

# **Independent Review of the safety of UK facilities handling foot-and-mouth disease virus**

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Presented to the Secretary of State for Environment,  
Food and Rural Affairs and the Chief Veterinary Officer

31 August 2007

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## FOREWORD BY PROF. BRIAN SPRATT

**A**nimal diseases are a continuing threat to our livestock and the devastating impact of the foot-and-mouth disease outbreak of 2001 is still strongly etched on our collective memory. Several other animal diseases remain a serious threat to this country and the Institute for Animal Health laboratories at Pirbright carry out essential research on these diseases, develop and implement the diagnostic tests required to rapidly identify infected animals, and provide the scientific understanding essential to effective disease control, including the potential for new vaccines. The Merial Animal Health vaccine production facility on the same site produces vaccines to assist in the control of foot-and-mouth disease and bluetongue. These are the only facilities in the UK that are authorised to work with live foot-and-mouth disease virus. Similar high containment facilities elsewhere in the UK work on other major animal diseases and the most dangerous pathogens of humans or plants, again to help understand, diagnose and protect against disease.

It is an irony that those facilities that help us to control outbreaks of disease have the potential to cause disease if organisms are accidentally released into the environment. Fortunately, the secure containment laboratories in which these dangerous pathogens are handled have had an excellent (albeit not perfect) safety record. This excellent recent safety record of UK laboratories, and the current safety procedures, derive in large part from investigating and understanding the causes of past accidents and acting promptly to eliminate any identified vulnerability.

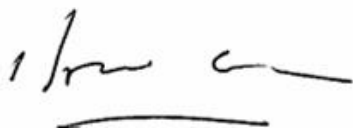
An accidental release from a high security containment facility is of obvious concern to the public and raises issues of public trust in both science and Government. It is for this reason that the Secretary of State and the Chief Veterinary Officer of the Department for Environment, Food and Rural Affairs (Defra) have acted with great speed to commission our review, to provide an independent view on whether the recent outbreak of foot-and-mouth disease at two farms near Pirbright was due to release of virus from one of the facilities at Pirbright, and to review their biosecurity arrangements and identify any breakdown in biosecurity that could have led to the outbreak. This is important not only to understand what went wrong and how to prevent it happening again, but also to allow consideration of any more widely applicable issues that this incident raises.

Our Review Group was assembled at very short notice and I am most grateful to the members of the group for clearing their busy diaries and agreeing to take part in the review. The group included international experts in foot-and-mouth disease, in molecular epidemiology and molecular biology, and in biosecurity and biosafety. I am particularly grateful to the two Swiss members of the group, who brought a most valuable international perspective on the biosecurity of laboratories handling foot-and-mouth disease virus, from their senior positions within the Swiss Institution that serves the equivalent function to the Institute for Animal Health laboratories at Pirbright.

Our Review Group was not responsible for the detailed investigation and testing of the safety equipment and protocols at the two Pirbright facilities. This work was co-ordinated by a group from the Health and Safety Executive and we are very grateful to them for keeping us updated on their thorough and professional investigations. At the request of Government, the delivery of our report has been delayed to allow us to consider and respond to the evidence contained within the final report of the Health and Safety Executive.

I would like to thank Defra scientists for regular updates on their outbreak investigations and the senior management at the Institute for Animal Health laboratories and Merial Animal Health who, under difficult circumstances, provided thorough access to their facilities and staff during our visits and have responded rapidly to our numerous queries.

Finally, this review could not have been carried out without the help of our efficient Defra secretariat and I thank them for their hard work.

A handwritten signature in black ink, appearing to read 'B. Spratt', with a horizontal line underneath.

**BRIAN SPRATT**

August 2007

# EXECUTIVE SUMMARY

## Overview

1. The recent outbreak of foot-and-mouth disease (FMD) at two farms near Pirbright, Surrey, was caused by a virus strain that is not believed to be circulating in nature, but had been used at the Institute for Animal Health (IAH), and at Merial Animal Health Limited on the Pirbright site, during the infection window (the most likely period in which the cattle were infected).
2. There is very little doubt that the FMD outbreak was caused by foot-and-mouth disease virus (FMDV) from one of these two facilities at Pirbright.
3. The Review Group was asked to look at biosafety at the Pirbright site and whether a breakdown in biosafety led to the FMD outbreak. We were unable to determine with confidence which of the two facilities, IAH or Merial, was the source of the outbreak virus. However, we did find several areas where biosafety and biosecurity at the site must be improved, and these are detailed in the recommendations.
4. No defects in the major safety features, including air handling and filtration, were identified within the high containment laboratories of IAH or Merial by the Health and Safety Executive (HSE) investigations.
5. The main focus of our concern was the old, poorly maintained and defective effluent system that is shared by the two very different types of facility at the Pirbright site. The industrial scale of virus production at Merial, and their procedures for inactivating virus before release to effluent, makes it very likely that infectious FMDV is released to the liquid effluent pipe. Maintenance of biosafety therefore requires that the effluent pipe is fully contained, which it was not.
6. The poor state of the IAH laboratories, and the effluent pipes, indicates that adequate funding has not been available to ensure the highest standards of safety for the work on FMDV carried out at this ageing facility. Adequate funding to ensure the safety of the important work on FMDV at IAH must be put in place until the new high containment laboratories are completed around 2012.
7. The Department for Environment, Food and Rural Affairs (Defra) licenses facilities to work with SAPO Category 4 animal pathogens, and also acts as regulator and inspector. However, it also funds about 65% of the work carried out at IAH Pirbright and is thus the major customer for research and services at IAH. This could be perceived as a conflict of interest, which would be avoided by having an arm's length body, such as the HSE, providing a

common framework for regulation and inspection of all laboratories working with animal or human pathogens at high containment.

### **The foot-and-mouth disease outbreak and the nature and source of the virus**

8. Epidemiological studies by Defra have indicated that the infection windows for the first and second infected farms overlap. This overlap indicates that both farms could have been infected at the same time, or that infection at one farm was transmitted to the other. The current evidence favours the latter interpretation, but is not conclusive.
9. During the infection window, the outbreak FMDV strain (O<sub>1</sub> BFS 1860) was being used in small-scale experiments within the Category 4 high containment laboratories at IAH and was being grown in extremely large amounts (12,000 litres) in the virus production facility at Merial to make vaccine, again under Category 4 containment conditions.
10. An independent laboratory determined the DNA sequences of the outbreak FMDV and the three stocks of the O<sub>1</sub> BFS 1860 virus being used at Pirbright during the infection window. The sequences indicate that the source of the outbreak was either the virus used for molecular biology and immunological research at IAH, or the virus used for vaccine production at Merial. These two viruses were extremely similar and the outbreak strain differed in sequence from the Merial virus at five positions and from the IAH virus at six positions; this very slight difference does not allow us to conclude which strain was the most likely source of the outbreak with any degree of confidence.

### **Security of access to the Pirbright site**

11. Biosecurity is maintained by preventing access to the Pirbright site by a perimeter fence (with razor wire and intruder detection systems) that extends around both the IAH and Merial facilities. There are separate entry gates but, as there is no fencing between the two facilities, access to the Merial site can be obtained through the IAH gate and vice versa. Prevention of illegal entry to each facility is therefore dependent on the security of their own entrance gate and that of the other facility. In our view illegal access to the Pirbright site is possible by determined individuals, particularly at the Merial entrance which is not manned during the day. Perimeter and access security is not as good as that in Category 4 facilities handling human pathogens.
12. Even if security of access to the Pirbright site is breached, illegal access to the high containment laboratories of IAH or Merial is very unlikely.

### **The Category 4 containment facilities at Pirbright**

13. Apart from the modern facilities for large animal work, the Category 4 containment laboratories at IAH are very old and are well short of the

standards expected of an internationally-important laboratory handling such livestock-threatening pathogens as FMDV. The vaccine production facilities at Merial are state-of-the-art. We would, however, stress that this does not mean the IAH facility is dangerous and the Merial site is safe. Safety depends not on the age of the facility but on the procedures that are carried out, approved safe protocols for those procedures that are appropriate for the facility, regular inspection and testing and a very strong culture of biological safety, biological security and management.

14. The type and scale of the procedures undertaken at IAH and Merial are very different. Most experiments at IAH involve small amounts of FMDV in Class II safety cabinets that prevent exposure of the worker. At Merial extremely large amounts of FMDV are regularly grown (6,000 litre batches) and further processed to produce vaccine.
15. At both sites, release of virus into the exterior environment following any accidental or inadvertent release into the laboratory is prevented by the negative pressure of the high containment laboratories and effective filtering of all air released to the environment.
16. Occasional exposure of laboratory staff to FMDV may occur at either site due to minor accidents (which are reported and logged, apparently without recrimination). Exposure of susceptible animals is then dependent on the required change of clothes and thorough showering on exiting the containment laboratories, and by quarantine rules that specify a period in which workers in high containment areas may not enter farms, visit zoos, or approach susceptible animals.

### **Inspection of facilities at Pirbright**

17. The HSE team has thoroughly examined the Category 4 high containment laboratories for vulnerabilities that could have led to release of virus as an aerosol into the environment. No problems were found at IAH or Merial with the negative pressure air handling system, the air filtration systems or the equipment used by Merial for large-scale virus growth and vaccine production.
18. There was some concern by HSE about an experimental procedure used by one group that could release some infectious O<sub>1</sub> BFS 1860 into the IAH laboratory. Any release of virus would be contained within the laboratory by negative pressure and air filtration. They were also concerned about the HEPA filter testing procedure at IAH, and the state of part of their laboratories. These problems are not considered to be a likely cause of the FMD outbreak.

## **The Pirbright liquid waste effluent system**

19. Separate effluent drainage pipes take liquid waste from IAH and Merial. These pipes join and enter a shared caustic soda treatment plant managed by IAH. This plant is over 50 years old but its operation is simple and robust, and has not been a cause for concern in the past. However, there was some water ingress during the exceptionally heavy rains of 20 July 2007 and the plant is in an area of the Pirbright site liable to flooding.
20. We were concerned that the effluent pipes from IAH and Merial are shared, particularly considering the huge differences in the amounts of virus being handled, with small-scale laboratory procedures at IAH compared to industrial procedures at Merial.
21. All contaminated liquid waste leaving IAH and Merial to the effluent drainage pipes is subject to chemical disinfection. Chemical disinfection is not considered to be a completely effective process but, before release to the sewer, effluent is treated further in the caustic soda treatment plant.
22. Chemical disinfection of the small amounts of FMDV being handled at the IAH facility should be highly effective, resulting in no significant amount of infectious virus being released into the effluent pipe. However, due to the industrial scale of production of FMDV at Merial, and the nature of the vaccine production process, we were not convinced that procedures to ensure chemical disinfection of FMDV before release to effluent were fully effective, or validated.
23. The possibility of infectious virus being discharged to the effluent pipes was recognised by the Defra inspectors and, for this reason, the drainage system that leads to the caustic soda final treatment plant is considered part of Category 4 containment at Pirbright. It must therefore be well maintained and contained, so that infectious virus in effluent cannot escape.
24. The Site Director of Merial agreed with the Review Group that infectious virus was likely to be in the effluent from Merial, but this did not appear to be known to the biological safety officer of IAH. Insufficient communication between the biological safety officers of IAH and Merial, and different perceptions of the risk from the effluent were apparent. We were also concerned that the Site Director of Merial took the lead role of biological safety officer, with the potential for a conflict of interest between biological safety and the commercial interests of the company.

## **Release of virus from Pirbright**

25. It is still not clear how virus from the Pirbright site reached the outbreak farm(s). Several possible routes have been considered; some are so unlikely



that they can be ruled out, others are very unlikely and some remain possibilities.

26. Release of virus into the environment due to an accidental aerosol release within IAH or Merial and windborne spread to the infected farms is very unlikely.
27. Contamination of a laboratory worker(s) with FMDV and transfer to the outbreak farm is another possible source of infection. This should not occur if strict showering on exiting the containment facility and quarantine procedures were adhered to. Staff at both facilities showed a thorough understanding of these biosecurity procedures and the potential consequences of non-compliance.
28. Malicious removal of small amounts of infectious agents from the high containment laboratories at IAH or Merial by a determined laboratory worker (or ex-worker) would be possible if the intent was present. However, it is recognised that it is difficult to prevent the removal of infectious agents from any Category 4 pathogen containment facility.
29. Although it is hard to rule out, there were no indications from IAH or Merial of staff or ex-staff who were considered capable of such an act. Discussions with the security services did not identify any security threats to the Pirbright site. Similarly, there were no indications of industrial sabotage against Merial or agroterrorism activity.
30. There was concern about one employee at Merial who had an allotment directly adjacent to one of the fields of the first outbreak farm. It is very unlikely that this individual was responsible for causing the outbreak.
31. The most likely cause of the outbreak is release of infectious FMDV from the effluent pipes.

#### **Lack of integrity of the effluent pipes from IAH and Merial**

32. The effluent pipes from IAH and Merial to the caustic soda final treatment plant are old and appear not to have been subject to regular thorough inspections to ensure their integrity. An inspection of the effluent pipes and manholes carried out for the HSE team showed deficiencies and it is considered very likely that they leak effluent. The effluent pipes are therefore not contained, as they should be as part of Category 4 containment at Pirbright.
33. There had been concern for several years that the effluent pipes were old and needed replacing but, after much discussion between IAH, Merial and Defra, money had not been made available.

34. The combination of incomplete inactivation of FMDV before it leaves Merial, and the deficiencies in the effluent pipes and drainage system, are likely to have released infectious virus into or onto the surrounding soil.
35. There has been much recent contractor activity at IAH, including around the area above the defective effluent pipes and drainage system. Mechanical spread by virus-contaminated contractors' vehicles to the first outbreak farm is a possibility.
36. Direct spread of virus by water to the first outbreak farm from any surface contamination with FMDV at Pirbright is highly unlikely. Spread of FMDV by deer from contamination at the Pirbright site is also unlikely.

### **Final remarks**

37. The cause of the escape of FMDV from Pirbright has still not been established, and may never be. No gross breach of biocontainment within the IAH or Merial laboratories was identified by the HSE investigations. Release of infectious virus from Merial and consequent surface contamination from the drainage system, and mechanical spread to the outbreak farm, is considered the most likely cause. However, it must be stressed that it is not certain that the virus causing the outbreak was from Merial, and experimental evidence is lacking to establish that infectious virus was released to the effluent pipe from Merial and contaminated the area around or above the effluent pipes.
38. The release of FMDV from Pirbright, and its consequences, highlights the risks of two facilities with very different missions and cultures being co-located and dependent on each other for aspects of their biosecurity and biosafety.
39. A number of recommendations are made to improve biosecurity and biosafety at the Pirbright site, and to reconsider the future safety of laboratories that work on FMDV and other important exotic animal pathogens.

# RECOMMENDATIONS

The recommendations are numbered as they appear in the text.

## Immediate action on containment

- As a matter of urgency, Defra should require that actions are taken to ensure the effluent drainage system at the Pirbright facility is fully contained and its continuing integrity confirmed by regular inspections. In the interim, we advise that work with infectious virus should only be allowed if effluent released into the pipes has first been completely inactivated (Recommendation 8).
- Merial should discuss with Defra how it plans to modify its procedures to minimise the possibility of release of infectious FMDV virus into the effluent pipe. Any new process should be validated (Recommendation 9).
- IAH should have a thorough review of the safety of all laboratory activities to ensure that procedures which could release infectious FMDV into the containment laboratories are eliminated. This is particularly important for aerosol-producing procedures (Recommendation 5).
- Entry to any facility handling Special Animal Pathogens Order (SAPO) Category 4 pathogens should require all visitors to sign in, obtain a numbered visitor pass, be escorted into the building and handed over to their host. Visitors (including all contractors) must be informed of the animal quarantine requirements and sign (and be given a copy of) a form accepting that these are understood. For all visitors, including contractors, requirements to prevent inadvertent infection of livestock should be based on an assessment of the risk of exposure to pathogens (Recommendation 2).

## Further action on biosecurity and biosafety

- The biological safety officers of IAH and Merial should institute regular meetings to improve communication and their understanding of the risks on the Pirbright site, particularly those that arise from the sharing of the effluent system (Recommendation 7).
- The responsibilities of the Site Director and Biological Safety Officer of Merial should be clearly separated. The Biological Safety Officer should not be subject to commercial pressures on matters of biosafety and biosecurity (Recommendation 4).
- Defra and the Veterinary Medicines Directorate (VMD) should work together more closely and exchange information about inspections at Merial. One of the two regulatory authorities should take responsibility for ensuring that all aspects of biocontainment and biosafety are thoroughly inspected (Recommendation 6).

- IAH and Merial should erect secure fencing to separate their two facilities, with swipe card entrances through the fencing between sites for those authorised to move between the facilities (Recommendation 3).
- If identifying the source of the virus is considered a priority, an independent group consisting of international experts in the molecular epidemiology of FMD, and in RNA virus molecular evolution, should convene to consider whether additional virus sequencing, or the passage of candidate viruses through cattle, could establish with confidence which was the cause of the outbreak (Recommendation 1).

### **Funding, design and governance**

- The construction of the new high containment laboratories at IAH should go ahead as a matter of urgency. Such facilities are expensive to construct and maintain and Government must ensure that adequate funds continue to be available to enable the highest standards of biological safety for dealing with FMDV and other high risk viruses. In the meantime, investment to ensure safety and public trust in the existing laboratories and the effluent system is needed (Recommendation 11).
- The plans for future development of the Pirbright site should be reviewed to ensure that all safety critical issues have been addressed. This should be carried out with the help of the full range of relevant experts and regulatory bodies (Recommendation 10).
- Biosecurity of laboratories that work with FMDV is of paramount importance. Therefore there should be a review of funding, governance and risk management at IAH Pirbright to ensure an appropriate focus on biosafety and biosecurity in the future (Recommendation 12).
- There should be shared governance for the management of risks to biosecurity and biosafety involving both IAH and Merial. The two facilities should ensure complete clarity of responsibility and liability for the biosafety and biosecurity of the whole site (Recommendation 13).

### **Regulatory and inspection framework**

- There should be a review of systems for regulation, inspection and enforcement of biosecurity for work on animal and human pathogens at containment level 4. This should consider whether there should be a common regulatory inspection framework overseen by an arm's length body such as the HSE (Recommendation 14).

