Prospects, including time-frames, for improved foot and mouth disease vaccines

M.J. Grubman & P.W. Mason

United States Department of Agriculture (USDA), Agricultural Research Service (ARS), North Atlantic Area (NAA), Plum Island Animal Disease Center (PIADC), Foot and Mouth Disease Research Unit, P.O. Box 848, Greenport, NY 11944, United States of America

Summary
Inactivated foot and mouth disease (FMD) vaccines have been used successfully as part of eradication programmes. However, there are a number of concerns with the use of such vaccines and the recent outbreaks of FMD in disease-free countries have increased the need for improved FMD control strategies. To address this requirement, new generation FMD vaccines are being developed. Currently, one of the most promising of these vaccine candidates utilises an empty viral capsid subunit delivered to animals by a live virus vector. This candidate, a replication-defective recombinant human adenovirus containing the capsid and 3C proteinase coding regions of FMD virus (FMDV), induces an FMDV-specific neutralising antibody response in inoculated animals. Upon challenge with a virulent animal-passaged homologous virus, swine and cattle vaccinated with this recombinant adenovirus are protected from clinical signs of FMD as well as from FMDV replication. One inoculation of a high dose of this vaccine candidate protected swine from challenge as early as seven days after vaccination.

Keywords
Empty viral capsids – Eradication – Foot and mouth disease – Replication-defective adenoviruses – Vaccination – Vaccines.

Introduction

Foot and mouth disease (FMD) is economically the most important animal disease of livestock world-wide. The disease, which is caused by a picornavirus, is characterised by debilitating oral and pedal vesicles which can result in a significant decline in production of meat and dairy products, but generally produces low mortality (4). However, in young animals, infection of the heart muscle may result in severe myocardial necrosis and death (12, 13).

Foot and mouth disease is highly contagious and can be spread by contact or aerosol. Outbreaks spread easily from farm to farm, either through movement of animals or animal products, or on personnel, transport vehicles, etc. Foot and mouth disease is highly infectious and countries that are not affected maintain rigid quarantine and import restrictions on animals and animal products from infected countries to prevent introduction of the disease since the presence of FMD would restrict their participation in international trade. Currently, the most effective controls in slowing spread of the disease through livestock populations include the inhibition of movement of animals and animal products, slaughter of infected and exposed animals, disinfection and vaccination using a killed FMD virus (FMDV)-based vaccine.

The alarming rate of spread of FMD was recently demonstrated during an outbreak in Taipei China in the spring of 1997. From the first reports of the outbreak in March 1997, the disease spread to almost the entire island within three weeks (26, 80). This outbreak resulted in the eventual death or destruction of four million swine and a multibillion-dollar loss due to clean-up costs, lost export revenue, and repopulation. The recent FMD outbreak in the United Kingdom (UK) and several other European Union (EU) countries as well as the reintroduction of FMD into Uruguay, Argentina and the southern part of Brazil, further emphasises the difficulty of controlling this disease and the severe economic consequences to livestock producers as well as the general population. In the UK outbreak, which

590

Rev. sci. tech. Off. int. Epiz., **21** (3)

lasted from February to September 2001, almost 4 million livestock were destroyed, including over 3 million sheep. Estimates of direct and indirect costs of this outbreak exceed US$15 billion (68).

Current foot and mouth disease vaccines

Inactivated whole virus vaccines are used in FMD control programmes, and more than a billion doses of this vaccine are estimated to be used annually world-wide (66). Whole virus vaccines require the production of large quantities of live virus in high containment facilities, followed by imine-inactivation. These vaccines have been utilised successfully in controlling the disease in many countries.

Foot and mouth disease virus is an antigenically variable virus consisting of seven serotypes and dozens of subtypes. Immunity to one serotype does not provide protection against the others and in some cases, immunity to one subtype will not protect against other members of the same serotype. A number of countries have established vaccine banks containing concentrated antigens that can be mobilised for use during an outbreak. These banks contain several subtypes and serotypes of virus to help ensure that they will be effective against the likely introduced strains of virus (24).

Increased volumes of trade as a result of new trade agreements (e.g. North American Free Trade Agreement [NAFTA] and the General Agreement of Tariffs and Trade [GATT]), the expansion of free trade in the EU, the opening of trade with Eastern Europe and the admission of the People's Republic of China into the World Trade Organization (WTO) increase the possibility of an FMD outbreak in FMD-free countries. Recent FMD outbreaks in Taipei China, the Republic of Korea, Japan, the UK, France, the Netherlands, and the Republic of Ireland that had been free of the disease for many decades, emphasise the increased risk of disease incursion and the need to be able to control an outbreak rapidly.

Although inactivated-virus vaccines are effective as part of eradication programmes in enzootic areas, vaccine production and use of these vaccines pose a number of problems that limit vaccination in emergency control programmes, as follows:

– security breaches in vaccine production plants have been implicated in FMD outbreaks (9, 36)

– residual live virus in vaccines has been implicated in outbreaks (9)

– presence of antibodies to vaccine, including antibodies to some viral non-structural proteins, can interfere with detection of convalescent animals or vaccinated animals that harbour the virus and display subclinical disease

– dozens of antigenically distinct subtypes of virus threaten FMD-free countries, requiring multiple vaccines

– new variants are evolving continuously, requiring adaptation of new field strains to high-level growth in cell culture, which can select antigenically altered variants that produce poor-quality vaccines

– repeated vaccinations are needed to maintain protective immunity.

Although several of these problems, notably those in the area of safety, production, inactivation and administration have been largely overcome using strict quality control methods in vaccine production (6), some countries, such as the United States of America, still do not permit the manufacture of FMD vaccines from live virus. In addition, despite the usefulness of current vaccines, several other particularly problematic concerns limit application of vaccination in outbreak situations as follows:

a) Current vaccines generally require at least seven days to protect animals from disease. Thus, a post-vaccination window of susceptibility to disease exists. This phenomenon was re-documented in the recent episode that took place in the Netherlands, where several outbreaks were observed on vaccinated farms between two and nine days post-vaccination (57).

b) Current vaccines do not prevent infection (hence vaccinated animals can become infected and may shed virus) and the vaccines cannot prevent animals from becoming long-term carriers of the virus (67). Current evidence suggests that this level of infection does not induce detectable levels of antibodies to non-structural proteins which makes detection of carrier animals difficult (38).

There are a number of long-term economic consequences in using current vaccines to control an outbreak of FMD. These are based, in part, on the belief that the presence of disease might be missed if a country suppresses FMD with vaccination, and the inability to reliably distinguish among vaccinated, convalescent and vaccinated/infected animals (see above). The Office International des Epizooties (OIE: World organisation for animal health) has established that if FMD vaccines are used during an outbreak, countries can only achieve an FMD-free status by documenting absence of disease for one year after the last cases were stamped-out, or two years after the last case if stamping-out is not employed (38, 54). In contrast, if an eradication programme specifies that affected animals and their contacts be slaughtered without vaccination, the disease-free status can be reviewed three months after the last stamping-out exercise. Recently, in the outbreak in the Netherlands, a fourth possible OIE-recognised control strategy was applied in which all vaccinated animals were destroyed. This eliminated the chance that any vaccinated animals could have been short-term shedders or long-term carriers, permitting the Netherlands to resume trade within the EU (and with OIE Member Countries) in three months (54). To control the FMD outbreaks in

Rev. sci. tech. Off. int. Epiz., **21** (3)

591

Argentina and Uruguay, these countries elected to slaughter infected and in-contact animals and institute a national vaccination programme for the remaining cattle in their herds. Consequently, Argentina and Uruguay are currently not considered FMD-free, and will not be allowed to export animals or animal products to FMD-free countries until they can document one year without disease after the vaccination programme has been completed (54).

