

## Synthetic Peptide-based Vaccine and Diagnostic System for Effective Control of FMD



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**Abstract.** We have designed synthetic peptides corresponding to two different regions of the genome of foot-and-mouth disease virus (FMDV) that are effective as (a) a vaccine or (b) a diagnostic reagent which differentiates convalescent from vaccinated animals, respectively. The peptide vaccine is based on a sequence from the prominent G-H loop of VP1, one of the four capsid proteins. The sequence was optimized by the inclusion of a cyclic constraint and adjoining sequences, and broader immunogenicity was obtained by the incorporation of consensus residues at hypervariable positions. The peptide also included a promiscuous T-helper epitope for effective immunogenicity in outbred populations of large animals.

The diagnostic reagent, a peptide based on non-structural (NS) protein 3B, is used in immuno-assays for the detection of antibodies. Antibodies to this NS protein are present in the sera of infected animals but not in the sera of vaccinated animals. The VP1 peptide can be used in complementary immuno-assays for confirmation of NS test results and to monitor for vaccination. This system for differential diagnosis is important to establish the disease-free status of a country. © 2001 The International Association for Biologicals

**Key words:** FMDV, peptides, vaccine, diagnostics, system, control.

### Introduction

Foot-and-mouth disease virus (FMDV) is the most contagious agent known to infect cloven-hoofed animals, including ruminants and swine. Control in those countries where the disease does not normally occur is by culling infected and exposed animals, quarantine, and vaccination. In addition to those expenditures, a significant cost for many affected countries is the loss of markets due to lengthy trade restrictions imposed by FMD-free countries. The period for costly control measures and the time needed for a country to establish or re-establish FMDV-free status is protracted because of the disadvantages of the currently accepted immuno-assays. These assays do not distinguish infected from vaccinated animals and consequently

cannot be used to identify asymptomatic carrier animals that may be present in vaccinated herds. Accordingly, a country with vaccinated animals cannot be proved to be FMD-free under the rules of international trade.<sup>1</sup> Under these rules, countries that would otherwise use vaccines to expedite eradication of FMD, while limiting the slaughter of healthy animals, are reluctant to use the vaccines that are available.

We have overcome the problem of differentiating infection from vaccination by designing a synthetic peptide antigen from FMDV NS 3B as the sensitizing agent for differential enzyme-linked immuno-assays (EIAs).<sup>2</sup> The 3B peptide is an immuno-dominant antigen that imparts the UBI NS FMDV EIA tests with high sensitivity and the precision to detect antibodies elicited by infection differentially while excluding antibodies to vaccination. A synthetic peptide EIA based on a structural protein determinant, in this case the immuno-dominant G-H loop from VP1,<sup>3</sup> has also

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been developed. It can be used both as a confirmatory test for the NS EIAs and as the means to monitor the effectiveness of vaccination campaigns by confirming vaccination status. The peptide-based tests are inexpensive and can be used safely to monitor infection or vaccination in any clinical laboratory equipped for the ELISA format.

The current vaccines are effective in preventing the disease but the antigenic variability of the virus demands that they must be closely matched with the circulating virus. Consequently, a strategy incorporating a peptide-based vaccine which provides a wider spectrum of protection against antigenic variants would have great advantages. The precise and rational approach of site-specific synthetic peptide immunogens has given us the means to produce a vaccine with a high concentration of a neutralizing epitope and allowed for an immunogen designed for broader cross-reactivity. Moreover, a site-specific vaccine also acts as an antigenic marker vaccine to which the immune response is readily differentiated from the immune response to infection. We have focused our attention on the G-H loop structure of the VP1 capsid protein. This is a surface-exposed immuno-dominant site and the target for neutralizing antibodies which was first described in the early 1980s.<sup>3</sup> VP1 loop peptide vaccines evoke neutralizing antibody responses and have provided protection from infection in small animal models. However, they have usually shown only limited immunogenicity in hoofed species. The VP1-loop region itself lacks T-helper cell epitopes for recognition by bovine and porcine MHC and needs the addition of promiscuous T-cell help from an outside source.<sup>3-6</sup> An effective VP1 peptide vaccine also needs potent B cell sites for the induction of high affinity neutralizing antibodies.<sup>7,8</sup> We have used peptide design technology to produce a synthetic peptide immunogen with T- and B-cell sites developed for broad immunogenicity and full protection in swine. The hypervariability and narrow type-specific protection of VP1 loop immunogens has been another problem. Synthetic peptide technology has been used here to design G-H loop immunogens with cross-reactivities to a broad spectrum of viruses. Type-specific hypervariable positions are substituted with the consensus amino acids of the desired serotype.