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# 1 INTRODUCTION

Following the unexpected outbreak of foot-and-mouth disease (FMD) confirmed on 3 August 2007, the Government commissioned an independent inquiry into the arrangements for biosecurity, that have been and are in place, at the two facilities handling foot-and-mouth disease virus (FMDV) at Pirbright, Surrey. These are the only facilities which handle infectious FMDV in the UK. The Review Group were tasked with evaluating whether a breakdown in biosecurity controls could have led to the outbreak of FMD in the UK and to make recommendations for further investigations if necessary.

The Pirbright site and facilities are owned by the Biotechnology and Biological Sciences Research Council (BBSRC). It houses two separate organisations: the Institute for Animal Health (IAH) Pirbright laboratories, which are sponsored and funded by the BBSRC, and Merial Animal Health Limited (Merial), a company making vaccines against FMD and Bluetongue. The BBSRC leases the Pirbright site to IAH who subleases part of it to Merial. The BBSRC provides the core funding for IAH Pirbright and most of the rest of their income is from Defra.

This report is one of two independent inquiries announced by the Government in August 2007. In parallel, the Health and Safety Executive (HSE) were asked to investigate any potential breaches of biosecurity at both sites, whether such breaches may have led to a release of any specified animal pathogen and whether any such breaches had been rectified to prevent future incidents.

This review, therefore, does not duplicate the checking of equipment, protocols and validation, and the forensic evidence gathered by the HSE, but rather considers the underlying science in order to advise the Government on the plausibility of the escape of the virus and to provide appropriate science-based recommendations on how to deal with the current situation and to ensure the future biosecurity of high containment facilities that work with FMDV.

It should, however, be stated at the outset that the amount of hard science that could be applied to our investigation of the source of the outbreak was very limited, and we have had to consider many possible scenarios and use our judgement and experience to rank their likelihood of causing the outbreak. This was unsatisfactory and frustrating but identifying the source of an outbreak of this kind with any certainty is always likely to be inconclusive, unless some gross and obvious breakdown in a safety critical feature has occurred.

## 2 CONDUCT OF THE REVIEW

The independent Review Group first met at the IAH, Pirbright, Surrey, on 7 August 2007. The morning session involved presentations on the FMD outbreak and the epidemiological investigations. In the afternoon, the Review Group met with the HSE team for updates on their investigations and main lines of enquiry. The Review Group interviewed Human Resources (HR) personnel from IAH to discuss the procedure for employing and vetting staff with access to the Level 4 containment facilities and their Biological Safety Officer.

On the morning of 8 August, the Review Group interviewed the IAH research workers who had handled the O<sub>1</sub> BFS 1860 virus (O<sub>1</sub> BFS for short) during the infection window. The FMD and biosafety experts in the Review Group carried out an inspection of IAH's high containment facilities. The group then met with the Director of IAH, followed by a Meteorologist from the Met Office, currently seconded to Defra, to discuss airborne transmission. The group received further updates from the HSE team and IAH Project Leaders.

In the afternoon, the Review Group moved to Merial Animal Health and met with their Site Director. The process of FMD vaccine production and the running of the Merial laboratories were discussed. Officials from the Veterinary Medicines Directorate (VMD) were also present. On the morning of 9 August, the interview resumed at Merial with the Site Director. The Review Group then divided into three to look at the quality assurance and the two separate virus production units within the high containment area. In the afternoon, the group interviewed the Quality Assurance Manager and personnel at Merial who had been involved with production of the O<sub>1</sub> BFS virus during the infection window.

The group then returned to IAH and received updates from the Environment Agency, the HSE team, and the IAH Biological Safety Officer on a number of matters, including the effluent treatment system.

On 10 August the group met with further project leaders at IAH. Once the interviews were complete, the group discussed the evidence available and the proposed structure and content of their report. The group left the Pirbright site in the afternoon.

Review Group discussions and report writing took place from 11 to 30 August. The group met regularly by teleconference to discuss the relevant science, and the unfolding evidence from the HSE inspection team, the Defra epidemiologists and others. One member of the Review Group inspected the surroundings of the first infected farm on 22 August. The group met again on 23 August in London to discuss and finalise their report.

Members of the Review Group were asked to declare any conflicts of interest. None were identified.



## **3 FOOT-AND-MOUTH DISEASE – THE BASICS**

This section provides a brief overview of FMD and FMDV. It is mostly drawn from the Defra Veterinary Surveillance Strategy provisional profile for FMD (Defra 2006) and the references therein. The AUSVETPLAN pages of the Animal Health Australia website gives additional useful information (Animal Health Australia 2002).

### **3.1 Characteristics of FMD and FMDV**

FMD is a highly contagious disease of cloven-hoofed animals including cattle, sheep, pigs, goats, other farmed mammals and wild ruminants, and is one of the most important diseases of livestock. On introduction to a herd or flock the virus can spread very rapidly by direct and indirect transmission. Affected animals have a high temperature, which is followed by the development of blisters chiefly in the mouth and on the feet. However, in some species (notably sheep and goats), the disease is frequently less severe or occurs as a sub-clinical infection. The disease is not usually fatal in adult animals, but can be in young stock. However, it causes severe pain and distress, especially in cattle; animals may be left permanently lame and the productivity of recovered animals may be reduced.

FMD is caused by a picornavirus. It is a non-enveloped, single-stranded, positive-sense RNA virus, 30 nm diameter, with a genome of ~ 8.4 kilobases. On the basis of cross-protection studies, seven serotypes of FMDV have been identified: O, A, C, South African Territories (SAT) 1, SAT 2, SAT 3 and Asia 1. There is no cross-protection between these seven serotypes and within each serotype different strains vary in antigenic characteristics. As a consequence of this antigenic variation vaccine strains must be chosen carefully to ensure maximum protection against current field strains.

### **3.2 Pathogenesis**

FMD is characterised primarily by the formation of vesicles (blisters) in and around the mouth and on the feet, but vesicles may also develop on the snout or muzzle, teats, mammary gland, vulva and other sites of the skin. Initially blanched areas of epithelium are observed which subsequently develop into fluid filled vesicles which soon rupture, leaving a raw red area. After rupture, regeneration of epithelia occurs resulting in a variable degree of scarring. On occasions secondary infections may prolong the healing process.

FMD lesions on the tongues and feet of cattle and pigs can be aged according to the following established criteria: vesicle development 0-2 days; rupture of vesicles (initially having fragments of epithelia attached); followed by sharply margined erosion 2-3 days; with the sharpness lost from day 3; serofibrinous exudation 4-6 days; and the beginning of repair with a marked fibrous tissue margin at 7 or more

days. Dating of lesions of animals from an outbreak farm, combined with the incubation period, provides important information about the likely window in which infection occurred. A high proportion of cattle become asymptomatic chronic carriers of the virus after recovery from acute infection.

### **3.3 Survival of FMDV in the environment**

Survival of FMDV in the environment will depend upon the nature of the material containing it; the initial concentration of virus in the material; the strain of virus; the humidity; the pH and the temperature, and will therefore be highly variable under field conditions. However some reported examples are: up to 20 weeks on hay or straw; up to 14 days in dry faeces; up to 39 days in urine; up to 6 months in slurry; up to 3 days on soil in summer and 28 days on soil in autumn. Survival has been reported up to 50 days in water (Mahnel et al. 1977).

The virus is progressively inactivated at temperatures above 50°C but sunlight has little or no direct effect on infectivity. It is preserved by refrigeration and freezing. Airborne virus is stable at humidities above 55-60% and drying will inactivate most but not all of the virus. FMDV is inactivated by acid pH (<6.0) or alkaline pH (>9.0), for example by 2% sodium hydroxide, 4% sodium carbonate and 0.2% citric acid. In laboratories handling FMDV, citric acid and FAM 30 (a propriety iodophor disinfectant approved by Defra for FMDV), at prescribed dilutions that have been demonstrated to be effective, are used for chemical disinfection.

### **3.4 Transmission and epidemiology**

Cattle, sheep, pigs, goats and wild ruminants, including all species of deer, are susceptible. The incubation period for FMD is highly variable, and depends on the strain and dose of virus, the route of transmission, the animal species and the husbandry conditions. It is usually stated as 2-14 days (including in guidelines from the World Animal Health Organisation – Office International des Épizooties [OIE]), but is more commonly 3-5 days, and may be as short as 24 hours. Essentially, for all species, the higher the dose or intensity of contact, the shorter is the observed incubation period.

Expired breath, saliva, nasal and lachrymal fluids, urine, faeces, milk, and semen all become infectious to a greater or lesser extent during the course of the disease and some contain significant quantities of virus before development of clinical signs. Animals become infected either by direct contact with infected animals, or by contact with contaminated material on vehicles, equipment, clothing, feedstuffs etc., or in the case of pigs, through the illegal feeding of contaminated meat. Infection may also occur indirectly as a result of airborne transmission of FMDV.

Pigs produce large amounts of virus in exhaled air and infected pig farms present the greatest risk of airborne spread. Because of their large respiratory volume, cattle are particularly susceptible to infection by virus inhalation. Sheep may show few clinical

signs and thus present the greatest risk of spread through animal movements between farms.

The minimum dose that causes infection in 50% of cattle by the respiratory route (inhalation) has been measured for some FMDV strains. For the strain causing the 2007 Pirbright outbreak (O<sub>1</sub> BFS), a value of 12.5 infectious units has been determined (Donaldson et al. 1987). An infectious unit is the minimal amount of virus required to infect highly susceptible tissue culture cells.

### **3.5 Viral evolution**

In common with other RNA viruses the replication of FMDV is highly error prone as it lacks a proof reading function. On average, approximately one mutation will occur each time a FMDV genome replicates and, as a consequence, most viral genomes within a population can be expected to differ from the others by at least one nucleotide substitution (Knowles and Samuel 2003). Therefore a virus sequence determined in the laboratory represents an average of a large number of individual sequences that existed in the infected animal (or in cell culture), a so-called quasi-species. The consensus sequence of a quasi-species is constrained by continual selection – for example, non-viable mutations are eliminated from the population as soon as they arise while highly beneficial features of the sequence will be selected and can be stably maintained over many generations. However, mutations with little or no selective disadvantage can accumulate (typically those that do not change an amino acid), thus leading to a continual drift in the consensus sequence in successive generations, and those mutations that confer new advantages to the virus will tend to increase as the population evolves.

These inherent properties of FMDV sequences can be used in two ways for epidemiological purposes:

Firstly, the sequence divergence in the RNA encoding the VP1 capsid protein can be used to classify FMDV into major groups and subgroups (Knowles and Samuel 2003). A very large database of VP1 sequences is available, and the VP1 sequence of a new FMDV strain is routinely compared to this database, to assign the new virus to a major group or subgroup, and to identify its closest relatives.

Secondly, the accumulation of differences in the consensus sequence of the whole viral genome during transmission from farm to farm can complement epidemiological information to track the spread of the virus during an epidemic. This was carried out in the 2001 FMD outbreak where small changes in the full genome sequences of viruses isolated during the developing epidemic provided evidence for the spatial and temporal links between individual outbreaks (Cottam et al. 2006).

## **4 THE 2007 FMD OUTBREAK**

### **4.1 Key events**

This section provides a summary of the outbreak. It is largely drawn from epidemiological reports (Defra 2007a) on Defra's website and briefings the Review Group received from Defra epidemiologists.

The first confirmation of FMD was on a beef farm near Guildford, in Surrey on Friday 3 August 2007. The field where the infected cattle were held is approximately 4.5 km south-west of the Pirbright site. On confirmation of disease Defra set up zones of movement controls and increased surveillance around the affected farm. These were a protection zone of at least 3 km radius and a surveillance zone of at least 10 km radius.

Suspect disease was investigated at another cattle premises on Monday 6 August and disease was confirmed on clinical grounds. This second outbreak farm is approximately 3 km south-west of the Pirbright site. The diagnosis was subsequently confirmed by laboratory tests. Infection at this second farm was not unexpected, being situated within the 3 km Protection Zone and was identified by a routine protection zone surveillance visit.

The location of both infected farms and the Pirbright site are shown in Figure 1. The Protection and Surveillance Zones around both infected farms and the Pirbright site are shown in Figure 2. On 14 August Defra records showed that the extended protection zone around the field where the infection was first detected, the Pirbright site, and the nearby second outbreak farm, included 59 holdings with livestock: 19 with cattle, 12 with pigs, 29 with sheep, 21 with goats and one with alpacas (some holdings had more than one type of livestock).

Animals on one farm adjacent to the first infected premises, though with no animal contact, were slaughtered on 9 August on suspicion of disease. There were 16 beef cattle, 3 sheep, 2 goats and approximately 280 pigs (sows and followers) on the premises. Disease was not confirmed by laboratory tests.

### **4.2 Infection window**

The timeline of an outbreak provides important evidence for the epidemiological investigation. Careful examination was carried out by experts from the IAH Pirbright on all clinically infected animals after slaughter to estimate the age of the lesions. The estimated age of the lesions, and the incubation period, was used to determine a source tracing window (or 'infection window'), which is the period within which infection is estimated to have been introduced onto a farm (Figure 3).

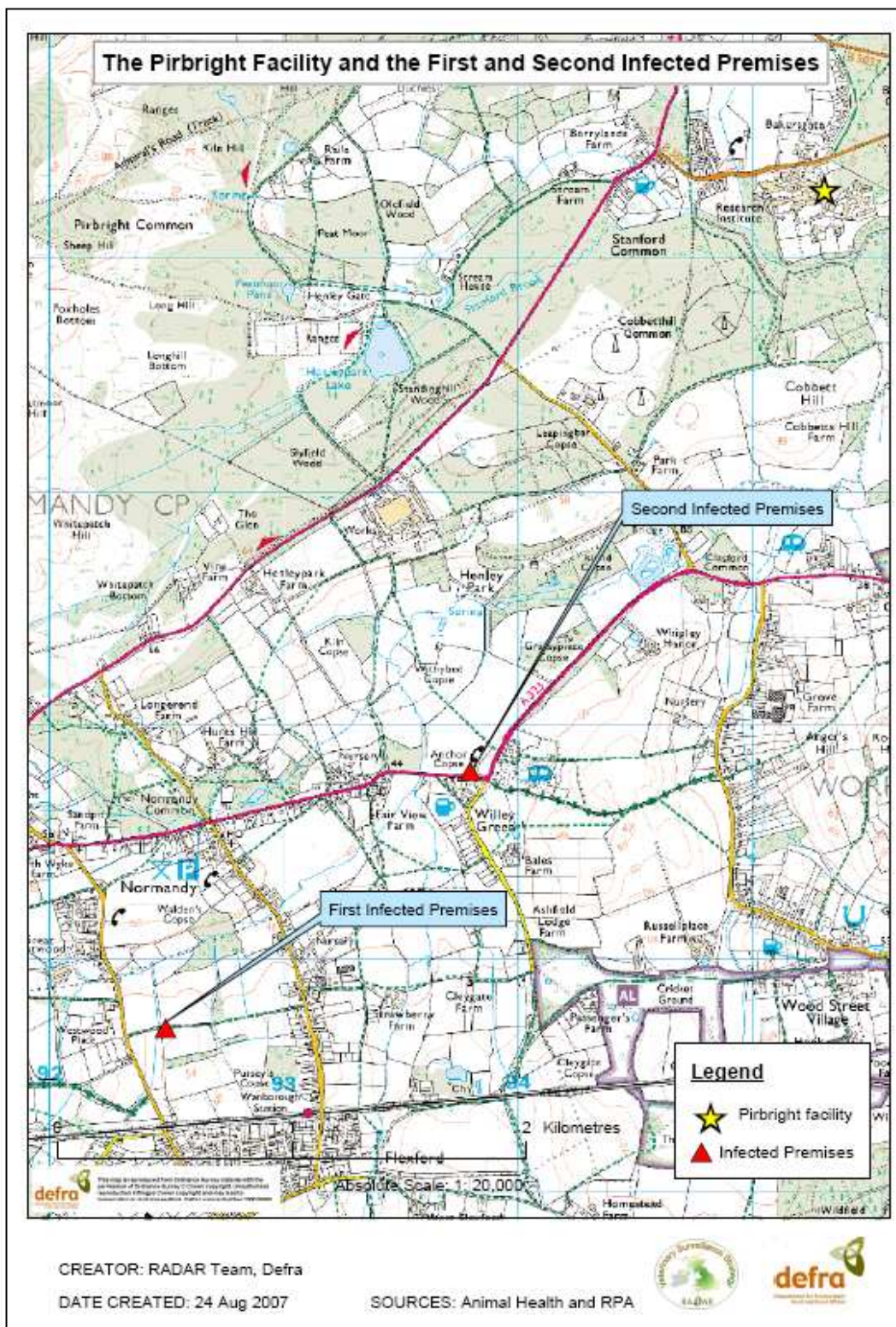
Defra epidemiologists have advised that the earliest the infection would have arrived at the first farm is 12 July. The latest time is estimated to be 25 July. The infection

window is therefore between the 12 and 25 July for the first farm and the earliest date for infection of the second farm is estimated to be the 17 July. There is thus a period of overlap between the infection windows of the two farms from 17 to 25 July. Both premises could therefore have been infected from a common source, or infection at the second farm could be due to secondary transmission from the first farm. It is unlikely that infection actually occurred first at the 'second' farm and then spread to the 'first' farm.

The genome sequence of a virus from the second infected farm shows an extra amino-acid changing mutation compared to virus from the first infected farm (IAH laboratories, communication to Review Group) which, together with the ages of the lesions and the differences in the infection window (although these overlap for the two farms), favours the view that virus was transmitted from the first farm to the second, although the evidence cannot yet be considered to be conclusive.

