J Vet Diagn Invest 18:545–552 (2006)

Development and evaluation of an indirect enzyme-linked immunosorbent assay for detection of foot-and-mouth disease virus nonstructural protein antibody using a chemically synthesized 2B peptide as antigen

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Abstract. Forty peptides were synthesized corresponding to hydrophilic clusters of amino acids within the sequences of foot-and-mouth disease virus (FMDV) nonstructural proteins (NSP). Six peptides were studied in more detail and the most promising, a 2B peptide, was evaluated in enzyme-linked immunosorbent assay (ELISA) using sera from naïve, vaccinated, and vaccinated-and-challenged cattle as well as bovine sera from field outbreaks. The performance of the new NSP peptide ELISA was compared to that of 4 commercial NSP ELISA kits. Antibody to 2B was detectable from the end of the first week to the second week after infection in most of the nonvaccinated animals and by the second to third week in vaccinated-and-challenged animals. The sensitivity of the 2B peptide ELISA was comparable to the 3ABC Ceditest (*Ceditest® FMDV-NS*, Cedi Diagnostics B.V.; Chung et al., 2002). With some modification and further validation, this 2B test could be useful as a screening or conformational NSP test in postvaccination surveillance for FMD.

Key words: 2B protein; foot-and-mouth disease virus; NSP ELISA; peptide antigens.

Introduction

Foot-and-mouth disease virus (FMDV) belongs to the family *Picornaviridae*, genus *Aphthovirus*, and causes a highly contagious disease of cloven hoofed animals that is of global importance.[19] The FMDV RNA genome encodes 12 viral proteins, L, 1A, 1B, 1C, 1D, 2A, 2B, 2C, 3A, 3B, 3C, and 3D; some of these (e.g., 3ABC) exist as uncleaved precursors. Four viral structural proteins 1A, 1B, 1C, and 1D make up the protein shell of the virion, which also contains traces of 3D. The other eight viral proteins (L, 2A, 2B, 2C, 3A, 3B, 3C, and 3D) play a role in replication and other functions within the host cell and are not part of the virion structure; hence their collective name, nonstructural proteins, or NSPs. An important method of FMD control is vaccination, using inactivated vaccines derived from whole virus virions.[4,15] However, vaccinated animals may on occasion become infected either with or without clinical signs, and in the case of ruminants this may result in persistent infection beyond 28 days postinfection.[5,12] Therefore, to monitor postvaccination infection or to substantiate its absence in order to re-establish FMD free status for purposes of international trade, postvaccination serosurveillance is required. A variety of serological tests are used to help diagnose FMD and to certify that animals or regions are free of FMDV infection. Some of these tests detect antibodies to viral structural proteins whereas other, so-called NSP tests, detect antibodies to the viral nonstructural proteins.[18] The NSP tests have two advantages over tests for antibodies to structural proteins: 1) they are effective at detecting infection with all FMDV serotypes; 2) they can be used to detect infection in vaccinated animals.[8,17]

Recently a number of in-house and commercial tests have been developed and evaluated to identify infection in vaccinated livestock, including the presence of viral carrier animals. Use of these tests in experimental vaccinated animals as well as animals from the field[1,12,14] failed to provide a categorical assurance of detecting infection; therefore, there is still a need to develop and validate more sensitive and specific tests that could be used either in their own right, or as the screening or confirmatory method alongside existing NSP tests. The potential for use of a 2B synthetic peptide ELISA for the detection of NSP antibodies in infected animals from an experi-

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mental vaccine challenge study[12] encouraged us to check the use of other NSP peptides as well as this 2B peptide with further field and experimental samples.

Material and methods

Reference sera

Known NSP 3ABC antibody negative (n = 30) and positive (n = 29) sera were available from earlier studies on 3ABC ELISA-based differentiation of vaccination and infection[11] and were used as reference sera in 2B ELISA. Detailed information on these sera have been given in a previous publication.[11] Briefly, the positive sera were collected from cattle 21–28 d after the animals had been intradermolingually inoculated with at least 1 representative strain from each of the 7 serotypes of FMDV (i.e., 119 ER42, Cruzeiro MR 88, Den 1/70 HF75, EYSTRUP, France L50, Mahmatli LO83, Pando JK30, Phillipine LV42, Turkey 1/69, and USSR 1/66 for type A; Israel 3/63 and Kemron for type Asia1;C997 EO26, C997 EP2, and Noville MR25 for type C; BFS 1860SG89, Israel 1/63, Lausanne, Pacheco, Swiss 1/66, Thai 1/80, and Brescia CQ97 for type O; Ghana 14/68, Israel 4/62, SA 13/61 for SAT1; NR 1/60, SA 2/67 and Uganda 6/70 for SAT2; and RV 7 for SAT3 respectively). All 30 negative sera were free of antibodies to structural proteins of FMDV and were obtained from serum bank, World Reference Laboratory, Pirbright, UK.