Alternative vaccines

The foot and mouth disease virus, the type species of the *Aphthovirus* genus of the family *Picornaviridae*, is the causative agent of FMD. The virus contains a single-stranded positive-sense ribonucleic acid (RNA) genome of approximately 8,300 bases surrounded by an icosahedral capsid composed of 60 copies each of four structural proteins, 1A, 1B, 1C, and 1D

(63, 64). The RNA genome is translated as a single, long open reading-frame and codes for the four structural proteins and a number of non-structural proteins, which function in various aspects of the virus replication cycle. The viral polyprotein is co-translationally processed by at least three viral-encoded proteinases (L^{pro}, 2A oligopeptide and $3C^{pro}$) (63, 74). Most of the cleavages are catalysed by $3C^{pro}$ or a $3C^{pro}$-containing precursor, including the processing of the capsid precursor polypeptide, P1, into 1AB, 1C, and 1D (Fig. 1). One exception is the maturation cleavage of the precursor capsid protein 1AB in the provirion to generate the capsid proteins 1A and 1B that occurs by an unknown mechanism. Only 1D, 1B, and 1C have been shown to be surface-exposed (2) and an immunologically important loop found between the G and H beta strands of 1D has been identified as a prominent surface structure on the viral capsid (43).
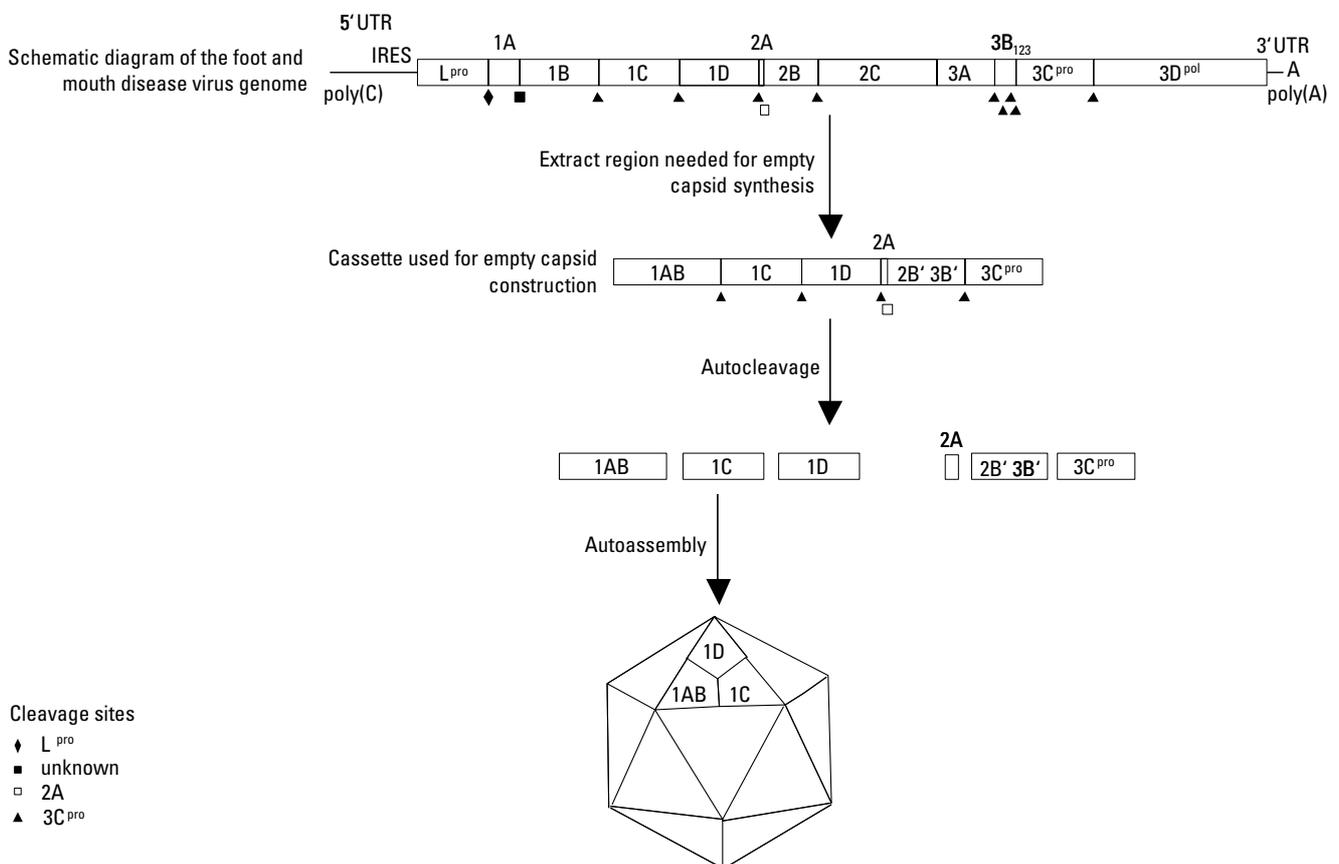

Fig. 1
Schematic diagram of foot and mouth disease virus empty capsid construction and autoassembly
The untranslated regions of the genome are represented by lines and the protein-encoding regions are represented by boxes. The symbols below the protein-encoding regions identify the proteinases responsible for cleavage of the viral polyprotein. The 2A autoproteinase and $3C^{pro}$ regions are included in the empty capsid construct since processing of the capsid proteins to 1AB, 1C, and 1D by $3C^{pro}$ and removal of P1-2A from the remainder of the viral polyprotein are required for autoassembly, and assembly is necessary for induction of neutralising and protective immunity

Based on information concerning the FMDV capsid structure, including the prominent immunogenicity of the 1D G-H loop, a number of strategies have been used to produce experimental FMD vaccines that do not require infectious virus (Table I).

Table I
Vaccine strategies and associated problems

Candidate	Immunogenicity	Efficacy	Drawbacks
Escherichia coli-expressed 1D	++	++	Limited number of epitopes producing breakthroughs
Synthetic peptide	++	+	Limited number of epitopes producing breakthroughs
Viral vector, DNA or plant-expressed 1D	+	+	Limited number of epitopes producing breakthroughs
Modified live virus	++	++	Reversion to virulence (host-dependent)
Escherichia coli-expressed empty capsids	+	+	Low immunogenicity
Baculovirus-expressed P1	+	+/–	Low immunogenicity
DNA-encoding empty capsids	+	+	Low immunogenicity
Adeno-vectored empty capsids	++	++	??