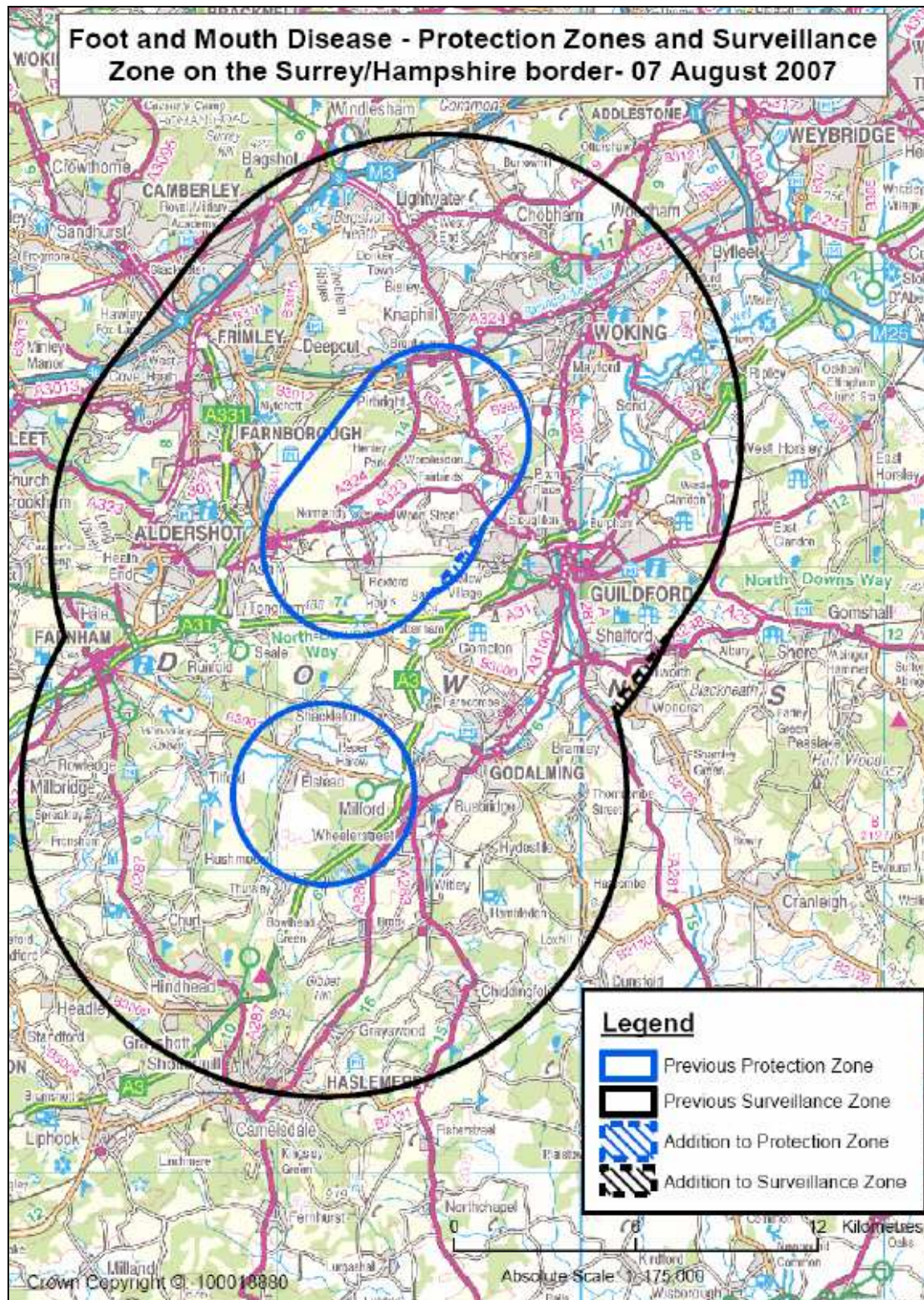


Figure 1. Location of the two infected farms relative to the Pirbright site

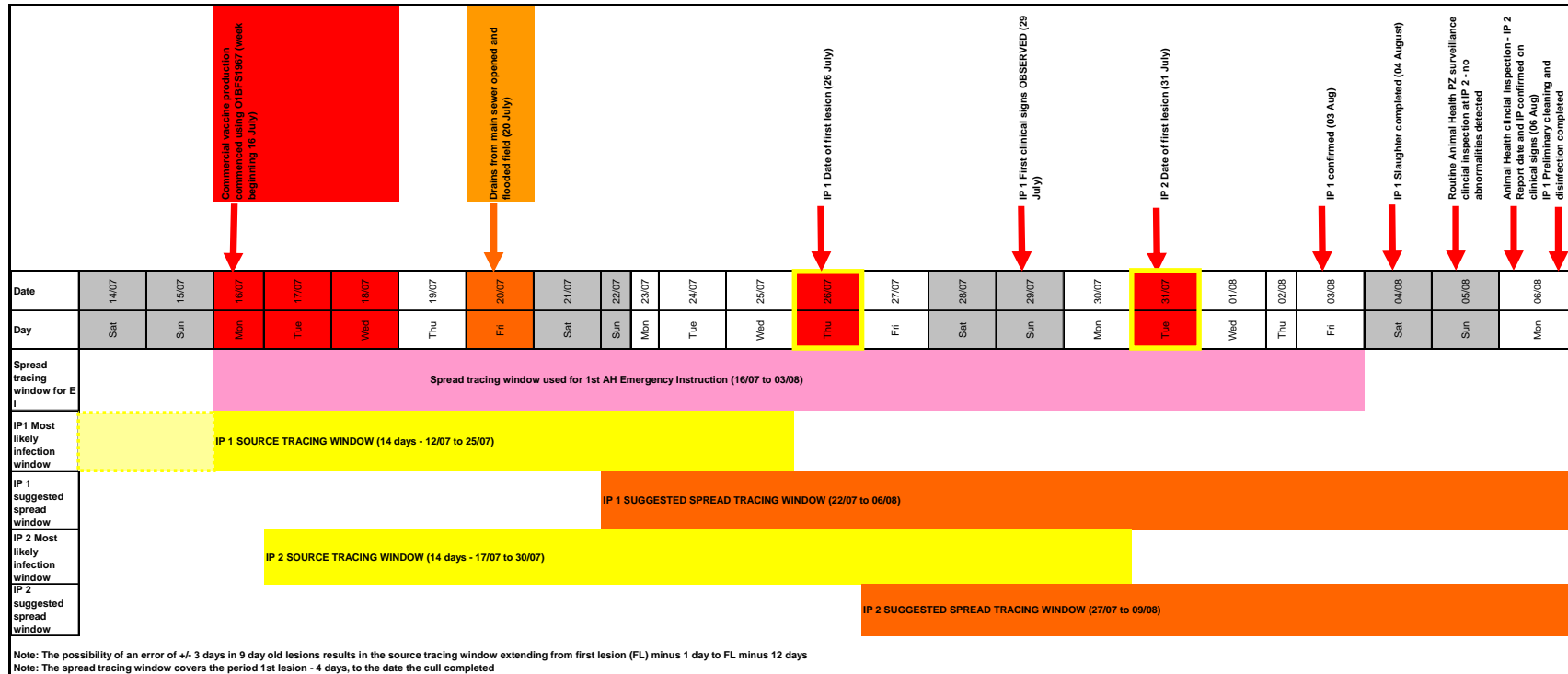




**Figure 2. Protection and surveillance zones around both infected farms and the Pirbright site**



**Figure 3. Infection time-line prepared by the Defra Epidemiology Team (Defra 2007a)**





## 5 POSSIBLE SOURCES OF THE OUTBREAK VIRUS

### 5.1 The outbreak strain

Sequencing of the VP1 coding region of three virus isolates from the first infected farm and one isolate from the second showed that they are identical to each other, and very closely related to the published sequences of the VP1 coding region of two O<sub>1</sub> BFS 1860 viruses, differing by one and two nucleotides, respectively. Further sequencing from other regions of the virus genome confirmed the near identity of the UK 2007 isolates to O<sub>1</sub> BFS 1860.

The O<sub>1</sub> BFS 1860 strain was originally isolated from the outbreak of FMD in the UK in 1967 and has been widely distributed to FMDV reference laboratories in many parts of the world for research purposes and is used to produce vaccines. The rapid accumulation of mutations in FMDV during its replication results in continuous diversification of the genome sequence. Consequently, viruses with a VP1 sequence that is very similar to O<sub>1</sub> BFS would not be expected to be circulating in the wild after 40 years. The only plausible way that the outbreak virus could be so similar to O<sub>1</sub> BFS is if it was from a laboratory stock of this virus that has not changed significantly in sequence as it has been maintained as a frozen archived stock in a laboratory.

There is anecdotal evidence that improperly inactivated O<sub>1</sub> BFS vaccines produced in India have in the past given rise to outbreaks of disease, providing a possible alternative source of viruses that are relatively closely related to the outbreak strain. This is not thought to have occurred in recent years and there is no evidence of viruses similar to O<sub>1</sub> BFS circulating currently in India according to information we obtained from the relevant authorities there.

The O<sub>1</sub> BFS virus was being used at both IAH and Merial during the infection window (section 5.2), and the close proximity of the infected farms to these facilities at Pirbright, makes it almost certain that the 2007 outbreak originated from virus that in some way escaped from the Pirbright site. This was unexpected as the site has a strong biosafety record and there has been no escape of FMDV from the site since 1960 (see annex 3). The only other possibility is that the same virus was introduced maliciously by someone from another laboratory who had access to the O<sub>1</sub> BFS strain, presumably to implicate Pirbright in the outbreak. We consider this highly unlikely but discuss malicious release further in section 10.1.

## **5.2 Use of outbreak strain in Pirbright during the infection window**

The infection window of the first farm has been estimated to have been between 12 and 25 July. The records of IAH and Pirbright were used to identify the usage of the O<sub>1</sub> BFS strain within this time period.

IAH reported that no animals were infected with the O<sub>1</sub> BFS strain in this period. However, their records show that five individuals, in four different research groups (Microbiology, Immunology, Biosafety and a small private company – Stabilitech Limited), used a total of about 12ml of O<sub>1</sub> BFS within the infection window. Stabilitech Limited is a private company with staff working within the containment facilities at IAH.

Two different stocks of O<sub>1</sub> BFS were used at IAH, one for molecular biology/immunology work (and by Stabilitech), and the other for disinfection tests. One individual used a total of ~ 400µl of virus over four days (18, 23, 24 and 25 July), a second used about 2ml between the 17-19 July, a third within the Stabilitech Group used about 2ml between 16/17 July. A fourth individual used about 1ml on 12 July. All of the above worked with the virus used for Molecular Biology/Immunology. About 7ml of the other virus was used on 24 July by a fifth individual in the Biosafety Group for disinfection tests. Assuming these virus stocks contained about 10<sup>7</sup> infectious units/ml, a total of about 1.2 x 10<sup>8</sup> infectious units were used at IAH during the infection window.

Records at Merial show that two 6,000 litre batches of the O<sub>1</sub> BFS strain were produced from their vaccine production stock during the infection window. Assuming the yield of virus in these batches was about 10<sup>7</sup> infectious units/ml, a total of about 1.2 x 10<sup>14</sup> infectious units were produced at Merial during the infection window.

## **5.3 Sequencing of the viruses - IAH or Merial as the source?**

Almost full length genome sequences were obtained at IAH for four stocks of O<sub>1</sub> BFS: those used at IAH for molecular biology and immunology (and by Stabilitech) (IAH-M) and for disinfection testing (IAH-D), that of the seed stock virus for vaccine production at Merial (MER), and virus taken from an infected animal at the first outbreak farm (OUT). The latter was sequenced directly from infected material whereas the other three viruses had had different numbers of passages through tissue culture cells. The same viruses were also sent for sequencing to an independent laboratory that has much experience of working with FMDV (National Veterinary Institute, Lindholm, Denmark). The IAH and Danish laboratories did not know the identity of the viruses they were sequencing as they were labelled A-D by the HSE, except that the Danish laboratory was told the identity of the outbreak virus as this was needed to assess which of the three laboratory viruses was the most likely to have given rise to the outbreak. The identities of the three laboratory viruses were also not available to the two

members of our Review Group who analysed the sequences and provided their conclusions to the HSE team. Once their interpretation and opinion had been transmitted to the HSE team the identities of the viruses were made known to the Review Group.

The IAH and Danish sequences were the same, except that the former laboratory had sequenced approximately 300 nucleotides more of the viral genomes than the latter. The extra 300 nucleotides of sequence made no material difference to the interpretation of the results. The independent sequencing in the Danish laboratory showed that the IAH-M and MER viruses were extremely similar, differing at only one out of the ~7,600 nucleotides sequenced. These two viruses were also the most similar to the outbreak virus, the MER virus differing from OUT at 5 nucleotides, and the IAH-M virus differing at 6 nucleotides. The IAH-D virus was less closely related to OUT, differing at 11 nucleotides. The number of nucleotide differences between the MER/IAH-M viruses and the OUT virus was considered, by both the IAH laboratory and the independent Danish laboratory, to be consistent with available data on the number of differences that accumulate when FMDV that has adapted to growth in tissue culture is transmitted through cattle. We agree with this view.

The outbreak was therefore caused by either the IAH-M or the MER virus. The MER virus is the most similar to the outbreak virus but there is sufficient uncertainty to prevent us from assigning the outbreak to the virus from Merial with a high degree of confidence. Due to the similarity in the sequences of the IAH-M and MER viruses it may not be possible through additional investigations to establish, with the required level of certainty, which virus caused the outbreak. A more technical explanation of the sequencing results and their interpretation is given in annex 4.

**Recommendation 1. If identifying the source of the virus is considered a priority, an independent group consisting of international experts in the molecular epidemiology of FMD, and in RNA virus molecular evolution, should convene to consider whether additional virus sequencing, or the passage of candidate viruses through cattle, could establish with confidence which was the cause of the outbreak.**

## 6. OVERVIEW OF SECURITY AT THE PIRBRIGHT SITE

There is very little doubt that the outbreak of FMD was caused by virus from one of the facilities on the Pirbright site and was therefore due to a breakdown in some aspect of biosecurity or biosafety at the site (see section 5.1). We therefore examine next the nature of the two facilities at Pirbright and their biosecurity and biosafety.

### 6.1 General principles of laboratory biosafety and biosecurity

Biosecurity/biosafety describes the containment principles, technologies and practices that are implemented to prevent inadvertent exposure to biological agents, or their release from the laboratory into the environment (World Health Organization 2004; Richmond and Nesby-O'Dell 2003). With human pathogens this includes protection of both the laboratory worker and the environment; with animal pathogens there are measures to prevent contamination of laboratory workers but the main emphasis is on preventing release to the environment.

The objective of a high containment facility is to keep pathogens inside a barrier e.g. inside a culture vessel, a biosafety cabinet or a closed fermentation system, whereby full control can be achieved during all phases of the process. The *primary barrier* is the one closest to the infected material as exemplified above, whereas the next layer, i.e. the walls of the containment facility, denotes the *secondary barrier*, the function of which is to prevent any pathogen release into the exterior environment. The ventilation system, waste treatment system and exit shower are key features of this secondary barrier. *Tertiary barriers* are those that are put in place around the perimeter of laboratories or facilities to prevent any unauthorised entry to the site (Security standards for FMD laboratories 1993; Mani and Langevin 2006).

Containment is applied in order to protect the laboratory/production worker and the community (the general population and the environment) against infection. In agricultural containment facilities, such as those at Pirbright, worker protection is also important but the emphasis is placed on reducing the risk of organisms escaping into the environment (U.S. Department of Health and Human Services 2007; Canadian Food Inspection Agency 1996; Frasier and Talka 2005).

### 6.2 Perimeter and access security at Pirbright

The two facilities at the Pirbright site, the Institute for Animal Health and the vaccine production facility of Merial, are adjacent. A security fence consisting of a single wire mesh fence topped by razor wire surrounds the two facilities. There is a trembler wire and a microwave beam to detect human movements inside the

fence. The site is under CCTV surveillance. Perimeter security at Pirbright is less than at the Health Protection Agency (HPA) site at Porton Down, which works on Category 4 human pathogens, where there are two separate razor wire fences and the area in between can be patrolled and there is CCTV surveillance. Perimeter security at the Defence Scientific and Technical Laboratories (DSTL) at Porton Down, which also handles Category 4 human pathogens, is even tighter and the Ministry of Defence police who guard the site are armed. However, the latter facility is involved in a large range of defence work that is highly sensitive.

There are two separate entrances, one for IAH, which is manned 24 hours, and one for Merial, which is manned only at night and controlled from their reception area during the day. The security patrol of IAH can cover the Merial site if needed. Both have normally closed gates, which minimises unauthorised access.

Visitors gain access to the IAH site on foot through the main gate (vehicles are parked in an area outside the security fencing) after being checked by the security staff. A form needs to be signed at the gate, and the visitor receives a 'visitor pass'. Contractors have their own passes but, according to HSE investigations, the number of vehicles identified by CCTV as coming on and off the site does not agree well with records at the gatehouse. We noted that there was no formal visitor control and handing over to a member of staff for escort at all times.

At the Merial site access is via a telephone interface. If the visitor is expected, or known, the metal gate slides back to allow entry on foot or by vehicle. Visitors walk to the main entrance and a check-in form is signed and they receive a numbered 'visitor pass'. A CCTV system to monitor the entrance is in place. Merial has only a few visitors with most people accessing the site being contractors or suppliers.

**Recommendation 2. Entry to any facility handling Special Animal Pathogens Order (SAPO) Category 4 pathogens should require all visitors to sign in, obtain a numbered visitor pass, be escorted into the building and handed over to their host. Visitors (including all contractors) must be informed of the animal quarantine requirements and sign (and be given a copy of) a form accepting that these are understood. For all visitors, including contractors, requirements to prevent inadvertent infection of livestock should be based on an assessment of the risk of exposure to pathogens.**

The lack of a consistent policy of formal escorting of visitors to their hosts at IAH contrasts with policy at the HPA site at Porton where visitors are escorted and

only have access to certain areas, and at DSTL Porton where visitors have to be escorted around the site at all times.

We were concerned that illegal vehicle access to the Merial site could be obtained by a vehicle following another into the site. Furthermore, as the Merial gate is unmanned during the day, it could be vaulted by an intruder. Once through the gate an intruder has access to the whole Pirbright site as there is no secure fencing between Merial and IAH.

Access to Merial therefore depends on the security of its own entrance gate and that of the IAH and vice versa. We consider it unwise to have access to one facility on a shared site being dependent on the security of the entrance gate of the other.

**Recommendation 3. IAH and Merial should erect secure fencing to separate their two facilities, with swipe card entrances through the fencing between sites for those authorised to move between the facilities.**

Although there are vulnerabilities that could allow intruder access to the Pirbright site, access to IAH and Merial buildings is by pin code or swipe card and, once inside the building, entry into the restricted high containment laboratories would be difficult due to additional access controls. However, access controls to the IAH laboratories could be improved.