Negative sera

Sera collected from healthy Japanese dairy cattle in 1996 (n = 100) were included as FMD seronegative sera. Their seronegative status was confirmed by virus neutralization test (VNT) and liquid phase blocking (LPB) ELISA using the international standard protocol[9] at the FMD Regional Reference Laboratory in Thailand.

Sera from vaccinated cattle

One hundred forty sera were collected from 20 vaccinated cattle at 7 time points before and after vaccination but prior to challenge at 3 wks after vaccination[5,12] and analyzed in 2B ELISA.

Sera from experimentally infected/challenged cattle

Sera from 3 studies were used. Sequentially collected sera (n = 85) from 5 unvaccinated infected cattle were obtained from FMD vaccine potency tests conducted at the FMD Regional Reference Laboratory in Thailand. All the cattle had shown typical FMD symptoms and lesions. Four cattle were infected with FMDV serotype O/ Udornthani/ 87 and 1 animal was infected with FMDV Asia1. Sera were collected weekly from 1 wk until 16 wks after infection. Preinfection sera were also collected before intradermolingual inoculation.

Another 96 sera were obtained from 6 cattle held at the Institute for Animal Health, Pirbright, UK. Two of these cattle were vaccinated with a commercial trivalent vaccine containing antigen of O, A, and C FMDV serotypes and Al $(OH)_3$ /Saponin as adjuvant. On 21 d postvaccination both vaccinated cattle along with a new unvaccinated animal were challenged by 5 d of direct contact with an intradermolingually inoculated ($10^{6.8}$ $TCID_{50}$ of FMDV O_1 Saudi 8/88) steer (RV19). One month later, 2 more steers were infected by contact exposure to 2 "donor" cattle that had been inoculated intradermolingually with O_1 Saudi 8/88 as described above. Twelve months later (on 60th wk after the first infection), all 6 cattle were rechallenged by intranasal inoculation of $10^{5.0}$ $TCID_{50}$ of FMD virus type O_1 Saudi 8/88. Sera were collected until 4 wks after rechallenge at an interval of 1 wk. All of the unvaccinated animals showed typical clinical signs of FMD following either direct inoculation or contact exposure. Neither of the vaccinated animals showed clinical signs. Animal 18, 34, and 35 were identified as carrier animals by virus isolation from probang fluid.

Sera (n = 450) from the third study had been collected from 9 vaccinated carrier cattle (virus detected by virus isolation and RT-PCR), 5 unvaccinated infected cattle, and 11 vaccinated cattle that had been challenged by direct contact with inoculated animals.[5,12]

Field sera

Four hundred and one sera collected from FMD affected cattle herds in Zimbabwe[14] were analyzed by the 2B ELISA to compare the rate of seroconversion in a field situation between this test and 4 commercial assays[14] i.e., Cedi,[a,3,19] Bommeli,[b,2] UBI,[c,15] and Svanova.[d,1] Briefly, representative sera had been collected from 6 farms in 3 provinces of Zimbabwe: farm A (129/1300 animals) and farm F (60/herd size not recorded) from Mashonaland East, farms C (65/307 animals), D (41/85 animals) and E (65/767 animals) from Masvingo, and farm B (41/75 animals) from Mashonaland west. Animals from farms A, E, and F had been vaccinated. The vaccination status of farm B was not known and the cattle of farms C and D had not been vaccinated. During the outbreaks, clinical signs were observed on all farms except farm F although some animals in this farm tested positive for FMDV NSP antibodies. Samples were collected within 1 to 5 months of the outbreaks, i.e., 2, 1, ~1, 4, and 5 mo afterwards from farms A, B, C, D, and E respectively. SAT I and SAT II serotype FMD virus and/or RNA were detected from the probang samples of 90 animals by virus isolation and/or reverse transcription-PCR.

