Comparative evaluation of serological tests for the detection of foot-and-mouth disease virus infection in vaccinated pigs

David Paton¹, Donal Sammin², Kitman Dyrtja³, Mary Chow³, Lucy Fleming¹, Blesilda Verin⁴, Nigel Ferris¹, Goretti McDonagh², Michael O'Connor², Pip Hamblin¹, Debbi Gibson¹ and Satya Parida¹.

¹Institute for Animal Health, Ash Road, Pirbright, Surrey, UK.
²Department of Agriculture and Food, Celbridge, Co. Kildare, IRELAND
³Agriculture, Fisheries and Conservation Department, HK-SAR.
⁴Bureau of Animal Industry, Department of Agriculture, PHILIPPINES.

Introduction: Serological diagnosis of foot-and-mouth disease (FMD) was investigated in Hong Kong where pig herds are known to be infected periodically with serotype O and preventive vaccination is widely used. The aim of the study was to identify herds containing either vaccinated or vaccinated and infected pigs and to collect blood samples to evaluate the specificity and sensitivity of tests for detection of antibodies to non-structural viral proteins (NSP) that are designed to identify infected animals within a vaccinated population.

Materials and methods: Four herds were selected in each of which a vaccine of European manufacture was used. Four hundred and five pigs were sampled and categorized into infected and uninfected classes on the basis of disease history, presence of hoof lesions at the time of sampling and results of conventional serological tests for antibodies to viral structural proteins. In a follow-up study to further assess specificity of NSP tests, ninety-nine Irish pigs (known with certainty not to be infected) were sampled and tested after vaccination against serotype O.

Results: The three NSP tests that were compared detected infection in all groups of pigs with clinical signs of FMD but with differing specificities and sensitivities; when used in uninfected vaccines, diagnostic specificity of the tests varied from 98% to 100%.

Discussion: Further work is required to determine the frequency with which vaccinated pigs become subclinically infected and to establish how readily these can be detected by serology.

Introduction:

If an outbreak of foot-and-mouth disease (FMD) were to occur within the European Union (EU), it is increasingly likely that emergency vaccination would be deployed to help control the spread of disease. The European Council directive 2003/85/EC on intra-community measures for control of FMD makes provisions for a “vaccinate to live” policy, and sets out the post-vaccination surveillance that should be conducted to demonstrate freedom from infection at the end of the control process. This surveillance would include serology using ELISAs, which detect antibodies to non-structural proteins of FMD virus (NSPEs), since these tests can discriminate between infected and vaccinated animals (DIVA tests). In order to develop and have confidence in vaccination and testing strategies it is essential that NSPEs are validated for DIVA purposes on all species of livestock that may be vaccinated.

A workshop was held at IZSLER, Brescia, Italy in May 2004 to compare the performance of six NSPEs when used to test sera from vaccinated and from vaccinated-and-infected animals. Data derived from the bovine sera tested at this workshop has been analyzed (Brocchi et al., 2006). Although some ovine and porcine sera were also tested as part of that comparative evaluation, insufficient data were generated to validate the use of NSPEs for DIVA purposes in those species. Other reports describe the evaluation of pig sera in NSPEs (Chung and others 2002b and 2003; Lee and others 2004; Bruderer and others 2004), but not all of the currently available tests have been included and the
circumstances of vaccination, exposure and sampling have not been fully relevant to emergency vaccine use in Europe.

It was considered that a cost-effective and humane alternative to experimental challenge studies, allowing sufficient sera to be sourced from FMD vaccinated pigs with and without infection, would be to conduct a prospective study in an FMD-endemic country where pigs are kept in relatively large numbers, where outbreaks of clinical FMD are regularly reported and where mass prophylactic vaccination of pigs still continues. Such conditions exist within the Hong Kong-Special Administrative Region (HK-SAR). Therefore, it was decided that clinical specimens and relevant epidemiological information should be collected from FMD-vaccinated and convalescent pigs in HK-SAR and that the specimens should be sent for testing to the FAO World Reference Laboratory for FMD based at the Institute for Animal Health’s Pirbright laboratory, UK (WRL). However, uncertainty about the vaccination and infection status of some of the pigs sampled in HK-SAR prompted an additional experimental study in Ireland (which is designated by the OIE as "FMD-free without vaccination").