DNA: deoxyribonucleic acid
+   : low
++ : significant
+/– : weak
?? : no current drawbacks

Initially, these included use of 1D, either isolated from purified virus or produced by recombinant DNA techniques (5, 40), use of 1D synthetic peptides (11, 23, 29, 53, 56), or use of live vectors expressing 1D fusion proteins (37, 39). More recently, transgenic plants expressing the entire 1D coding region or plants infected with a recombinant tobacco mosaic virus expressing 1D, have been used to immunise mice (76, 77) and a DNA-based inoculation strategy containing 1D epitopes has been used to inoculate both mice and swine (15, 78). All of these strategies present a limited subset of viral immunogens to the vaccinated animal, and although they often induce high titres of neutralising antibodies in vaccinated animals, these antibodies are not always protective in livestock (23, 50, 51, 52). The immunogenicity of these subunit vaccines appears to be due to their ability to present sequential epitopes from the immunologically important G-H surface loop. Although these epitopes appear to be immunodominant in many assay systems, they are neither the only neutralising epitopes on the virion (7, 21), nor are they uniformly recognised in all host species (45). With the known variability of the FMDV genome (25), the use of immunogens containing a limited subset of epitopes in a vaccination programme appears to invite selection of antigenic variants from the challenge virus that cause disease among animals vaccinated with these products

(41, 48). In a large-scale vaccination study in bovines using synthetic peptides, Taboga *et al.* (72) demonstrated that the peptides were highly immunogenic, but that the vaccinated animals were poorly protected from disease and that disease-causing antigenic variants of the challenge virus were detected in many of the unprotected animals.

Development of empty capsid vaccines

Other experimental FMD vaccines have targeted immunogens that contain the entire repertoire of immunogenic sites present on intact virus, but lack infectious nucleic acid (1, 32, 33, 42, 61) (Table I). This strategy involves utilising recombinant deoxyribonucleic acid (DNA) methods to produce a subunit vaccine comprised only of the regions of the viral genome necessary for the synthesis, processing, and assembly of the viral structural proteins into empty viral capsids (P1/2A and 3C^{pro}) (Fig. 1). Empty capsids appear to provide an excellent subunit vaccine candidate, since empty capsids produced in FMDV-infected cell cultures are antigenically similar to virus particles, and are as immunogenic as virions in animals (32, 62, 65). Moreover, animals inoculated with this type of subunit vaccine could be easily distinguished from infected or convalescent animals using current technology, such as the virus infection associated antigen (VIAA) assay (20), because the regions of the genome coding for non-structural proteins used in diagnostic assays (VIAA, also termed 3D^{pol}) as well as for other non-structural proteins are not encoded in empty capsid constructs. In preliminary studies, FMDV empty capsid structures were expressed in *Escherichia coli* or in recombinant baculovirus infected cells and inoculated into animals. Although these products offered some protection from FMD, they did not reach the efficacy of imine-inactivated virus vaccine because only small amounts of capsids were obtained (33, 42).

To enhance the expression and delivery of empty capsid constructs, alternative delivery systems have been examined that allow expression of FMDV capsid structures in cells of vaccinated animals, which could potentially induce both humoral and cell-mediated immune responses to FMDV. A DNA inoculation-based strategy designed to produce empty capsids in inoculated animals was utilised (8, 10, 14, 18). Initial studies in mice inoculated either intradermally by gene gun or intramuscularly (IM) with empty capsid constructs resulted in the induction of a FMDV-specific neutralising antibody response after two gene gun inoculations, or four to five IM inoculations (8, 18). The presence of an active viral 3C^{pro} in the empty capsid construct was required for induction of neutralising antibody (8, 18). Studies in swine required large amounts of DNA, at least two inoculations, induced a low FMDV-specific neutralising antibody response, and variable levels of protection (8, 10, 14). Although naked DNA vaccines offer numerous advantages, the efficiency of DNA uptake is low

Rev. sci. tech. Off. int. Epiz., **21** (3)

593

and the mechanism of action is still not well understood. Cedillo-Barron *et al.* (14) found that co-administration of a plasmid containing porcine granulocyte-macrophage colony-stimulating factor with the empty capsid construct increased the FMDV-specific antibody response and the level of protection in some swine. These authors suggested that the use of this cytokine might enhance antigen presentation, thereby increasing antibody induction.

Delivery of foot and mouth disease virus empty capsids by adenovirus

The use of adenovirus vectors for delivery of protective immunogens from numerous pathogens is being explored as a vaccine strategy for several diseases (3, 16, 17, 30, 34, 44, 55, 58, 59, 71). Adenoviruses target the upper respiratory and gastrointestinal tracts, thereby inducing local mucosal immune responses and triggering cellular immunity (75). Since the initial site of FMDV infection is the upper respiratory tract, the ability to deliver FMDV nucleic acid to this area would resemble natural infection and could be important in induction of protective immunity. Human adenoviruses possess low pathogenicity in man and animals. Wild-type virus has been safely and successfully used in oral immunisation of millions of US military recruits as prevention against acute respiratory disease (19, 73). As an additional safety feature, replication-defective adenovirus vectors, which lack an essential portion of the adenovirus genome, have been produced. Although these vectors can only grow productively in specific cell cultures that provide the missing functions, i.e. 293 cells (31), they can infect cells of several animal species, including cattle and swine (58). Effective immunisation of animals has been achieved with replication-defective adenovirus expressing proteins from other pathogens (27, 28, 35, 60, 79).

Replication-defective human adenovirus type 5 (Ad5) vectors encoding the FMDV serotype A12 capsid with either wild-type (Ad5-3CWT) or inactive 3C^{pro} (Ad5-3CMUT) (MUT: mutant) have been constructed (46, 47). In these viruses, the FMDV coding regions are under the control of the cytomegalovirus immediate early gene promoter. Inoculation of mice with these viruses resulted in an FMDV-specific neutralising antibody response only in the group given the Ad5-3CWT vaccine (46). Likewise, swine inoculated IM with either one or two doses of Ad5-3CWT developed an FMDV-specific neutralising antibody response and after virulent virus challenge, showed either significantly reduced clinical disease as compared to uninoculated co-housed control animals or were completely protected (46, 47). However, swine inoculated with Ad5-3CMUT (47) or swine and cattle inoculated with a replication-competent recombinant Ad5 virus containing only the FMDV capsid coding region from C1 Oberbayern (69, 70) did not develop FMDV-specific neutralising antibodies and

were not protected after challenge. These results indicate that processing of the P1 capsid precursor protein by WT 3C^{pro} is necessary for production of potent and efficacious empty capsid vaccines.

The studies of Mayr *et al.* (46, 47) also indicated that there was no spread of the recombinant virus to uninoculated swine in the same room, as demonstrated by the absence of FMDV or Ad5-specific neutralising antibody responses in unvaccinated, co-housed animals. The most efficacious vaccine regimen in these studies was shown to be a low dose initial inoculation (1×10^8 plaque-forming unit [pfu]/animal), followed four weeks later by a high dose boost (5×10^8 pfu/animal). Using this protocol, five of six inoculated animals were completely protected from contact challenge, while the remaining animal in this group demonstrated only limited disease (47). The five protected animals showed no detectable level of challenge virus replication as demonstrated by the absence of antibodies against the viral non-structural proteins. All the non-inoculated control animals developed severe disease (47). The increased efficacy of the two-dose regimen as compared to a low, one-dose inoculation schedule was associated with heightened levels of FMDV-specific IgG1 and IgG2 antibodies, while one-dose inoculated animals developed an IgM response, which reappeared after challenge indicating that the low, one-dose regimen was suboptimal for protection (47).