Peptides

Hydrophilic clusters on the amino acid sequence of FMDV serotype O1K NSPs[7] were analyzed using the SDC-GENETYX program.[e] Forty peptides, of identical sequence to individual hydrophilic clusters were synthesized by the F-moc solid-phase protocol with Beckman 990B[f] or Advanced ChemTeck ACT90 peptide synthesizer[g] at National Institute of Animal health (NIAH), Japan. The purity of peptides, analyzed by C18 HPLC column[h] was maintained at >90%. They were subsequently conjugated with Cohn fraction II and III of horse γ globulin (HGG)[i] at a 60:1 molecular ratio of peptide to carrier protein, by the modified glutaraldehyde method.[20] Later on, the promising 2B RSTPEDLERAEKQ (#7) peptide was synthesized with

higher purity i.e., > 97% and conjugated with key-hole limpet hemocyanin (KLH) by PEPTIDE Institute.[i] The molar ratio of induced free peptide against KLH carrier protein was 160–166 nmol/mg. The conjugates were lyophilized and stored at −20°C; master solutions were made up to 1 mg/ml with distilled water and stored at −20°C.

Virus infection associated (VIA) antigen for ELISA

VIA antigen was used as a control ELISA antigen at the initial stage of screening of peptides. It was prepared from O1K FMDV infected BHK-21 cells, as previously described.[10] VIA antigen was diluted 1:15 with carbonate buffer and used to coat the ELISA plate. The concentration was adjusted to show clear precipitation line in agar gel immuno-diffusion test at 1:16 dilution against a FMDV type Asia1 convalescent serum.

Commercial NSP tests

Results of 4 commercial assays (Cedi,[a,3,17] Bommeli,[b,2] UBI,[c,16] and Svanova[d,1]) used in a previous study[14] were compared to the current results of 2B ELISA using the same field sera. Three tests detect antibodies to viral nonstructural poly peptide 3ABC whereas the UBI test detects antibody to a 3B synthetic peptide. The Cedi test is a competitive ELISA and can be used for cattle, sheep, and pigs and the other 3 are indirect ELISAs and use antispecies conjugates to detect bound antibodies. Purified antigens are directly coated to the plates in Bommeli, UBI, and Svanova tests whereas the antigen is captured using a monoclonal antibody in the Cedi test.

Peptide ELISA

Ninety-six-well Maxisorp Nunc Immuno plates[k] were coated overnight at 4°C with 50 µl/well of 2B peptide at a concentration of 250 ng/ml in carbonate/bicarbonate buffer[j]. The following day, the plates were washed 3 times with PBS and blocked with 50 µl/well of blocking buffer containing 5% nonfat dried milk powder and 1% Tween 20[j] in PBS for 1 h at 37°C on an orbital shaker. A 1:10 dilution of test serum was made directly in the plates by adding 5 µl of serum to 45 µl of dilution buffer (1% nonfat dried milk powder, 1% Tween 20 and 1% normal horse serum[j] in PBS) and incubated for 1 h at 37°C on an orbital shaker. After washing 3 times with PBS, peroxidase-conjugated anti-bovine IgG[j] was added at 1:5,000 in dilution buffer (total volume 50 µl/well) and again incubated for 1 h at 37°C. After 3 final washes, the color reaction was developed for 10 min by adding a chromogen/substrate mixture (50 µl/well) containing 5.05 mM ortho-phenylene-diamine dihydrochloride[j] 30% (w/w/) hydrogen peroxide[j] diluted 1:2,000. The reaction was stopped with 50 µl/well 1 M sulfuric acid. The optical density was measured using a multichannel spectrophotometer at 492 nm (A_{492}). The cut-off point for the test was established by calculating the mean optical density (OD) plus 2 standard deviations for test results from sera collected from the 20 vaccinated Cattle[5,12] at 7 time points before and after vaccination but prior to challenge exposure. In this indirect ELISA, all the peptides and VIA antigen were used in the same way as 2B.

Results

Screening of NSP peptides

Antigenic reactivity of 40 different FMD NSP peptides measured by indirect ELISA is summarized in Table 1. Six peptides, #7(2B), #10(2C), #24 (3C), #34 (3D), #35 (3D), and #39 (3D), were identified with an equivalent or higher OD value than the VIA antigen (VIA OD value = 0.6). In particular, the #7 peptide of 2B and #10 peptide of 2C showed substantially higher OD values i.e., >2.0. To evaluate the reactivity of both peptides #7 and #10, a 2-fold dilution series of each screening serum was applied in the indirect ELISA (Fig. 1). An OD = 1.00 was observed at serum dilutions of 1:6,000, 1:400, and 1:30 for the peptides #7, #10, and VIA antigen, respectively. Thus, the #7 peptide (2B) yielded an OD value 200 times higher than the OD of the conventional VIA antigen.

