THE USE OF NON-STRUCTURAL PROTEINS OF FMDV TO DIFFERENTIATE BETWEEN VACCINATED AND INFECTED ANIMALS IN THAILAND

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Abstract

The detection of antibody to non-structural proteins (NSP) of foot and mouth disease virus (FMDV) using various types of NSP kits produced from World Reference Laboratory (WRL), United Biomedical Inc.(UBI), Bommeli/Intervet, CEDI and other research institutes, has been studied. The Liquid Phase blocking ELISA (LPBE) for measurement of antibody to FMDV type O, A and Asia1 was used in parallel with the NSP tests to study the specificity and sensitivity of each NSP test kit. Three thousand nine hundred and twenty two serum samples from cattle, buffaloes and pigs were grouped according to known history comprising: non vaccinated, single vaccinated, multiple vaccinated and field infected animals. The NSP tests all gave showed high specificity in non vaccinated cattle of 100%, 100%, 99% and 100% respectively. In pigs both the WRL and UBI kits gave 100%. In the single vaccination group, the specificities were WRL = 99.5% and UBI = 98.7%. The NSP test in multiple vaccinated cattle and buffaloes gave specificities of WRL = 97.73%, and UBI = 100%. In the FMD field outbreak area, clinically distinguishable infected cattle gave sensitivity results by NSP tests of WRL, 98.3%; UBI, 88.9%; Bommeli/Intervet, 84.6%; CEDI, 90.1%; Inoue/Japan, 93.8%; USDA, 88.3%; and Pen Side/Korea, 84.6%. This study indicates that the NSP kits from WRL, UBI, Bommeli/ Intervet, and CEDI had a high specificity for cattle and pigs from 99-100% and a high sensitivity from 84.6-93.8% in cattle and 73.33-80% in pigs. In addition, the NSP tests have been applied to FMD sero surveillance in animals being quarantined at the international quarantine station in a multilateral Malaysia Thailand Myanmar (MTM) project and also used in sero-monitoring of field animals in the Thailand national plan for the FMD vaccination campaign programme in the country. Hence, the use of NSP test to differentiate between vaccinated and infected animals would be used as a standard diagnostic test for the control and eradication of FMD in the region.

1. INTRODUCTION

Foot and mouth disease (FMD), an acute, highly contagious disease of cloven-hoofed animal, is an important economic disease of livestock in Thailand. There are three serotypes of O, A and Asia1 that are considered endemic in the country and cause economic losses due to decreased production and export trade restrictions. Rapid and accurate diagnosis of the disease is very important to prevent spread and assist with the selection of the appropriate vaccines. The Regional Reference Laboratory for FMD in South East Asia (RRL) at Pakchong Nakhonratchasima Province, Thailand, provides the FMD diagnosis service to the region.

The standard ELISA typing test [1] is used to assay field samples submitted for type identification. Other serological tests include the virus neutralization (VN) test [2] and the liquid phase blocking ELISA (LPBE) [3] have been used for disease surveillance and sero-monitoring of vaccinated animals in parallel with the virus infection associated agar gel immunodiffusion (VIA-AGID) test [4]. Cowan and Graves [5] proposed this test for differentiating infected from infected animals. The VIA antigen has been identified as RNA-

dependent RNA polymerase which is presented in virus preparations used for vaccine production and is also a constituent of the 146S viral particles which is a major immunogenicity of viral harvests [6].

Recently an advanced biotechnology application of developed 2B, 3B, 3AB, 3ABC and the 3D non-structural protein of FMDV have been used to replace the VIA-AGID test [7], [8], [9]. This test uses the non-structural proteins of FMDV in an indirect ELISA [10] or competitive ELISA [11] and tests have demonstrated as showing a higher specificity and sensitivity as compared to existing methods for differentiating the vaccinated from naturally infected animals [12].

The Department of Livestock Development (DLD) has been established the national FMD vaccination campaign over the country. A trivalent vaccine FMD type O, A and Asia1 has been used for vaccinating field animals, twice a year. The purified vaccine is produced by the Bureau of Veterinary Biologics (BVB) and contains FMDV type O, A and Asia1. The seed virus vaccine strains were selected from local strains causing outbreaks in Thailand. The serum samples were collected one month after vaccination in each round, to determine the antibody titre against FMDV type O, A and Asia1 using the LPBE and also to determine the antibodies to FMD NSP.

The IAEA Coordinated Research Project (CRP) provided NSP kits from World Reference Laboratory (WRL), Pirbright and various other commercial NSP kits that were used to detect antibodies against 2B, 3B, 3AB and 3ABC in animal sera from many sources. In addition the assays were used to examine sera sampled from sero monitoring and disease surveillance campaigns at the national level during the FMD vaccination campaign programme. The tests were also compared in surveillance of animals along the border or being at quarantine stations supporting the bilateral and multilateral project on the establishment of disease free zone in Malaysia Thailand Myanmar (MTM), and the Upper Mekong and Lower Mekong projects in SEAFMD countries.

The objectives were:

- To study the specificity of various NSP reagent kits in difference animal status.
- To study the sensitivity of various NSP reagent kits in nationally infection and experimental animals.
- Application of the NSP test in sero-monitoring and surveillance in field animals at national and regional level.