## **6.3 Merial Animal Health Limited**

### **6.3.1 The facility**

The Merial facility on the Pirbright site manufactures FMD and Bluetongue vaccines. The FMD vaccine is manufactured on an industrial scale and involves a number of steps, including large-scale virus growth in cell culture, centrifugation to remove cell debris, virus inactivation and chromatographic purification. The resulting concentrated inactivated virus (referred to as antigen) is stored, and can be formulated into vaccine lots as required. The facility dates back to the 1960s when it was developed for FMD vaccine production by Wellcome Foundation Laboratories (subsequently Wellcome Biotech). It has since changed ownership and from the 1990s has been developed into a modern vaccine production plant by Merial Animal Health Limited, the UK arm of Merial Limited, a joint venture between Rhône Mérieux (now part of Sanofi Aventis) and MSD AgVet (a division of Merck Sharp and Dohme).

### **6.3.2 Biosecurity procedures**

#### **Personnel recruitment**

Recruitment of new personnel is controlled by the HR department. There are no security background checks and HR only checks the curriculum vitae and references of applicants and assesses suitability at interview. The Site Director reported that he checks at interview the length of time applicants have remained in previous jobs, their understanding of the quarantine requirements and nature of the job, and reaction to questions about animal rights issues. Advertisement is mainly in the local press.

#### **Entry and exit procedures to the restricted area**

Approximately 80 staff are employed at Merial of which only about 45 have access to the restricted high containment area. The restricted area is accessed through airlocks (swipe card access) with an outside change room for street clothes, showers and an inside change room where laboratory clothing is put on. Visitors receive single use overalls whereas permanent staff have their own set of company clothing that never leaves the restricted area. Clothing is washed inside the restricted area.

Personnel leaving the restricted area need to remove all clothes and shower. There is no time indication for how long people should shower in the two virus production areas (VP1 and VP2). In the quality assurance (QA) facility visitors are instructed to shower for 5 minutes.

#### **Description of the restricted high containment areas**

The restricted area consists of a quality control (QC) area and two virus production areas, with up to 6,000 litre stainless steel virus growth vessels, similarly sized holding and processing units in which virus inactivation occurs, and an industrial Westphalia centrifuge. Each of these areas has dedicated staff who do not move between areas.

Surfaces, walls and ceilings are water-resistant and easy to clean. The surface coatings are impervious to liquids and chemicals. Entrances into the restricted area are sealed to facilitate cleaning and chemical decontamination. Biological safety cabinets for virus preparation or QC procedures are in dedicated areas. Special gowning is a requirement for work with FMDV that involves the opening and manipulation of tissue culture flasks, culture bottles etc. These procedures are carried out within Class II biological safety cabinets.

The stainless steel virus growth vessels are interconnected with a closed piping system. Pipes are all hard ducted, no flexible tubes are used. The stainless steel vessels are equipped with anti-backflow dampers. Inlet air to the vessels passes through a 0.2µm filter and exhaust air passes through two 0.2µm filters which from their specification should prevent any release of virus into the room.



The restricted area is maintained under negative air pressure and exhaust air from the room is passed to the exterior through two HEPA filters.

### **6.3.3 Production procedures – QC procedures**

#### **Production**

Tissue culture cells for virus growth are produced outside the restricted area and then pumped into the vessels in the production areas.

The working seed virus is grown in roller bottles the contents of which are then pooled to provide a 4 litre starter culture to infect the 6,000 litre vessels containing the media and tissue culture cells. After virus growth the cell debris is removed by a centrifugation step and the washings containing cell debris are pumped into a holding tank. The virus preparation is pumped back into a second stainless steel vessel for the first inactivation step with binary ethyleneimine (BEI). Samples are taken at intervals to follow the virus inactivation kinetics. There is then a second inactivation treatment with BEI and for further removal of debris the virus suspension is passed through a series of filtration steps.

#### **Processing**

Additional filtration steps take place in the processing area where the virus suspension is concentrated to a final volume of approximately 50 litres. The concentrated inactivated purified FMD antigen is tested in the QC unit to ensure that the final product contains no live virus. The inactivated virus antigen is then stored in the vaccine bank in liquid nitrogen.

#### **Cleaning of vessels**

The vessels are cleaned with 0.8% citric acid and heated up to 60°C. The solution is passed into the centrifuge and approximately 100 litres goes to the holding tank with the cell debris, to give a final concentration of 0.4% citric acid. The pipe to the holding tank is closed and the cell debris is held for 2-3 days in citric acid (0.4%) without agitation.

The culture tank, associated pipe work and the centrifuge is then cleaned with Parabrite, a propriety sodium hydroxide based product, to neutralise the citric acid and flushed through with water. The centrifuge is steam sterilised before being re-used.

#### **Quality control department**

The QC department of Merial carries out all testing in a restricted area which is physically separated from the virus production facilities. A QC manager is responsible for the staff (about 8 people). Access to the restricted area is by swipe card; cross contamination of the vaccine antigen with live virus is prevented by prohibiting movement of personnel from the virus production and QC areas to vaccine production areas.



The laboratories are all well maintained and equipped. The staff spoken to appeared to have a thorough knowledge of biosafety procedures and their importance. Procedures for spill management are in place. Staff are well trained and knowledgeable about these procedures.

### **Biosafety/biosecurity management**

The role of biosafety/biosecurity officer is shared by the Site Director and the QA manager. In discussions the Site Director stated that the majority of biosafety/biosecurity issues went to him. The QA manager is responsible for all good manufacturing practice on site and spends approximately 10% of his time on biosafety/biosecurity issues.

Biosafety/biosecurity seems not to be a well integrated part of the management system and it would be expected that a company producing FMDV in industrial quantities would have a single biosafety/biosecurity officer with clear authority over biosafety matters and who was not potentially subject to commercial pressures.

**Recommendation 4. The responsibilities of the Site Director and Biological Safety Officer of Merial should be clearly separated. The Biological Safety Officer should not be subject to commercial pressures on matters of biosafety and biosecurity.**

### **Quarantine**

All visitors and employees entering the restricted areas of the site are bound by the Disease Security Regulations and must submit to a 5-day quarantine where they must not visit farms or zoos or be within 10 metres of FMD-susceptible animals.

### **Training**

New staff are trained by their responsible supervisor on the job (shadowing). There is no separate formal biosafety/biosecurity training to raise awareness of biosafety/biosecurity issues. However, personnel appeared knowledgeable and appropriately trained.

### **Air handling**

Buildings are equipped with double HEPA filters on the extract air. The first HEPA filter is in most cases located directly in the ceiling of the room. The second filter is located just before the point of exit of all exhaust air from the restricted area. Each unit of the restricted area has its own air handling system. A contractor does integrity testing on a yearly basis. All activities involving live virus are carried out in a biological safety cabinet. Air within restricted areas is always at negative pressure.

## **Waste handling**

All solid waste is decontaminated in a double-ended autoclave and discharged as special waste. Fumigation chambers (formalin) used to decontaminate heat sensitive equipment that leaves the restricted area. Liquid waste from the restricted areas goes to the effluent holding plant on Merial's site before it is discharged into the effluent pipes and to the IAH caustic soda final effluent treatment plant. IAH is responsible for the effluent treatment plant. The storage capacity in the holding tank is approximately 3-4 cubic metres. An average of 20 cubic metres of effluent waste is discharged by Merial every day to the caustic soda treatment plant.

## **Sample transfer**

Samples are decontaminated on transfer out of the restricted area by passage through either a dunk tank with citric acid or a fumigation chamber.

## **6.4 Institute for Animal Health laboratories**

### **6.4.1 The facility**

The IAH facility at Pirbright carries out research and develops diagnostics for exotic diseases of farm animals (i.e. diseases that are not endemic to the UK). It also provides the testing facility for FMDV and other agents in suspect animals. IAH Pirbright is the National Reference Laboratory, and is designated as the World Reference Laboratory for FMD by the Food and Agriculture Organization of the United Nations and as a reference laboratory by the Office International des Épizooties (OIE). The current facility is a mixture of old (dating from the early 1950s) and newer buildings. The site has undergone several changes and renovations since its establishment.

The state of the laboratories at Pirbright was raised in the 2002 Review of the Institute for Animal Health - Pirbright Laboratory – commissioned by the BBSRC Council and carried out by a Review Group chaired by Prof. Keith Gull. This report considered that “some of the laboratories ... are not of a standard that would be expected in a modern bio-medical research facility” and described parts of the facility as “shabby”. We fully concur with this view.

A letter on the 28 March 2007 from the Director of IAH Pirbright to the Chief Veterinary Officer at Defra stresses that “the laboratories at Pirbright are old and in urgent need of replacement” and continues “the overall infrastructure does not allow IAH to maintain a risk profile as low as other sister laboratories in Europe”.

The £121 million Pirbright Site Redevelopment Scheme (funded by Defra and the Department for Business, Enterprise and Regulatory Reform [formerly the Department of Trade and Industry]) was agreed in 2005, following the recommendations of the 2003 Review of the UK's national facilities for infectious animal disease research, surveillance and diagnosis, chaired by Dr. Richard

Cawthorne. The new facility at Pirbright will bring together the work on exotic viruses carried out at both IAH Pirbright and the Veterinary Laboratories Agency (VLA) at Weybridge. Work has started but the new laboratories will probably not be completed and commissioned until 2012.

#### **6.4.2 Biosecurity procedures**

##### **Personnel recruitment**

Normal HR procedures take place when new staff are recruited, including interviews and taking up of references. With non-EU citizens, visa and work permits are also checked. There are no other background checks of personnel. The HR section keeps records of all employees and temporary workers.

##### **Entry and exit procedures to the high containment restricted areas**

The restricted area is accessed through airlocks with an outside changing room for street clothes, showers and an inside changing room where laboratory clothing is put on. Access to the wardrobes is controlled by a pin code. Outside working hours laboratories are operated by key lock.

Clothes worn in the restricted area must be completely removed before showering. The shower procedure on exiting should take approximately 5 minutes, but is not monitored.

##### **Description of restricted laboratory area**

Several buildings are within the restricted area such as the main research laboratory, the epidemiology laboratory including the world reference laboratory for FMD, the Central Service Unit (CSU), the Bluetongue and other reference laboratories, a section of the canteen, and some engineering space. These different buildings are not interconnected and share a common courtyard which is separated from the non-restricted area by a fence. Entrance to the courtyard of small animals, birds or insects cannot be prevented. The walls or ceilings are not easy to clean.

Within the restricted area staff can move from one building to another without any restriction, except for the CSU building where the master virus stocks are held in locked freezers. Access to the master FMDV stocks is restricted to key personnel. The virus repositories allow access to named people from each research group and first aiders. A number of engineers also have access for emergency use. The total number of named card holders who can access master virus stocks is 41, plus an additional number of technical staff with access to all areas. Working stocks are held in the different laboratories in unlocked freezers. The laboratory staff are in charge of record keeping of the working stocks. Overall, 120 people have access to the restricted area where work on FMDV is conducted.

Unlike modern containment facilities, the laboratories at IAH are not airtight but they are operated under negative air pressure to prevent any release of organisms. The exhaust air is filtered through a double HEPA filter.

The canteen is divided into an outer zone, outside the restricted area and an inner zone for people working in the restricted area (see section 8.4). Meals for all staff are served from a common kitchen area.

### **Laboratory procedures**

The laboratories are equipped with modern research and safety equipment. Procedures are in place for handling spills and other incidents. Laboratory staff are well trained and seem very competent in laboratory knowledge and procedures. The biosafety/biosecurity procedures are well known.

However, HSE investigations identified areas of concern about biological safety procedures in some critical areas. This included the suitability of some parts of the laboratories for Category 4 work, and the procedures used to test the integrity of the HEPA filters. Additionally, freeze-drying of high-titre O<sub>1</sub> BFS was being carried out by one group using the IAH laboratory facilities without any filtering of the exit air or exhausting of the air into a safety cabinet. Freeze-drying is known to be an aerosol-producing procedure and the process being used may result in some release of infectious O<sub>1</sub> BFS virus into the laboratory. We do not believe that these procedures were responsible for the outbreak.

Only small volumes of live FMDV are usually handled in the laboratory. Personnel handling live FMDV wear special protective aprons and gloves. Contaminated materials and liquids are decontaminated in citric acid or FAM 30, according to a defined procedure, before release via the effluent pipe to the caustic soda treatment plant. Solid waste is autoclaved before disposal and water from showers etc. are chemically treated in the caustic soda treatment plant.

**Recommendation 5. IAH should have a thorough review of the safety of all laboratory activities to ensure that procedures which could release infectious FMDV into the containment laboratories are eliminated. This is particularly important for aerosol-producing procedures.**

### **Biosafety/biosecurity management**

IAH Pirbright has a Biosafety Department that provides training and advice on risk assessment, regulatory issues and other biosafety/biosecurity related topics. Procedures covering all biosafety/biosecurity steps within restricted areas are in place and subjected to regular review. The Biosafety Officer has his own team (5 people) and is well integrated in the management structure of the laboratories. He reports to the Director.

The current structure is in accordance with the Laboratory Biorisk Management Standard European Committee for Standardisation (CEN, Brussels) that is being elaborated at the present time by the European Biosafety Association, American Biological Safety Association, Asia Pacific Biological Safety Association and the World Health Organisation.

### **Quarantine**

All staff are subject to varying periods of quarantine from susceptible farm animals depending on the nature of their work. This varies from a 24-hour period for those staff who work outside the restricted area (including contractors), to 5 days for those at the highest risk of virus contamination.

Due to the current situation the quarantine has been set at 5 days for all, including contractors entering the IAH site. However it would be difficult to ensure compliance with this new requirement.

### **Training**

An introduction to biosafety/biosecurity is provided by the biosafety officer. Due to the specialised nature of the diseases with which the staff will work, on the job training is a major component. Each laboratory supervisor is in charge of the training of their team of staff. New staff receive a one-week introduction. Training is up-dated on a regular basis by the Biosafety Officer. The responsibility for the competency assessment lies with the supervisor.

All visitors receive formal biosafety/biosecurity training before entering the restricted area. Visitors must sign a disease security form informing them about the quarantine rules.

### **Air handling**

Buildings have double HEPA filters on the extract air. A contractor does integrity testing on a yearly basis. All aerosol-producing activities are carried out in Class II biosafety cabinets, with the exception of freeze-drying of FMDV, as mentioned above.

### **Waste handling**

All solid waste is decontaminated in a double-ended autoclave and discharged as special waste. Fumigation chambers (formalin) are used to decontaminate heat sensitive equipment that has to leave the restricted area. Liquid waste is discharged to an effluent pipe and thence to the final effluent treatment plant.

### **Sample transfer**

Prior to any transfer of samples out of the restricted area the primary containers of samples are required to be wiped with citric acid. Samples are transferred in double containers. Transfers of samples out of the restricted area are recorded.

### **Large animal isolation units**

The large animal isolation units were built in the 1990s and are considered to be a state-of-the-art high containment building comparable with other similar international agricultural facilities. The supply air is filtered through a single HEPA filter and the exhaust air is filtered through two double HEPA filters. Liquid waste is subject to a thermal inactivation procedure prior to discharge to a private sewer. The waste treatment plant of the animal housing unit is therefore completely independent of the site's liquid effluent caustic soda treatment plant.

For vaccine safety and potency studies the animal isolation unit is shared with Merial. Merial is charged a fee for the usage of the facilities. There were animal experiments carried out within the infection window, but none of these involved O<sub>1</sub> BFS. Sequencing of the VP1 gene of the viruses that were used has confirmed their identity. Therefore this unit was not inspected and is not further discussed.

## **6.5 Licensing and inspection by Defra and the Veterinary Medicines Directorate**

IAH and Merial are licensed by Defra to handle FMDV under the Specified Animal Pathogens Order 1998 (SAPO). The general requirements for licensing by Defra to handle Category 4 animal pathogens are shown at annex 5.

The separate licences for the two facilities set out strict requirements for laboratory containment. Amongst other things, both require that "all work must be carried out in facilities under negative pressure and with HEPA filtration of all exhaust air". For IAH, there is a requirement that "all materials leaving the laboratory where the specified animal pathogens are handled must be autoclaved, incinerated or undergo chemical disinfection". For Merial, the corresponding requirement is that "all materials leaving the laboratory where the specified animal pathogens are handled must be autoclaved or incinerated or undergo a process of chemical inactivation such that the specified animal pathogen is no longer infectious".

The Merial laboratory is additionally licensed by the Veterinary Medicines Directorate (VMD) for the production of veterinary medicinal products including FMD vaccines and is subject to specific Good Manufacturing Practice (GMP) requirements.

The IAH has been inspected annually by Defra and has been required to provide quarterly reports on issues relating to the conditions of the licence. The most

recent Defra inspection was in December 2006. Overall the findings were satisfactory and a number of ongoing biocontainment issues were reviewed. The frequency of reports from IAH to Defra was reduced to biannually from the end of 2006.

VMD inspect Merial for compliance with Good Manufacturing Practice (GMP). These biannual GMP inspections also cover containment issues to the extent needed by GMP requirements (e.g. the adequacy of containment between virus production and vaccine production areas to prevent contamination of vaccine with live virus). The most recent, in July 2006, raised one area of concern relating to the balancing of air pressures but this has now been rectified.

Defra also carries out inspections at Merial but, because VMD inspects the vaccine production process, they concentrate on the handling of liquid and solid waste. Defra also carries out periodic inspections of the Defra FMD antigen store and informed the Review Group that general biosecurity issues are discussed during these inspections. The last inspection was in February 2007.

Defra and VMD appear to have arranged to share responsibilities for inspection at Merial. However, their reports are not exchanged and therefore there is no assurance that all aspects of biocontainment are covered.

The laboratories are also subject to inspection by the HSE with respect to workplace safety and COSHH (Control of substances hazardous to health) and the Home Office with respect to anti-terrorism legislation. The primary focus of these inspections is not biocontainment as such and so they are not discussed further here. However, this further complicates the regulatory framework.

**Recommendation 6. Defra and the Veterinary Medicines Directorate (VMD) should work together more closely and exchange information about inspections at Merial. One of the two regulatory authorities should take responsibility for ensuring that all aspects of biocontainment and biosafety are thoroughly inspected.**



## **7 The effluent treatment system at the Pirbright site**

All effluent from the high containment areas of the Merial facility is collected in a single effluent sump in a building maintained under Category 4 containment on the part of the site leased by Merial. A pipe from this effluent sump then joins the system of pipes which serves the IAH laboratories. Thus effluent from both facilities passes through a shared pipe system to the final caustic soda effluent treatment plant operated by IAH. The effluent pipes from the IAH laboratories and the pipe from the Merial sump to the shared system are entirely on the IAH site (see Figure 4).