The primary purpose of this study was to generate information on the performance of NSPEs when used to test porcine sera, so that these tests could be used with confidence to identify infected pigs in the aftermath of a future FMD outbreak in Europe where emergency vaccination had been deployed.

**Materials and methods:**

**Initial investigation of FMD outbreaks in HK-SAR:**

During the two-month period from the end of December 2004 until the end of February 2005, veterinary staff of the HK-SAR Agriculture, Fisheries and Conservation Department (AFCD) investigated all reported outbreaks of FMD in pigs with a view to facilitating the present study. On-farm investigations consisted of: (i) an interview of the owner or keeper of the pigs, during which a questionnaire was used to obtain relevant epidemiological information; (ii) clinical examination of affected animals and (iii) collection of lesion material (epithelial fragments from vesicular lesions) from acutely affected animals. If the owner of the pigs agreed to participate in the present study, clinically affected and in-contact animals were individually identified with numbered ear tags. Lesion material was submitted to the AFCD’s Veterinary Laboratory Division (VLD) to confirm the diagnosis of FMD.

**Collection of clinical specimens in HK-SAR:**

Serum samples were collected not sooner than 30 days after clinical disease was first noticed in each herd. All "sampled" pigs had been vaccinated at least once against FMD with a double-oil emulsion vaccine containing purified, inactivated antigen from two different serotype O viruses, O1 Manisa and O 3039 ("Aftopor", Merial Animal Health Limited, Pirbright, UK); all were between three and twelve months of age at the time of sampling. Pigs were restrained by mouth-snaring and blood was collected from the jugular vein or anterior vena cava into a vacutainer tube and allowed to clot. At VLD (HK-SAR), serum was harvested from clotted blood samples and heat-treated at 56°C for 30 minutes before storing at -20°C. When sampling was completed at all locations a single shipment of all specimens was sent on dry ice by airfreight to WRL (UK).

**Experimental study of vaccinated pigs in Ireland:**

Ninety-nine Irish pigs sourced from a minimal disease herd were vaccinated on two occasions with monovalent vaccine of the same manufacture and serotype/strain as that used in HK-SAR. The second vaccination was administered 28 days after the first. Serum was obtained from each pig immediately prior to each administration of vaccine and again 28 days after second vaccination; pigs were vaccinated and sampled during February-April 2006.

**Virus detection:**

A suspension was prepared from lesion material collected during outbreak investigation and a serotyping antigen capture ELISA was used to test for the presence of FMD viral antigen as described in the OIE Manual (Ferris and Dawson, 1988; OIE, 2004). The remainder of the suspension was inoculated onto IB-RS-2 cell cultures. Supernatant fluids were harvested from cultures showing cytopathic effects (CPE) and tested for the presence
of FMDV by a serotyping ELISA (Ferris and Dawson, 1988). Supernatant fluid from cultures which had not shown CPE by 48 hours after inoculation was passaged for a further 48 hours on fresh cultures of the same cell-line. The complete VPI gene of virus isolates was sequenced using reverse transcriptase, polymerase chain reaction (RT-PCR) amplification and cycle sequencing and these sequences were used to compare the HK-SAR isolates with previously characterized FMD viruses of the same serotype from Asia. In addition, epithelial suspensions were tested for virus by real-time RT-PCR using the procedure of Reid and others (2003).

Serology:
Sera from HK-SAR were tested at WRL for antibodies to structural proteins of the virus by serotype O specific assays, the virus neutralization test (VNT) and the solid-phase competition ELISA (SPCE), developed at WRL (Anderson and others, 2002). The VNT employed the O Manisa virus and the procedures described in the OIE Manual (OIE, 2004) with titres of greater than or equal to 1 in 45 being considered positive, as this is the cut-off appropriate for herd-based evaluation of serological status. SPCE used O Manisa antigens, a fixed test serum dilution, polyclonal antibody reagents specific for serotype O and a 60% cut-off as described by Palba and others (2004). Sera from vaccinated Irish pigs were tested at CVRL for antibodies to structural proteins of the virus by a serotype O specific ELISA (Ceditest® FMDV type O; Cedi Diagnostics B.V., Lelystad, the Netherlands).