Specificity of #7 (2B) and #10 (2C) peptides against reference and negative sera

The 2B and 2C peptides were tested in indirect ELISA as described in materials and methods. The results are illustrated as frequency distributions of the OD values of each serum group (Fig. 2). With the 2B ELISA, the OD values for the negative sera (n = 100 negative sera from Japanese cattle and n = 30 negative reference sera) were <0.8 and 90% of sera were in the range of OD 0.2–0.4. The positive sera (n = 29 positive reference sera) ODs were all >0.8. The frequency distribution of the positive sera was well separated from that of the negative sera. On the other hand, in the 2C ELISA, the distribution of values for the positive sera was shifted to the left and overlapped that of the negative sera.

The 140 sera collected from 20 vaccinated cattle before the challenge were analyzed only in 2B ELISA and the cut-off point was calculated as >0.79, representing the mean OD value of these 140 sera in ELISA plus 2 standard deviations.[13]

Kinetics of antibodies to the 2B peptide

The kinetics of antibody appearance and persistence in the 2B ELISA was studied using the sera collected sequentially from 11 experimentally infected/challenged cattle. Seroconversions of infected cattle against 2B were first detected within 1 to 2 weeks after FMDV infection (Fig. 3).

The duration of antibodies detected by the 2B ELISA was variable among individual cattle (Fig. 4). In noncarrier cattle (RV19, RV21) the antibody level decreased to the negative level approximately 30–40 weeks after infection. On the other hand, the carrier cattle (RV34, RV35) maintained high anti-

Table 1. Antigenic reactivity of FMDV NSP peptides tested by indirect ELISA.

Region	Location*	Peptides No.	Sequence	OD†
L	33	#1	LYNGEKKTFY SRPNNHDN	<0.1
	161	#2	AIDDEDFYPW TPDPSDVLF	0.45
2A	938	#3	NFDLLKLAGD VESNPG	<0.1
2B	1,000	#4	VKAIRTGLDE AKP	<0.1
	1,027	#5	AAVAARSKDP VLVA	<0.1
	1,056	#6	VVKKISDSLS SL	<0.1
	1,094	#7	RSTPEDLERA EKQ	>2.0
2C	1,108	#8	LKARDINDIF AILKNGE	<0.1
	1,141	#9	IASEEKFVT	<0.1
	1,158	#10	LEKQRDLNDP SKYKEAKE	>2.0
	1,199	#11	APAPSKSRPE PVV	<0.1
	1,214	#12	CLRGKSGQGK SFLA	<0.1
	1,248	#13	CPPDPDHFDG YNQ	<0.1
	1,265	#14	VMDDLGQNPD GKDFKY	<0.1
	1,295	#15	ASLEDKGKPF NSKV	<0.1
	1,377	#16	NGMAVEMKRM QQDMFKPQ	<0.1
3A	1,453	#17	GMVHDSIKEE LRPLI	<0.1
	1,471	#18	SFVKRAFKRL KENFEIVA	<0.1
	1,499	#19	IMIRETRKRQ KMVDDAVNE	<0.1
	1,519	#20	IEKANITTDD KTLDEAEKSP L	<0.1
3B	1,583	#21	GPLERQKPLK VRAKLPQQEG	<0.1
	1,606	#22	GPMERQKPLK VKAKA	<0.1
3C	1,692	#23	VPRHLFAEKY DKIM	<0.1
	1,707	#24	DGRAMTDSDY RVFEFEIKVK GQD	0.7
	1,739	#25	LHRGNRVRDI TKHFRDTARM KKGTPV	<0.1
	1,781	#26	EALTYKDIVV CMDGDTMPGL	<0.1
	1,815	#27	AVLAKDGADT FIVG	<0.1
3D	1,863	#28	GLIVDTRDVE ERVHVMRKTK LAP	<0.1
	1,899	#29	AALSNKDPRL NEGVVLD	<0.1
	1,912	#30	VVLDEVIFSK HKGDTKMSEE DKALFR	<0.1
	1,960	#31	IYEAIKGVDG LDAMEPDTAP GL	<0.1
	1,986	#32	QGKRRGALID FENGT	<0.1
	1,997	#33	ENGTVGPEAA LKLMEKREYF KVCQ	<0.1
	2,006	#34	EAALKLMEKR EYFKVCQ	0.8
	2,023	#35	TFLKDEIRPL EKVRAGKTRI VDVL	0.78
	2,138	#36	TLVNTEHAYE NKRITVGGGM P	<0.1
	2,177	#37	VLYALRRHYE GVELDTYTMI SYGDDIVV	<0.1
	2,206	#38	SDYDLDFEAL KPHFKSLG	<0.1
	2,225	#39	TITPADKSDK GFVLGHSITD V	0.8
	2,290	#40	AGLAVHSGPD EYRRLFEPGQ GL	<0.1

* Amino acid number on polyprotein.