Initial information about the effluent pipes was obtained in discussions with the IAH site engineers. This was then clarified and more detailed information provided by the report of the HSE investigation into the condition of the drainage pipes.

It became apparent from our discussions with IAH staff that the structure of the effluent pipes is not well understood. The majority of the pipes are clay, although some are cast iron. The pipe work is old with some believed by the IAH site engineers to be up to 50 years old. The initial section of the pipe from Merial is cast iron (and probably about 30 years old) and cased at least in part by concrete.

The HSE investigation provided a comprehensive account of the effluent handling system and the condition of most of the pipes. Effluent is pumped uphill from the Merial sump to manhole FM1 (Figure 4). The pumps are submersed in the Merial sump and each has a capacity of 25,000 litres per minute. Under normal circumstances, the pump will run for a few minutes at a time. The sump has a capacity of a few cubic metres and during a normal production day, an average of 20 cubic metres per day is discharged. The sump will not retain effluent for significant periods. From manhole FM1 the effluent then travels by gravity across IAH land to the final effluent plant. Part of the pipe exiting Merial's holding tank is also cast iron cased in concrete (a recently dug trench has exposed this pipe) and this must join to a clay pipe at some stage on its route across IAH land to the treatment plant. The older pipes are 4 inch diameter, the more recent ones, which are about 10 years old, are 6 inch. Several manholes are distributed over the site of IAH. Some of these are not tightly sealed with the possibility of water ingress into the effluent pipes or effluent egress. IAH staff reported that during heavy rains, such as on 20 July, they had not observed effluent overflow from the manholes.



As the pipe from Merial to the manhole FM1 runs uphill some liquid effluent is always in this pipe. The influence on the integrity of the cast iron of the frequent discharge of citric acid is unclear.

The final caustic soda treatment plant is over 50 years old and is a simple and robust mechanical device that treats incoming effluent with a metered quantity of caustic soda to raise the pH to 12. Effluent is then transferred to holding tanks where it is maintained at pH 12 for 24 hours and then released to a private sewer that runs to the local sewage treatment plant. The volume of liquid effluent leaving Merial is monitored and they are charged by IAH per cubic meter for effluent treatment.

It has long been recognised that the 50 year old caustic soda treatment plant needs to be replaced with a more modern system and an electric thermal treatment plant has been built but was unreliable. It is now being extensively modified but is not yet in use.

It is surprising that two Category 4 high containment laboratories rely on an effluent treatment system major parts of which are 50 years old. However, the treatment plant has worked well in the past with inevitable occasional problems. During the last eighteen months there have been two incidents reported to Defra, plus the problem with flooding mentioned below. In February 2006, a valve failure led to a holding tank overflowing and "a small amount of effluent" was released into the public sewer without being held at pH12 for 24 hours (letter from IAH to Defra, 24 February 2006), and in September 2006 there was an outlet valve failure on a holding tank, also resulting in effluent being released to the sewer without having been maintained at pH12 for 24 hours (report from IAH to Defra for period January to March 2006). Neither of these incidents was considered by IAH to result in any risk of infection.

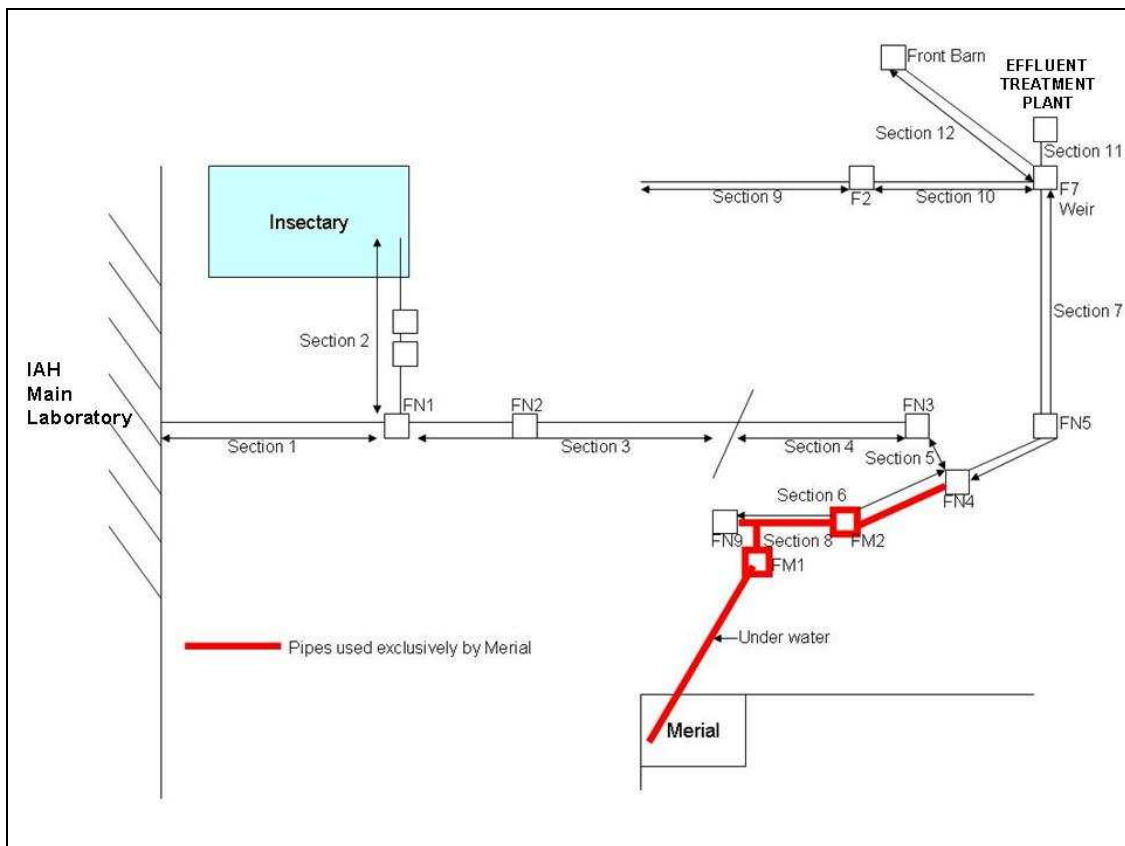
### **Flooding**

The treatment plant is on one of the lowest parts of the Pirbright site and is liable to flooding in extreme weather. During the week of 20 July, heavy rainfall caused flooding in the Pirbright area. The area of the caustic soda effluent treatment plant was not flooded whereas the parking lot of Merial was. IAH staff found that rain water was entering the effluent system and the water level did rise in the pit that contains the treatment plant, but according to IAH no ingress of rain water directly into the plant or overflow of the plant is believed to have occurred.

Merial was informed by IAH not to discharge any further waste water to the caustic soda effluent treatment plant until the problem of the increased amount of water could be resolved. As a result of additional water the effluent in the holding tanks was held at very slightly less than pH12 but after 24 hours was considered safe for release. The biosafety officer inspected all manholes on the site during the period of heavy rain and reported that they were full but not overflowing.

Problems with the final effluent treatment plant and the potential problem of flooding had been raised previously. A letter dated 1 November 2006 from IAH to Defra refers to extreme rainfall on 13 August 2006 that overloaded the effluent treatment plant to the point that work in the restricted areas had to stop on the 14 August to allow the effluent plant to catch up. Documents show similar problems occurred following heavy rains on 22/23 November 2003.

**Figure 4. Diagram of the shared liquid effluent pipe system**



## **8 POSSIBLE ROUTES OF VIRUS ESCAPE**

### **8.1 Airborne transmission by aerosols**

Aerosols are an efficient means of long distance transmission of micro-organisms. If aerosols are generated when working with micro-organisms, and these aerosols contain particles of small size, they have the capacity to travel many kilometres aided by the wind. Larger particles such as droplets fall to the ground very quickly and do not present such a risk.

There is strong circumstantial evidence for long distance airborne transmission of FMDV. For example, this route of transmission is considered to have been important in the spread of virus between farms in the 1967 and 2001 FMD outbreaks (Gloster et al. 2005; Gloster et al. 2003), and as a likely route by which virus moved through Normandy to the Channel Islands and on to the Isle of Wight in 1981 (Donaldson et al. 1982).

Aerosol release has to be considered as a source of the recent outbreak, since the infection window for the two infected farms overlaps, and both are in a line to the south-west of Pirbright, indicating that both farms could have been infected by an aerosol travelling to the south-west from Pirbright.

The risk of aerosol transmission from high containment laboratories is well recognised and these facilities are designed to prevent this from happening. In the recent FMD outbreak, aerosol transmission would require a release of virus as an aerosol into the containment laboratory, escape of the aerosol from the laboratory to the exterior, and transfer on the wind to the infected farm.

During the estimated infection window small amounts of the O<sub>1</sub> BFS strain were used at IAH within Class II safety cabinets designed to contain any aerosols generated by the manipulation of the fluids. These biosafety cabinets are within a high containment laboratory that is under negative pressure to prevent the release of any aerosol, and finally air that leaves the laboratory passes through two HEPA filters. The risk of aerosol production is very low using most of the procedures at IAH, except for during freeze-drying of virus which, as discussed above (section 6.4.2), is being further investigated by the HSE. There is no evidence from the HSE investigations of any problems with the negative pressure in the containment laboratories or of the HEPA filtration of air released to the environment.

At Merial, large amounts of O<sub>1</sub> BFS were produced for vaccine production during the infection window. The seed stock of virus was opened and the initial cell cultures were inoculated in a Class II safety cabinet in a high containment laboratory. The risk of aerosol production is very low and no spills were reported. This initial starter culture was tested in the quality control laboratory and then

transferred by a closed system to the culture vessels which each have a capacity of 6,000 litres. Following large-scale virus production, the virus was separated from cell debris by centrifugation and the virus supernatant transferred to new vessels for virus inactivation. Both culture growth and centrifugation are aerosol-generating procedures and the equipment is designed to prevent any aerosol release. There are no indications from the HSE investigations that the procedures to prevent aerosol release were compromised. If any aerosol was released into the containment area it would be contained by negative pressure and by air exiting to the exterior being passed through two HEPA filters. There were no deviations of the negative pressure, there were no spills reported, and the integrity of all HEPA filters have been tested by an independent contractor and met the standards required.

If an aerosol was released from either IAH or Merial, the prevailing wind would usually take the virus in the opposite direction to the two infected farms. IAH Pirbright have used the Meteorological Office's main atmospheric dispersal model (NAME) and records of wind direction to determine the days on which wind dispersal to the two infected farms could have occurred. They have concluded that there were only four days in the time window for the first infected farm that airborne transmission might have occurred: 15 July, 19, 20 and 23 July. Only on 23 July was there an extended period (approximately 14 hours) when the wind was in the right direction. On the other three days the wind was only in the required direction for a short period and modelling estimated a much lower likelihood of spread to the farms on these days. The modelling methodology and conclusions were discussed between IAH and Prof. Neil Ferguson of Imperial College, who agreed with their conclusions.

Centrifugation of one of the 6,000 litre batches of O<sub>1</sub> BFS was being carried out at Merial on 23 July when the wind was in the correct direction to pass over the infected farms but, as mentioned above, there is no evidence of any equipment malfunction that might have generated an aerosol in the containment laboratory. We conclude that it is very unlikely that an aerosol was generated within the containment facility, escaped into the outside air, and virus was spread in the aerosol to the infected premises on the wind. For this to happen it requires a combination of unlikely events – release of aerosol into the laboratory, a breakdown in the double HEPA filtration, and the wind being in the correct direction. The chance of these three events coinciding, and the lack of evidence of any failure of safety systems, makes aerosol release from the containment laboratories at Merial (or IAH) and airborne spread to the infected farm(s) very unlikely.

## **8.2 Contamination of laboratory staff**

It has been shown during previous outbreaks of highly contagious animal diseases that biological agents can be spread mechanically by human movements. Investigations of laboratory-acquired infections with human

pathogens have revealed that even though the source of most infections was unclear, aerosol-generating procedures may account for most of them (Collins and Kennedy 1999). In some cases, personnel were not aware of any spill occurring during handling of biological agents. The dangers of aerosol release have resulted in strict procedures for protecting laboratory workers from infection with highly infectious human pathogens. These procedures are not so rigorously applied to those working with Category 4 animal pathogens that are not infectious to humans, such as FMDV, and in laboratories handling high-titre virus suspensions unintentional exposure may occasionally occur (Murray 1998). This applies also for production facilities in which large amounts of high-titre virus are produced and processed. Spills of high-titre FMDV or inadvertent exposure to aerosols in laboratories or production facilities may contaminate workers' hands, exposed skin, hair, nasal cavities or clothing. Similarly, during activities with infected large animals personnel come into contact with large amounts of virus (Amass et al. 2004).

Without any specific safety precautions there is a risk of unintentional spread of virus from contaminated laboratory workers into the environment. Thus facilities working with FMDV assume exposure may sometimes occur and have specific safety measures to prevent transmission to susceptible animals. These measures are implemented at both facilities on the Pirbright site.

The following safety precautions were in place at IAH and Merial to prevent FMDV from leaving the laboratory in this way.

### **Laboratory clothing**

Protective laboratory clothing could be contaminated and is therefore either autoclaved before being passed out of the containment laboratory for washing, or is washed inside the restricted area. Washing water is inactivated prior to discharge to the sewer.

### **Spillages clean-up procedures**

In both facilities spill clean-up procedures are in place and the staff interviewed were aware of how a spill has to be treated and to whom they should report it.

### **Showering at exit**

A thorough full body shower, 3 to 5 minutes, has to be taken before leaving the high containment laboratories at both IAH and Merial. The shower timing is not automated or monitored at either facility and it is the responsibility of each individual to shower thoroughly before exiting the containment laboratory. There is a report that FMDV can be carried in the nose for up to 48 hours and it has been considered as an unusual route of mechanical transmission in FMD outbreaks (Sellers 1971). Clearing of the nose during the showering process is not included in the protocols at either site.

### **Quarantine procedures**

At both sites, staff working with FMDV are quarantined and not allowed to visit farms, zoos, agricultural premises or be within 10 metres of susceptible animals, for a period of 3 days if they worked in the laboratory area and 5 days if they worked in any of the restricted areas where FMDV is used. At IAH, contractors or visitors not entering the restricted area, nor visiting the animal facility, are submitted to a 1 day quarantine. Quarantine is considered an efficient way to prevent any disease transmission of FMDV to a susceptible animal. There do not appear to be any checks at IAH or Merial on whether staff or construction workers strictly comply with these regulations.

It is difficult to exclude the possibility of laboratory workers failing to shower adequately, or to ignore quarantine regulations, and maintaining compliance require a constant reinforcement of the importance of these procedures and a strong safety culture.

The aim of the above two procedures is to have a double mechanism by which any laboratory workers who do get contaminated (as presumably would occur when FMDV was freeze-dried at IAH) do not transfer FMDV to susceptible animals. Thus virus contamination should be removed by thorough showering and animals are further protected from any remaining virus by the quarantine rules.

### **8.3 Escape in laboratory solid waste**

Everything leaving a high containment laboratory could be contaminated and needs to be properly decontaminated, either by heat or chemical treatment. All of these procedures for waste treatment should be validated and thus proven to be effective.

#### **Heat treatment**

The standard procedure for solid waste treatment is autoclaving using a double-door autoclave, so that waste enters the autoclave from within the containment laboratory and after treatment exits to the exterior. These are used on a regular basis on both sites and have mechanisms that prevent both entry and exit doors being open at the same time. Autoclaved waste goes for incineration. Autoclaves at both facilities are calibrated using multiple thermocouples on a yearly basis.

### **8.4 Other mechanical transmission**

#### **Movement of equipment, etc.**

Movement of equipment out of the high containment areas is kept to a minimum but when unavoidable approved disinfection protocols are implemented. At both facilities these involve fumigation with formaldehyde gas in a humidified atmosphere.



### **Movement of samples**

Samples are occasionally removed from within the restricted area of IAH for transport within the Institute or to other laboratories. Shipments to other countries have to comply with international transport regulation. Sealed sample containers are externally decontaminated by swabbing or dunking in approved disinfectant before being placed within a metal container. This is also chemically disinfected before placing within a second metal container within a double-ended cupboard spanning the interface of the restricted and non-restricted areas. Non-infectious materials such as DNA or serum samples are appropriately inactivated (chemically or by heat treatment) using approved protocols before removal from the restricted high containment area. Only chemically inactivated samples of virus (antigen) are removed from the Merial facility for final bovine safety (innocuity) testing in the IAH large animal isolation facility.

### **Carcass removal**

Animals are only used within the IAH premises. Large animal experiments are conducted within the animal isolation facility which is modern and self contained with respect to biosecurity measures. Experimental animals are incinerated within this facility. Carcasses of small animals are autoclaved out from the high containment area of the Biological Service Unit. The bags are then either directly moved to the incinerator in the Large Animal Isolation units or are frozen until the incinerator becomes available.

### **Canteen**

At Merial, kitchen and rest areas are provided within the high containment area and staff are encouraged to consume their own food without leaving the containment laboratory.

IAH has a canteen which serves both the restricted high containment laboratories and outside areas. The 'inside' restricted part of the canteen is within the same building as the kitchen and 'outside' eating area, and staff within the restricted area are provided with food across a serving hatch, which acts as a biosecurity barrier. The movement of plates, food, etc. is strictly unidirectional, from outside the restricted zone to within. Staff entering the restricted area part of the canteen will have removed laboratory coats, gloves, etc. as part of the normal procedures for exiting laboratories. One member of the cleaning team who does not work in the laboratories rinses all cutlery/crockery in detergent before loading them into a dishwasher. Clean cutlery/crockery from the dishwasher is then fumigated out from the restricted area and washed again. This split canteen arrangement has been in place for many years without any problems, but it is highly unlikely that it would be approved in a modern Category 4 high containment facility.

## **8.5 Release of infectious virus into the effluent pipes**

Chemical disinfection differs from sterilisation in that the former aims to reduce the amount of infectious pathogen to a very low level, whereas the latter aims to

inactivate 100% of the pathogen. The failure of chemical disinfection to completely inactivate viruses (or bacteria) is recognised in work with human pathogens, where work at Category 3 or 4 containment requires heat sterilisation rather than chemical disinfection before release as effluent.