Three different commercially available NSPE test-kits were used to assay antibodies to non-structural proteins (NSP) of FMD virus: the Ceditest® FMDV-NS (Cedi Diagnostics B.V.; Sorensen and others 1998; Chung and others, 2002b), the UBI® FMDV NS ELISA (SWINE), (United Biomedical Inc., NY, USA; Shen and others, 1999; Wang and others, 2001) and the CHEKIT-FMD-3ABC po (Bommeli diagnostics, Bern, Switzerland; Bruderer and others, 2004). Each ELISA was conducted in accordance with the manufacturer’s instructions. The UBI ELISA was used in the first instance as a “screening assay” (UBI-S). Sera that tested positively in the UBI-S but gave a negative test result with the Ceditest® FMDV-NS were retested by UBI “neutralization assay” (UBI-N). The UBI-N was performed in the same way and with the same test kit as UBI-S after preincubation of each test serum with a buffer containing the 3B peptide (UBI® FMDV NS 3B NEUTRALIZATION BUFFER, United Biomedical Inc.). As per the manufacturer’s instructions, only those sera for which the UBI-N result was less than or equal to 50% of the UBI-S result were considered to be truly positive; the remainder were considered to be “false positives” and due to non-specific reactivity.

Results:

Information on herds and animals sampled in HK-SAR:
Serum was obtained from a total of 405 pigs from one vaccinated but uninfected “control” herd (herd A) and three vaccinated, “infected” herds (herds B, C and D), as summarized in Table 1. The proximity of these herds to each other and their location within the New Territories district of HK-SAR is illustrated in Figure 1. All sera were collected between 8 and 23 March 2005.

No clinical signs of FMD had been observed in herd A during the 12 months prior to sampling. All fattening pigs in this herd were vaccinated twice, at 11 and 20 weeks of age, whilst breeding pigs were vaccinated three times per year.

Herds B and C were located 0.3 km apart, were in the same ownership and had a similar vaccination regime; fattening pigs were vaccinated once at 80 days of age and breeding pigs were vaccinated once annually. According to the owner, FMD was clinically apparent in herd B from 28 December 2004 until 27 February 2005, when the last new case was observed. The morbidity and mortality reported by the owner were 80% and 5%, respectively and the clinical signs in affected pigs included the presence of vesicular lesions on the snout and lameness associated with hoof damage. Although pigs were sorted and moved from pen-to-pen during the outbreak, the owner claims to have enhanced on-farm cleaning and disinfection procedures in an effort to prevent the spread of disease. The owner also claimed that FMD had occurred in herd C between 30 December 2004 and 5 January 2005 and that affected pigs were lame and developed vesicular lesions on their snouts. Lesion material was collected from two affected pigs during the outbreak in herd B but no specimens were submitted from herd C. At the time of sampling, most of the
sampled pigs in herd B had hoof lesions (linear breaks in the walls of their hooves) consistent with recovery from FMD within previous weeks/months (Figure 2) but similar lesions were not apparent in any of the pigs sampled in herd C. The 72 pigs sampled in herd B consisted of 52 fatteners (sampled at approximately 60 days post-infection) and 20 replacement gilts (sampled at approximately 30 days post-infection); infection appears to have spread from the fatteners to the gilts during February 2005.

In herd D, all fattening pigs were vaccinated twice, at 75 days and again at 110 days of age whilst breeding pigs were vaccinated twice per year. FMD was clinically apparent in this herd between 16 and 24 February 2005. Clinical signs in affected pigs included the presence of vesicular lesions on the snout and mild foot lesions, resulting in an observed morbidity of 13% and a reported mortality of 0.4%. The owner did not take any biosecurity precautions to prevent spread of the disease. Pigs were swill-fed with the swill being cooked adjacent to where pigs were housed. Lesion material was collected from one affected pig during the outbreak. The 151 pigs sampled from this herd consisted of 50 “convalescent” and 101 “in contact” pigs; 61 of the in-contacts had been vaccinated once only and 40 had been vaccinated on two occasions. At the time of sampling, most of the convalescent pigs but none of the in-contacts had hoof lesions consistent with recent recovery from clinical FMD.