A system that uses vaccination by synthetic peptides combined with immuno-surveillance by the differential peptide-based tests would be a complementary programme. It would be free of biohazard and could be used to expedite eradication of FMD

while minimizing blind culling and the need to slaughter vaccinated animals.

## Materials and methods

### *Peptide synthesis*

The peptide antigens for immuno-assays and the peptide immunogens for vaccines were produced by synthesis on a solid-phase support using an Applied Biosystems Peptide Synthesizer Model 430A, and Fmoc protection for the  $\alpha$ -NH<sub>2</sub> terminus and side-chain protecting groups of tri-functional amino acids. Peptides with combinatorial library Th and B-cell target sites were prepared by providing a mixture of the desired amino acids at the specified positions. Completed peptides were cleaved from the solid support and side chain protecting groups removed by 90% trifluoroacetic acid. Synthetic peptide preparations, except for the library immunogen (see Fig. 2B), were characterized for correct composition by Matrix-Assisted Laser Desorption Time-of-Flight Mass Spectrometry using a PerSeptive Biosystems/Vestec LaserTec Benchtop 11 Mass Spectrometer, and by Reverse Phase HPLC.

### *Serum samples*

The bovine and swine seroconversion panels (Tables 1 and 2B) were serial bleeds collected following in-contact or inoculated infections at the biocontainment facilities at the USDA Plum Island Animal Disease Center (PIADC), Greenport, NY, U.S.A. These and other sera from PIADC were drawn from the reagent repository at the Foreign Animal Disease Diagnostic Laboratory. An abattoir in New Jersey (U.S.A.) provided normal swine sera. Sera for field trials of the immuno-assays were supplied by the National Institute of Animal Health Taiwan and the Council of Agriculture, Taiwan. Sera from the vaccine trial were collected and analysed at the containment facilities of the National Institute of Animal Health Taiwan, Tanshui.

### *EIA method*

The EIA tests were done by the ELISA procedures as reported,<sup>2</sup> except that the VP1 EIA used the serotype O consensus peptide without the UBITH<sup>®</sup>1 site as antigen; results are reported as Signal/Cutoff ratios; and the chromagen is 3,3',5,5'-tetramethyl benzidine (TMB). The signal is the absorbance of the sample at 450 nm and the cut-off value is 0.23  $\times$  Abs of reactive control.

**Table 1.** Results of immunogenicity/challenge trial in pigs. Pigs were immunized at weeks 0 and 4 with UBITH<sup>®</sup>1-VP1 peptide immunogen (Fig. 2A) in indicated formulations with ISA51, except for group 4 which also received a VP1 immunogen with the target VP1 sequence of FMDV<sub>O1Taiwan</sub> without UBITH<sup>®</sup> and linker (Fig. 2C). Neutralizing antibody (NA) titres, expressed as reciprocal dilutions, were determined by quantitative VN microtest.<sup>14</sup> Protection was scored by absence of clinical signs of FMD

Group	Vaccine	Tag no.	NA titre (day 0)	NA titre (day 40)	Protection
1	UBITH <sup>®</sup> 1-VP1 100 µg/0.5 ml/dose	368	≤3	64	+
		369	≤3	4	+
		370	≤3	23	+
2	UBITH <sup>®</sup> 1-VP1 50 µg/0.5 ml/dose	373	≤3	32	-
		374	≤3	91	+
		375	≤3	64	+
3	UBITH <sup>®</sup> 1-VP1 50 µg/1.0 ml/dose	377	≤3	45	+
		378	≤3	362	+
		379	≤3	362	+
4	UBITH <sup>®</sup> 1-VP1 <sup>a</sup> 50 µg/1.0 ml/dose	394	≤3	181	+
		395	≤3	256	+
		397	≤3	32	+
5	UBITH <sup>®</sup> 1-VP1 50 µg/1.5 ml/dose	381	≤3	256	+
		382	≤3	362	+
		383	≤3	64	+
6	UBITH <sup>®</sup> 1-VP1 25 µg/0.5 ml/dose	386	≤3	32	+
		388	≤3	181	+
		389	≤3	91	+
7	UBITH <sup>®</sup> 1-VP1 12.5 µg/0.5 ml/dose	390	≤3	45	+
		391	≤3	64	+
		392	≤3	45	+
8	Adjuvant 0.5 ml/dose	371	≤3	≤3	-
		372	≤3	≤3	-
		387	≤3	≤3	-
9	Commercial FMDV O <sub>1Taiwan</sub> vaccine	376	≤3	512	+
		380	≤3	≥724	+
		393	≤3	≥724	-