In modern Category 4 FMDV containment facilities liquid waste should preferably also be sterilised before release (as occurs in the large animal isolation units at IAH) and can then directly enter the sewage system. However, the liquid effluent released from the main IAH laboratories and from Merial is not sterilised but is subject to chemical disinfection before release. After disinfection this liquid effluent is still considered to be potentially infective and is transferred along the effluent pipes to be treated with caustic soda at the shared final treatment plant, before release to the local sewage treatment plant.

Citric acid (0.4%) or FAM 30, an iodophor disinfectant, both of which are approved for FMDV decontamination, are used at Pirbright to treat liquid waste before release to the effluent pipes. Validation of the effectiveness of chemical disinfection is labour intensive and in most cases efficacy is based on published data; validation is not done on a regular basis at either facility at Pirbright.

Contact times for chemical disinfection are specific for each type of waste. Disinfection of virus suspensions, or virus growing in tissue culture flasks, can be assumed to be highly effective, provided the stipulated concentrations of chemical disinfectant are used, but semi-solid material, cell debris, soil or organic matter can interfere with the effectiveness of disinfectants (Sonder et al. 1990; Sellers 1968).

The amounts of O<sub>1</sub> BFS virus being used in experiments at IAH during the infection window should have only resulted in very small amounts of virus surviving chemical disinfection and entering their effluent pipe. However, in some of the industrial scale processes at Merial it is unclear whether the citric acid treatment that is used will completely inactivate virus, for example within the released semi-solid waste derived from the removal of cell debris from the virus batches during centrifugation.

Large-scale growth of two 6,000 litres batches of O<sub>1</sub> BFS virus was initiated by Merial on 17 and 19 July with centrifugation to remove cell debris on 19 and 23 July. Cleaning of the tanks and pipework, and release of effluent, followed centrifugation. Cell debris from the two centrifugations was maintained in holding tanks and release of this debris to the effluent occurred on 22-23 July and 25-26 July, respectively. There are no validation data available from Merial or Defra on the efficacy of the citric acid treatment at inactivating FMDV within this semi-solid proteinaceous waste. In our view a failure to completely inactivate FMDV in the semi-solid waste from the centrifugation steps may have resulted in considerable amounts of infectious virus entering the effluent pipe from Merial.

The Site Director of Merial agreed with this assessment and stated that he thought it very likely that some infectious virus entered the effluent pipe from Merial. This issue had been raised in a letter from Defra to Merial on 2 August 2004 which stated that "...although there is preliminary treatment of the liquid waste, it is still regarded as potentially contaminated, though less of a hazard than if there was no such preliminary treatment. Thus the subsequent handling of this waste requires the appropriate level of containment until it is satisfactorily sterilised at the IAH treatment plant".

The Site Director of Merial indicated that perhaps as much as 10% of the FMDV from the 6,000 litre culture vessels would remain in the debris after the centrifugation step. Even if disinfection with citric acid reduced the amount of infectious virus by 99.99% there would still be a large amount of infectious virus released into the effluent pipe (1 billion infectious units) from the two 6,000 litre batches of O<sub>1</sub> BFS produced during the infection window. As the disinfection process has not been validated for the semi-solid waste from the centrifugation step, we cannot quantify the extent of virus inactivation, and thus the amount of infectious virus that may be released to the Merial effluent pipe.

Surprisingly, the biological safety officer of IAH was unaware that Merial considered infectious virus would be entering the effluent pipe. There was evidence from our interviews that there was a lack of communication between the biological safety officers of IAH and Merial.

**Recommendation 7. The biological safety officers of IAH and Merial should institute regular meetings to improve communication and their understanding of the risks on the Pirbright site, particularly those that arise from the sharing of the effluent system.**

## **8.6 Integrity of effluent pipes and potential leakage of FMDV**

As infectious FMDV is very likely to be released into the effluent, the drainage system and the final treatment plant are considered to be an integral part of Category 4 containment, thus preventing any release of live virus into the environment. If infectious FMDV travels along the effluent pipe from Merial (or from IAH) to the final treatment plant it is crucial that the system does not leak.

The effluent pipes at Pirbright are old and there have been concerns about their integrity for several years. Discussions about replacing these pipes are documented in a series of letters between IAH, Merial, Defra and the BBSRC extending back to 2003. Progress has been slow due to concerns over costs, specification, the extent of Merial's responsibility for the pipe from their laboratories and changes to plans due to the Pirbright Site Redevelopment Scheme.

As part of their investigations of the 2007 FMD outbreak, the HSE commissioned a full survey of the effluent pipes. Whilst there were no defects that would be a major concern in a standard drainage system the report identifies problems with the pipe work and manholes that indicates that it cannot be considered to be contained. These include joint misalignment, root penetration, water ingress and corrosion. All of these problems were found in the shared pipes that carried effluent from both Merial and IAH to the effluent treatment plant, the worst affected being the section from manhole FM1 towards F7, which carries the effluent from Merial. The report concludes that there is a strong possibility that material has leaked from the buried pipes into the surrounding ground.

The inspection also looked in detail (including the use of Computational Fluid Dynamics analysis) at the arrangements for effluent pumped from the Merial sump into manhole FM1 (Figure 4); it concluded that there was potential for that manhole to fill and overflow, depending on the rate of discharge from Merial (one or two pumps operating) and the ability of the pipe system to carry away the effluent.

**Recommendation 8. As a matter of urgency, Defra should require that actions are taken to ensure the effluent drainage system at the Pirbright facility is fully contained and its continuing integrity confirmed by regular inspections. In the interim, we advise that work with infectious virus should only be allowed if effluent released into the pipes has first been completely inactivated.**

If infectious FMDV is released into the effluent pipe (as suggested by their Site Director), leakage into the soil surrounding the effluent pipe from Merial is likely to have occurred for months and probably years, and yet the 2007 FMD outbreak is the only one that has occurred around Pirbright. Thus for this scenario there may have been some special feature that resulted in live FMDV being released from the drainage system and becoming a hazard.

One possibility is that FMDV was brought to the surface by a rise in the water table during the exceptionally heavy rains on and around the 20 July. FMDV has been shown to be able to survive in soil for three days in warm weather and about four weeks in cold weather (Animal Health Australia 2002). In the relatively cool summer weather during the infection window (the highest temperature recorded in the Pirbright area over this period was 22.5°C) the virus should have been able to survive in soil for several days.

A further possibility, identified by the HSE inspection of the drainage system, is contamination resulting from overflow of effluent from a manhole onto the surface of the ground. The period that discharge of O<sub>1</sub> BFS virus into the effluent system may have occurred coincided with groundwork on the site, including heavy lorry traffic.

Sharing of a common effluent system, or any other safety critical element, should be avoided where high containment facilities that handle virus on hugely different scales, using very different procedures, are co-located. Where it does happen, it requires a good understanding of the nature of the different risks from both facilities and good communications between senior management and in particular the Biological Safety Officers.

**Recommendation 9. Merial should discuss with Defra how it plans to modify its procedures to minimise the possibility of release of infectious FMDV virus into the effluent pipe. Any new process should be validated.**

**Recommendation 10. The plans for future development of the Pirbright site should be reviewed to ensure that all safety critical issues have been addressed. This should be carried out with the help of the full range of relevant experts and regulatory bodies.**

**Recommendation 11. The construction of the new high containment laboratories at IAH should go ahead as a matter of urgency. Such facilities are expensive to construct and maintain and Government must ensure that adequate funds continue to be available to enable the highest standards of biological safety for dealing with FMDV and other high risk viruses. In the meantime, investment to ensure safety and public trust in the existing laboratories and the effluent system is needed.**

## **9 MOVEMENT OF VIRUS FROM PIRBRIGHT TO INFECTED FARM**

Release of FMDV through defective effluent pipes and movement to the surface is considered to be the most likely mechanism by which infectious virus escaped from Pirbright. A number of possible routes by which virus could have travelled from soil contaminated with FMDV to the infected farm(s) can be considered.

### **9.1 Transfer by human activity**

Preliminary work for the construction of new IAH laboratories has commenced and contractors have been on-site during the last months. The area above the effluent pipe that leads from the Merial site to IAH's caustic soda treatment plant is not a restricted area and heavy trucks and diggers have recently been traversing this area. When the Review Group visited there were still trucks and a digger parked on the latter area and the recent wheel tracks of heavy vehicles.

An inspection trench had been dug immediately above the effluent pipe on the IAH side of Merial's holding tank. This trench was dug between 24 and 26 July and has exposed the concrete drain casing around the pipe, and parts of the concrete casing have been broken away to expose the cast iron effluent pipe. Several other inspection trenches have been made in this area, but not directly above the line of the effluent pipe.

The clear evidence of heavy plant traversing this area and the exposure of the effluent pipe raises the possibility that the footwear of contractors (or others crossing this area), and the wheels of contractors' vehicles, became contaminated with FMDV if this was present in the upper areas of the soil. The HSE investigations have logged approximately 1000 movements of vehicles on and off the IAH site from CCTV recordings during the infection window providing many opportunities for any contamination to be spread from the site.

FMD is known to spread rapidly, and to be difficult to control, and the route by which many transmissions between farms occurs during an epidemic is often not understood, as presently is the case for transmission between the two infected farms in the 2007 outbreak. The possibility of spread of infection between farms by contaminated vehicles and people is well established and disinfection at the entrances to farms is a standard precaution to prevent such spread. However, the risk of infection of cattle by this route is considered unlikely by FMD epidemiologists, unless contaminated vehicles or people actually enter a farm, with a low risk if such vehicles or people just pass the farm by road or footpath. Tracing the movements of vehicles and people from Pirbright to the first outbreak farm is still ongoing but at present there are no indications that any of the



contractors' vehicles or people working at Pirbright entered the first outbreak farm.

However investigations by the HSE and Defra's National Emergency Epidemiological Group (NEEG) have confirmed that during the period of the infection window a number of contractors' vehicles from the Pirbright site travelled down Westwood Lane, from which the farmer at the first infected farm gained access to his cattle. In particular, HSE report that there were several 32 ton lorries carrying unsheeted subsoil from Pirbright that travelled along Westwood Lane on 20 and 25 July. The NEEG consider that contaminated lorries are the most likely means of transmission from the Pirbright site to this farm and their conclusions are in a separate report (Defra 2007b).

One individual at Merial did work an allotment that is immediately adjacent to one of the fields of the first infected farm. However, there was a thick bramble hedge and barbed wire fence between the allotment and adjacent field. Furthermore, cattle had not been grazing in this field during the infection window and this individual stated that he did not visit the allotment within this period. He had regularly taken containers from Merial to his allotment but these were destined for landfill and were therefore not considered to be an infection risk. It is not considered likely that this individual was involved in the spread of infection to the farm.

The leakage of FMDV into soil surrounding the effluent pipes and its appearance at or near the soil surface, or overflow from the drainage system, is at present the most plausible reason for the accidental release of FMDV from the Pirbright site into the environment. The route by which virus moved from any contaminated areas to the outbreak farm(s) is less clear and is still being actively investigated by the Defra epidemiology team. However, mechanical transmission is considered the most likely route.

It should be stressed that, at present, clear evidence of FMDV in effluent and of virus contamination of soil around the effluent pipes, or on or near the ground surface, and the extent of any virus contamination, are still lacking. Nevertheless, on the basis of the HSE investigation, the effluent pipe is not considered to be fully contained and virus leakage is very likely.

## **9.2 Transfer by water**

Virus in the upper layers of soil following the heavy rains of 20 July could have entered the stream alongside the Pirbright site and moved by water flow to the first outbreak farm. This mechanism can be ruled out as the Environment Agency confirmed that any water flow between Pirbright and the infected farms would be in the wrong direction, since the first and second infected farms are both at least 10 metres higher than the Pirbright site.

### 9.3 Transfer by animals

There is some possibility of mechanical transmission by small animals (including birds) and deer from contaminated areas to the outbreak farm. This risk is hard to quantify but is probably low.

There could also be biological transmission by deer infected close to the Pirbright site. Deer are common around Pirbright but should be kept off the site by fencing and if they did enter should trigger an alarm to the gatehouse. Infection of deer by ingestion or contact might have been possible during the period of exceptional rain, from water running off any contaminated ground around the effluent pipes and final treatment plant. Each of the five species of deer native to the UK can be infected by diseased cattle, under laboratory conditions, and the infected deer can then transmit to cattle (McVicar et al. 1974; Gibbs et al. 1975; Thomson et al. 2003). However, it has been suggested that under natural conditions deer are unlikely to have sufficiently close contact with cattle for transmission to occur, and in Europe deer are believed not to act as disseminators of FMDV. In the UK during periodic outbreaks of FMD over the past fifty years, there has never been any suggestion that deer have been directly involved. In an area such as the New Forest in the south of England, which is over 1000 square miles in extent, cattle and pigs share the forest grazing areas with at least four species of deer but, despite outbreaks in farm livestock, no deer has ever been seen to be infected clinically. Similarly, during the 2001 FMD outbreak in the UK and the Netherlands no deer were found to be infected (A I Donaldson and A J Garland, personal communication, cited in a document provided to Review Group by Dr. R. F. Sellers; Elbers et al. 2003). Transfer of disease by deer is therefore considered unlikely.

## **10 OTHER POSSIBLE SOURCES OF THE OUTBREAK**

There are at least three other routes that may explain the appearance of FMD at the infected premises. These are probably highly unlikely but nevertheless have to be considered. These involve the deliberate spread of FMDV from either of the Pirbright facilities by disgruntled employees or ex-employees through malicious intent, industrial sabotage by a Merial competitor, or a deliberate act of agroterrorism by individuals or groups wishing to cause harm to the United Kingdom.

One member of the Review Group discussed these possibilities with the National Counter Terrorism Security Office, the Centre for the Protection of National Infrastructure and the Joint Terrorism Analysis Centre. These discussions involved details that for reasons of national security are not reported here.

### **10.1 Malicious release**

About 120 staff have access to FMDV at the IAH. The master seed stocks are in a secure area with key access. The working seed stocks for virus work are held in -70°C freezers in the laboratory corridor where workers in the restricted area can have access them.

The records and stocks reconciled, although somebody who knew the inventory system could remove virus without anybody noticing and in talking with staff this possibility was recognised. We were also told by one member of staff that they could not remember the access code to allow entry into the containment area being changed, increasing the risk that an ex-worker could gain access. Some staff who had been working with the O<sub>1</sub> BFS strain were interviewed and we could find no cause for concern and none of those interviewed had concerns about their co-workers. It was accepted that there were a number of people who had worked at IAH who were disgruntled as the Institute had to lay off staff in 2005; the quarterly return from IAH to Defra for the period September to December 2005 mentions that there were two disgruntled ex-staff members who had made allegations about breaches of security but these were considered by IAH to be “for vindictive motives rather than honest concern over disease security”.

About 45 staff at Merial have access to the restricted areas. On this site the virus seed stocks and records also reconciled and staff were interviewed; again we found no cause for concern. The Site Director identified two recent employees with access to the high containment areas who had been unhappy in their employment but neither was considered likely to cause malicious harm.

It was noted that there was little background checking at either facility of new staff who were to work in the restricted areas, beyond what is standard for any laboratory position.

We concluded that there were no suspicions that any staff were likely to be so angry with IAH or Merial that they would cross the huge gulf between being disgruntled to maliciously causing release of a virus that they knew could cause massive economic cost to the country.

Although we have no reason to believe the source of the outbreak was FMDV maliciously removed from the laboratory, or any evidence from our interviews of any individual who was of serious concern to IAH or Merial, it is well recognised that removal of virus from a high containment laboratory by a determined person is very difficult to prevent.

## **10.2 Industrial sabotage**

Industrial sabotage by a competitor is a very unlikely explanation for the appearance of FMDV on the infected premises. Importantly, no competitor would know that they should release O<sub>1</sub> BFS to implicate Merial as the company was preparing batches of O<sub>1</sub> BFS vaccine at that time; this information is kept secret and would require collusion with someone in the company who had this knowledge.

## **10.3 Deliberate release – agroterrorism**

The view of the security services was that this outbreak did not look like terrorism and did not have indicators of terrorist activity. A small isolated group of cattle were infected, whereas multiple sites of release across the country, possibly using a number of different serotypes, with the probability of a rapidly spreading epidemic would be the hallmark of terrorist activity. Furthermore there has been no claim of responsibility. Additionally, a terrorist group would need access to virus and, if they somehow had access, the chance that their source of virus matched any strain being used in the infection window by IAH and Merial is very low.

There is no evidence to suggest that the 2001 epidemic of FMD in the UK and its subsequent spread to continental Europe were caused by agroterrorism. In the 1970s the Irish Republican Army threatened to release FMD virus in the UK; in the 1980s Australia had to respond to an extortionist who similarly threatened to use FMD virus, and recently there was a hoax threat to New Zealand. No such threats to the UK have been received in recent years. We consider it highly unlikely that this outbreak is associated with terrorist activity.

## 11 FINAL REMARKS

Accidental releases of highly infectious animal pathogens from secure containment facilities have the potential to cause devastating outbreaks of disease. The Pirbright FMD outbreak appears to have been contained, largely because by its nature it was highly localised, and since Government responded rapidly and appropriately to prevent opportunities for further spread. If infection had not been rapidly identified, or the response to the outbreak was slow and ineffectual, the outbreak could have spread more widely and become out of control. The potential consequences of such a disaster are well known from our experiences of the 2001 FMD epidemic, which besides its devastating effects on agriculture, farmers and rural communities, is estimated to have cost the country around £8 billion.

Given the scale of the dangers of an epidemic resulting from the accidental release of FMDV, it is surprising that the IAH laboratories charged with the safe handling of FMDV and other exotic animal viruses are so old. Old laboratories are not necessarily unsafe, as safety depends not on the age of the facility, but on the procedures that are carried out, approved protocols that are appropriate for the facility, regular inspection and testing and a very strong culture of biological safety and management. However, as with a fifty year old car, an old containment facility is much more likely to go wrong, requires more repairs, more extensive preventative maintenance and more regular safety inspections than a modern facility. This makes an old facility expensive to maintain and puts high demands on the biological safety officer and management.

The new containment laboratories that are a central component of the Pirbright Site Redevelopment Scheme are urgently needed, but they are not likely to be commissioned until 2012. The prospect of this new state-of-the-art facility cannot be an excuse for failing to invest in the existing IAH containment laboratories and effluent system, to ensure the safety of FMD work at Pirbright until the new facility is ready. Neither should there be any attempt to save money on biosecurity and biosafety to help cover increasing costs of the Pirbright Site Redevelopment Scheme.