**Virus detection:**
The two epithelial specimens collected from Herd B tested positive for FMD virus serotype O by antigen capture ELISA, RT-PCR and virus isolation. The two isolates, designated O/HKG/09/2005 and O/HKG/10/2005, had identical VP1 sequences to one another and phylogenetic analysis showed them to be of the pig-adapted “Cathay topotype” of FMDV observed for many years in Hong Kong (Samuel and Knowles, 2001), with closest similarity to a Hong Kong isolate from 2004 (99.4% nucleotide identity). Moderate antigenic matches to the O Manisa and O 3039 vaccine viruses were demonstrated ($r_1 = 0.4$ and 0.5 respectively) by in vitro neutralization test comparing neutralization of vaccine virus and O/HKG/09/2005 by bovine antiserum against the relevant vaccine ($r_1 > 0.3$ considered significant; Rwemiyama, 1984). The single epithelial specimen collected from Herd D tested positive for virus by both ELISA (serotype O) and RT-PCR (and was designated O/HKG/11/2005), although virus was not isolated in tissue culture.

**Serology**

**Virus neutralisation test (VNT):**
In herd A, only 13 of the sampled pigs were seropositive in the VNT, even though 50 of these pigs were sampled between 20 and 50 days after receiving a booster dose of vaccine and the VNT virus was homologous to one of the vaccine strains. In herd B, replacement gilts displayed noticeably higher VN antibody titres than fattening pigs and a higher proportion of the gilts were considered seropositive (85% as opposed to 44% of the fattening pigs). Overall, only 7% of the pigs in herd C were seropositive and the VN antibody profile of this herd was very similar to that in herd A (Figure 3). In herd D, all 50 convalescent pigs but only 31 of 101 “in-contacts” were seropositive; VN antibody profiles were distinctly different for convalescent pigs, “in contact” pigs that had been vaccinated once only and “in contact” pigs that had been vaccinated on two occasions (Figure 4).

**Solid phase competitive ELISA (SPCE):**
In all herds a higher proportion of the sampled pigs were seropositive by SPCE than by VNT. Overall 83% of the sampled animals tested positive in each of herds B and D whilst 47% and 24% were seropositive in herds A and C, respectively. In herd D, all of the convalescent pigs, 62% of once-vaccinated “in-contacts” and 93% of the “in-contacts” that had been vaccinated twice, were seropositive.

**Ceditest® FMDV type O ELISA:**
None of the Irish pigs were seropositive prior to vaccination but 97 of 99 were seropositive at 28 days after first vaccination and all 99 pigs were seropositive at 28 days after second vaccination.

**Ceditest® FMDV-NS:**
None of the pigs in herds A or C and none of the vaccinated Irish pigs were seropositive. The proportion seropositive in herd B was 49%; 29 of 52 (56%) fattening pigs but only 6
of 20 (30%) older replacement gilts tested positive. In herd D, 82% of convalescent pigs were seropositive, but all of the “in contacts” were seronegative.

**UBI® FMDV NS ELISA (SWINE):**
Five (5%) of the pigs sampled in Herd A and 22 (27%) of the pigs sampled in herd C tested positive on the initial screening assay (UBI-S). Overall, 53% of herd B (62% of the fatteners and 30% of the gilts) and 46% of herd D (88% of convalescent pigs and 25% of “in contacts”) were seropositive by UBI-S. A total of 64 sera tested positive in the UBI-S but negative by Ceditest® FMDV-NS. Only 14 of these sera yielded a positive result when retested by the neutralisation assay (Table 3). None of the Irish pigs tested positive by UBI-S at any timepoint pre- or post-vaccination.

**CHEKIT-FMD-3ABC po:**
All of the pigs in herds A and C and all of the “in contacts” in herd D were seronegative. Only 21% and 42% of convalescent pigs were seropositive in herds B and D, respectively; in herd B, this consisted of 25% of the fatteners and 10% of the gilts. Two of 99 (2%) vaccinated Irish pigs tested positive 28 days after second vaccination.