2. MATERIALS AND METHODS

2.1. Serum samples

A total of 3922 serum samples from various groups of animal were studied including non vaccination, single vaccination, multiple vaccinations, experimental infection and nationally infected with vaccination. Field sera from FMD vaccination campaign and regional project were also tested. Those samples were used to detect antibodies to structural protein of FMDV type O, A and Asia1 by the LBPE and to detect antibody to NSP of FMDV by indirect ELISA and competitive ELISA.

2.2. Purified vaccine

FMD trivalent vaccine containing type O, A and Asia1 was produced by the Bureau of Veterinary Biologics (BVB), Department of Livestock Development, and Thailand. The cattle $Al(OH)_3$ adjuvant vaccine and pig oil adjuvant vaccine were prepared by the suspension method using 146S antigen as the major antigenic component.

2.3. Liquid phase blocking ELISA (LPBE)

The LPBE measuring total antibodies against FMDV O, A and Asia1 was performed in duplicate, using two-fold dilution series method described by [3] and 13].

2.4. Non-structural protein (NS) test

NSP test by using various type of NSP kits were listed as this following:

2.4.1. WRL NSP reagents

A NSP reagent set produced by World Reference Laboratory (WRL)/Brescia, Pirbright Laboratory, United Kingdom was used to detect antibody to 3ABC NSP of FMDV by indirect ELISA, the test procedure was described in the instruction manual of WRL.

2.4.2. UBI® FMDV NS EIA (UBI)

A commercial NSP kit produced by United Biochemical Inc. USA was used to detect antibodies to FMDV 3B by indirect ELISA, the test procedure was described in the instruction manual of the manufacturer.

2.4.3. CHEKIT FMDV-3ABC Bommeli

A commercial NSP kit produced by Bommeli Switzerland, was used to detect antibodies to FMDV 3ABC by indirect ELISA, the test procedure was described in the instruction manual of the manufacturer.

2.4.4. CEDI Test®FMDV-NS (CEDI)

A commercial NSP kit from CEDI Diagnostic was used to detect antibody to 3ABC NSP of FMDV by competitive ELISA method, as described by instruction manual.

2.5. Other NSP reagents

2.5.1. 3AB NS reagent.

Developed by USDA, Plum Island Animal Disease Center, USA, the test procedure was described by protocol of USDA.

2.5.2. 2B NS reagent

Developed by Dr. Toru Inoue, NIAH, Japan, the test procedure was described in the instruction manual of NIAH.

2.5.3. ABC rapid pen-side test

A commercial NSP kit produced by Korea, the test procedure was described in the instruction manual of the manufacturer.

TABLE I. SERUM SAMPLES USED FOR TESTING OF NSP KITS AND PEN-SIDE TEST. (TOTAL SAMPLE = 3922)

Immunization status	Species	No. of sample
Non vaccination and non infection	Cattle/pig	380
Single vaccination and non infection	Cattle/pig	395
Multiple vaccination and non infection	Cattle/buffalo	220
Nationally infection and vaccination	Cattle/pig	360
1 month post vaccination from the national FMD control programme	Cattle/buffalo	1515
Animal movement at quarantine station	Cattle/buffalo	1052

3. RESULTS

3.1. Data from cattle and pigs

In order to illustrate the extent of testing, the data summarising a variety of tests is show in the next series of tables. A = agreement of all tests was positive. D = disagreement in ELISA results. A = agreement between ELISA test data as negative, but AGID positive.

TABLE II. DATA FROM CATTLE COMPARING LPBE, VIA AGID, WRL AND UBI TESTS

Exp.no	Serum history	LP ELISA			VIA			
		O	A	AS1	Test	WRL	UBI	
1110	Field sera	2560	160	640	+	+	+	A
1111	2 weeks post infection	2560	320	640	+	+	+	A
1112	from field outbreak of	5120	2560	640	+	+	+	A
1113	FMDV	5120	2560	2560	+	+	+	A
1114	"	2560	2560	2560	+	-	-	A-
1115	"	5120	2560	2560	+	+	+	A
1116	"	5120	2560	640	+	+	+	A
1117	"	5120	640	640	+	+	+	A
1118	"	5120	640	2560	+	+	+	A
1119	"	5120	640	640	+	+	+	A
1120	"	5120	640	2560	+	+	+	A
1121	"	5120	160	320	+	+	+	A
1122	"	5120	2560	2560	+	+	+	A
1123	"	5120	2560	5120	+	+	+	A
1124	"	5120	2560	1280	+	+	+	A
1125	"	5120	2560	2560	+	-	-	A-
1126	"	5120	1280	640	+	+	+	A
1127	"	5120	5120	5120	+	-	-	A-
1128	"	5120	2560	2560	+	-	-	A-
1129	*"*	*2560*	*2560*	*640*	+	+	-	*D*
1130	"	5120	2560	5120	+	-	-	A-
Number		O	A	Asia1	VIA	WRL	UBI	
1131	"	5120	640	2560	+	+	+	A
1132	"	5120	5120	5120	+	+	+	A
1133	"	5120	640	640	+	+	+	A
1134	"	5120	5120	2560	+	+	+	A
1135	"	5120	2560	2560	+	+	+	A
1136	"	5120	320	640	+	+	+	A
1137	"	5120	320	320	+	+	+	A
1138	"	5120	2560	320	+	+	+	A
1139	"	5120	160	160	+	+	+	A
1140	"	5120	1280	1280	+	+	+	A
1141	"	5120	2560	2560	+	+	+	A
1142	"	5120	2560	1280	+	+	+	A
1143	2 weeks post infection from	5120	1280	1280	+	+	+	A
1144	field outbreak of FMDV	5120	1280	1280	+	+	+	A
1145	Type O	5120	5120	5120	+	+	+	A
1146	"	5120	5120	2560	+	+	+	A
1147	"	5120	5120	2560	+	+	+	A
1148	"	5120	5120	5120	+	+	+	A
1149	"	5120	5120	2560	+	+	+	A
1150	"	5120	2560	1280	+	+	+	A
1151	*"*	*5120*	*5120*	*5120*	+	+	-	*D*
1152	"	5120	5120	5120	+	+	+	A
1153	*"*	*2560*	*5120*	*5120*	+	+	-	*D*
1154	"	5120	5120	1280	+	-	-	A-
1155		5120	5120	5120	+	+	+	A
1156		5120	5120	5120	+	+	+	A