**Recommendation 12. Biosecurity of laboratories that work with FMDV is of paramount importance. Therefore there should be a review of funding, governance and risk management at IAH Pirbright to ensure an appropriate focus on biosafety and biosecurity in the future.**

There was evidence of a lack of urgency and ownership of risk at all levels, resulting in the failure to take appropriate decisions on the funding for essential improvements in safety critical infrastructure. This was particularly documented in

the series of letters and reports from the biological safety officer of IAH in his attempts over four years to get agreement on funding for the replacement of the effluent pipes. The fact that animal viruses like FMDV do not infect humans, and that there has not been any previous problems with infections of animals on farms around Pirbright for nearly 50 years, may have led to some complacency about safety. This needs to be addressed.

Historically, the vaccine production area and the diagnostic and research laboratory evolved as one unit and therefore shared infrastructure including the effluent disposal system. The IAH and Merial facilities are now very clearly separate, with different missions and cultures, but still share some of the same infrastructure. The vaccine production unit has greatly expanded into a modern high volume unit producing much greater amounts of waste than was envisaged when the effluent system was designed.

This raises the issue of whether it is sensible for two such different facilities to depend on each other for any aspect of their security or safety. It is not for the Review Group to apportion blame for the accidental release of FMDV, but the relationship between IAH and Merial brings risks to both parties. These include the responsibility of what is essentially a government laboratory for elements of the biocontainment risks of a commercial company, the impact of a safety breakdown at one facility preventing work at the other, and the potential loss of public trust in a government laboratory due to incidents that are beyond their control. In future we believe that these risks should be avoided by each facility at Pirbright being entirely responsible for all aspects of their own safety.

**Recommendation 13. There should be shared governance for the management of risks to biosecurity and biosafety involving both IAH and Merial. The two facilities should ensure complete clarity of responsibility and liability for the biosafety and biosecurity of the whole site.**

We note a potential conflict of interest between the role of Defra as regulator, licensor and inspector of SAPO 4 regulation and as a major customer of research and diagnostics related to exotic animal pathogens.

**Recommendation 14. There should be a review of systems for regulation, inspection and enforcement of biosecurity for work on animal and human pathogens at containment level 4. This should consider whether there should be a common regulatory inspection framework overseen by an arm's length body such as the HSE.**



## **12 ACKNOWLEDGEMENTS**

We would like to thank the Directors and staff at the Institute for Animal Health and Merial Animal Health for their co-operation throughout the inquiry.

We would also like to thank the teams we have worked with in the Department for Environment, Food and Rural Affairs (Defra), the Health and Safety Executive (HSE), the Veterinary Medicines Directorate (VMD), the Environment Agency (EA) and the following for their expert advice: Prof. Neil Ferguson Imperial College London, Prof. Soren Alexandersen, National Veterinary Institute, Lindholm, Denmark and Dr. R. F. Sellers (Former Director of Animal Virus Research Institute now IAH, Pirbright Laboratory).

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# Annex 1

## Review Group Terms of Reference

### **An Independent Review of arrangements for biosecurity that have been and are in place in UK facilities handling FMD virus**

To review arrangements for biosecurity that have been and are in place in UK facilities handling foot-and-mouth disease virus in the UK. To evaluate if a breakdown in these controls could have led to the outbreak of FMD in the UK in August 2007.

#### **Timings**

To make a preliminary evaluation as to whether a more detailed investigation is required. If there is a need to undertake a full investigation provide recommendations to the Secretary of State and the Chief Veterinary Officer on the conduct of a more detailed investigation.

#### **Membership**

##### **Chair**

Prof. Brian G Spratt FRS FMedSci  
Head, Department of Infectious Disease Epidemiology, Imperial College London

##### **Members**

- Prof. Nigel Lightfoot  
Director, Emergency Response, Health Protection Agency.
- Dr. Christian Griot, Director, Institute of Virology and Immunoprophylaxis, Swiss Federal Veterinary Office, Mittelhäusern, Switzerland.
- Dr. Kathrin Summermatter  
Deputy Director, Head Biosafety, Institute of Virology and Immunoprophylaxis, Swiss Federal Veterinary Office, Mittelhäusern, Switzerland.
- Prof. David Rowlands  
Professor of Molecular Virology, Leeds

##### **To be advised by:**

- HSE investigation team led by Paul Logan
- Officials from the Veterinary Medicines Directorate and Defra familiar with containment of pathogens, as required

##### **Secretariat**

Nigel Gibbens	Department for Environment, Food and Rural Affairs
Yvette Balbaligo	Department for Environment, Food and Rural Affairs
Edward Currie	Department for Environment, Food and Rural Affairs

### Review Group Biographies

#### **Prof. Brian G Spratt FRS FMedSci**

Brian Spratt is Head of the Department of Infectious Disease Epidemiology and Wellcome Trust Principal Research Fellow at Imperial College London. He is an expert in the epidemiology and evolution of bacterial pathogens. He has previously carried out an independent review for the Ministry of Defence of the health consequences of the large-scale releases of bacteria in the Dorset Defence Trials and Chaired the Royal Society independent review of the health hazards of depleted uranium munitions. He is a Fellow of the Royal Society, the Academy of Medical Sciences and the American Academy of Microbiology.

#### **PD Dr. Christian Griot Dr. med. FVH, MPA**

Christian Griot is a veterinarian and Director of the Institute of Virology and Immunoprophylaxis (IVI), the Swiss National Reference Laboratory for Foreign Animal Diseases, and a senior lecturer at the Vetsuisse Faculty, University of Bern and Zürich. He is an expert on foreign animal diseases and currently project leader on a multi-national research project on avian influenza in the 'Lake of Constance' region. He has previously carried out independent laboratory and research reviews for the USDA (APHIS and ARS). Internationally, he is a member of the scientific advisory board of the European 'Network of Excellence EPIZONE', a member of the expert panel of the Seventh EU Research Framework and a member of the scientific advisory board of the Friedrich Löffler Institute (FLI), Federal Research Institute for Animal Health, Germany.

#### **Prof. Nigel Lightfoot MB BS FRCPath**

Nigel Lightfoot is Director of Emergency Response and Head of the Influenza and Respiratory Viruses Programme Board at the Health Protection Agency. He is an expert on chemical, biological and radionuclear terrorism preparedness and response. He is the expert UK delegate to the Global Health Security Action Group, the G8 Bioterrorism Working Group and the Health Security Committee of the European Commission. He is a visiting professor to Cranfield University and provides expert input to several government departments. He sits on several national expert committees and was a member of the Royal Society Working Group on Detection and Decontamination.

#### **Prof. David J. Rowlands FRSA**

David Rowlands is Emeritus Professor of Molecular Virology in the Institute of Molecular and Cellular Biology at the University of Leeds. He is an expert in the molecular biology of RNA viruses, including foot-and-mouth disease virus. He has had many years of research experience with FMDV at Pirbright, both in the research institute and in the commercial facility. He regularly reviews and advises on foot-and-mouth disease research funded by Defra. He is a Fellow of the Royal



Society of Arts, a member and past council member of the Society for General Microbiology and a member of the American Society of Virology.

**Dr. Kathrin Summermatter Dr.rer.nat.**

Kathrin Summermatter is a molecular biologist and Deputy Director of the Institute of Virology and Immunoprophylaxis (IVI) and Head of the biosafety department of the IVI. She is an expert in biosafety and biosecurity and currently project leader of Biosafety-Europe. She has previously served as biosafety advisor to the WHO for the polio eradication programme. She is involved in national and international biosafety teaching. She is an external advisor to the Institutional Biosafety Committee of GlaxoSmithKline. She is a member of the International Veterinary Biosafety Workgroup, the American Biosafety Association (ABSA) and the past president of the European Biosafety Association (EBSA).

## Annex 3

### Previous incidents of FMDV release from Pirbright Laboratory

This summary is drawn from information provided by Dr. R. F. Sellers (former Director of Animal Virus Research Institute, now IAH, Pirbright Laboratory).

#### Outside the Institute area

In January 1960, an outbreak at a farm 1.5 km from the Institute was attributed to virus escape via the ventilation system from an isolation compound housing infected animals. No exhaust air filtration system was in place at the time. After this incident filtration units were installed in the vaccine production unit and animal compounds and later in all buildings where live virus was handled.

Since 1960, no further cases of FMD have occurred on farms surrounding Pirbright, until 2007.

#### Within the Institute area

Since the 1960 escape, there have been two incidents limited to unintentional infection of animals housed on the IAH Pirbright site.

In 1967, virus from infected pigs was carried through the ventilation system from one of the animal isolation units to infect sheep, and subsequently cattle, in a separate holding unit about 50 metres away. This was attributed to a filter failure.

In 1970, cattle vaccinated against another FMDV type being held in barns became infected with the O<sub>1</sub> BFS 1860 virus. Several sources were considered: infected animals in an isolation unit; contaminated materials from the vaccine Department; and the effluent collection point next to the barns. It was thought that east winds blowing over the isolation unit could have caused negative pressure on the lee side of the unit leading to virus escape.

In each of these three cases action was taken at the time to prevent further escapes, for example use of better isolation facilities, more effective filter systems, and testing of filters.

No incidents attributable to Merial have been recorded since they commenced vaccine production on the Pirbright site.

## Annex 4

### Analysis of the sequences of the O<sub>1</sub> BFS viruses

Section 5.3 briefly describes how the nucleotide sequences of FMDV RNAs can be used to both identify strains of the virus and provide forensic evidence of temporal and spatial transmission between outbreaks. In an attempt to obtain further information on the precise origin of the virus responsible for the 2007 UK outbreak, four virus samples were selected for near full length sequence determination by scientists at IAH and independently at the National Veterinary Institute, Lindholm, Denmark. The samples were all of type O<sub>1</sub> BFS as initial sequencing of the VP1 coding region of the outbreak virus clearly showed it to be closely related to this strain. The samples examined were:

- 1) Virus from epithelium of a tongue lesion on one of the cattle (animal number UK262726300944) at the first infected farm.
- 2) Tissue culture grown virus used at IAH for disinfection studies.
- 3) Tissue culture grown virus used at IAH for molecular biology/immunology investigations and by the Stabilitech Group.
- 4) Tissue culture grown virus used at Merial for vaccine production.

The samples were coded A-D and sequenced blind. The code identification was held by one member of the HSE team and was not revealed until all of the sequence data had been obtained and interpreted.

#### IAH results

Sequences were obtained from fragments amplified from specifically designed primers by RT-PCR using high fidelity enzymes to minimise the introduction of errors. Each virus genome was amplified as separate overlapping fragments to ensure reliability of the origins of the sequences obtained. At least two independent sequence determinations were made for all parts of the genome included in the analysis. The number of nucleotides compared for the four samples was 8,028. This is slightly smaller than the entire genome sequence (~8,400 nucleotides) due to the exclusion of regions used for primer amplification.

Three other sequences were included in the IAH data analysis. These were:

- 1) Published 1 – a sequence published by the Plum Island Animal Disease Center (PIADC) of an O<sub>1</sub>BFS strain they originally obtained from IAH in 1993. This virus is thought to have been passed only three times in primary tissue cultures (bovine thyroid and lamb kidney cells).
- 2) Published 2 – also published by PIADC and with the same origin as published 1 but receiving two passages in baby hamster kidney (BHK) cells instead of primary lamb kidney cells.

- 3) The sequence of virus derived from epithelial tissue of another infected animal (animal number UK26276300958) from the first infected farm. This had been obtained before the coded samples had been distributed for analysis.

All sequences were aligned and phylogenetic relationships predicted using Neighbor-joining and Minimum Evolution trees implemented using Mega3 (Kumar et al. 2004).

### **Mutations associated with adaptation of serotype O viruses to growth in tissue culture**

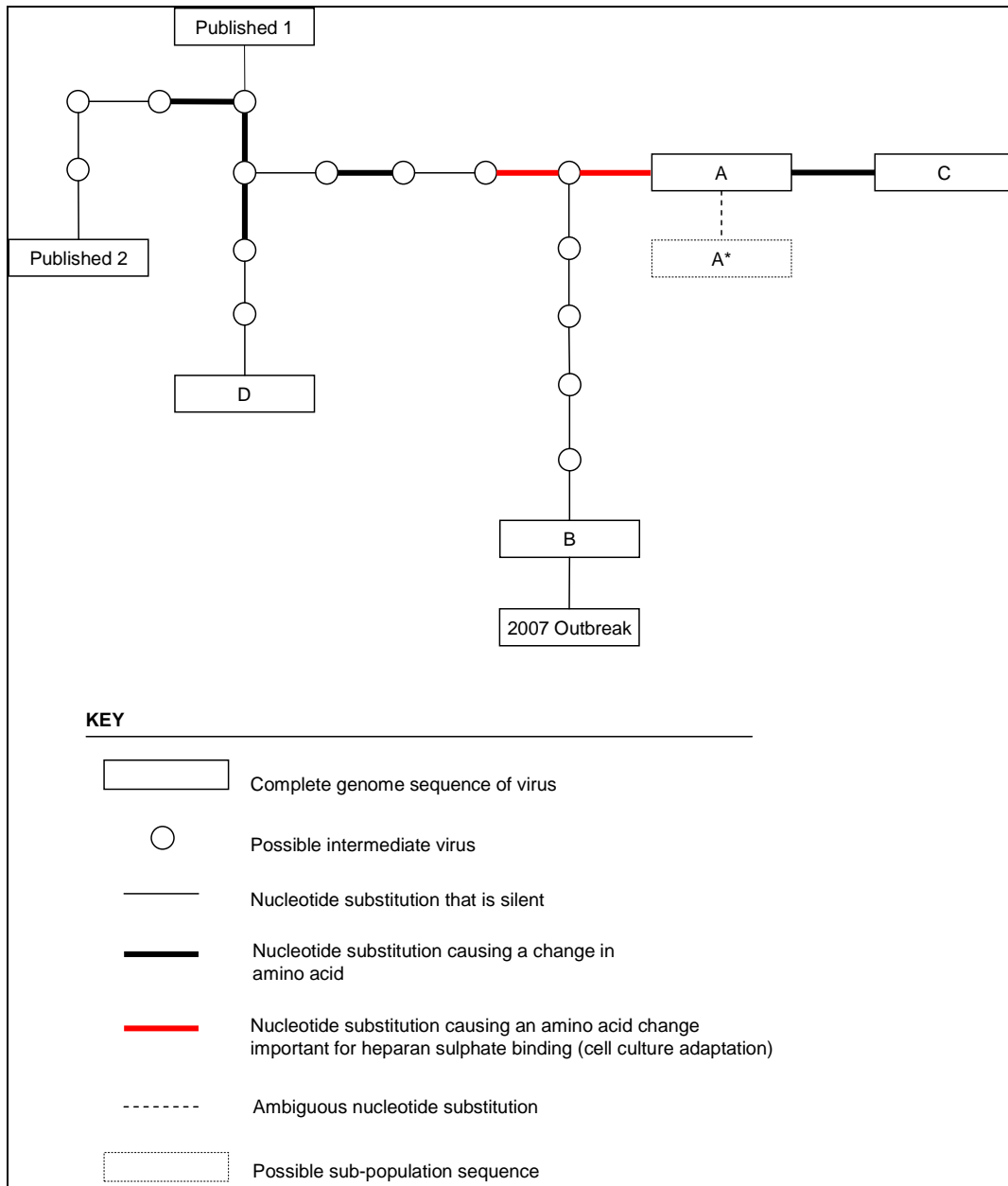
Integrins are the natural receptor molecules used by FMDVs to attach to their host cells (Jackson et al. 2000). However, serial passage in tissue culture cells frequently leads to the selection of mutations that allow the virus to attach to heparan sulphate as an alternative receptor (Fry et al. 1999). Two mutations are necessary for this functional adaptation and a third is not essential but has been observed. The presence of these mutations is a hallmark of viruses that have been adapted for growth in tissue culture. However, there is strong selective pressure for the reversion of one of the mutations essential for heparan sulphate binding when tissue culture adapted virus is passaged in cattle (Sa-Carvalho et al. 1997).

### **Results of the sequence comparisons.**

Both Neighbor-joining and Minimum Evolution trees showed that viruses A and C were most closely related to the outbreak virus, and that virus D was more distantly related and similar to the two viruses from Plum Island.

The sequence comparisons allow the minimum mutational path (i.e. the most parsimonious path) from each laboratory virus (and the Plum Island isolates) to the outbreak virus to be predicted using the method of Templeton et al. (1992), as implemented in the TCS program of Clement et al. (2000). Figure 5 below shows the analysis carried out by IAH using this method. We believe this is a good and valid representation of the likely evolutionary history of the viruses.

**Figure 5. Minimum mutational path from laboratory viruses to the outbreak viruses**



**Genealogy of the sequences examined**

Each line in Figure 5 represents a nucleotide substitution and each circle a putative intermediate virus. Those substitutions which lead to an amino acid change are shown by thicker lines and amino acid mutations involved in

adaptation of the virus to use heparan sulphate as a cellular attachment receptor are shown in red.

### **Lindholm results**

Aliquots of the same virus samples were sequenced in Denmark at the National Veterinary Institute, Lindholm. The same codes were used to distinguish the samples but the identity of sample B as the outbreak virus was revealed for analytical purposes. As at IAH, cDNAs synthesised from the virus RNAs were amplified using specific primers and the products were sent for sequencing to two sequencing facilities. As with the IAH analysis multiple reads were obtained for each nucleotide position. The results obtained were in accordance with those produced by the IAH and fully supported the original conclusions.

When the confirmatory sequences had been reported from Lindholm the identities of the coded samples were revealed:

A = Merial virus (MER)

B = Outbreak virus (OUT)

C = IAH virus used for molecular biology/immunology work and by Stabilitech (IAH-M)

D = IAH virus used for disinfectant studies (IAH-D)

### **Specific Conclusions**

1. The sequences obtained by IAH and the National Veterinary Institute, Lindholm were the same, except that the IAH sequences showed one additional synonymous substitution in the OUT virus that was not in the other viruses, due to the extra region of sequence that they determined.
2. The two published sequences and IAH-D are predicted to be non-heparan binding, indicative of low tissue culture passage.
3. IAH-M and MER are predicted to have the heparan sulphate binding phenotype, characteristic of tissue-culture adapted FMDV.
4. All of the sequences have at least one mutation associated with adaptation to heparan sulphate binding.
5. The sequence previously determined by IAH for a separate virus isolate from the outbreak differs from the outbreak virus OUT at only a single nucleotide site.
6. The sequence previously determined by IAH for a separate virus isolate from the outbreak differs from the outbreak virus OUT at only a single nucleotide site. MER and IAH-M differ from OUT by 5 and 6 (Lindholm results), or 6 and 7 nucleotide substitutions (IAH results), respectively. The differences are due to the IAH laboratory sequencing about 300 additional nucleotides compared to the Lindholm laboratory.
7. IAH-D differs from OUT by 11 (Lindholm laboratory) or 12 (IAH laboratory) nucleotide substitutions and is therefore about twice as distantly related to OUT as are viruses IAH-M and MER.

