Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.
Protection of swine by live and inactivated vaccines prepared from a leader proteinase-deficient serotype A12 foot-and-mouth disease virus

Jarasvech Chinsangaram, Peter W. Mason and Marvin J. Grubman

Previously, we demonstrated that a genetically engineered variant of foot-and-mouth disease virus (FMDV) serotype A12 lacking the leader proteinase-coding region (A12-LLV2) was attenuated and induced an immune response that partially protected cattle from FMD. In this study, A12-LLV2 was tested in swine as a live or chemically inactivated vaccine. Animals vaccinated with chemically inactivated A12-LLV2 or wild-type (WT) virus in oil adjuvant developed high levels of neutralizing antibodies and were protected from FMD upon challenge. Animals vaccinated with live A12-LLV2 did not exhibit signs of FMD, did not spread virus to other animals, developed a neutralizing antibody response and antibodies to nonstructural protein 3D, and were partially protected from FMD. Animals given a similar dose of chemically inactivated A12-LLV2 in the absence of adjuvant developed a poor immune response and were not protected from FMD, indicating that limited replication was responsible for the improved immune response found in animals vaccinated with live A12-LLV2.

The results demonstrate the potential of A12-LLV2 as a live-attenuated vaccine as well as a safe source of antigen for chemically inactivated vaccines. © 1998 Elsevier Science Ltd. All rights reserved

Keywords: foot-and-mouth disease; attenuated vaccine; swine

Foot-and-mouth disease (FMD) is a debilitating disease of cloven-hoofed animals including swine and cattle. The disease is highly contagious and characterized by fever, vesicular lesions (mainly on the feet, but also on the mouth, nose, and sometimes teats), as well as abortions, and neonatal deaths. Infected animals rapidly spread the virus via aerosol to others, resulting in a dramatic loss of production by the herd. FMD outbreaks are so devastating that countries which do not have the disease restrict the importation of animals and animal products from countries where the disease is present. When FMD is found, infected and exposed animals are slaughtered and ring vaccination surrounding the affected area may be implemented. Vaccines prepared by chemical inactivation of FMDV have been used to successfully control FMD. However, there is an association of disease outbreaks with incomplete chemical inactivation and escape of virus from vaccine manufacturing facilities. Furthermore, this conventional vaccine induces relatively short-lived immunity and some vaccinated animals can develop a carrier state following contact with FMDV. Over the last 20 yr, a number of alternative approaches in FMD vaccine development have been examined. One of the approaches we have examined is the live-attenuated vaccine. A vaccine based on stably attenuated FMDV could provide improved safety over the existing products. Moreover, work on other viral diseases has shown that live-attenuated vaccines can induce longer lasting immunity, present a greater antigenic mass, as a result of limited replication, than inactivated vaccine, and if delivered by the natural route can induce mucosal immunity.

FMDV is an *Aphthovirus* in the Family *Picornaviridae*. The single-stranded positive-sense RNA genome is surrounded by an icosahedral capsid composed of four structural proteins, VP1, VP2, VP3 and VP4. The RNA genome contains a long translational open reading frame that codes for a polyprotein which is processed by virally encoded enzymes into mature structural and nonstructural proteins. The FMDV leader (L) protein, a papain-like proteinase, is associated with viral virulence. In infected cells, the L proteinase autocatalytically cleaves itself from the
Table 1  Summary of immune responses to vaccination and challenge