Exp.no	Serum history	LP ELISA			VIA			
		O	A	AS1	Test	WRL	UBI	
1157		5120	5120	5120	+	+	+	A
1158		5120	2560	2560	+	+	+	A
1159		5120	5120	5120	+	+	+	A
1160	"	*5120*	*5120*	*5120*	+	+	-	*D*
1161	"	5120	5120	5120	+	-	-	A-
1162	"	5120	5120	2560	+	+	+	A
1163	"	5120	5120	5120	+	+	+	A
1164	"	5120	5120	5120	+	+	+	A
1165	"	5120	5120	2560	+	+	+	A
1166	"	5120	640	640	+	+	+	A
1167	"	5120	5120	5120	+	+	+	A
1168	"	5120	1280	1280	+	+	+	A
1169	"	5120	5120	5120	+	+	+	A
1170	"	5120	1280	5120	+	+	+	A
1171	"	5120	2560	5120	+	+	+	A
1172	"	2560	640	1280	+	+	+	A
1173	"	5120	5120	5120	+	+	+	A
1174	"	5120	320	320	+	+	+	A
1175	"	5120	2560	2560	+	+	+	A
1176	"	5120	5120	5120	+	+	+	A
Number		O	A	Asia1	VIA	WRL	UBI	
1177	"	5120	640	1280	+	+	+	A
1178	2 weeks post infection from	1280	640	320	+	+	+	A
1179	field outbreak of FMDV	5120	320	1280	+	+	+	A
1180	Type O	5120	5120	1280	+	+	+	A
1181	"	2560	640	1280	+	+	+	A
1182	"	5120	2560	1280	+	+	+	A
1183	"	2560	640	640	+	+	+	A
1184	"	5120	320	640	+	+	+	A
1185	"	5120	1280	2560	+	+	+	A
1186	"	5120	1280	2560	+	+	+	A
1187	"	5120	1280	2560	+	+	+	A
1188	"	5120	5120	5120	+	+	+	A
1189	"	5120	1280	5120	+	+	+	A
1190	"	5120	2560	2560	+	+	+	A
1191	"	5120	1280	640	+	+	+	A
1192	"	640	1280	640	+	+	+	A
1193	"	*5120*	*5120*	*5120*	+	+	-	*D*
1194	"	5120	1280	2560	+	+	+	A
1195	"	5120	5120	2560	+	+	+	A
1196	"	5120	2560	5120	+	-	-	A-
1197	"	*5120*	*320*	*640*	-	+	-	*D*
1198	"	5120	1280	5120	+	+	+	A
1199	"	2560	320	1280	+	+	+	A
1200	"	5120	2560	2560	+	+	+	A
1201	"	5120	5120	5120	+	+	+	A
1202	"	5120	640	2560	+	+	+	A

Exp.no	Serum history	LP ELISA			VIA			
		O	A	AS1	Test	WRL	UBI	
1203	"	5120	1280	5120	+	+	+	A
1204	"	5120	1280	5120	+	+	+	A
1205	2 weeks post infection from	1280	320	320	+	+	+	A
1206	field outbreak of FMDV	5120	640	640	+	+	+	A
1207	Type O	5120	320	1280	+	+	+	A
1208	"	5120	640	1280	+	+	+	A
1209	"	5120	5120	5120	+	+	+	A
1210	"	80	320	320	+	+	+	A
1211	"	320	5120	5120	+	+	+	A
1212	"	80	2560	2560	+	+	+	A
1213	"	160	640	1280	+	+	+	A
1214	"	80	5120	5120	+	+	+	A
1215	"	5120	320	640	+	+	+	A
1216	"	5120	320	2560	+	+	+	A
1217	"	5120	5120	5120	+	+	+	A
1218	"	5120	320	640	+	+	+	A
1219	"	5120	640	640	+	+	+	A
1220	"	5120	5120	5120	+	+	+	A
1221	"	5120	5120	2560	+	+	+	A
1222	"	5120	1280	320	+	+	+	A
1223	"	5120	5120	1280	+	+	+	A
1224	"	2560	2560	1280	+	+	+	A
1225	"	5120	320	320	+	+	+	A
1226	"	5120	5120	640	+	+	+	A
1227	"	5120	5120	640	+	+	+	A
1228	"	5120	5120	2560	+	+	+	A
1229	"	2560	2560	2560	+	-	-	A-
1230	"	2560	1280	2560	+	+	+	A
1231	"	5120	2560	1280	+	+	+	A
1232	"	5120	640	1280	+	+	+	A
1233	*"*	*640*	*640*	*2560*	+	+	-	*D*
1234	"	2560	160	640	+	+	+	A
1235	*"*	*640*	*640*	*640*	+	+	-	*D*
1236	"	5120	1280	2560	+	+	+	A
1237	"	5120	5120	5120	+	+	+	A
1238	"	2560	160	640	+	+	+	A
1239	"	640	80	320	+	+	+	A
1240	"	2560	2560	1280	+	+	+	A
1241	"	1280	320	640	+	+	+	A
1242	"	5120	2560	2560	+	+	+	A
1243	"	5120	320	640	+	+	+	A
1244	"	2560	640	2560	+	+	+	A
1245	"	2560	160	640	+	+	+	A
1246	"	5120	80	320	+	+	+	A
1247	"	2560	160	320	+	+	+	A
1248	"	5120	160	640	+	+	+	A