<sup>a</sup>Consensus FMDV O peptide immunogen (Fig. 2A) and O1 Taiwan peptide immunogen (Fig. 2C) in 1:1 ratio.

## Results and Discussion

### Synthetic peptide vaccine

A series of peptides to imitate the well-known G-H loop of VP1 was synthesized with varying lengths of the VP1 sequence, with and without cyclic constraint, to retain the flexible loop conformation,

and additional immunostimulatory sequences. The candidate peptide immunogens were used to immunize guinea pigs. The most potent immunogens with functional cross-reactivity to the virus were selected by testing the immune sera for neutralizing antibodies. It was found in a study to be published elsewhere for an FMDV PanAsia O isolate that: (1)

**Table 2.** Sensitivity of UBI FMDV NS (swine) EIA during disease and convalescence of experimentally infected swine

Days post-infection	No. positive	No. tested	Sensitivity (%)
1-4	1	12	8.3
5-13	6	19	31.5
14-98	13	14	93.3
105-210	12	15	83.3
217-301	11	13	86.7

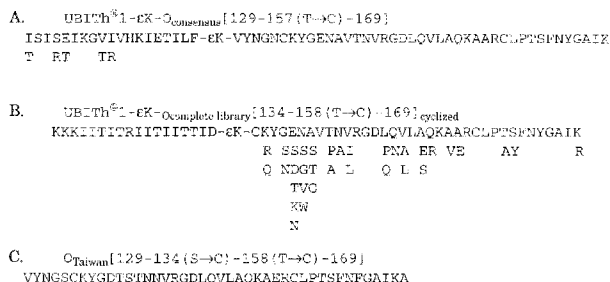
Seven swine were exposed (contact and inoculation). Four contact swine were maintained after acute infection for long term study.<sup>13</sup> Overall sensitivity of the NS test, excluding days 1-13 was 85.7%.

Consensus O VP1 site	VYNGCKYGENAVTVNRGDLQVLAQKAARCLPTSPNYGAIK
Corresponding VP1 sequences from FMDV O isolates	
○ TMN/ 1/97 (O1 Taiwan)	VYNGSSKYGCTSTNNVRGDLQVLAQKAERKLTPTSPNYGAIK
○ TMN/ 2/99	VYNGCKYGESVTVNRGDLQVLAQKAARLTPTSPNYGAIK
○ UK/ 2/01	VYNGCKYGESVTVNRGDLQVLAQKAARLTPTSPNYGAIK
○ CHN/GD/86	VYNGCKYSEKAVTVNRGDLQVLAQKAARLTPTSPNYGAIK
○ Campos/BR	VYNGCRYSKAVTVNRGDLQVLAQKAARLTPTSPNYGAIK
○ Mansfa/TK/69	VYNGCKYGGTVANVRGDLQVLAQKAARLTPTSPNYGAIK

**Figure 1.** Alignment of corresponding VP1 immunogenic sites from prevalent FMDV O isolates and comparison to O consensus site.

an extended G-H loop domain peptide having the loop and flanking segments from amino acid residues 129-169 provided for stronger neutralizing activity than shorter peptides; (2) the introduction of cyclic constraint by cysteine residues positioned 8 Å apart at residues 134 and 158 was necessary for high neutralization activity; and (3) chimaeric constructs having an extrinsic T-helper epitope (Th) were more effective than immunogens which relied on the Th intrinsic to the loop domain. Other design elements were then tested to obtain synthetic immunogens that would not be strain-specific.