| Vaccine preparation/route | Animal no. | Prechallenge<sup>a</sup> | Postchallenge<sup>b</sup> | Clinical signs<sup>c</sup> |
|--------------------------|------------|--------------------------|--------------------------|--------------------------|
|                          |            | Serum NT<sup>d</sup>     | RIP<sup>e</sup>          | Fever<sup>f</sup>        | Score<sup>g</sup> |
| Live A12-LLV2/SC         | 14         | 1.9                      | ++                      | 5.1                      | +/+                  | 5 |
|                          | 15         | 2.5                      | ++                      | 4.8                      | +/+                  | 1 |
|                          | 16         | 2.5                      | ++                      | 4.8                      | +/+                  | 2 |
|                          | 17         | 1.5                      | --                      | 4.8                      | +/+                  | 11 |
| BEI-A12-LLV2/SC          | 18         | 0.9                      | --                      | 4.8                      | ++                   | 15 |
|                          | 19         | <0.6                     | --                      | 3.9                      | ++                   | 14 |
| BEI-A12-LLV2-oil/IM      | 20         | 3.9                      | --                      | 3.9                      | ++                   | 0  |
|                          | 21         | 4.2                      | ++                      | 4.5                      | ++                   | 0  |
|                          | 22         | 4.6                      | ++                      | 4.5                      | ++                   | 0  |
| BEI-A12-IC-oil/IM        | 23         | 4.2                      | ++                      | 4.2                      | +/+                  | 0  |
|                          | 24         | 3.6                      | ++                      | 4.2                      | ++                   | 0  |
|                          | 25         | 3.9                      | ++                      | 4.2                      | ++                   | 0  |
|                          | 26         | <0.6                     | --                      | 3.9                      | ++                   | 16 |
| None                     | 27         | <0.6                     | --                      | 3.9                      | ++                   | 15 |
|                          | 34         | <0.6                     | --                      | 4.2                      | ++                   | 10 |
|                          | 35<sup>i</sup> | <0.6                  | --                      | 4.5                      | ++                   | 13 |

<sup>a</sup> Serum collected at 56 days postvaccination.
<sup>b</sup> Serum collected at 12 days postchallenge.
<sup>c</sup> Clinical signs were observed for 12 days after challenge.
<sup>d</sup> Vaccine was administered either subcutaneously (s.c.) or intramuscularly (i.m.).
<sup>e</sup> Log of serum dilution giving a 70% reduction in p.f.u. (see Section 2).
<sup>f</sup> Antibodies to capsid proteins as detected by RIP.
<sup>g</sup> Temperature over 40.0°C for at least 2 days.
<sup>h</sup> Number of digits with vesicles.
<sup>i</sup> Animal no. 35 was challenged at 56 days postvaccination with 5 x 10⁶ infectious units of virulent cattle-passaged strain of FMDV A12.

Figure 1  Immunoprecipitation of viral proteins with serum from swine vaccinated with BEI-inactivated A12-LLV2 or A12-IC in the presence of adjuvant. Cytoplasmic extracts from FMDV-infected LK cells radiolabeled with [35S]methionine were immunoprecipitated with 14 and 56 dpv serum from swine vaccinated with: lanes 3–8, BEI-inactivated A12-LLV2 (no. 20–22); lanes 9–14, BEI-inactivated A12-IC (no. 23–25); lanes 15–18, control swine (no. 20 and 27). Lane 1, immunoprecipitation with bovine convalescent serum. Lane 2, immunoprecipitation with 0 dpv serum from swine no. 20. The products were examined by SDS–PAGE on a 15% gel.
MATERIALS AND METHODS

Cell lines and viruses

Baby hamster kidney (BHK) cells (strain 21, clone 13) were used to propagate virus stocks and for plaque reduction neutralization (PRN) assays. Secondary lamb kidney (LK) cells (provided by Dr C. House, Foreign Animal Disease Diagnostic Laboratory, USDA, Plum Island, USA) were used to produce antigen for radioimmunoprecipitation (RIP). Parental wild-type (WT) FMDV A12 119 (A12-IC), A12-LLV2, and a virulent cattle-passaged strain of FMDV A12 were described previously. The A12-IC and A12-LLV2 viruses were purified by sucrose-gradient centrifugation and chemically inactivated by treatment with binary ethylenimine (BEI) as previously described.

Animal experiment

Sixteen 20–30 kg Yorkshire cross or Yorkshire/bluepoint gilts were divided into four vaccine groups of three animals each and two groups of two control animals. These animals were housed in two separate rooms. The first room contained one group of animals vaccinated subcutaneously with 2 µg of live A12-LLV2 (10⁶ p.f.u.) and two control animals. The second room contained three groups of animals and the remaining two control animals. Each group of animals in the second room was vaccinated either subcutaneously with 2 µg of BEI-inactivated A12-LLV2 or intramuscularly with 2 µg of BEI-inactivated A12-LLV2 in mineral oil (9:1 Marcol 52/Montanide 888) or 2 µg BEI-inactivated A12-IC in the above adjuvant. At 56 days postvaccination (dpv), all animals were combined into a single room and challenged with live FMDV by inoculating a single control animal intradermally in the snout and by a combination of intradermal (coronary band and heel bulb) and skin scarification (coronary band) on one foot, with a total of 5 × 10⁴ infectious units of virulent cattle-passaged FMDV type A12. Rectal temperature data and clinical signs, including lameness and vesicular lesions were recorded daily. Temperature of over 40°C for two or more consecutive days was considered to constitute a fever. At the time of necropsy, the number of digits affected by the disease were recorded as a score for each animal (see Table I). Serum samples were collected every week and used for PRN and RIP assays.