Exp no	Serum history	LP ELISA			VIA			
		O	A	AS1	Test	WRL	UBI	
1249	"	2560	160	640	+	+	+	A
1250	"	5120	2560	5120	+	+	+	A
1251	***"***	***5120***	***5120***	***5120***	+	+	-	***D***
1252	"	5120	2560	2560	+	+	+	A
1253	"	5120	640	1280	+	+	+	A
1254	"	5120	160	640	+	+	+	A
1255	"	*5120*	*2560*	*2560*	+	+	-	*D*
1256	"	5120	160	320	+	+	+	A
1257	"	5120	160	640	+	+	+	A
1258	***"***	***5120***	***1280***	***1280***	+	+	-	***D***
1259	"	5120	160	640	+	+	+	A
1260	"	5120	320	320	+	+	+	A
1261	"	5120	5120	5120	+	+	+	A
1262	"	5120	640	320	+	+	+	A
1263	"	5120	5120	5120	+	+	+	A
1264	"	5120	5120	5120	+	+	+	A
1265	"	5120	5120	1280	+	+	+	A
1266	"	1280	640	640	+	+	+	A
1267	"	2560	640	640	+	+	+	A
1268	"	5120	5120	640	+	+	+	A
1269	"	5120	1280	160	+	+	+	A
1270	"	5120	640	320	+	+	+	A
1271	"	5120	1280	2560	+	+	+	A
1272	1 month post infection from	2560	640	1280	+	+	+	A
1273	field outbreak of FMDV	2560	160	320	+	+	+	A
1274	Type O	5120	1280	640	+	+	+	A
1275	Field serum	5120	160	320	+	+	+	A
1276		2560	640	160	+	+	+	A
1277	"	5120	5120	2560	+	+	+	A
1278	"	5120	2560	5120	+	+	+	A
1279	"	5120	1280	2560	+	+	+	A
1280	"	5120	320	320	+	+	+	A
1279	"	5120	1280	2560	+	+	+	A
1280	"	5120	320	320	+	+	+	A
1281	"	2560	320	160	+	+	+	A
1282	"	2560	640	320	+	+	+	A
1283	"	5120	2560	640	+	+	+	A
1284	"	640	160	160	+	+	+	A
1285	"	2560	160	160	+	+	+	A
1286	"	1280	80	160	+	+	+	A
1287	"	640	80	80	+	+	+	A
1288	"	2560	160	320	+	+	+	A
1289	"	1280	1280	640	+	+	+	A
1290	"	2560	160	160	+	+	+	A
1291	"	1280	160	320	+	+	+	A
1292	***"***	***640***	***160***	***40***	+	+	-	***D***

Exp no	Serum history	LP ELISA			VIA			
		O	A	AS1	Test	WRL	UBI	
1293	"	1280	640	640	+	+	+	A
1294	"	5120	1280	640	+	+	+	A
1295	"	1280	640	320	+	+	+	A
1296	"	5120	640	1280	+	+	+	A
1297	"	5120	320	320	+	+	+	A
1298	"	2560	640	640	+	+	+	A
1299	"	1280	320	320	+	+	+	A
1300	"	2560	1280	1280	+	+	+	A
1301	"	2560	640	1280	+	+	+	A
1302	"	1280	640	640	+	+	+	A
1303	"	5120	1280	640	+	+	+	A
1304	"	*2560*	*640*	*640*	+	+	-	*D*
1305	"	5120	640	640	+	+	+	A
1306	"	2560	320	160	+	+	+	A
1307	"	*160*	*80*	*80*	+	+	-	*D*
1308	"	5120	640	1280	+	+	+	A
1309	"	2560	640	320	+	+	+	A
1310	"	2560	1280	1280	+	-	-	A-
1311	"	*640*	*640*	*1280*	+	+	-	*D*
1312	"	2560	640	1280	+	-	-	A-
1313	"	640	160	80	+	+	+	A
1314	"	2560	640	640	+	+	+	A
1315	"	2560	640	640	+	+	+	A
1316	"	*2560*	*640*	*1280*	+	+	-	*D*
1317	"	640	160	160	+	+	+	A
1318	"	*2560*	*160*	*160*	+	+	-	*D*
1319	"	*2560*	*160*	*640*	+	+	-	*D*
1320	"	*640*	*80*	*640*	+	-	+	*D*
1321	"	1280	640	1280	+	-	-	A-
1322	"	320	320	640	+	+	+	A
1323	"	2560	160	640	+	+	+	A