We aligned VP1 sequences from historic and prevalent serotype O isolates and consensus amino acids were selected for each variable position. The derived O consensus sequence is shown at the top of Figure 1 where it is compared to a PanAsia sequence found in a U.K. isolate from the outbreak of February 2001<sup>9</sup> and other prevalent serotype O strains. The final consensus peptide immunogen (Fig. 2A) was provided with extrinsic T-cell help by attachment to an epsilon-Lys linker and to a proprietary combinatorial library T-helper site (UBIth<sup>®</sup>1). The Th site was derived from measles virus fusion protein.<sup>10</sup> Guinea pig antibodies evoked by the chimaeric consensus peptide displayed cross-

**Figure 2.** UBI FMDV O peptide immunogens.

serotype neutralizing indices against FMDV A<sub>12</sub>, two O<sub>1</sub> isolates, and an Asia<sub>1</sub> isolate.

We also designed for broad cross-reactivity by a combinatorial library approach to the VP1 domain of serotype O isolates that provides every alternative residue,<sup>11</sup> as shown in Figure 2B. A peptide immunogen with this site was used in pigs in an immunogenicity study. It did not provide broader neutralizing activity than consensus immunogens and was discontinued because it would be impractical to manufacture such a peptide as a large-scale cGMP product. A Taiwan O<sub>1</sub>-specific sequence (Fig. 2C) was included as a vaccine component together with a consensus peptide in a vaccine challenge trials in pigs (Table 1, group 4). It too was discontinued because it did not contribute to neutralizing activity and protection above that of the consensus peptide (see below).

A vaccine trial done at the Pig Research Institute Taiwan and the National Institute of Animal Health (Taiwan) included nine groups of three pigs each. Experimental groups 1-8 were given UBIth<sup>®</sup>1 peptide immunogens prepared as water-in-oil emulsions in adjuvant Montanide ISA 51 (Seppic, Paris, France), in doses ranging from 12.5 to 100 µg (Table 1). The peptide for groups 1-3 and 5-7 was the consensus peptide immunogen shown in Figure 2A. Group 4 was immunized with the consensus peptide (Fig. 2A) mixed 1:1 with the Taiwan O<sub>1</sub>-specific peptide (Fig. 2C). Group 9 was given a commercial killed virus vaccine specific for FMDV O<sub>1</sub> Taiwan for comparison. Group 8 received adjuvant only. The pigs were immunized on days 0 and 28 and challenged with O<sub>1</sub> Taiwan virus on day 56.

By day 40, most immunized animals had attained significant levels of neutralizing antibodies against FMDV O<sub>1</sub> Taiwan and all but one of the 21 peptide-immunized animals were protected from disease (Table 1). The group 4 pigs that received a vaccine spiked with the peptide specific for the challenge virus did not develop higher neutralizing titres

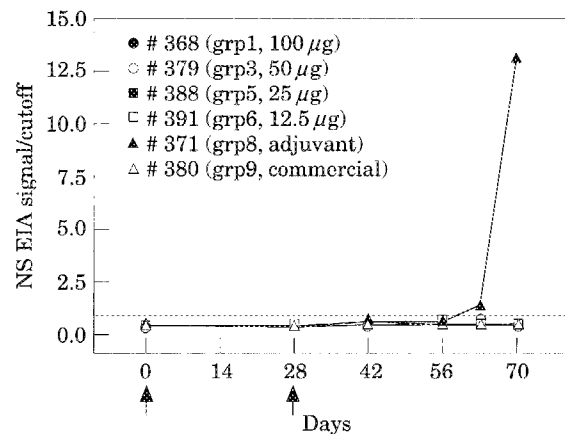
**Table 3.** Sensitivity of UBI FMDV NS (ruminant) EIA on bovid FMD serum panels

A	Disease and convalescence	No. positive	No. tested	Sensitivity (%)
	Experimental infection (Brazil)	12	12	100
	Experimental infection (Brazil)	29	29	100
	Gaur	3	3	100
	Buffalo (Brazil)	23	23	100
	Buffalo (Brazil)	8	8	100
B	Experimental infection (PIADC) days post-infection			
	1-4	0	2	0
	5-13	NT	NT	
	14-98	14	14	100
	105-210	19	19	100
	217-301	11	15	73.3
	308-365	7	11	63.6

against the O1 Taiwan virus than did the groups given the O consensus immunogen alone. The peptide-based vaccines were effective throughout the dose range.