Radioimmunoprecipitation assay

RIP was performed using cytoplasmic extracts from [³⁵S] methionine-labeled FMDV-infected LK cells as antigen. Serum samples were screened for antibodies to FMDV structural and nonstructural proteins at 1:40 dilution by RIP and the precipitated products were analyzed by SDS–PAGE on 15% gels.

![Graph](https://via.placeholder.com/150)

**Figure 2** Mean serum neutralization titers. The neutralizing antibody titers of the swine were determined at 0, 7, 14, 21, 28, 42, and 56 dpv, and 12 dpc. The arrow indicates the day of challenge. Error bars are based on the data variability determined for the animals in each vaccination group.
**Plaque reduction neutralization assay**

Serum samples were serially diluted and assayed for neutralizing antibodies by PRN in BHK cells. Neutralization titers were reported as the log of the serum dilution yielding a 70% reduction in p.f.u. (PRN).

**RESULTS**

**Immune response to vaccination with inactivated viruses**

Intramuscular vaccination of BEI-inactivated virus in adjuvant (both A12–LLV2 [no. 20–22] and A12–1C [no. 23–25]) induced high neutralizing antibody titers (Table 1) and these animals had antibodies to the viral structural proteins as detected by RIP (Figure 1). Neutralizing antibody was first detected in these animals at 7 dpv and plateaued at 14–21 dpv (Figure 2). Subcutaneous vaccination of BEI-inactivated virus in the absence of adjuvant (no 17–19) induced a lower neutralizing antibody response (Table 1), the titer appeared to decline slightly over time (Figure 2). This latter group of animals did not develop antibodies to the viral structural proteins that could be detected by RIP (lanes 9–14 in Figure 3). As expected all animals given inactivated viruses did not produce any detectable antibodies to nonstructural proteins, including 3D (Figures 1 and 3, Table 1).

**Replication and safety of live-attenuated A12–LLV2**

In contrast to animals given inactivated virus preparations, animals vaccinated with live A12–LLV2 (no. 14–16) developed antibodies to nonstructural protein 3D and the structural proteins at 14 dpv (lanes 3–8 in Figure 3). The presence of antibodies to a viral nonstructural protein is indicative of virus replication in these animals. However, these animals did not show any clinical signs of FMD including lameness, fever or any apparent lesions on the feet or mouth. In addition, the two control animals (no. 34 and 35) which were housed in the same room with swine no. 14–16 for 56 days did not seroconvert or show any signs of FMD, indicating that there was no spread of virus from A12–LLV2-vaccinated animals (Table 1, lanes 15–18 in Figure 3).

**Protection from challenge**

Following commingling of all animals in a single room, one control animal (no. 35) was infected with virulent cattle-passaged A12 virus on the snout and one foot. Vesicular lesions were observed on the foot and snout at 1 and 2 days postchallenge (dpc), respectively, and vesicles developed in the following days on all four feet of this animal, and temperatures over 40°C persisted from two to four and from seven to 10 dpc [Figure 4(C)]. All the remaining control animals (no. 26, 27, and 34) developed fever [Figure 4(C)] and vesicular lesions at 3 dpc; one of these animals (no. 34) also developed a vesicle on the snout.

Animals vaccinated subcutaneously with BEI-inactivated A12–1.I.V2 in the absence of adjuvant (no. 17–19) were not protected from challenge (Table 1), although all three animals had a delayed appearance of vesicles (4–5 dpc) as compared to control swine (no. 26, 27, and 34). Animals vaccinated subcutaneously with live A12–LLV2 (no. 14–16) were partially protected from challenge (Table 1). These animals did not develop a fever [Figure 4(A)], had a delayed appearance of vesicles (5–6 dpc), and only one animal in this group (no. 14) developed vesicles on all four feet (although with a low score; see Table 1). No fevers [Figure 4(B)] or lesions (Table 1) were found in any of the animals vaccinated with BEI-inactivated A12–1C (no. 20–22) or A12–LLV2 (no. 23–25) in adjuvant for the period of observation (12 days).