**Discussion:**
The primary purpose of this study was to validate serological testing with ELISAs that detect antibodies to non-structural proteins of FMDV (NSPE) so as to substantiate freedom from infection with the virus in vaccinated pigs. This is required in order to help regions that are FMD free without vaccination to adopt a vaccinate-to-live policy in the face of a FMD outbreak and to subsequently regain their FMD free status as quickly as possible post-outbreak. Such a validation requires sera from pigs that are on the one hand vaccinated but not infected, and on the other hand vaccinated and infected. In Hong Kong, where FMD is endemic and vaccination is regularly performed there is a good prospect of obtaining the required categories of pig but there is also a danger that the sampled pigs may have been exposed to unrecognized infection, especially where control and infected herds are located close together. To alleviate this concern, a herd was selected in which no clinical disease had been recognized in the twelve months preceding the study and all animals sampled were less than 12 months old. The virus-neutralizing (VN) antibody profiles of the herds are consistent with herds A and C being uninfected and herds B and D being infected. The presence of FMD virus was also confirmed in herds B and D and both of these herds, but not the other two, had pigs with horizontal hoof breaks suggestive of healed coronary band lesions. The original “infected” status of herd C does not seem to be correct. It was based upon the information supplied by the owner, who claimed to have observed signs of disease and it appeared to be consistent with the fact that herds B and C were in the same ownership, were located only 0.3 to 0.4 km apart and employed an overlapping work-force. However, no veterinary investigation was conducted at the time of the outbreak in herd C and no lesion material was available for virus detection. The fact that the owner was paid for every pig sampled may have encouraged him to see disease where it did not really occur.

Based on the results of analysing 100 sera from herd A, the specificities of the Cedi and Chekit ELISAs were both 100%, regardless of whether pigs had been vaccinated once or twice. In contrast, the UBI screening assay (UBI-S) had a specificity of 95%. Brocchi and others (2006) tested the specificity of these ELISAs on between 152 and 184 sera from non-vaccinated pigs and found values of 100% for Cedi, 97% for Chekit and 95% for UBI-S. In the present study, if groups of pigs without hoof lesions from herds C and D are considered as uninfected (n = 183), then again the Cedi and Chekit assays have 100% specificity, whereas the UBI-S has a specificity of only 74%. Other than the possibility that these pigs had been subclinically infected, there is no obvious explanation for the poorer specificity of the UBI-S in these herds compared to the results obtained for herd A and by Brocchi and others. Using the UBI neutralization assay (UBI-N) to retest all sera which tested positive by UBI-S but negative by Cedi ELISA appeared to greatly improve the specificity of the UBI “test system” but a system that employs a screening test with specificity as low as 74% (based on the results from herd D) and requiring a second test to confirm all positives is not really suitable for large-scale, post-outbreak serosurveillance, as it would add considerably to the workload of the serodiagnostic laboratory. However, it should be noted that the UBI-S performed with a diagnostic specificity of 100% when used to test sera from 99 vaccinated Irish pigs, sampled and tested on three separate
occasions. This may reflect modification of the UBI test kit by the manufacturer during the interval of approximately one year between testing of sera obtained from pigs in HK-SAR and production/supply of a different batch of the test-kit to test sera obtained from the Irish pigs.

Considering only herd B and the convalescent subgroup in herd D, the Cedi ELISA and the UBI "test system" showed equivalent sensitivity (herd B, n = 72, Se Cedi = 49% and Se UBI-S = 53%; herd D convalescent pigs, n = 50, Se Cedi = 82% and Se UBI-S = 88%), whereas the Chekit ELISA only detected half as many positives as the other two assays. The present study was not designed to evaluate different periods of elapsed time between infection and sampling, but this may also influence the relative sensitivity of the tests. Lee and others (2004) studied FMD-vaccinated pigs that were experimentally challenged with virus and then sequentially bled and assayed for NSP antibodies using the UBI ELISA, Chekit ELISA and the prototype of the Cedi ELISA. In that study, the UBI ELISA detected more seropositive pigs than the Cedi ELISA up to seven weeks post-challenge but the reverse was true thereafter.