TABLE III. DATA FROM PIGS COMPARING LPBE, VIA AGID, WRL AND UBI TESTS

PIGS		O	A	AS1A 1	VIA	WRL	UBI	Compare
678	3W Vacc+ challenge O	320	320	320	-	-	-	A-
679	3W Vacc+ challenge O	80	80	160	-	-	-	A-
708	3W Vacc+ challenge O	2560	320	320	-	-	-	A-
709	3W Vacc+ challenge O	640	320	640	-	-	-	A-
710	3W Vacc+ challenge O	640	640	320	-	-	-	A-
711	3W Vacc+ challenge O	2560	640	2560	-	-	-	A-
712	3W Vacc+ challenge O	160	160	160	-	-	-	A-
686	3W Vacc+ challenge A	1280	5120	2560	-	+	-	D
687	3W Vacc+ challenge A	1280	5120	2560	+	+	+	A+
688	3W Vacc+ challenge A	1280	5120	2560	+	+	-	D
689	3W Vacc+ challenge A	2560	5120	2560	+	+	-	D
690	3W Vacc+ challenge A	2560	5120	2560	+	-	-	A-
697	3W Vacc+ challenge As1	320	160	1280	-	-	-	A-
698	3W Vacc+ challenge As1	1280	320	2560	-	-	-	A-
699	3W Vacc+ challenge As1	640	320	1280	-	-	-	A-
700	3W Vacc+ challenge As1	640	1280	1280	-	-	-	A-
701	3W Vacc+ challenge As1	1280	1280	1280	-	-	-	A-
754	3W vacc+ 1 w PC As1	320	320	640	-	+	-	D
755	3W vacc+ 1 w PC As1	640	640	1280	-	-	-	A-
756	3W vacc+ 1 w PC As1	320	320	2560	-	-	-	A-
757	3W vacc+ 1 w PC As1	640	1280	1280	-	-	-	A-
758	3W vacc+ 1 w PC As1	320	320	640	+	-	-	A-
764	3W vacc+ 2 w PC As1	1280	320	1280	-	-	+	D
765	3W vacc+ 2 w PC As1	1280	320	2560	-	-	-	A-
766	3W vacc+ 2 w PC As1	1280	320	1280	-	-	-	A-
767	3W vacc+ 2 w PC As1	1280	640	1280	-	-	-	A-
768	3W vacc+ 2 w PC As1	320	320	640	+	+	-	D
789	3W vacc+ 3 w PC As1	80	160	320	-	+	+	A+
790	3W vacc+ 3 w PC As1	320	160	1280	-	-	-	A-
791	3W vacc+ 3 w PC As1	160	160	640	-	+	+	A+
792	3W vacc+ 3 w PC As1	320	320	640	-	-	+	D
793	3W vacc+ 3 w PC As1	160	160	320	+	+	-	D
799	3W vacc+ 4 w PC As1	160	160	320	-	+	+	A+
800	3W vacc+ 4 w PC As1	320	160	640	-	-	-	A-
801	3W vacc+ 4 w PC As1	640	160	640	-	+	+	A+
802	3W vacc+ 4 w PC As1	640	80	640	-	-	+	D
803	3W vacc+ 4 w PC As1	320	160	320	-	-	-	A-
809	3 W vacc+ 5 w PC As1	160	80	320	-	-	+	D
810	3 W vacc+ 5 w PC As1	640	640	640	-	-	-	A-
811	3 W vacc+ 5 w PC As1	320	320	640	-	-	+	D
812	3 W vacc+ 5 w PC As1	640	640	640	-	-	+	D
813	3 W vacc+ 5 w PC As1	320	160	640	-	+	-	D
684	1W postchallenge O	<40	<40	<40	-	+	+	A+
685	1W postchallenge O	<40	<40	<40	-	-	-	A-
713	1W postchallenge O	1280	160	40	-	-	-	A-
714	1W postchallenge O	320	160	80	-	-	-	A-
715	1W postchallenge O	320	80	40	-	-	-	A-
716	1W postchallenge O	640	320	80	+	-	-	A-
717	1W postchallenge O	1280	320	160	-	+	-	D
718	1W postchallenge O	40	40	<40	-	-	-	A-
691	1W postchallenge A	160	640	320	-	+	-	D
692	1W postchallenge A	160	640	640	-	+	-	D
693	1W postchallenge A	40	640	80	+	+	-	D
694	1W postchallenge A	<40	320	40	-	-	-	A-
695	1W postchallenge A	40	1280	80	-	-	-	A-
696	1W postchallenge A	80	640	160	-	+	-	D

Exp		O	A	AS1	VIA	WRL	UBI	Compare
719	710-733 Infected area	80	40	40	+	-	-	A-
720	2 weeks post infection with	40	<40	<40	-	-	-	A-
721	In field outbreak	160	80	80	-	-	-	A-
722	of FMDV type O	5120	2560	640	+	-	-	A-
723		5120	5120	5120	+	+	-	D
724		40	40	<40	-	-	-	A-
725		1280	1280	1280	-	-	-	A-
726		160	320	160	-	-	-	A-
727		2560	1280	2560	+	+	-	D
728		1280	1280	1280	+	-	-	A-
729		5120	1280	1280	-	+	-	D
730		5120	5120	5120	-	-	-	A-
731		5120	5120	5120	+	-	-	A-
732		2560	5120	5120	-	+	-	D
733		320	320	640	+	-	-	A-
734	Field sample serum 734-743	ND	ND	ND	-	-	-	A-
735	Post O outbreak	2560	640	1280	-	-	-	A-
736		2560	1280	320	-	-	-	A-
737		640	320	640	-	-	-	A-
738		3940	320	960	-	-	-	A-
739		5120	2560	1280	+	+	-	D
740		5120	2560	1280	+	-	-	A-
741		5120	2560	2560	-	-	-	A-
742		5120	2560	2560	+	+	+	A+
743		5120	2560	1280	+	+	-	A+