Sera collected 12 dpc was evaluated to determine the extent of challenge virus replication in the exposed animals. The results revealed a strong boost in neutralizing antibody titers in animals vaccinated subcutaneously with either live or BEI-inactivated A12–LLV2 and no increase in neutralizing titers in animals vaccinated intramuscularly with BEI-inactivated A12–LLV2 or A12–1C (Figure 2, Table 1). Moreover, RIP analyses revealed that only the latter two groups of animals failed to develop antibodies to the nonstructural protein 3D following exposure, indicating that replication of the challenge virus was absent or limited in these animals (Figure 3).
Proteinase-deficient FMD vaccines: J. Chinsangaram et al.

Figure 4  Temperatures postchallenge. Groups of swine were vaccinated with different antigen preparations, challenged at day 56, and rectal temperatures recorded daily. Panel A, vaccinated with live A12-LLV2, no. 14-16, (---) or BEI-inactivated A12-LLV2 without adjuvant, no. 17-19, (---); Panel B, vaccinated with BEI-inactivated A12-LLV2, no. 20-22, (---) or BEI-inactivated A12-IC with adjuvant, no. 23-25, (---); Panel C, control animals, no. 26 (---), no. 27 (---), no. 34 (---), and no. 35 (---). The solid line represents the cut-off threshold at 40°C.

DISCUSSION

In this study, we have shown that a leader proteinase-deficient derivative of FMDV serotype A12 can be safely administered to swine, and that vaccinated swine produce neutralizing antibodies and are partially protected from virulent virus challenge. Replication of live A12-LLV2 in swine was confirmed by the detection of antibody to nonstructural protein 3D, and presumably improved the immune response relative to that observed in animals vaccinated with inactivated A12-LLV2 by the same route (subcutaneous vaccination) in the absence of adjuvant. In spite of the evidence of virus replication, vaccination with live A12-LLV2 did not produce signs of disease and A12-LLV2 did not spread to control animals. These results together with our previous results in cattle (see Section 1) demonstrate the safety of A12-LLV2 as a live-attenuated vaccine candidate for livestock. However, an extensive study involving animals of various ages and immune status is required to unequivocally address the safety of this type of vaccine candidate.

The severity of disease judged by lesion score and rectal temperature correlated well with the neutralizing antibody titer measured at the day of challenge (Table 1). Animals vaccinated with BEI-inactivated A12-IC or A12-LLV2 in an oil adjuvant developed high titers of neutralizing antibody and were all protected against challenge. Thus, as expected, the deletion of the L proteinase coding region did not alter the antigenic structure of the virion. Animals vaccinated with live A12-LLV2 had lower levels of neutralizing antibody as compared to animals vaccinated with BEI-treated virus in the presence of adjuvant and were partially protected from virulent virus challenge, as determined by the absence of fever, delayed lesion appearance, and low lesion score. Animals vaccinated with BEI-inactivated A12-LLV2 in the absence of adjuvant displayed low or non-detectable neutralizing antibody titers at the day of challenge and although lesions appeared later in these animals than in control animals, by the end of the challenge period, no difference in disease severity was noticed between these two groups.

Subcutaneous vaccination of live A12-LLV2 was chosen for this study to allow a comparison with our previous study in cattle. In addition, this route of inoculation was selected based on a study in cattle indicating that subcutaneous was better than intramuscular vaccination in terms of ability to induce neutralizing antibodies (unpublished observation). Subcutaneous inoculation in cattle induced neutralizing antibody titers (PRN₉₀) of 2.0, 2.6 and 2.9 and the animal with the highest titer (2.9) was protected against challenge, the animal with the PRN₉₀ 2.6 developed a fever but no pedal lesions, and the third animal (PRN₉₀ 2.0) developed fever and mild lesions. In the present study, we showed that vaccination of swine by the same route induced similar neutralizing antibody titers, and these swine were partially protected from challenge (animals did not develop fever and displayed a low lesion score). Since the immune response to live A12-LLV2 given by the

1520  Vaccine 1998 Volume 16 Number 16
cytoplasmic extracts from FMDV-infected LK cells radiolabeled with [35S]methionine were immunoprecipitated with 12 dpc serum from swine no. 14-27, 34, and 35, respectively. Lane 1, immunoprecipitation with bovine convalescent serum. Lane 2, immunoprecipitation with 6 dpc serum from swine no. 14. The products were examined by SDS-PAGE on a 15% gel.

