km up to 9 km. One of the farms which was most probably infected by airborne virus from Burnside Farm was Prestwick Hall Farm, which is believed to have been key to the rapid spread of the disease throughout the country. In contrast, the results of detailed atmospheric modelling, based on a combination of clinical evidence from the field and laboratory experiments have shown that by assuming a relationship between the 24-hour average virus concentrations and subsequent infection, threshold infection levels were seldom reached at the farms close to Burnside Farm. However, significant short-term fluctuations in the concentration of virus can occur, and short-lived high concentrations may have increased the probability of infection and explain this discrepancy.

Zhang, Z. D., Hutching, G., Kitching, P., and Alexandersen, S. 2002. **The effects of gamma interferon on replication of foot-and-mouth disease virus in persistently infected bovine cells**. Arch.Virol., 147, 2157-2167.
Abstract: Foot-and-mouth disease virus (FMDV) causes a highly contagious viral disease of cloven-hoofed animals, which has a considerable socio-economic impact on the countries affected. In addition, persistent infection can occur following clinical or sub-clinical disease in either vaccinated or non-vaccinated cattle. The mechanism(s) by which FMDV persistence is established and maintained is not fully understood. To better understand the basic mechanisms controlling the virus infection in cattle, the effects of interferon gamma (IFN-gamma) on the replication of FMDV was evaluated in vitro in persistently infected-epithelial cells isolated from FMDV infected cattle. Initially primary bovine thyroid (BTY) cells were treated with varying doses of bovine recombinant IFN-gamma. The cytokine activity was measured by detection of viral antigen in cell supernatants and viral RNA expression compared with cells without INF-gamma treatment. Pretreatment with IFN-gamma profoundly affected FMDV growth in BTY cells. The replication of FMDV was affected in the presence of more than 2.5 u/ml of IFN-gamma and the effect was both dose-dependent and related to the time of exposure. Analysis of the mechanism of inhibition suggests that IFN-gamma did not inhibit the viral replication through induction of nitric oxide. More interesting is the finding that continuous treatment with IFN-gamma severely restricts FMDV replication or even cures persistently infected bovine epithelial cells, indicating that a cytokine-mediated pathway may be involved in the in vivo clearance of persistent FMDV.

Mason PW, Pacheco JM, Zhao QZ, Knowles NJ. 2003. **Comparisons of the complete genomes of Asian, African and European isolates of a recent foot-and-mouth disease virus type O pandemic strain (PanAsia).** J Gen Virol 84: 1583-93.

During the last 12 years, a strain of foot-and-mouth disease (FMD) virus serotype O, named PanAsia, has spread from India throughout Southern Asia and the Middle East. During 2000, this strain caused outbreaks in the Republic of Korea, Japan, Russia (Primorsky Territory), Mongolia and South Africa (KwaZulu-Natal Province), areas which last experienced FMD outbreaks in 1934, 1908, 1964, 1974 and 1957, respectively. In February 2001, the PanAsia strain spread to the United Kingdom where, in just over 7 months, it caused outbreaks on 2030 farms. From the UK, it quickly spread to the Republic of Ireland, France and the Netherlands. Previous studies that utilized RT-PCR to sequence the VP1-coding region of the RNA genomes of approximately 30 PanAsia isolates demonstrated that the UK virus was most closely related to the virus from South Africa (99.7 % nucleotide identity). To determine if there was an obvious genetic reason for the apparently high level of fitness of this new strain, and to further analyse the relationships between the PanAsia viruses and other FMDVs, complete genomes were amplified using long-range PCR techniques and the PCR products were sequenced, revealing the sequences for the entire genomes of five PanAsia isolates as well as an animal-passaged derivative of one of them. These genomes were compared to two other PanAsia genomes. These analyses revealed that all portions of the genomes of these isolates are highly conserved and provided confirmation of the close relationship between the viruses responsible for the South Africa and UK outbreaks, but failed to identify any genetic characteristic that could account for the unprecedented spread of this strain.

Bastos AD, Haydon DT, Sangare O, Boshoff CI, Edrich JL, Thomson GR. 2003. **The implications of virus diversity within the SAT 2 serotype for control of foot-and-mouth disease in sub-Saharan Africa.** J Gen Virol 84: 1595-606.

SAT 2 is the serotype most often associated with outbreaks of foot-and-mouth disease (FMD) in livestock in southern and western Africa and is the only SAT type to have been recorded outside the African continent in the last decade. Its epidemiology is complicated by the presence of African buffalo (Syncerus caffer), which play an important role in virus maintenance and transmission. To assess the level of genetic complexity of this serotype among viruses associated with both domestic livestock and wildlife, complete VP1 gene sequences of 53 viruses from 17 countries and three different host species were analysed. Phylogenetic analysis revealed eleven virus lineages, differing from each other by at least 20 % in pairwise nucleotide comparisons, four of which fall within the southern African region, two in West Africa and the remaining five in central and East Africa. No evidence of recombination between these lineages was detected, and thus we conclude that these are independently evolving virus lineages which occur primarily in discrete geographical localities in accordance with the FMD virus topotype concept. Applied to the whole phylogeny, rates of nucleotide substitution are significantly different between topotypes, but most individual topotypes evolve in accordance with a molecular clock at an average rate of approximately 0.002 substitutions per site per year. This study provides an indication of the intratypic complexity of the SAT 2 serotype at the continental level and emphasizes the value of molecular characterization of diverse FMD field strains for tracing the origin of outbreaks.