Potency and efficacy of a single-dose regimen with an adenovirus type 5 vector containing the P1 region of a foot and mouth disease virus field strain

To be useful in emergency outbreak situations such as those that occurred in Taipei China and the UK, vaccines need to be given in a single dose and induce rapid immunity. To test these criteria with an Ad5-FMDV vectored vaccine, Moraes *et al.* (49) constructed an Ad5-vector containing the P1 region of an FMDV field strain, A24 Cruziero, which is currently used as a vaccine in several countries in South America, and the A12 3C^{pro}.

Evaluation of the Ad5-A24 virus in cell culture showed that A24 capsid proteins were synthesised and processed by the A12 3C^{pro} (49). The potency and efficacy of this virus was examined in swine using the optimal two-dose vaccination regimen (see above) and a single inoculation at a higher dose (Table II) (49).

594

Rev. sci. tech. Off. int. Epiz., **21** (3)

Table II
Potency and efficacy of adenovirus type 5-A24 Cruziero virus (Ad5-A24) in swine: one-dose versus two-dose regimen

Group [a]	Vaccine	Boost	Challenge	Protection (P/T)[b]	Viraemia[c] No.	Score[d] mean
Control	PBS[e]	No	42 dpv[f]	0/4	4	15.25
Low/Hi	1×10^8 pfu[g] Ad5-A24	5×10^9 pfu	14 dpv	4/4	0	0
Hi1	5×10^9 pfu Ad5-A24	No	14 dpv	4/4	0	0
Hi2	5×10^9 pfu Ad5-A24	No	42 dpv	4/4	0	0
Vac1	BEI[h]-A24	No	14 dpv	4/4	0	0
Vac2	BEI-A24	No	42 dpv	4/4	0	0

(a) Four animals/group inoculated intramuscularly
(b) Number of animals protected/total number of animals
(c) Determined by virus isolation (number of positive animals)
(d) The score is the number of digits and snouts with lesions; the maximum score is 17
(e) Phosphate-buffered saline
(f) Days post-vaccination: the animals were challenged either 42 or 14 days post-vaccination by direct inoculation with 10^5 bovine infectious doses$_{50}$ (BID$_{50}$) virulent A24 in the rear feet
(a) Plaque-forming unit
(b) Binary ethylenimine-inactivated vaccine

Twenty-four swine were divided into six groups and each group housed in separate rooms and assigned to the treatment groups shown in Table II. All animals were challenged by direct inoculation with virulent animal-passaged A24 six weeks after the first vaccination.

The control group developed viraemia by three days post-challenge and signs of severe FMD, including fever and vesicular lesions on all four feet and on the snout, lips, and tongue of some animals. Animals vaccinated with Ad5-A24 by any regimen as well as the animals vaccinated with commercial binary ethylenimine (BEI)-inactivated vaccine developed an FMDV-specific neutralising antibody response prior to challenge and were completely protected. In addition, challenge virus replication was not detected in Ad5-A24 inoculated animals.

Since these experiments demonstrated that a single high-dose approach with Ad5 vectors was protective, a second experiment was performed to examine how rapidly Ad5-A24 vaccination could induce protective immunity. In this experiment, twelve swine were divided into three groups and housed in separate rooms as shown in Table III. These animals were challenged by direct inoculation either 7 or 14 days after vaccination. All control animals developed severe disease including viraemia and virus in nasal swabs (Table III). The groups challenged by direct inoculation 7 or 14 days after vaccination with one injection of a high dose of Ad5-A24 were all protected from disease, showed no viraemia or virus in nasal swabs, and no evidence of virus replication.

These series of experiments demonstrate that the Ad5-vectored FMDV empty capsid approach successfully addresses many of the concerns with the current inactivated vaccine. This strategy does not require infectious FMDV, the replication-defective Ad5-vector does not spread to co-housed control animals and the vector lacks the coding regions of many FMDV non-structural proteins and thus companion diagnostic assays using

Table III
Potency and efficacy of adenovirus type 5-A24 Cruziero virus (Ad5-A24) in swine showing how rapidly protective immunity can be induced

Group [a]	Vaccine	Boost	Challenge	Protection (P/T)[b]	Viraemia[c] No.	Score[d] mean
Control	PBS[e]	No	14 dpv[f]	0/2	2	17
Ad5-A24-1	5×10^9 pfu[g]	No	7 dpv	4/4	0	0
Ad5-A24-2	5×10^9 pfu	No	14 dpv	4/4	0	0

(a) Four animals/group inoculated intramuscularly
(b) Number of animals protected/total number of animals
(c) Determined by virus isolation (number of positive animals)
(d) The score is the number of digits and snouts with lesions; the maximum score is 17
(e) Phosphate-buffered saline
(f) Days post-vaccination: the animals were challenged either 14 or 7 days post-vaccination by direct inoculation with 10^5 bovine infectious doses$_{50}$ (BID$_{50}$) virulent A24 in the rear feet
(g) Plaque-forming unit

Rev. sci. tech. Off. int. Epiz., **21** (3)

595

currently approved protocols are already available. Furthermore, the potency, efficacy and rapidity of induction of protective immunity is equivalent to that observed with the current vaccine.

The adenovirus type 5-A24 Cruziero empty capsid candidate can protect cattle from foot and mouth disease

In a preliminary study in cattle, Ad5-A24 was administered to two cattle (5×10^9 pfu/animal) which were co-housed with an animal inoculated with phosphate-buffered saline (PBS) (M.P. Moraes and M.J. Grubman, unpublished data). Nine weeks later, the vaccinated cows were boosted with the same dose of Ad5-A24 and all animals were challenged by intradermal inoculation in the tongue with virulent, animal-passaged A24 (Table IV). The Ad5-A24 vaccinated animals developed a significant FMDV-specific neutralising antibody response after the first vaccination and this was increased 10 to 20-fold after the booster while the control animal did not seroconvert. After challenge, the control animal developed severe clinical signs of FMD, including fever and lesions on all four feet, and showed a high neutralising antibody response to FMDV. In contrast, both Ad5-A24 vaccinated animals were completely protected from FMD, even in the presence of the actively infected animal.

Additional large-scale studies, including single, high-dose inoculations, are needed to confirm this initial experiment. Nevertheless, the ability to effectively revaccinate animals nine weeks after the initial inoculation indicates that the Ad5-vectored approach can also be used in vaccination campaigns in enzootic areas where semi-annual or annual revaccinations are required.