The unprotected peptide-immunized animal (number 373) had been given 50 µg of peptide in 0.5 ml so that the one vaccine failure was not related to dose size; in addition, the animal had developed neutralizing antibodies so that failure was not simply because the animal failed to respond. Pig numbers 371 and 387 of adjuvant control group 8 developed vesicles by the fourth day post-challenge and developed further signs of FMD through day 8. Pig number 372 of the control group had a raised temperature and lameness by day 4. One of the pigs in killed virus vaccine group 9 developed vesicles on the leg by day 11 post-challenge, typical of delayed FMD, despite having a neutralization titre higher than the peptide-immunized animals that were fully protected against disease. The other two pigs of the conventionally vaccinated group were protected (Table 1).

The pigs were also monitored throughout the trial for reactivity to the VP1 consensus peptide by UBI VP1 peptide ELISA and for reactivity to 3B NS peptide by UBI NS peptide ELISA. All the immunized animals had measurable anti-VP1 peptide antibodies by day 28 which peaked by day 42 for the peptide groups and by day 56 for the killed vaccine group. The control group had no anti-VP1 reactivity until after challenge (data not shown). The two control animals with the most signs of disease, numbers 371 and 387, developed sustained antibody responses against the NS peptide following exposure. NS reactivities for representative animals



**Figure 3.** Seroconversion to NS reactivity. Sera were prepared and tested by the UBI<sup>®</sup> NS (swine) EIA having a 3B non-structural peptide as antigen for antibody capture.<sup>2</sup>

of the groups are shown in Figure 3. The readily measurable antibody responses of the peptide-immunized animals by the VP1 EIA, and the correlation of that response to protective immunity, suggests that the VP1 EIA (using consensus VP1 loop peptide) is a useful assay for monitoring the efficacy of the peptide vaccines. The lack of seroconversion by the protected animals to reactivity to the NS peptide ELISA suggested that the synthetic peptide vaccines strongly limited viral replication as well as providing protection against the disease.

The consensus peptide approach has provided for broadly neutralizing activities without careful molecular modelling of the G-H loop antigenic site. This empirical approach for selecting residues was successful for the cyclized peptide. Cyclic constraint of

the loop conformation was a possible key contributor to effective immunogenicity, as had been suggested for G-H loop immunogens.<sup>12</sup> Extending the peptide to include the complete VP1 G-H loop and flanking sequences, amino acid residues 129-169, also contributed to the protective effect of the peptide-generated antibodies. Furthermore, the use of promiscuous foreign T-cell help has immunopotentialized the VP1 peptide immunogen sufficiently to provide full protection from infection in an economically significant host species. This T helper site has also been shown to be immunogenic in goats and cattle. By May 2001 in five separate immunization/challenge trials, we have protected 45 out of 46 pigs from FMDV infection with the UBITH<sup>®</sup>1 consensus peptide immunogen. In these studies peptide doses ranging from 12.5 to 300 µg were protective for swine and the oil-in-water vaccine delivery system has not caused adverse localized reactions. These results are predictive of success in field trials in terms of efficacy, regulatory approvals, and for achieving a marketable vaccine product.

#### *Synthetic peptide ELISAs*

UBI has developed peptide-based enzyme immuno-assays (EIAs) for FMDV that differentiate the antibody response to infection from the antibody response to vaccination. These were developed by adapting our technology for indirect ELISAs that use synthetic peptide as a solid-phase immunosorbent. We selected a peptide for the 3B non-structural (NS) protein of the virus as the sensitizing agent for the differential test for the detection of infection.<sup>2</sup> 3B is expressed at high levels in infected animals but non-structural proteins are at worst minor components of the killed virus vaccines,<sup>13</sup> and are not at all present in synthetic peptide vaccines. Also, NS proteins vary little among the diverse serotypes of FMDV so that antigenic variability is not an obstacle.