8. Both laboratories reported ambiguities at a single nucleotide for sample A although the position at which a mixed population was seen was different in the two sequences.
9. OUT has an amino acid substitution compared to IAH-M and MER at a position known to be involved in heparan sulphate binding. This non-synonymous substitution in the OUT virus was probably selected during passage in cattle of a tissue-culture adapted virus originating from Pirbright.
10. IAH-M has a unique amino acid coding mutation compared to all the other viruses, which would have had to revert to the consensus sequence for O<sub>1</sub> BFS if it were the virus causing the outbreak. This is unlikely to have occurred by chance, but reversion could have been selected if this mutation is advantageous in cattle. There is no evidence that it is, but also no evidence that it is not.

### **General Conclusions**

The two viruses from IAH that were sequenced were the stocks used in their experiments, whereas the Merial virus that was sequenced was from their vaccine seed stock, and there would have been virus replication to produce the two 6,000 litre batches of high-titre virus from the seed stock. If release had occurred from Merial, it would most likely be virus from one of the 6,000 litre batches that infected cattle, and not the seed stock virus that was sequenced. Unfortunately, virus samples from the 6,000 litre batches were not available for sequencing as inactivation with BEI had taken place.

Based on these sequence comparisons the IAH-M or the MER viruses are the most likely of those investigated to be the progenitor of the outbreak virus. The number of changes between IAH-M or MER and OUT is within the range observed during natural transmission events (Carrillo et al. 2007; Cottam et al. 2006), whereas the number of changes between IAH-D and OUT is significantly greater. IAH-M and MER differ from OUT and the other outbreak virus sequence by a substitution that reverts the heparan sulphate binding phenotype and which has been reported to occur on passage of tissue culture adapted virus to growth in cattle. Although IAH-M is more distant in sequence from OUT this is only by a single nucleotide substitution. However, this non-synonymous substitution distinguishes IAH-M from all the other sequences examined and would have had to revert to the consensus sequence if IAH-M is the progenitor virus. This is unlikely unless there is a strong selective advantage for the back mutation in cattle.

### **Recommendations**

The sequence analyses done so far are suggestive of the identity of the outbreak virus but are by no means conclusive. Several further studies are needed to try and increase the confidence in conclusions that might be drawn from them. The most important of these are:



- 1) To sequence viruses from additional infected animals at the first infected farm and those from the second infected farm. FMDV populations exist as quasi-species and the sequences obtained are a consensus. Further sequences of outbreak viruses would confirm whether the sequences already obtained are representative of the outbreak viruses and it might be possible to identify viruses that are closer to the original source of the outbreak, especially with respect to the heparan binding motifs and the unique substitution found in sample IAH-M. These data could also add further support for the favoured view that the second infected farm was infected from the first.
- 2) The IAH-M virus should be used to infect a number of cattle to determine whether the non-synonymous substitution unique to this virus reverts to the consensus sequence in cattle. This is perhaps the most critical experiment. If the unique non-synonymous substitution present in IAH-M is never seen to revert to the consensus in cattle it indicates that there is not strong selection for this to occur. This would considerably strengthen the view that the reversion of this residue (which is required for IAH-M to be the outbreak virus) is a very unlikely event, and would make the Merial virus more likely to be the source of the outbreak.
- 3) Sequences of other (older) samples of O<sub>1</sub> BFS from IAH and Merial should be determined to define (if possible) the origin of the difference between IAH-M and MER.

However, given the near identity of the IAH-M and MER viruses, even with additional studies it may well not be possible to identify the source of the outbreak with a high degree of certainty.

## Annex 5

### **Containment Requirements for Laboratories to be Licensed to Handle Defra Category 4 Pathogens under the Specified Animal Pathogens Order 1998**

- The laboratory - siting and structure
- Laboratory facilities
- Protective clothing
- Safety Officer
- Training in handling specified pathogens
- Supervision
- Laboratory discipline
- Handling of specimens
- Security
- Standard Operating Procedures
- Animal room
- Arthropods

The following describes the physical features and operating conditions which would be required by Defra of any laboratory to be licensed to hold or work with Defra Category 4 pathogens. It is concerned with preventing the escape of pathogens from the laboratory and not primarily with ensuring the safety of the workers. It does not in any way limit the obligations placed upon employers and employees by the Health and Safety at Work etc. Act 1974 in general and COSHH in particular, or the Health and Safety Executive's duty to enforce these obligations. Extra precautions will often be necessary for the safety of the staff.

The Containment requirements in this Appendix are based on those published by the Advisory Committee On Dangerous Pathogens (ACDP) as being suitable for ACDP Category 4 pathogens ("The Management, Design and Operation of Microbiological Containment Laboratories", 2001). However, it should be noted that the Defra Categorisation of pathogens and conditions of containment differ in points of detail from those published by ACDP. The reason for this is that ACDP is concerned with protection of workers in the workplace, whereas Defra is concerned with protection of livestock and the environment. Laboratories must meet DEFRA containment requirements to be considered for licensing under the Specified Animal Pathogens Order 1998 (SAPO). In addition, the relevant ACDP requirements apply.

## **The laboratory - siting and structure**

1. Whereas the laboratory need not be physically separated from other laboratories it should not be sited next to a known fire hazard (e.g. the solvent store) or be in danger of flooding.
2. The laboratory should be isolated by an air lock and provided with a suitably placed shower. Air locks and rooms must be ventilated by an exhaust air system. The air pressure in the laboratory should be monitored and displayed both within and immediately outside the laboratory. The laboratory should be maintained at a differential negative pressure of 75 Pascal's (Pa) (0.3 inches or 7.6 mm water pressure) to ambient. An alarm should sound if the air pressure falls below this.
3. The exhaust air must be filtered before discharge through two HEPA filters. The system must include a device to prevent back flow through the filters. The air intake should be protected by a single HEPA filter in case of power failure.
4. The laboratory must be sealable so as to permit fumigation.
5. The laboratory must be proofed against entry or exit of animals or insects. This is particularly important in the case of diseases which can be spread by insect vectors.
6. Effluent should be sterilised by a procedure known to kill the relevant pathogens. This procedure must be confirmed as having operated satisfactorily before the effluent is discharged to the public sewer, e.g. if heat sterilisation is to be used, temperature recording facilities should be provided to monitor the process. Since sterilisation and tests may take some time, it may be necessary to have more than one standing tank if work is to be carried out continuously. The standing tank(s) and recording equipment form parts of the facilities of the laboratory, so the Safety Officer is responsible for ensuring their proper functioning.

## **Laboratory facilities**

1. The laboratory must be equipped with a Class I/II/III exhaust protective cabinet. All laboratory manipulations with live pathogens should be carried out in the cabinet in any mode with the exception of homogenisation which should be carried out with the cabinet in the Class I or Class III mode.
2. All waste biological material must be sterilised prior to removal from the laboratory. Therefore, each laboratory should have direct access to an autoclave which should have double doors. There should be no possibility of removing the load without the autoclave cycle having been completed. As soon as practicable after the completion of the autoclave cycle the load should be taken to an incinerator and immediately incinerated. Autoclaves should be monitored to ensure that time / temperature cycles are completed and records should be kept.
3. All material must be made safe before being removed from the laboratory unit. A double ended dunk tank filled with an effective disinfectant is required for the removal of materials that cannot be autoclaved. The dunk tank should be sealed during fumigation if the disinfectant is incompatible with the fumigant.
4. Each member of staff working in the laboratory must have adequate working space.

5. Specified pathogens should be stored in the laboratory and in suitable containers (depending on the mode of storage, frozen or freeze-dried) in a cabinet reserved for specified pathogens and kept under lock and key. A key should be available on demand only to nominated individual(s).

### **Protective clothing**

1. Laboratory gowns must wrap over the chest and fit tightly at the wrists. Ordinary white laboratory coats are UNSUITABLE. Staff should have a clean gown for each uninterrupted period spent in the laboratory. Other types of clothing giving the same degree of protection may be acceptable.
2. Gowns must be autoclaved before they are removed from the laboratory.
3. Gloves must be worn for all work with infective materials and workers must shower before leaving the laboratory.

### **Safety Officer**

NOTE: Throughout this document the term Safety Officer refers to a person having responsibility for work with specified pathogens.

1. A Safety Officer able to advise on infectious hazards, and a deputy, must be appointed or designated. The establishment may have a Safety Officer with general responsibility for such hazards. If not, an additional individual must be designated.
2. A Safety Officer should have appropriate qualifications and laboratory experience in working with specified pathogens.
3. The Safety Officer will act as adviser to the Head of the Department in all matters which may affect the containment of the pathogens, and should be authorised to stop practices considered unsafe, pending guidance when necessary, from the laboratory Head.
4. He or she will take control, implement first aid in, and investigate, all accidents in laboratories and take what other action he considers necessary.
5. Where their responsibilities are not sufficient to warrant full-time employment as Safety Officer, provided that they are readily accessible to the laboratory during normal hours, they may hold another appointment.
6. He or she will be responsible for the safe storage of specified pathogens and the maintenance of the inventory.
7. He or she will be responsible for organising the admission to the laboratory of cleaners and maintenance personnel and for the disinfection of any apparatus, etc. which is to be removed.
8. He or she will be responsible for advising staff on all aspects of the application of these Safety Precautions.

## **Training in handling specified pathogens**

1. The Safety Officer will organise the initial training of staff in the safe handling of specified pathogens.
2. Training will cover, e.g. the correct use of safety hoods, exhaust protective cabinets, pipettes, syringes / needles, hot / cold rooms, centrifuges, blenders, freeze-driers, shaking machines, ultrasonic disintegrators, glassware and the disposal of contaminated protective clothing and laboratory materials.
3. Staff should only work with specified pathogens if they have some previous experience in microbiology and have had a course of training supervised by the Safety Officer.

## **Supervision**

1. Work in the laboratory must, at all times, be carried out by or be supervised by a senior, trained and experienced member of the staff.
2. The supervisor will be personally responsible to the Safety Officer for the safety of the work actually in progress at any time, although he or she may not be responsible for the overall project.

## **Laboratory discipline**

1. The containment area of each laboratory must be identified clearly with appropriate warning notices.
2. When unoccupied, the laboratory must be locked. The key(s) must be kept under the supervision of the Safety Officer, and released only to authorised persons. A key, however, should be kept at a secure control point, available at all times, in case of emergency.
3. In normal hours the supervisor will be responsible to the Safety Officer for ensuring that no unauthorised person enters the laboratory.
4. Only the Safety Officer or their deputy may authorise staff to enter the laboratory, and he or she will hold a list of names of personnel so authorised.
5. Unlisted persons (e.g. visitors, observers, cleaners or maintenance / repair personnel must not enter the laboratory unless they have received a signed statement from the Safety Officer that it is safe for them to do so.
6. The Safety Officer will be responsible for confirming when a laboratory and its apparatus have been disinfected.
7. The laboratory must be entered through a 'clean-side' changing area (locker room) separated from the 'dirty-side' by a shower and an airlock. All clothing, rings, watches, etc. must be removed into a locker. No food, drink, tobacco, make-up, etc. may be taken through the airlock. Clean protective clothing should be put on. The 'clean' and 'dirty' areas should be clearly distinguished physically.
8. On the way out, over garments should be placed in a bin on the 'dirty-side' of the showers and all remaining clothing also removed to a bin. The individual must then shower, transfer to the 'clean-side' and dress.
9. This procedure should be adhered to whenever, and for whatever purposes, the room is vacated.

10. All accidents or spillage of potentially dangerous material in the laboratory must be reported IMMEDIATELY to the Safety Office. EVERY SUCH INCIDENT MUST BE REGARDED AS A FULL MEDICAL OR ANIMAL DISEASE HAZARD.

11. The day-to-day cleanliness of the laboratory is the responsibility of those working in it. Only when the Safety Officer has confirmed that it has been disinfected can other cleaning / maintenance work be carried out.

12. At the end of a working day benches and working surfaces should be disinfected.

13. Work on specified animal pathogens must be kept separate at all times from other work in the laboratory.

14. Periodically, the rooms and everything in them must be fumigated with gaseous formaldehyde.

### **Handling of specimens**

1. All in-coming packages which may contain specified pathogens must be opened by trained staff in the laboratory.

2. Senders should be advised that a liquid sample should be externally identified and sealed in a can filled with sufficient absorbent material wholly to mop up a spill. The can may, if necessary, be cooled in solid carbon dioxide or liquid nitrogen. Similarly solid samples should be double wrapped so that, in the event of the outer container rupturing, there can be no leakage of contents.

3. Chapter 6 of "Laboratory-Acquired Infections" by C H Collins (4th edition, Butterworth and Co. 1999) gives general advice on packing and unpacking specimens, but in the present context all such unpacking must be carried out in the containment facility.

4. Particular care must be taken when biological material which cannot be autoclaved, is to be removed from the laboratory. The Safety Officer must be consulted before unsterilised material is removed. Precautions must be taken to sterilise the outer surface of containers and to sterilise the material itself, as far as possible.

5. The movement of specified pathogens from an approved laboratory to any other premises is prohibited except under the provisions of a licence issued by Defra.

### **Security**

1. It is imperative that the laboratory and animal rooms must be secure against intruders or vandals. An intruder alarm system must be fitted.

2. Security patrols, etc. must not enter laboratories, or animal rooms. If it appears that an adjacent fire or water hazard threatens the room then the Safety Officer should be informed immediately.

3. A key to the laboratory should be held centrally for emergency access but must only be released on the instruction of the Safety Officer or their deputy.

4. The Safety Officer must maintain a list of the specified pathogens used at the laboratory. This list must indicate the number of vials of pathogen under storage.

## **Standard Operating Procedures**

1. SOPs must be written and issued to staff covering-
  - (i) receipt and unwrapping of incoming specimens;
  - (ii) handling of specified pathogens in vitro;
  - (iii) handling of specified pathogens in vivo (where appropriate);
  - (iv) disposal of all waste and surplus pathogens;
  - (v) storage of specified pathogens; and
  - (vi) emergency procedures.
2. All staff must be familiar with these SOPs and have access to them on a day to day basis. Adherence to the SOPs will be a condition of a licence issued under the Specified Animal Pathogens Order 1998 and they must not be altered without prior approval from the Defra licensing office. Any plans to amend SOPs must be forwarded, via the Defra inspector, to the appropriate HQ licensing office.

## **Animal room**

NOTE: All relevant regulations in these Safety Precautions apply to any room in which animals are in contact with specified pathogens. There are, in addition, hazards arising from the natural diseases of animals which may be transmissible to man. Diseases can be contacted following bites, scratches, droplet infection or the bites of insect vectors. There are particular hazards associated with the generation of aerosols in animal rooms.

In addition to the staff utilising the animals, others may be engaged to clean and feed them and the Safety Precautions also apply to them.

1. DUST: Pre-filters are required to protect the HEPA filters and should be changed as necessary with the air-steam working. Used filters should be immediately placed into bags, autoclaved and then incinerated.
2. DRAINS: See THE LABORATORY - SITING AND STRUCTURE paragraph 6.
3. DEAD ANIMALS, BEDDING, DUNG etc.: see LABORATORY FACILITIES paragraph 2. Where autoclaving followed by incineration would create a radiological hazard, carcasses must be first sealed in a suitable bag.
4. CAGES AND ASSOCIATED EQUIPMENT: must be autoclaved or disinfected before being cleaned and returned to store.
5. ESCAPES: in no circumstances should there be a direct exit to the outside. The Safety Officer and the licensing authority of Defra must be informed if an animal cannot be accounted for.
6. VERMIN: suspected or obvious infestation with insects or wild rodents must be reported at once to the Safety Officer and the licensing authority of Defra.
7. MONKEYS: the principal hazard in monkey handling not common to the handling of other animals is the risk of infection with monkey viruses which can produce serious disease in man. The established basic rules for handling must be observed.
8. RESPONSIBILITY: servicing of specified pathogen rooms in the animal house must not be carried out by general animal house staff. Suitably trained staff approved by the Safety Officer should carry out these duties under the day-to-day supervision of the person in charge of the animal house.



## **Arthropods**

See separate containment requirements for laboratories to be licensed to handle arthropods under the Specified Animal Pathogens Order 1998.

## Annex 6

### Glossary of terms

BBSRC	Biotechnology and Biological Sciences Research Council
Biosafety	Laboratory biosafety describes the action taken to prevent unintentional exposure to biological agents and toxins, or their accidental release
Biosecurity	Laboratory biosecurity describes the action taken to prevent unauthorised access, loss, theft, misuse, diversion, or intentional release of biological agents and toxins
CCTV	Closed-circuit television
CSU	Central Service Unit
COSHH	Control of substances hazardous to health
Defra	Department for Environment, Food and Rural Affairs
DNA	Deoxyribonucleic acid
DSTL	Defence Science and Technology Laboratory
FAM 30	A propriety iodophor disinfectant
FMD	Foot-and-mouth disease
FMDV	Foot-and-mouth disease virus
GMP	Good Manufacturing Practice
HEPA filter	High efficiency particulate air filter
HPA	Health Protection Agency
HR	Human Resources
HSE	Health and Safety Executive
Infectious unit	Minimal amount of virus necessary to initiate an infection in a given system. For example to infect a tissue culture or a particular species by a particular route
IAH	Institute for Animal Health
NEEG	National Emergency Epidemiological Group
OIE	Office international des Épizooties (OIE, French for International Épizootic Office) now known as the World Organization for Animal Health
RNA	Ribonucleic acid
RT-PCR	Reverse transcription polymerase chain reaction – laboratory technique for copying ribonucleic acid into deoxyribonucleic acid and amplifying it
SAPO	Specified Animal Pathogens Order
SOP	Standard Operating Procedure
QA	Quality Assurance
QC	Quality Control
VLA	Veterinary Laboratories Agency
VMD	Veterinary Medicines Directorate

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