In this study, the presence or absence of horizontal breaks in the hooves of pigs appeared to be a useful indicator of convalescence from clinical FMD and the presence of lesions correlated with serological evidence of infection. Seroconversion to NSP has been observed in cattle that were subclinically infected after vaccination (Parida and others, 2005) but this remains to be demonstrated in pigs.

It is interesting to speculate on the efficacy of the vaccination regimes employed in the Hong Kong herds. All four herds used the same batch of bivalent serotype O vaccine for which a moderate serum neutralizing match was demonstrated between the vaccine strains and one of the viral isolates from herd B. Similar matches have been obtained for other Hong Kong field isolates with the same vaccine strains (Paton, unpublished results). As with other field studies, one cannot be certain that the vaccine had been properly stored and administered. When a similar vaccine was used to vaccinate Irish pigs in near ideal experimental conditions, almost all of the vaccines seroconverted by 28 days after first vaccination and all were strongly seropositive 28 days after booster vaccination. A low VN antibody response was measured in pigs within herds A, C and D that had only been vaccinated on one occasion and had been blood sampled at between 16 and 115 days post vaccination. As expected, a second vaccination increased the proportions of pigs scored seropositive by VNT on farm D (Figure 4). The optimal timing for FMD vaccination of fattening pigs in endemic areas is a first dose between 10 and 12 weeks of age followed by revaccination four weeks later (Chung and others, 2002a). Accordingly, a near optimal strategy was used in herds A and D, who also revaccinated sows twice (herd A) or three times (herd D) per year. In contrast, in herds B and C young pigs only received a single dose of vaccination at 11 weeks of age and breeding pigs were only vaccinated annually. In herds B and D vaccination did not prevent infection nor the occurrence of moderate to severe clinical signs. However, infection did not appear to have spread to all of the pig units on the farm and in the case of herd B, did not appear to have spread to a nearby farm in the same ownership. This suggests that the vaccination regime may have offered some protection and that the initial introduction of virus to herds B and D represented either a particularly severe challenge or else occurred at a time between vaccinations when immunity was particularly low.

Although all pigs in herd B were only vaccinated on one occasion, affected gilts displayed higher VN antibody titres than fattening pigs (85% seropositive compared to 44% for fatteners). However a lower percentage of the gilts tested positive for NSP antibodies (30% as opposed to 56-62% for the fattening pigs). NSP responses take a longer time to develop than SP antibody responses and the gilts were more recently infected than the fatteners, although still at least 30 days after infection. An alternative explanation is that less viral replication may have occurred in infected gilts possibly because of higher protective antibody levels pre-outbreak. Wang and others (2001) measured the ratio of antibodies to structural versus non-structural viral proteins as detected by two different ELISAs, one based on 3B and the other on VP1. This ratio was used with field sera collected from pigs to discriminate between responses to vaccination and responses to infection. It was found that a structural protein antibody: non-structural protein antibody ratio of < 1.7 was indicative of infection. In the present study antibody titres were only evaluated for VNT and therefore, such a comparison was not possible.
The study also afforded an opportunity to compare antibody detection rates for the VNT and SPCE when using the cut-off values employed at the WRL FMD for herd-based testing; that is for establishing the status of a group of animals tested together as opposed to individual animal certification (herd-based cut-offs are 1 in 45 for VNT and 60% inhibition for SPCE; OIE Manual, 2004). More pigs were scored positive by SPCE in all categories including both vaccinated and vaccinated-and-infected animals. This is in contrast to the results of a study where sera from cattle that had been vaccinated and in some cases subsequently infected with SAT type FMD viruses were tested by both of these methods (Sammin and others, in press).

In conclusion, all of the NSPE tests evaluated could detect infection in groups of vaccinated pigs with hoof lesions consistent with FMD convalescence, in herds where acute FMDV had been observed prior to sampling. The Cedi test was relatively sensitive and specific, whereas the UBI test was less specific in the absence of a confirmatory method and the Chekit test was relatively less sensitive. The frequency with which truly subclinical infection occurs in vaccinated pigs remains to be demonstrated and if it occurs, the ease with which it can be detected by NSPE tests also needs to be established.