† The reactivity of each peptide is shown as a corrected optical density (OD) value after subtracting the OD value for preinfection serum from the OD value of 9-weeks postinfection serum of animal #214. Test sera were diluted 1:40 in normal horse serum.

body levels during the course of the experiment. In the vaccinated and challenged cattle (RV17 RV18), though higher antibody levels were observed, the duration was limited in comparison to unvaccinated infected animals. An increased antibody response after second challenge was observed in some animals.

Application of 2B ELISA to sera collected from cattle in the field after FMD outbreaks

The proportions of seropositive cattle detected overall were 75%, 56%, 61%, and 56% in Cedi, Bommeli, UBI, and Svanova NSP 3ABC ELISAs respectively. With the 2B NSP ELISA the proportion was 79.2%. The proportions of seropositive cattle found in each herd by all 5 tests is shown in Table 2. Cedi and 2B detected 257 and 270 seropositive cattle respectively out of a total of 341 cattle tested in the farms A, B, C, D, and E. 238 (69.8%) out of 341 cattle were found seropositive in both Cedi and 2B NSP ELISAs. Nineteen animals seropositive by Cedi test were scored negative in 2B and 32 animals seropositve in 2B were scored negative in Cedi. Furthermore, the 2B test scored 84 out of 90 virus carrier animals as positive (93.3%) whereas Cedi detected 81 (90%) virus carriers. However, both Cedi and 2B detected 78 (86.7%) common virus carriers. Twenty percent of

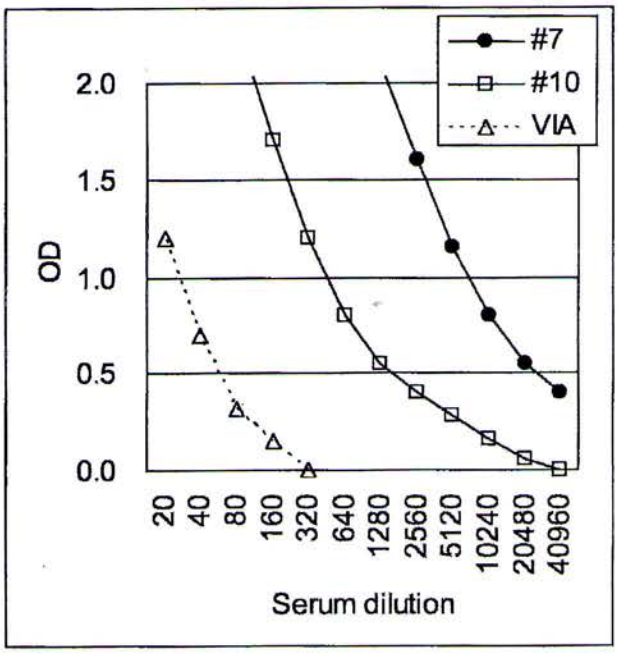

Figure 1. Reactivity of 2B #7 and 2C #10 peptides on indirect ELISA: Screening serum collected postinfection from animal #214 was used in a 2-fold dilution series. Reactivity is shown as corrected OD values of 9-week postinfection serum of animal #214. Test sera were diluted 1:40 in normal horse serum.

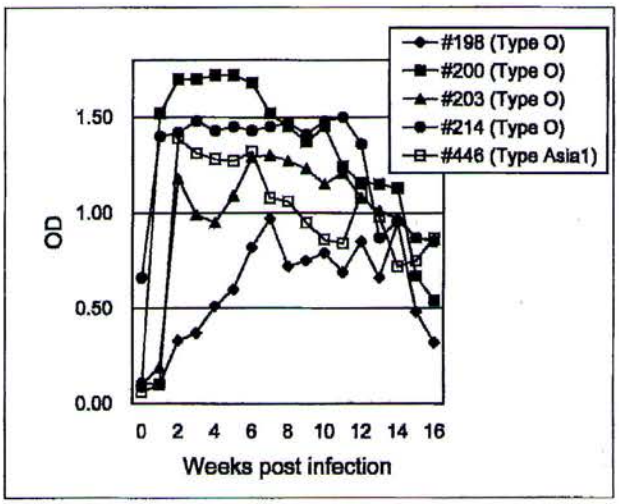

Figure 3. 2B antibody response in experimentally infected cattle measured by 2B peptide ELISA.

animals from farm F, where clinical signs were not observed, were seropositive for 2B antibody, which was the same proportion as detected by the Cedi test. The comparison of performance between Cedi and 2B ELISA for detecting seropositive as well as carrier animals by using Chi2 test is shown in Table 2. In general no significant difference ($P > 0.05$) was found in these tests for detecting seropositive or carrier animals.