A combination antiviral and subunit vaccine approach

The experiments described above demonstrate the potential of Ad5-vectored FMDV empty capsid vaccines as a safe method for inducing rapid protective immunity in swine, and preliminary results suggest their utility in cattle. However, there is a window after vaccination and prior to development of the FMDV-specific, adaptive immune response when animals are susceptible to disease. During control of an outbreak, this window of susceptibility is of great concern. To address this situation, the possibility of a combination approach utilising antiviral and vaccine strategies is being examined. This approach could provide antiviral-based short-term protection against all FMDV serotypes and allow the induction of a specific adaptive response against the co-administered vaccine. Thus, this combination strategy could potentially provide both immediate as well as long-term protection. In addition, the co-administration, via Ad5-vectors or by other means, of various cytokines could be used to enhance the protective immune response to the vaccine antigens.

An additional concern with current vaccines is the inability to block virus infection and establishment of the carrier state. Carrier animals may be 'cured' by utilising an antiviral approach and delivery of appropriate compounds to the privileged sites of virus replication. Similar antiviral therapies are currently in use to treat hepatitis C virus (HCV)- and human immunodeficiency virus (HIV)-infected humans. A 'cure' rate of over 40% has been obtained in HCV-infected individuals treated with interferon alpha and ribavirin (22).

Time-frame for application of new disease control strategies

The development and experimental testing of Ad5-vectored FMD empty capsid vaccines have demonstrated that this

Table IV
Preliminary cattle experiment with adenovirus type 5-A24 Cruziero virus (Ad5-A24)

Vaccine	Dose[a]	Boost[b]	0 dpb[d]	PRN$_{70}$[c] 0 dpc[e]	14 dpc[e]	Protection[f]
Control	PBS[g]	PBS	<8	<8	3,200	Lesions, fever
Ad5-A24	5×10^9 pfu[h]	5×10^9 pfu	160	1,600	12,800	No lesions or fever
Ad5-A24	5×10^9 pfu	5×10^9 pfu	80	1,600	3,200	No lesions or fever

(a) All animals in same room and inoculated intramuscularly
(b) Boosted 63 days after primary vaccination
(c) Plaque reduction number$_{70}$: FMDV-specific neutralising antibody titre reported as the serum dilution yielding a 70% reduction in the number of plaques
(d) Days post-boost: FMDV-specific titre 0 days post-boost, 63 days after the initial inoculation
(e) Days post-challenge: FMDV-specific titre 0 or 14 days post-challenge
(f) Challenged intradermally in the tongue with 10^5 bovine infectious doses$_{50}$ (BID$_{50}$) virulent A24
(g) Phosphate-buffered saline
(h) Plaque-forming unit

596

Rev. sci. tech. Off. int. Epiz., **21** (3)

approach is safe and efficacious and overcomes many of the limitations of current FMD vaccines. The combination of vaccination and antiviral treatment introduces the possibility of both immediate and long-term protection, and may address concerns with establishment of carrier animals. However, to demonstrate that these approaches are viable, further research, including work with other FMDV serotypes, which could take two to four years, and large-scale trials that need an additional five to ten years, will be required.

In addition, the use of recombinant molecules as potential vaccines introduces a number of concerns that need to be addressed by regulatory agencies. Although recombinant live-viral vectored vaccines have already been approved for several animal diseases, the use of these vaccines, even if they are replication-defective, in food-producing animals, may require additional safety testing. Nevertheless, the recent spread of FMD to several disease-free countries and the objections expressed in the UK and elsewhere about slaughter of large numbers of animals, indicate that the public and private sectors need to find alternative disease control strategies to address the scientific limitations of current vaccines.

■

Perspectives et délais de mise au point de meilleurs vaccins contre la fièvre aphteuse

M.J. Grubman & P.W. Mason

Résumé
Des vaccins à virus inactivé ont été utilisés avec succès dans le cadre de programmes d'éradication de la fièvre aphteuse. Toutefois, leur emploi suscite quelques inquiétudes et les récentes épizooties de fièvre aphteuse apparues dans des pays indemnes de la maladie ont souligné la nécessité de déployer des stratégies de lutte plus efficaces contre cette maladie. C'est pour répondre à ces exigences qu'une nouvelle génération de vaccins de la fièvre aphteuse a été mise au point. L'un des candidats vaccins actuellement les plus prometteurs est constitué d'une sous-unité de capside virale vide, administrée aux animaux par un vecteur viral vivant. Ce candidat, un adénovirus recombinant de type humain non réplicatif contenant les gènes codant la capside et la protéinase 3C du virus de la fièvre aphteuse, induit une réponse en anticorps neutralisants spécifiques du virus de la fièvre aphteuse chez les animaux vaccinés. Lors d'une épreuve virulente avec un virus homologue passé sur animaux, les porcins et les bovins vaccinés avec cet adénovirus recombinant n'ont présenté aucun signe clinique de la fièvre aphteuse et n'ont pas permis la réplication du virus de la fièvre aphteuse. Une seule injection de ce vaccin candidat à forte dose a protégé des porcins lors de l'épreuve virulente dès le septième jour après la vaccination.

Mots-clés
Adénovirus non réplicatif – Capsides virales vides – Éradication – Fièvre aphteuse – Vaccination – Vaccins.

■

Rev. sci. tech. Off. int. Epiz., **21** (3)

597

Perspectivas y plazos para mejorar las vacunas contra la fiebre aftosa

M.J. Grubman & P.W. Mason

Resumen

Las vacunas inactivadas contra la fiebre aftosa han sido utilizadas con éxito como parte de programas de erradicación. Pero estas vacunas pueden crear problemas, y los recientes brotes de fiebre aftosa en países libres de la enfermedad han hecho tanto más necesaria la elaboración de mejores estrategias de lucha contra la fiebre aftosa. Para obedecer a este requisito se está elaborando una nueva generación de vacunas. Una de las más prometedoras hasta la fecha utiliza una subunidad de nucleocápside viral vacía que se introduce en los animales por medio de un vector vírico vivo. Esta vacuna consiste en un adenovirus humano recombinante sin capacidad de replicación, provisto de las regiones que codifican la nucleocápside y la proteinasa 3C del virus de la fiebre aftosa, que induce en el animal una respuesta de anticuerpos neutralizantes específicos del virus de la fiebre aftosa. Los porcinos y bovinos vacunados con este adenovirus recombinante, al ser expuestos a un virus homólogo virulento cultivado por pase previo en un animal, no manifiestan signos clínicos de la enfermedad y exhiben protección contra la replicación del virus de la fiebre aftosa. La inoculación de una sola dosis elevada de esa vacuna candidata protegió de la enfermedad a los porcinos transcurridos apenas siete días después de la vacunación.

Palabras clave

Adenovirus sin capacidad de replicación – Erradicación – Fiebre aftosa – Nucleocápside viral vacía – Vacunación – Vacunas.

■

References

1. Abrams C.C., King A.M. & Belsham G.J. (1995). – Assembly of foot-and-mouth disease virus empty capsids synthesized by a vaccinia virus expression system. *J. gen. Virol.*, **76**, 3089-3098.

2. Acharya R., Fry E., Stuart D., Fox G., Rowlands D. & Brown F. (1989). – The three-dimensional structure of foot-and-mouth disease virus at 2.9 A resolution. *Nature*, **337**, 709-716.