3.2. Vaccine/challenge on selected data

TABLE IV. VACCINATED PIGS AT DIFFERENT DAYS AFTER CHALLENGE WITH ASIA 1

Pig	Treatment	O	A	ASIA1	VIA	WRL	UBI	Compare
91	3W vacc+ 1 w PC	320	320	640	-	+	-	D
91	3W vacc+ 2 w PC	1280	320	1280	-	-	+	D
91	3W vacc+ 3 w PC	80	160	320	-	+	+	A+
91	3W vacc+ 4 w PC	160	160	320	-	+	+	A+
91	3 W vacc+ 5 w PC	160	80	320	-	-	+	D
92	3W vacc+ 1 w PC	640	640	1280	-	-	-	A-
92	3W vacc+ 2 w PC	1280	320	2560	-	-	-	A-
92	3W vacc+ 3 w PC	320	160	1280	-	-	-	A-
92	3W vacc+ 4 w PC	320	160	640	-	-	-	A-
92	3 W vacc+ 5 w PC	640	640	640	-	-	-	A-
93	3W vacc+ 1 w PC	320	320	2560	-	-	-	A-
93	3W vacc+ 2 w PC	1280	320	1280	-	-	-	A-
93	3W vacc+ 3 w PC	160	160	640	-	+	+	A+
93	3W vacc+ 4 w PC	640	160	640	-	+	+	A+
93	3 W vacc+ 5 w PC	320	320	640	-	-	+	D
94	3W vacc+ 1 w PC	640	1280	1280	-	-	-	A-
94	3W vacc+ 2 w PC	1280	640	1280	-	-	-	A-
94	3W vacc+ 3 w PC	320	320	640	-	-	+	D
94	3W vacc+ 4 w PC	640	80	640	-	-	+	D
94	3 W vacc+ 5 w PC	640	640	640	-	-	+	D
95	3W vacc+ 1 w PC	320	320	640	+	-	-	A-
95	3W vacc+ 2 w PC	320	320	640	+	+	-	D
95	3W vacc+ 3 w PC	160	160	320	+	+	-	D
95	3W vacc+ 4 w PC	320	160	320	-	-	-	A-
95	3 W vacc+ 5 w PC	320	160	640	-	+	-	D

Pigs vaccinated with trivalent O, A and Asia 1 vaccine.
At three weeks the pigs were challenged with Asia 1 virus.
Animals were sampled (serum) at 1 w, 2 w, 3 w, 4 w and 5 w after challenge (PC)
Sera were tested by L PBE (O, A, Asia1); NSP ELISAs and VIA AGID.

3.3. Comparisons of data

The results of NSP test using various NSP kits were shown in the Table IV.

TABLE V. THE NSP TEST AND LP ELISA TITER IN NON-VACCINATED CATTLE AND PIGS. NSP REAGENT KITS WERE USED FROM WRL AND UBI

Species	Total sample	NSP Negative				LP ELISA titer		
		WRL	UBI	Bomm	CEDI	O	A	Asia1
Cattle	279	0/272 100%	0/272 100%	1/100 99%	0/100 100%	1.348± 0.265	1.602± 0.307	1.381± 0.311
Pigs	101	0/101	0/56			1.165± 0.237	1.025±0.244	1.126± 0.263
Specificity	380	100%	100%	99%	100%			

TABLE VI. RESULT OF NSP TEST AND LP ELISA TITER IN CATTLE AND PIGS RECEIVING SINGLE VACCINATION

Species	Total sample	NSP negative		LP ELISA titre		
		WRL	UBI	O	A	Asia1
Cattle	272	270/272 99/26%	0/112 100%	1.813±0.269	1.99±0.367	1.756±0.321
Pig	123	0/123	5/123	2.178±0.445	2.064±0.451	2.011±0.476
Specificity	395	99.5%	98.7%			

TABLE VII. NSP TEST AND LP ELISA TITERS IN CATTLE AND BUFFALOS RECEIVING MULTIPLE VACCINATION 5 TIMES OR MORE

Species/history	Total sample	NSP negative		LP ELISA titer		
		WRL	UBI	O	A	Asia1
Cattle	180	175/180 97.22%	180/180 100%	2.399 ± 0.246	2.315 ± 0.456	2.415 ± 0.456
Buffalo	40	0/40 100%	0/40 100%	2.685 ± 0.554	2.226 ± 0.353	2.35 ± 0.389
Specificity	220	97.73%	100%			

Status: non infected , multiple vaccination animals from bleeding station, receiving vaccination every 6 m.