Discussion

In FMD free countries, there is a heightened interest in vaccinate-to-live policies so as to reduce the need to stamp out susceptible as well as infected animals.[12] In order to regain the FMD free status soon after an FMD outbreak has been brought under control, countries have to demonstrate the absence of FMDV infection, including use of serosurveillance. If

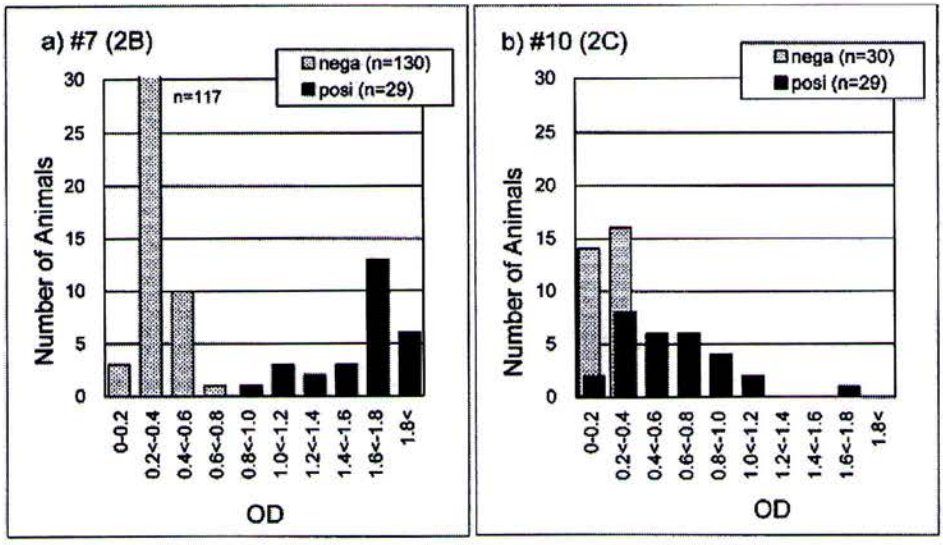

Figure 2. Frequency distribution of the OD values of cattle reference sera tested against peptides #7(2B) and #10(2C) in ELISA: **a**, #7(2B), nega (n = 130); negative reference sera (n = 30) and sera collected from healthy Japanese dairy cattle (n = 100), posi (n = 29); positive reference sera (n = 29). **b**, #10(2C), negative (n = 30); negative reference sera (n = 30), posi (n = 29); positive reference sera (n = 29).