3. Alkhatib G. & Briedis D.J. (1988). – High-level eucaryotic *in vivo* expression of biologically active measles virus hemagglutinin by using an adenovirus type 5 helper-free vector system. *J. Virol.*, **62**, 2718-2727.

4. Bachrach H.L. (1978). – Foot-and-mouth disease: world-wide impact and control measures. *In* Viruses and environment (E. Kurstak & K. Maramorosch, eds). Academic Press, Inc., New York, 299-310.

5. Bachrach H.L., Moore D.M., McKercher P.D. & Polatnick J. (1975). – Immune and antibody responses to an isolated capsid protein of foot-and-mouth disease virus. *J. Immunol.*, **115**, 1636-1641.

6. Barteling S.J. (2002). – Development and performance of inactivated vaccines against foot and mouth disease. *In* Foot and mouth disease: facing the new dilemmas (G.R. Thomson, ed.). *Rev. sci. tech. Off. int. Epiz.*, **21** (3), 577-588.

7. Baxt B., Vakharia V., Moore D.M., Franke A.J. & Morgan D.O. (1989). – Analysis of neutralizing antigenic sites on the surface of type A12 foot-and-mouth disease virus. *J. Virol.*, **63**, 2143-2151.

8. Beard C., Ward G., Rieder E., Chinsangaram J., Grubman M.J. & Mason P.W. (1999). – Development of DNA vaccines for foot-and-mouth disease, evaluation of vaccines encoding replicating and non-replicating nucleic acids in swine. *J. Biotechnol.*, **73**, 243-249.

9. Beck E. & Strohmaier K. (1987). – Subtyping of European foot-and-mouth disease virus strains by nucleotide sequence determination. *J. Virol.*, **61**, 1621-1629.

598

Rev. sci. tech. Off. int. Epiz., **21** (3)

10. Benvenisti L., Rogel A., Kuznetzova L., Bujanover S., Becker Y. & Stram Y. (2001). – Gene gun-mediated DNA vaccination against foot-and-mouth disease virus. *Vaccine*, **19**, 3885-3895.

11. Bittle J.L., Houghten R.A., Alexander H., Shinnick T.M., Sutcliffe J.G., Lerner R.A., Rowlands D.J. & Brown F. (1982). – Protection against foot-and-mouth disease by immunization with a chemically synthesized peptide predicted from the viral nucleotide sequence. *Nature*, **298**, 30-33.

12. Brooksby J.B. (1982). – Portraits of viruses: foot-and-mouth disease virus. *Intervirology*, **18**, 1-23.

13. Callis J.J. & McKercher P.D. (1986). – Foot-and-mouth disease. *In* Diseases of swine, Sixth Ed. (A. Leman, B. Straw, R.D. Glock, W.L. Mengeling, R.H.C. Penny & E. Scholl, eds). Iowa State University Press, Ames, 337-347.

14. Cedillo-Barron L., Foster-Cuevas M., Belsham G.J., Lefevre F. & Parkhouse R.M. (2001). – Induction of a protective response in swine vaccinated with DNA encoding foot-and-mouth disease virus empty capsid proteins and the 3D RNA polymerase. *J. gen. Virol.*, **82**, 1713-1724.

15. Chan E.W., Wong H.T., Cheng S.C., Yan W.Y., Zheng Z.X., Sheng Z.T., Zhu L.Q. & Xie Y. (2000). – An immunoglobulin G based chimeric protein induced foot-and-mouth disease specific immune response in swine. *Vaccine*, **19**, 538-546.

16. Cheng S.M., Lee S.G., Ronchetti-Blume M., Virk K.P., Mizutani S., Eichberg J.W., Davis A., Hung P.P., Hirsch V.M., Chanock R.M., Purcell R.H. & Johnson P.R. (1992). – Coexpression of the simian immunodeficiency virus Env and Rev proteins by a recombinant human adenovirus host range mutant. *J. Virol.*, **66**, 6721-6727.

17. Chengalvala M.V., Bhat B.M., Bhat R., Lubeck M.D., Mizutani S., Davis A.R. & Hung P.P. (1994). – Immunogenicity of high expression adenovirus-hepatitis B virus recombinant vaccines in dogs. *J. gen. Virol.*, **75**, 125-131.

18. Chinsangaram J., Beard C., Mason P.W., Zellner M.K., Ward G. & Grubman M.J. (1998). – Antibody response in mice inoculated with DNA expressing foot-and-mouth disease virus capsid proteins. *J. Virol.*, **72**, 4454-4457.

19. Couch R.B., Chanock R.M., Cate T.R., Lang D.J., Knight V. & Heubner R.J. (1963). – Immunization with types 4 and 7 adenovirus by selective infection of the intestinal tract. *Am. Rev. intestin. Dis.*, **88**, 394-403.

20. Cowan K.M. & Graves J.H. (1966). – A third antigenic component associated with foot-and-mouth disease infection. *Virology*, **30**, 528-540.

21. Crowther J.R., Farias S., Carpenter W.C. & Samuel A.R. (1993). – Identification of a fifth neutralizable site on type O foot-and-mouth disease virus following characterization of single and quintuple monoclonal antibody escape mutants. *J. gen. Virol.*, **74**, 1547-1553.

22. Davis G.L. (2001). – Treatment of chronic hepatitis C. *BMJ*, **323**, 1141-1142.

23. DiMarchi R., Brooke G., Gale C., Cracknell V., Doel T.R. & Mowat N. (1986). – Protection of cattle against foot-and-mouth disease by a synthetic peptide. *Science*, **232**, 639-641.

24. Doel T.R. (1999). – Optimisation of the immune response to foot-and-mouth disease vaccines. *Vaccine*, **17**, 1767-1771.

25. Domingo E., Mateu M.G., Matnez M.A., Dopazo J., Moya A. & Sobrino F. (1990). – Genetic variability and antigenic diversity of foot-and-mouth disease virus. *In* Virus variability, epidemiology and control, Vol. 2 (E. Kurstak, R.G. Marusyk, F.A. Murphy & M.H.V. Van Regenmortel, eds). Plenum Publishing Corporation, New York, 233-266.

26. Dunn C.S. & Donaldson A.I. (1997). – Natural adaption to pigs of a Taiwanese isolate of foot-and-mouth disease virus. *Vet. Rec.*, **141**, 174-175.

27. Eloit M., Gilardi-Hebenstreit P., Toma B. & Perricaudet M. (1990). – Construction of a defective adenovirus vector expressing the pseudorabies virus glycoprotein gp50 and its use as a live vaccine. *J. gen. Virol.*, **71**, 2425-2431.

28. Fooks A.R., Jeevarajah D., Lee J., Warnes A., Niewiesk S., ter Meulen V., Stephenson J.R. & Clegg J.C. (1998). – Oral or parenteral administration of replication-deficient adenoviruses expressing the measles virus haemagglutinin and fusion proteins: protective immune responses in rodents. *J. gen. Virol.*, **79**, 1027-1031.

29. Francis M.J., Hastings G.Z., Brown F., McDermed J., Lu Y.A. & Tam J.P. (1991). – Immunological evaluation of the multiple antigen peptide (MAP) system using the major immunogenic site of foot-and-mouth disease virus. *Immunology*, **73**, 249-254.