TABLE VIII. RESULT OF NSP TEST AND LP ELISA TEST IN CATTLE AND PIG SERA FROM FIELD OUTBREAK. (TOTAL SAMPLE = 360)

Serum history	NSP test (positive/total)						
	WRL	UBI	Bommeli/ Intervet	CEDI	Inoue (Japan)	USDA	Pen-side (Korea)
1 M PI + vaccinated, cattle	177/180 (98.3%)	160/180 (88.9%)	126/149 (84.6%)	163/180 (90.1%)	61/65 (93.8%)	159/180 (88.3%)	55/65 (84.6%)
2 M PI + vaccinated, cattle	ND	123/165 (74.5%)	121/165 (73.3%)	136/165 (82.4%)	135/165 (81.8%)	ND	ND
Infected pig	10/15 (73.33%)	12/15 (80%)	ND	ND	ND	ND	ND

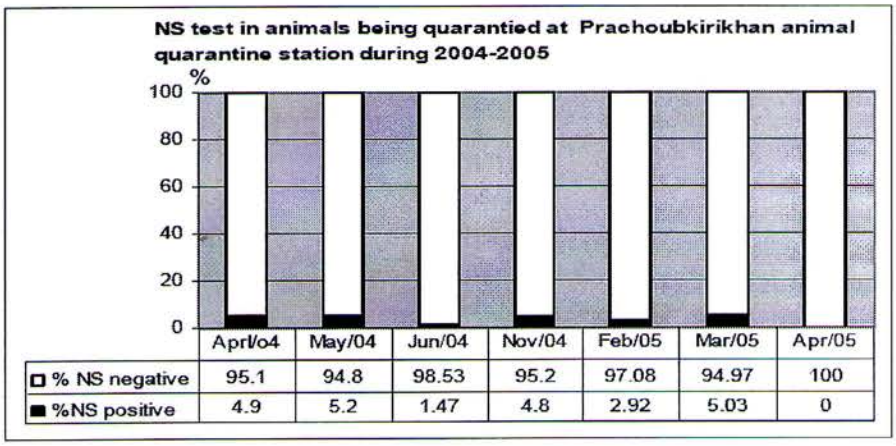
1 M = 1 m post infected and vaccinated cattle with trivalent vaccine , field outbreak type O in 2000
2 M =2 m post infected and vaccinated cattle with trivalent vaccine, field outbreak type A in 2004

WRL = 3ABC NSP World Reference Laboratory, UK; UBI = 3B NSP United Biomedical Inc., USA;
USDA =3AB NSP Plum Island Animal Disease center, USA; Bomm = 3ABC NSP Bommeli;
Inoue/Japan = 2B NSP T. Inoue, NIAH, Japan; CEDI = 3ABC NSP CEDI Diagnostic, Holland;
Pen-Side = 3ABC NSP rapid test from Korea; ND = Not done

TABLE IX. RESULTS ON ANNUAL VACCINATED ANIMALS TWICE A YEAR

Province	Total sample	NSP positive	LP ELISA positive (≥ 1:80)		
			O	A	Asia1
Burirum	231	11/231 (4.8%)	170/231(73.6%)	172/231 (74.5%)	181/231 (78.4%)
Ubonratchathani	259	24/259 (9.3%)	181/259 (69.9%)	181/259 (69.9%)	192/259 (74.1%)
Umnadchareon	349	5/349 (1.4%)	268/349 (76.8%)	273/349 (78.2%)	263/349 (75.4%)
Srisaked	120	7/120 (5.83%)	80/120 (66.7%)	84/120 (70%)	97/129 (80.83%)
Khonkhaen	76	1/76 (1.3%)	74/76 (97.36%)	70/76 (92.1%)	73/76 (96.05%)
Yasothon	120	3/120 (2.5%)	77/120 (60.2%)	67/120 (55.8%)	81/120 (67.5%)
Chaiyaphum	120	12/120 (10%)	80/120 (66.67%)	57/120 (47.5%)	69/120 (57.5%)
Surin	120	17/120 (14.2%)	109/120 (90.83%)	100/120 (83.3%)	111/120 (92.5%)
Roi et	120	4/120 (3.33%)	61/120 (50.8%)	63/120 (53%)	61/120 (50.8%)
Total	1515	84 (5.5%)	1100 (72.6%)	1067 (70.4%)	1128 (74.5%)

TABLE X. RESULTS ON CATTLE AND BUFFALOES IN BUFFER ZONE IN MTM PROJECT



	Aprl/o4	May/04	Jun/04	Nov/04	Feb/05	Mar/05	Apr/05
% NS negative	95.1	94.8	98.53	95.2	97.08	94.97	100
%NS positive	4.9	5.2	1.47	4.8	2.92	5.03	0

Blood samples were collected at the international quarantine station in Prachubkirikhan Province during year 2004–2005. (Total sample =1052)

3.4. Comparison of analytical sensitivity data on selected cattle sera

Selected cattle and pig sera were titrated in twofold dilution ranges and kits used to detect the signal. Comparison of analytical sensitivities was made by comparing the titration curves and end points (dilution at which the sample became negative in tests).

3.4.1. Comparison of early UBI and IAH kits using cattle sera

Table X shows data for comparison of tests at two defined OD values for the Indirect ELISAs from IAH and UBI. The figures represent the values obtained for the recommended dilution in test; the reciprocal of the dilution to achieve the OD designated (0.6) and the endpoint (reciprocal of the last dilution positive). The ratios dividing the IAH test results by the UBI are also shown.

3.4.2. Comparison of later CEDI, Bommeli and UBI kits using cattle sera

Figs 1–3 show the twofold titration curves for selected cattle sera using different kits.