Figure 5 Immunoprecipitation of viral proteins with postchallenge serum from swine vaccinated with all vaccine formulations. Lanes 3–19, cytoplasmic extracts from FMDV-infected LK cells radiolabeled with [35S]methionine were immunoprecipitated with 12 dpc serum from swine no. 14–27, 34, and 35, respectively. Lane 1, immunoprecipitation with bovine convalescent serum. Lane 2, immunoprecipitation with 6 dpc serum from swine no. 14. The products were examined by SDS-PAGE on a 15% gel.

subcutaneous route was superior to that seen in animals given the same antigenic mass of inactivated A12–LLV2 by the same route, these studies demonstrated that replication of live A12–LLV2 virus in these animals was responsible, in part, for the increased immune response.

Problems associated with the conventional FMD vaccine have led us to search for safer vaccine candidates. The development of attenuated FMD vaccines by passage in unnatural hosts to obtain viruses that are both innocuous and yet able to induce a protective immune response has proven difficult. In addition, viruses which are selected by this procedure can revert to virulence. We have taken the approach of deleting the entire L coding region of FMDV to produce an attenuated virus suitable for use as a live vaccine. This virus has a deletion of a complete coding region which significantly reduces the risk of reversion to virulence as compared to attenuated viruses produced by conventional means. Moreover, we have demonstrated that this virus can be used as a source of antigen in traditional BEI-inactivated vaccines since A12–LLV2 is non-pathogenic and does not spread between animals, its use in vaccine production could reduce the risk associated with current vaccine manufacture.

The results of this study, in conjunction with our previous results, suggest that vaccination of cattle and swine with live A12–LLV2 is feasible. However, the limited replication of this attenuated virus resulted in an increase in antigenic mass which was insufficient to induce a completely protective immune response. Additional studies are currently ongoing to further demonstrate safety, improve efficacy, and more completely understand the host response to attenuated virus infection.

ACKNOWLEDGEMENTS

We thank Marla Zellner for expert technical assistance and the Plum Island animal caretakers for assistance with the animals.