30. Gonin P., Fournier A., Oualikene W., Moraillon A. & Eloit M. (1995). – Immunization trial of cats with a replication-defective adenovirus type 5 expressing the ENV gene of feline immunodeficiency virus. *Vet. Microbiol.*, **45**, 393-401.

31. Graham F.L., Smiley J., Russell W.C. & Nairn R. (1977). – Characteristics of a human cell line transformed by DNA from human adenovirus 5. *J. gen. Virol.*, **36**, 59-74.

32. Grubman M.J., Morgan D.O., Kendall J. & Baxt B. (1985). – Capsid intermediates assembled in a foot-and-mouth disease virus genome RNA-programmed cell-free translation system and in infected cells. *J. Virol.*, **56**, 120-126.

33. Grubman M.J., Lewis S.A. & Morgan D.O. (1993). – Protection of swine against foot-and-mouth disease with viral capsid proteins expressed in heterologous systems. *Vaccine*, **11**, 825-829.

34. Jacobs S.C., Stephenson J.R. & Wilkinson G.W. (1992). – High-level expression of the tick-borne encephalitis virus NS1 protein by using an adenovirus-based vector: protection elicited in a murine model. *J. Virol.*, **66**, 2086-2095.

35. Juillard V., Villefroy P., Godfrin D., Pavirani A., Venet A. & Guillet J.G. (1995). – Long-term humoral and cellular immunity induced by a single immunization with replication-defective adenovirus recombinant vector. *Eur. J. Immunol.*, **25**, 3467-3473.

Rev. sci. tech. Off. int. Epiz., **21** (3)

599

36. King A.M., Underwood B.O., McCahon D., Newman J.W. & Brown F. (1981). – Biochemical identification of viruses causing the 1981 outbreaks of foot and mouth disease in the UK. *Nature*, **293**, 479-480.

37. Kit M., Kit S., Little S.P., DiMarchi R.D. & Gale C. (1991). – Bovine herpesvirus-1 (infectious bovine rhinotracheitis virus)-based viral vector which expresses foot-and-mouth disease epitopes. *Vaccine*, **9**, 564-572.

38. Kitching R.P. (1998). – A recent history of foot-and-mouth disease. *J. comp. Pathol.*, **118**, 89-108.

39. Kitson J.D., Burke K.L., Pullen L.A., Belsham G.J. & Almond J.W. (1991). – Chimeric polioviruses that include sequences derived from two independent antigenic sites of foot-and-mouth disease virus (FMDV) induce neutralizing antibodies against FMDV in guinea pigs. *J. Virol.*, **65**, 3068-3075.

40. Kleid D.G., Yansura D., Small B., Dowbenko D., Moore D.M., Grubman M.J., McKercher P.D., Morgan D.O., Robertson B.H. & Bachrach H.L. (1981). – Cloned viral protein vaccine for foot-and-mouth disease: responses in cattle and swine. *Science*, **214**, 1125-1129.

41. Krebs O., Ahl R., Straub O.C. & Marquardt O. (1993). – Amino acid changes outside the G-H loop of capsid protein VP1 of type O foot-and-mouth disease virus confer resistance to neutralization by antipeptide G-H serum. *Vaccine*, **11**, 359-362.

42. Lewis S.A., Morgan D.O. & Grubman M.J. (1991). – Expression, processing, and assembly of foot-and-mouth disease virus capsid structures in heterologous systems: induction of a neutralizing antibody response in guinea pigs. *J. Virol.*, **65**, 6572-6580.

43. Logan D., Abu-Ghazaleh R., Blakemore W., Curry S., Jackson T., King A., Lea S., Lewis R., Newman J., Parry N., Rowlands D., Stuart D. & Fry E. (1993). – Structure of a major immunogenic site on foot-and-mouth disease virus. *Nature*, **362**, 566-568.

44. Lubeck M.D., Davis A.R., Chengalvala M., Natuk R.J., Morin J.E., Molnar-Kimber K., Mason B.B., Bhat B.M., Mizutani S., Hung P.P. & Purcell R.H. (1989). – Immunogenicity and efficacy testing in chimpanzees of an oral hepatitis B vaccine based on live recombinant adenovirus. *Proc. natl Acad. Sci. USA*, **86**, 6763-6767.

45. Mateu M.G., Camarero J.A., Giralt E., Andreu D. & Domingo E. (1995). – Direct evaluation of the immunodominance of a major antigenic site of foot-and-mouth disease virus in a natural host. *Virology*, **206**, 298-306.

46. Mayr G.A., Chinsangaram J. & Grubman M.J. (1999). – Development of replication-defective adenovirus serotype 5 containing the capsid and 3C protease coding regions of foot-and-mouth disease virus as a vaccine candidate. *Virology*, **263**, 496-506.

47. Mayr G.A., O'Donnell V., Chinsangaram J., Mason P.W. & Grubman M.J. (2001). – Immune responses and protection against foot-and-mouth disease virus (FMDV) challenge in swine vaccinated with adenovirus-FMDV constructs. *Vaccine*, **19**, 2152-2162.

48. Meloen R.H., Casal J.I., Dalsgaard K. & Langeveld J.P. (1995). – Synthetic peptide vaccines: Success at last. *Vaccine*, **13**, 885-886.

49. Moraes M.P., Mayr G.A., Mason P.W. & Grubman M.J. (2002). – Early protection against homologous challenge after a single dose of replication-defective human adenovirus type 5 expressing capsid proteins of foot-and-mouth disease virus (FMDV) strain A24. *Vaccine*, **20**, 1631-1639.

50. Mulcahy G., Gale C., Robertson P., Iyisan S., DiMarchi R.D. & Doel T.R. (1990). – Isotype responses of infected, virus-vaccinated and peptide-vaccinated cattle to foot-and-mouth disease virus. *Vaccine*, **8**, 249-256.

51. Mulcahy G., Pullen L.A., Gale C., DiMarchi R.D. & Doel T.R. (1991). – Mouse protection test as a predictor of the protective capacity of synthetic foot-and-mouth disease vaccines. *Vaccine*, **9**, 19-24.

52. Mulcahy G., Reid E., DiMarchi R.D., Gale C. & Doel T.R. (1992). – Maturation of functional antibody affinity in animals immunised with synthetic foot-and-mouth disease virus. *Res. vet. Sci.*, **52**, 133-140.

53. Nargi F., Kramer E., Mezencio J., Zamparo J., Whetstone C., Van Regenmortel M.H., Briand J.P., Muller S. & Brown F. (1999). – Protection of swine from foot-and-mouth disease with one dose of an all-D retro peptide. *Vaccine*, **17**, 2888-2893.

54. Office International des Epizooties (OIE) (2001). – Foot and mouth disease. *In* International animal health code: mammals, birds and bees, Tenth Ed. OIE, Paris, 63-75.

55. Papp Z., Middleton D.M., Mittal S.K., Babiuk L.A. & Baca-Estrada M.E. (1997). – Mucosal immunization with recombinant adenoviruses: induction of immunity and protection of cotton rats against respiratory bovine herpesvirus type 1 infection. *J. gen. Virol.*, **78**, 2933-2943.