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References:


assay using nonstructural protein 3AB as the antigen and application to an eradication program. *Journal of Clinical Microbiology* 40: 2843-8.


Table 1: FMD-vaccinated and infected herds sampled in HK-SAR

<table>
<thead>
<tr>
<th>Herd</th>
<th>Herd size</th>
<th>Number of pigs sampled</th>
<th>Age of sampled pigs (months)</th>
<th>Vaccination status*</th>
<th>Clinical history</th>
<th>Time of sampling post-outbreak (days)</th>
<th>Foot lesions at time of sampling</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>6000</td>
<td>100</td>
<td>3-6</td>
<td>Once/twice</td>
<td>Not infected</td>
<td>-</td>
<td>None</td>
</tr>
<tr>
<td>B</td>
<td>2800</td>
<td>72</td>
<td>5-12</td>
<td>Once only</td>
<td>Infected</td>
<td>30-64</td>
<td>72/72</td>
</tr>
<tr>
<td>C</td>
<td>4100</td>
<td>82</td>
<td>4-7</td>
<td>Once only</td>
<td>Infected</td>
<td>?</td>
<td>None</td>
</tr>
<tr>
<td>D</td>
<td>1900</td>
<td>151</td>
<td>3-5</td>
<td>Once/twice</td>
<td>Infected</td>
<td>29-30</td>
<td>50/151</td>
</tr>
</tbody>
</table>

*In Herd A, 50 pigs were vaccinated once only and 50 on two occasions; in Herd D 86 pigs were vaccinated once only and 65 on two occasions

Table 2: Number (and percentage) of sampled pigs seropositive to serotype O FMD virus

<table>
<thead>
<tr>
<th>Herd</th>
<th>Hoof lesions at time of sampling</th>
<th>VNT (n = 100)</th>
<th>SPCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>NO</td>
<td>13 (13%)</td>
<td>47 (47%)</td>
</tr>
<tr>
<td>B</td>
<td>YES</td>
<td>40 (56%)</td>
<td>60 (83%)</td>
</tr>
<tr>
<td>C</td>
<td>NO</td>
<td>6 (7%)</td>
<td>20 (24%)</td>
</tr>
<tr>
<td>D</td>
<td>YES</td>
<td>81 (54%)</td>
<td>115 (83%)</td>
</tr>
</tbody>
</table>

Table 3: Number (and percentage) of sampled pigs seropositive by NSP-AB detection ELISAs

<table>
<thead>
<tr>
<th>Herd</th>
<th>Ceditest® FMDV-NS</th>
<th>UBI® FMDV NS ELISA (SWINE)</th>
<th>UBI neutralisation assay*</th>
<th>CHEKIT-FMD-3ABC po</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>(n = 100)</td>
<td>0</td>
<td>5 (5%)</td>
<td>0</td>
</tr>
<tr>
<td>B</td>
<td>(n = 72)</td>
<td>35 (49%)</td>
<td>38 (53%)</td>
<td>36 (50%)</td>
</tr>
<tr>
<td>C</td>
<td>(n = 82)</td>
<td>0</td>
<td>22 (27%)</td>
<td>1 (1.2%)</td>
</tr>
<tr>
<td>D</td>
<td>(n = 151)</td>
<td>41 (27%)</td>
<td>69 (46%)</td>
<td>47 (31%)</td>
</tr>
</tbody>
</table>

*Number (and percentage) of pigs positive after retesting sera that tested positive on UBI test and negative on Ceditest by the UBI neutralisation assay
Figure 1  Location of study (red) and other (green) pig herds in HK-SAR. Study farms: 819, Herd A; 1023, Herd B, 649, Herd C; 12, Herd D.

Figure 2  Photograph of hoof breaks below coronary band of an FMD-convalescent pig
Figure 3  FMD virus neutralizing antibody profiles for Herds A, B and C

Figure 4  FMD virus neutralizing antibody profiles for Herd D