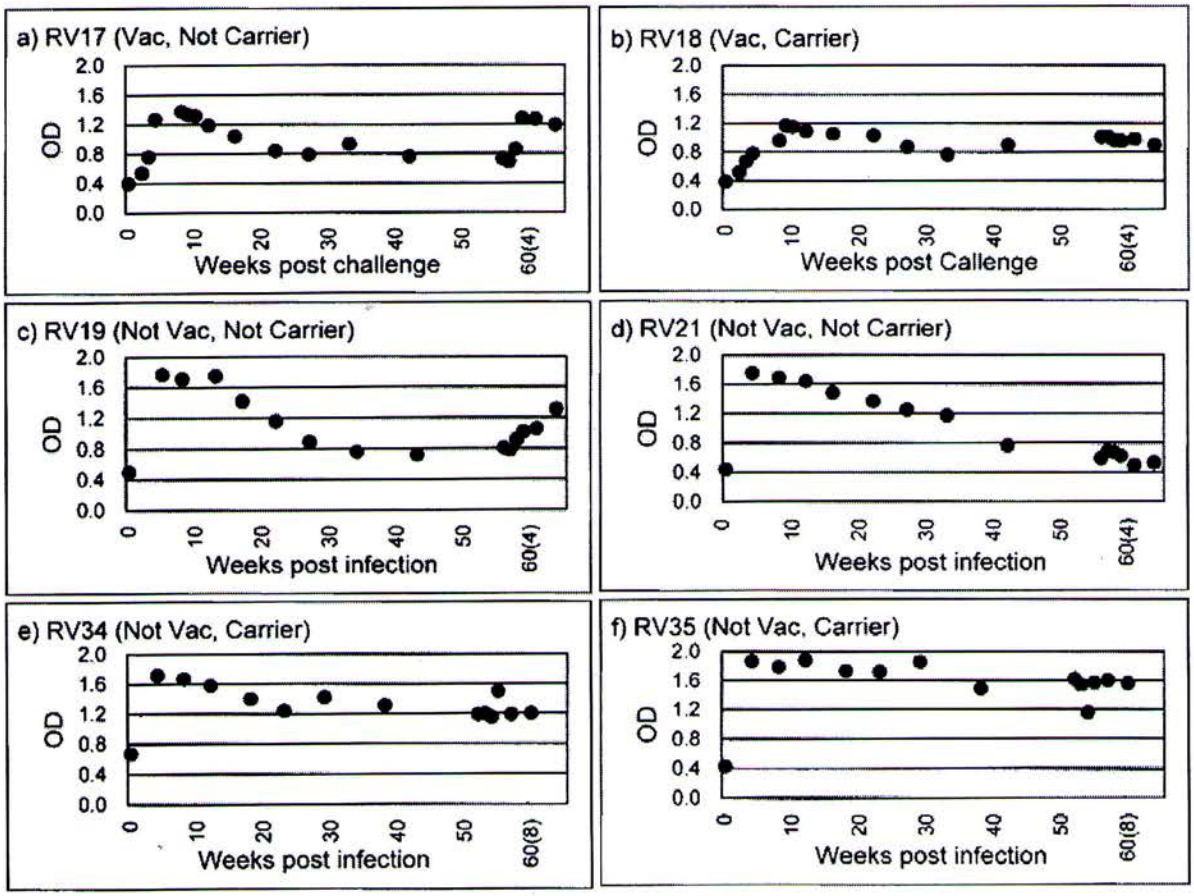

Figure 4. 2B antibody response in experimentally infected or vaccinated/challenged cattle with tandem infection: **a**, RV17, vaccinated, not carrier; **b**, RV18, vaccinated, carrier; **c**, RV19, infected, not carrier; **d**, RV 21, infected, not carrier; **e**, RV34, infected, carrier; **f**, RV35, infected, carrier; RV17, RV18, RV19, RV20 received a second challenge at 56 weeks after the first infection/challenge. RV34 and RV 35 received the second challenge at 52 weeks after the first infection/challenge.

vaccination has been used then NSP tests are the only currently available tool for this as both natural infection and vaccination elicit antibodies against structural proteins.[6,11,17] Though 4 commercial tests and some in-house NSP tests are available at the moment, their sensitivity and specificity are insufficient to demonstrate absence of infection with a high level of confidence in small herds.[13] After a recent international NSP test validation at Brescia,[1] it has been suggested to use more than one NSP test to increase the efficiency of detection. Keeping these requirements in mind, a preliminary 2B NSP peptide assay was developed[12] with promising results. In this communication, further trials have been undertaken to evaluate more nonstructural peptides from hydrophilic clusters on the amino acid sequence of FMDV and also to evaluate further the existing 2B peptide assay using sera collected from both the field and from experimental cattle.

In the present study, though 6 promising nonstructural peptides out of 40 were found with a high reaction profile to convalescent reference sera, the existing 2B peptide #7 worked better to differentiate between the positive and negative samples as depicted in Fig. 2a. From the preliminary specificity study it is evident that at a cut off OD 0.79, all the 100 negative samples and 140 vaccinated samples were scored negative. However, in the previous report[12] 2 animals were scored positive even before vaccination. Therefore, it will be necessary to test more sera from naïve and vaccinated cattle to more accurately determine the specificity and the cut off for the assay.

From the preliminary kinetics study presented here, it is evident that 2B NSP antibodies can develop very quickly (as early as 1–2 weeks after infection), although, as for other NSP tests, the development of NSP antibody was slightly delayed in vaccinated infected animals. This confirms earlier observations,[9] where the 2B antibody developed by the end of the first week in unvaccinated infected animals but by the end of the second week in vaccinated infected animals. Carrier animals maintained the 2B NSP

Table 2. Comparison of performance of 2B ELISA with commercially available ELISAs for detection of seropositive cattle.