REFERENCES

1. Bachrach, H.L. Foot-and-mouth disease: world-wide impact and control measures. In Viruses and Environment, eds E. Kurstak and K. Maramorosch. Academic Press, New York, 1978, pp. 299–310.
2. McCauley, E.H., Aulaqi, N.A., New, J.C., Sundquist, W.B. and Miller, W.M., A Study of the Potential Economic Impact of Foot-and-mouth Disease in the United States. US Government Printing Office, Washington, D.C., 1979.
3. Brown, F. New approaches to vaccination against foot-and-mouth disease. Vaccine 1992, 10, 1022–1026.
4. King, A.M.O., Underwood, B.O., McCahon, D., Newman, J.W.I. and Brown, F. Biochemical identification of viruses causing the 1981 outbreaks of foot-and-mouth disease in the U.K. Nature 1981, 293, 479–480.
5. Beck, E. and Strohmeier, K. Subtyping of European foot-and-mouth disease virus strains by nucleotide sequence determination. Journal of Virology 1987, 61, 1621–1629.
6. Bachrach, H.L. Foot-and-mouth disease. Annual Reviews in Microbiology 1968, 22, 201–244.
7. Salt, J.S. The carrier state in foot-and-mouth disease — an immunological review. British Veterinary Journal 1993, 149, 207–223.
8. Mason, P.W., Piccone, M.E., McKenna, T.St.C., Chinsangaram, J. and Grubman, M.J. Evaluation of a live-attenuated foot-and-mouth disease virus as a vaccine candidate. Virology 1997, 227, 96–102.
9. Murphy, S.R. and Chenock, R.M., Immunization against virus disease: In Virology, eds B.N. Fields, D.M. Knipe and P.H. Howley. Lippincott-Raven Publishers, New York, 1996, pp. 467–497.
10. Rueckert, R.R. Picornaviridae: the viruses and their replication. In Virology, eds B.N. Fields, D.M. Knipe and P.H. Howley. Lippincott-Raven Publishers, New York, 1996, pp. 609–654.
11. Gorbalenya, A.E., Koonin, E.V. and Lai, M.M.C. Putative papain-related thiol proteases of positive-strand RNA viruses: identification of rubi- and aphthovirus proteases and delineation of a novel conserved domain associated with proteases of rubi-, 2a- and coronaviruses. FEBS Letters 1991, 288, 201–205.
12. Kleina, L.G. and Grubman, M.J. Antiviral effects of a thiol protease inhibitor on foot-and-mouth disease virus. Journal of Virology 1992, 66, 7168–7175.
13. Piccone, M.E., Zellner, M., Kurmosinski, T.F., Mason, P.W. and Grubman, M.J., Identification of the active-site residues of the L proteinase of foot-and-mouth disease virus. Journal of Virology 1995, 69, 4950–4956.
14. Roberts, P.J. and Belsham, G. Identification of the critical amino acids within the foot-and-mouth disease virus leader protein, a cysteine protease. Virology 1995, 213, 140–146.
15. Piccone, M.E., Rieder, E., Mason, P.W. and Grubman, M.J. The foot-and-mouth disease virus leader proteinase gene is not required for viral replication. Journal of Virology 1995, 69, 5376–5382.
16. Brown, C.C., Piccone, M.E., Mason, P.W., McKenna, T.St.C. and Grubman, M.J., Pathogenesis of wild-type and leaderless foot-and-mouth disease virus in bovines. Journal of Virology 1996, 70, 5632–5641.
17. Strebel, K. and Beck, E. A seroantibody profile of foot-and-mouth disease virus. Journal of Virology 1986, 58, 893–899.
18. Devaney, M.A., Vakharia, V.N., Lloyd, R.E., Ehrenfeld, E. and Grubman, M.J., Leader protein of foot-and-mouth disease virus is required for cleavage of the p220 component of the cap-binding protein complex. Journal of Virology 1988, 62, 4407–4409.
Proteinase-deficient FMD vaccines: J. Chinsangaram et al.

19 Medina, M., Domingo, E., Brangwyn, J.K. and Belsham, G.J. The two species of the foot-and-mouth disease virus leader protein, expressed individually, exhibit the same activities. Virolgy 1993, 194, 355-359.

20 Kirchweger, R., Ziegler, E., Lamphear, B.J., Waters, D., Liebig, H.D., Sommergruber, W., Sobrino, F., Hohenadl, C., Blass, D., Rhodius, R.E. and Skerritt. T. Foot-and-mouth disease virus leader proteinase: purification of the Lb form and determination of its cleavage site on eIF-4. Journal of Virology 1994, 68, 5677-5694.

21 Doel, T.R., Williams, L. and Barnett, P.V. Emergency vaccination against foot-and-mouth disease: rate of development of immunity and its implications for the carrier state. Vaccine 1994, 12, 592-600.

22 Barteling, S.J. and Vreeswijk, J. Development in foot-and-mouth disease vaccines. Vaccine 1991, 9, 75-87.

23 Dunn, C.S. and Donaldson, A.I. Natural adaption to pigs of a Taiwanese isolate of foot-and-mouth disease virus. The Veterinary Record 1997, 141, 174-175.

24 Cottral, G.E., Patty, R.E., Galliunas, P. and Scott, F.W. Sensitivity of cell cultures, cattle, mice, and guinea-pigs for detection of nineteen foot-and-mouth disease viruses. Bulletin of the Office of International Epizootiology 1965, 63, 1607-1625.

25 Rieder, E., Bunch, T., Brown, F. and Mason, P.W. Genetically engineered foot-and-mouth disease viruses with poly(C) tracts of two nucleotides are virulent in mice. Journal of Virology 1993, 67, 5139-5145.

26 McKenna, T.S.C., Lubroth, J., Rieder, E., Baxt, B. and Mason, P.W. Receptor binding site-deleted foot-and-mouth disease (FMD) virus protects cattle from FMD. Journal of Virology 1995, 69, 5787-5790.

27 Bahnemann, H.G. Inactivation of virus antigens for vaccine preparation with particular reference to the application of binary ethylenimine. Vaccine 1990, 8, 299-303.

28 Brooksby, J.B. Portraits of viruses: foot-and-mouth disease virus. Intervirology 1982, 18, 1-23.