NS protein 3B was selected for our test through a process of site-directed serology that showed 3B to be the most immunogenic of the NS proteins. The UBI NS FMDV EIAs are provided in two formats, one for swine only and one for ruminants and other species. The 3B peptide-based immuno-assays have been shown to have 99.6-100% specificity in naïve populations of swine and cattle and 100% sensitivity on a serum panel from animals infected by all seven FMDV serotypes, and panels from experimentally infected swine and cattle.<sup>2</sup> The sensitivity of the UBI FMDV NS (swine) EIA was

evaluated further in a 1999 survey of 73 swine sera collected by the Foreign Disease Diagnostic Laboratory (FADDL) at the USDA Plum Island Animal Disease Center (PIADC) from a seroconversion study on seven experimentally infected animals (Table 2). The sensitivity on the samples collected from days post-infection 14 to 301 was 85.7% (36/42). Sensitivity was 93.3% for days post-exposure 14 to 98. In a survey for specificity, of 704 samples collected by a slaughterhouse in New Jersey (U.S.A.), two were initially reactive. These two were re-tested for confirmation by repeat reactivity and were non-reactive, for a final specificity on those 704 sera of 100%.

The sensitivity of the UBI FMDV NS (ruminant) EIA was further determined on sera panels in the FADDL collection obtained from experimentally infected cattle, and convalescent buffalo (*Bubalus bubalis*) and gaurs (*Bos frontalis*) (Table 3A). The test sensitivity on these panels was 100%. Sensitivity results are also presented (Table 3B) from additional seroconversion panels collected from six bovines experimentally infected at PIADC. Four of the five animals that were sampled long-term had been infected by contact, in mimicry of natural infection.<sup>13</sup> From days post-infection 14 to 210, sensitivity was 100%. The ability of the ruminant NS immuno-assay to detect infection was somewhat reduced beyond 210 days. Seventy-four cattle given multiple vaccinations with conventional killed virus vaccines, from an experimental trial and from the field, showed a specificity of 100% (data not shown).

In field trials, sensitivities and specificities have varied due to the uncontrolled factors of time of infection, lower infectious doses, and the various compositions of killed virus vaccines used in countries that vaccinate. However, field trials show that the peptide-based NS EIA has sensitivity and specificity, in the absence or presence of vaccination, sufficient to ensure that a herd is uninfected or has a history of exposure. The NS EIA test can be used in conjunction with the peptide-based VP1 EIA for confirmation of NS results. Animals whose sera show ratios of VP1 reactivities to NS reactivities of >1.7 can be identified as vaccinates and not as infected because the antibody response to natural infection is predominantly directed against non-structural proteins.<sup>2,13</sup> The VP1 peptide is serotype-specific so that the particular test will vary by the serotypes of vaccines used and prevalent isolates.

The performance characteristics for the UBI FMDV NS and VP1 EIAs were defined in field trials

**Table 4.** Reactivities by UBI NS and VP1 EIAs of Swine Herds in Taiwan

Herd status	n	N EIA reactive (%)				
		NS	VP1	NS & VP1	Ref* (>1.7)	Neg
Infection suspected	212	120 (56.6)	65 (30.7)	39 (18.4)	0	85
Unexposed vaccinates	481	16 (3.3)	198 (41.2)	11 (2.3)	8	279
Exposed vaccinates	973	213 (21.9)	330 (33.9)	138 (14.2)	32	556
Normal	167	3 (1.8)	4 (2.4)	2 (1.2)	0	162
Unknown	40	12 (30.0)	20 (50.0)	11 (27.5)	3	19

\*When samples are found to be reactive with both VP1 and NS EIAs, the Ref value VP1/S/C/NSS/C is determined (S/C=signal/cutoff). If Ref value is >1.7, the animal is considered to be effectively vaccinated rather than infected.

conducted in Taiwan, coordinated by the Republic of China Council of Agriculture in 1998. The field trials included the screening of 26 herds of swine of varying status with respect to FMDV exposure and vaccination (Table 4). Of 212 unvaccinated swine from two herds suspected to have had exposure to FMDV O1 Taiwan, 56.6% were reactive in the NS EIA (swine) and 30.7% were reactive in the VP1 EIA for serotype O. For sera having a dual VP1/NS reactive pattern, their Ref values (i.e. VP1 S/C to NS S/C ratios) were all <1.7. The patterns of NS and VP1 reactivities reflect the fact that the predominant antibody response during natural infection is directed against non-structural proteins and that the animals had not been vaccinated.