56. Pfaff E., Mussgay M., Bohm H.O., Schulz G.E. & Schaller H. (1982). – Antibodies against a preselected peptide recognize and neutralize foot and mouth disease virus. *EMBO J.*, **1**, 869-874.

57. Pluimers F.H., Akkerman A.M., van der Wal P., Dekker A. & Bianchi A. (2002). – Lessons from the foot and mouth disease outbreak in the Netherlands in 2001. *In* Foot and mouth disease: facing the new dilemmas (G.R. Thomson, ed.). *Rev. sci. tech. Off. int. Epiz.*, **21** (3), 711-721.

58. Prevec L., Schneider M., Rosenthal K.L., Belbeck L.W., Derbyshire J.B. & Graham F.L. (1989). – Use of human adenovirus-based vectors for antigen expression in animals. *J. gen. Virol.*, **70**, 429-434.

59. Prevec L., Campbell J.B., Christie B.S., Belbeck L. & Graham F.L. (1990). – A recombinant human adenovirus vaccine against rabies. *J. infect. Dis.*, **161**, 27-30.

60. Ragot T., Finerty S., Watkins P.E., Perricaudet M. & Morgan A.J. (1993). – Replication-defective recombinant adenovirus expressing the Epstein-Barr virus (EBV) envelope glycoprotein gp340/220 induces protective immunity against EBV-induced lymphomas in the cottontop tamarin. *J. gen. Virol.*, **74**, 501-507.

600

Rev. sci. tech. Off. int. Epiz., **21** (3)

61. Roosien J., Belsham G.J., Ryan M.D., King A.M. & Vlak J.M. (1990). – Synthesis of foot-and-mouth disease virus capsid proteins in insect cells using baculovirus expression vectors. *J. gen. Virol.*, **71**, 1703-1711.

62. Rowlands D.J., Sangar D.V. & Brown F. (1975). – A comparative chemical and serological study of the full and empty particles of foot-and mouth disease virus. *J. gen. Virol.*, **26**, 227-238.

63. Rueckert R.R. (1996). – Picornaviridae: the viruses and their replication. *In* Virology, Third Ed. (B.N. Fields, D.M. Knipe & P.H. Howley, eds). Lippencott-Raven, Philadelphia, 609-654.

64. Rueckert R.R. & Wimmer E. (1984). – Systematic nomenclature of picornavirus proteins. *J. Virol.*, **50**, 957-959.

65. Rweyemamu M.M., Terry G. & Pay T.W. (1979). – Stability and immunogenicity of empty particles of foot-and-mouth disease virus. *Arch. Virol.*, **59**, 69-79.

66. Rweyemamu M.M. & Leforban Y. (1999). – Foot-and-mouth disease and international development. *Adv. Virus Res.*, **53**, 111-126.

67. Salt J.S. (1993). – The carrier state in foot and mouth disease – an immunological review. *Br. vet. J.*, **149**, 207-223.

68. Samuel A.R. & Knowles N.J. (2001). – Foot-and-mouth disease virus: cause of the recent crisis for the UK livestock industry. *Trends Genet.*, **17**, 421-424.

69. Sanz-Parra A., Jimenez-Clavero M.A., Garcia-Briones M.M., Blanco E., Sobrino F. & Ley V. (1999). – Recombinant viruses expressing the foot-and-mouth disease virus capsid precursor polypeptide (P1) induce cellular but not humoral antiviral immunity and partial protection in pigs. *Virology*, **259**, 129-134.

70. Sanz-Parra A., Vazquez B., Sobrino F., Cox S.J., Ley V. & Salt J.S. (1999). – Evidence of partial protection against foot-and-mouth disease in cattle immunized with a recombinant adenovirus vector expressing the precursor polypeptide (P1) of foot-and-mouth disease virus capsid proteins. *J. gen. Virol.*, **80**, 671-679.

71. Sheppard M., Werner W., Tsatas E., McCoy R., Prowse S. & Johnson M. (1998). – Fowl adenovirus recombinant expressing VP2 of infectious bursal disease virus induces protective immunity against bursal disease. *Arch. Virol.*, **143**, 915-930.

72. Taboga O., Tami C., Carrillo E., Nunez J.I., Rodriguez A., Saiz J.C., Blanco E., Valero M.L., Roig X., Camarero J.A., Andreu D., Mateu M.G., Giralt E., Domingo E., Sobrino F. & Palma E.L. (1997). – A large-scale evaluation of peptide vaccines against foot-and-mouth disease: lack of solid protection in cattle and isolation of escape mutants. *J. Virol.*, **71**, 2606-2614.

73. Top F.H. Jr, Buescher E.L., Bancroft W.H. & Russell P.K. (1971). – Immunization with live types 7 and 4 adenovirus vaccines. II. Antibody response and protective effect against acute respiratory disease due to adenovirus type 7. *J. infect. Dis.*, **124**, 155-160.

74. Vakharia V.N., Devaney M.A., Moore D.M., Dunn J.J. & Grubman M.J. (1987). – Proteolytic processing of foot-and-mouth disease virus polyproteins expressed in a cell-free system from clone-derived transcripts. *J. Virol.*, **61**, 3199-3207.

75. Wesseling J.G., Godeke G.J., Schijns V.E., Prevec L., Graham F.L., Horzinek M.C. & Rottier P.J. (1993). – Mouse hepatitis virus spike and nucleocapsid proteins expressed by adenovirus vectors protect mice against a lethal infection. *J. gen. Virol.*, **74**, 2061-2069.

76. Wigdorovitz A., Carrillo C., Dus Santos M.J., Trono K., Peralta A., Gomez M.C., Rios R.D., Franzone P.M., Sadir A.M., Escribano J.M. & Borca M.V. (1999). – Induction of a protective antibody response to foot and mouth disease virus in mice following oral or parenteral immunization with alfalfa transgenic plants expressing the viral structural protein VP1. *Virology*, **255**, 347-353.

77. Wigdorovitz A., Perez Filgueira D.M., Robertson N., Carrillo C., Sadir A.M., Morris T.J. & Borca M.V. (1999). – Protection of mice against challenge with foot and mouth disease virus (FMDV) by immunization with foliar extracts from plants infected with recombinant tobacco mosaic virus expressing the FMDV structural protein VP1. *Virology*, **264**, 85-91.

78. Wong H.T., Cheng S.C., Chan E.W., Sheng Z.T., Yan W.Y., Zheng Z.X. & Xie Y. (2000). – Plasmids encoding foot-and-mouth disease virus VP1 epitopes elicited immune responses in mice and swine and protected swine against viral infection. *Virology*, **278**, 27-35.

79. Xiang Z.Q., Yang Y., Wilson J.M. & Ertl H.C. (1996). – A replication-defective human adenovirus recombinant serves as a highly efficacious vaccine carrier. *Virology*, **219**, 220-227.

80. Yang P.C., Chu R.M., Chung W.B. & Sung H.T. (1999). – Epidemiological characteristics and financial costs of the 1997 foot-and-mouth disease epidemic in Taiwan. *Vet. Rec.*, **145**, 731-734.