Cedi	Bommeli	UBI	Svanova	2B		Chi2
Cut off ≥ 50 PI*	Cut off ≥ 20 PP*	Cut off ≥ pc × 0.23*	Cut off ≥ 48 PP*	Cut off > .79 OD	Herd	cedi/2B
118 (91%)	85 (66%)	100 (78%)	88 (68%)	123(95.3%)	A $n = 129$	0.3155
29 (71%)	21 (51%)	25 (61%)	25 (61%)	34(82.9%)	B $n = 41$	0.2951
63 (97%)	65 (100%)	65 (100%)	65 (100%)	65(100%)	C $n = 65$	0.4961
17 (41%)	10 (24%)	7 (17%)	4 (10%)	22(53.65%)	D $n = 41$	0.3764
30 (46%)	10 (15%)	10 (15%)	8 (12%)	26(40%)	E $n = 65$	0.5952
12 (20%)	4 (7%)	7 (12%)	2 (3%)	12(20%)	F $n = 60$	0.8149
257 (75%)	191 (56%)	207 (61%)	190 (56%)	270(79.2%)	A + B + C + D + E $n = 341$	0.2729
81 (90%)	66 (73%)	67 (74%)	63 (70%)	84(93.3%)	Carrier (PCR + VI) $n = 90$	0.5896
46 (92%)	35 (70%)	37 (74%)	33 (66%)	47(94%)	Vaccinated carrier(A + E) $n = 50$	1.0000

* PI = percentage inhibition, PP = percentage positivity, PC = positive control OD and Chi2-Chi square test.

antibody status for a longer time (Fig. 4) than the noncarriers as seen in a previous experiment.[13] Both the Cedi and 2B tests were comparable in their ability to detect NSP antibody in the early period of infection and later on after infection. In this report the 2B ELISA detected all of the 3 experimentally derived carriers and this ability has also been seen in a previous experiment where both Cedi and 2B tests detected 7 carriers out of 9.[12]

The proportion of seropositive reactors amongst 341 field samples was 79.2% for the 2B NSP ELISA, which is higher than most of the commercial kits or similar to the Cedi test ($P > 0.05$). All of the NSP tests detected more positive reactors in herds A, B, and C than herds D and E. It appears from this that for optimal sensitivity, serosurveillance should be done within 2 months of an outbreak, as late serosurveillance as in herds D and E reduced the efficiency of detection of NSP antibody. The 2B test also detected the highest number (93.3%) of virus carrier animals confirmed by virus isolation and RT-PCR,[14] which is not significantly different ($P > 0.05$) from the performance of the Cedi test.

In conclusion, the 2B NS peptide ELISA has the potential to identify infection in vaccinated herds. The sensitivity of the 2B peptide ELISA for detecting confirmed carriers is comparable ($P > 0.05$) to the Cedi NSP test. As the comparison between the tests has been done with field samples, the scope for defining the real sensitivity of the assays is limited. However, considering the results from animals confirmed to be carriers by virus isolation and RT-PCR, the sensitivity of Cedi and 2B tests (90% and 93.3%, respectively) were not significantly different ($P > 0.05$). By combining the results of the 2 tests, 87 carriers were detected in the population. With some modification and further validation, this 2B test could be useful in postvaccination surveillance perhaps as either a screening or confirmatory test in conjunction with other NSP tests. However, more sera need to be analyzed from vaccinated and naïve animals to determine the specificity of the test. Modification of the 2B #7 peptide without changing the epitope may be useful in avoiding nonspecific reactions. It would also be useful to evaluate the assay in future using sera from FMDV vaccinated and infected pigs and sheep.

Acknowledgements

The work was funded partly by NARO Japan (project No. 12), JICA (Project on Animal Disease Control in Thailand and Neighboring Countries), Department for Food and Rural Affairs, United Kingdom through the grant SE1122, European Commission (FMD_Improcon project of the EU 6th Framework Programme, SSPE-CT-2003-503603) and Regional Reference Laboratory for FMD in South East Asia, Thailand. The authors thank Payont Sinsuwongwat (Bureau of Veterinary Biologics, Thailand) for providing us animals for FMD vaccine potency test.

Sources and manufacturers

a. *Ceditest® FMDV-NS*, Cedi Diagnostics B.V. Lelystad, The Netherlands.
b. *CHEKIT-FMD-3ABC bo-ov* Bommeli diagnostics, Liebefeld-Bern, Switzerland.

c. *UBI® FMDV NS ELISA (CATTLE)*, United Biomedical Inc, Hauppauge, NY.
d. *SVANOVIR™ FMDV 3ABC-Ab ELISA*, Svanova Biotech AB, Uppsala, Sweden.
e. Software Development Co., Ltd. Tokyo Japan.
f. Beckman Coulter, Inc. Fullerton CA.
g. Advanced ChemTeck, Louisville, KY.
h. PEPTIDE Institute Co. Ltd, Osaka, Japan.
i. TOSOH Corp, Tokyo, Japan.
j. Sigma-Aldrich Company Ltd, Poole, Dorset, UK.
k. Nalge Europe Limited, Hereford, UK.

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