In contrast, when 481 swine from nine herds assumed to have received vaccines of varying qualities against FMDV O but no exposure to FMDV were tested for reactivity in the NS EIA, 16 (3.3%) were reactive. Eight of these reactive animals had Ref values >1.7 consistent with vaccinate status, leaving a predominant NS reactivity rate of only 1.6%. In keeping with the presumed vaccinate status of these herds, 41.2% were reactive in the VP1 EIA specific for serotype O. These two reactivity rates are consistent with uninfected vaccinate status for these herds.

When a third category of 10 swine herds comprising 973 animals from farms with previous FMDV infection history and assumed to have received various FMDV O vaccines were screened with the peptide-based NS EIA, 213 (21.9%) were reactive. A more significant fraction of these vaccinated herds with potential exposure had NS reactivities than the above group of unexposed vaccinated herds. The results of the VP1 EIA show that 33.9% of the animals were reactive, consistent with them having been vaccinated. However, of the 138 animals with dual VP1/NS reactivities, 106 had Ref values >1.7.

This pattern is evidence for the presence of infected animals in these vaccinated herds. The post-infection interval varied greatly in this sample population so it is probable that some long-term convalescent animals were not identified by the NS EIA.

The NS EIA detected the herds from farms with histories of FMD, in the presence or absence of vaccination. In general, VP1 EIA reactivity rates did not differ greatly between the vaccinated, infected, or infected/vaccinated populations screened during field trials, emphasizing the need for the differential NS EIA to distinguish the antibody response to vaccination from that of infection. From the data of individual herds (not shown), the herds with the most complete histories for vaccine implementation had the highest rates of VP1-reactive animals. The low rates of VP1 reactivity in certain herds assumed to have been vaccinated was evidence for failure to implement, or the failure of certain vaccines.

Serological surveillance using the UBI NS EIA should be incorporated into national FMDV control programmes. The test can be used to promote and evaluate vaccination while minimizing the use of culling as a control measure.

The 3B peptide-based NS EIA alone and in combination with the VP1 peptide-based EIA is an ideal differential immuno-assay system when combined with the UBITH<sup>®</sup>1 synthetic peptide vaccine. Vaccinated but uninfected animals will be positive for VP1 reactivity but invariably non-reactive by the NS EIA because the chemically-defined VP1 immunogen of the vaccine is a marker antigen with no NS component. In contrast, vaccinated animals exposed to replicating virus will be positive for VP1 and will also have seroconverted to reactivity on the NS EIA (Fig. 3). The VP1 test can also be useful to confirm the NS test when testing non-vaccinated

animals. The UBI VP1 EIAs are simple and inexpensive methods to monitor the efficacy of vaccination campaigns.

### Conclusions

An effective synthetic peptide-based vaccine against FMD has been designed and tested in pigs. It includes the design elements of (1) cyclization to constrain a loop conformation like the one found on the VP1 capsid protein; (2) extensions to include sequences both upstream and downstream of the loop; (3) a consensus VP1 sequence for broad cross-reactivity; and (4) a UBITH<sup>h</sup>1 helper site for potent and promiscuous T cell help that will engender immune responsiveness in outbred populations and in multiple species.

The FMDV peptide vaccine was shown to provide reliable protection from disease of swine, a natural host species. The vaccine delivery system is effective without local adverse reactions. Therefore, regulatory approvals and customer acceptance are anticipated. It can be produced and sold at low cost because small peptide doses in the range of 12.5 µg to 50 µg have been effective.

A complementary synthetic peptide-based system for differential immuno-diagnostics has also been provided. The NS peptide test detects infection and differentiates infection from vaccination. The VP1 peptide test can provide confirmation of NS results and can be used to monitor the effectiveness of national vaccine campaigns.

A synthetic peptide-based vaccine in combination with the peptide-based tests is an integrated system for FMD control. It provides an ideal marker antigen vaccine system for the unambiguous differentiation of vaccinated from infected and carrier animals.

The absolute non-infectivity and differential capability of a synthetic peptide-based system can encourage the use of vaccines and immunosurveillance to expedite FMD eradication and the establishment of a disease-free status.

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