TABLE XI. UBI AND IAH TESTS TITRATING SELECTED CATTLE SERA

	OD at Recommended dilution for test	**Result at rec. dilution**	**0.6 OD**	**E.P. (0.2)**	**Ratio IAH/UBI At 0.6OD**	**Ratio IAH/UBI E.P**
IAH	OD (1/200)					
6 weeks post challenge	1.23	+	900	5000	900/25 = 36	5000/130 = 38
6 weeks post challenge	2.97	+	9000	45,000	9000/220 = 45	45,000/900 = 50
1 week post challenge	1.19	+	600	2000	600/20 = 30	2000/90 = 22
1 week post challenge	2.78	+	6000	30,000	6000/10 60	30,000/700 = 43
1 week post challenge	0.8	-	0	450	1/10 = 0.1	450/50 = 9
vacc	0.28		0	0	0/0	0/0
UBI	OD (1/20)					
6 weeks post challenge	0.71	+	25	130		
6 weeks post challenge	2.85	+	220	900		
1 week post challenge	0.67	+	20	90		
1 week post challenge	2.25	+	180	700		
1 week post challenge	0.3	+	10	50		
vacc	0.08		0	0		

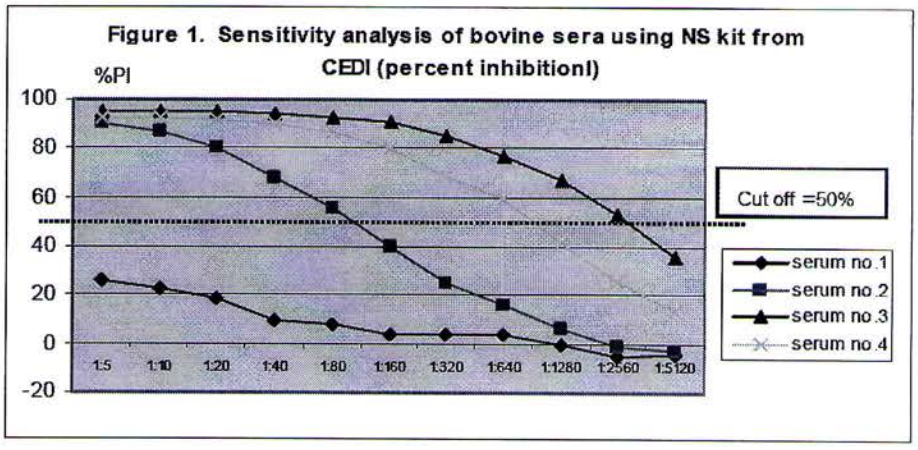


FIG. 1. Analysis of bovine serum titration curves usng CEDI kits.

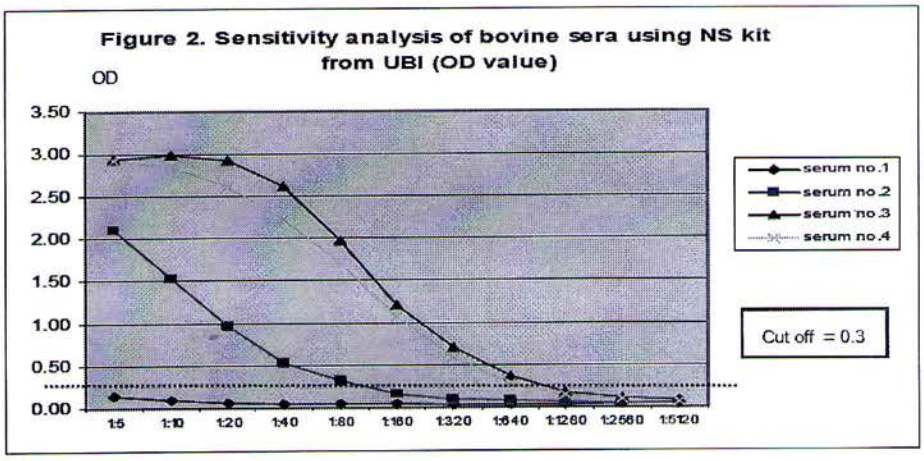
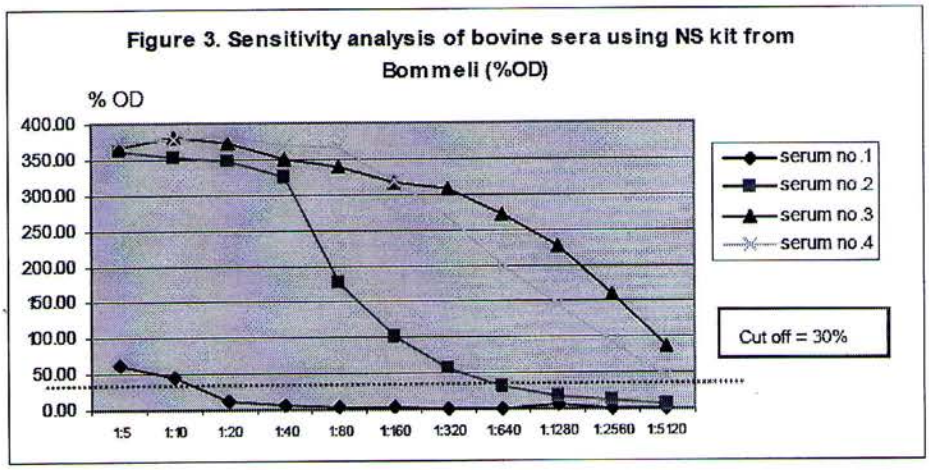


FIG.2. Analysis of bovine serum titration curvesuisng UBI kit.



Serum 1 = post vaccinated cow, serum 2 = 6 weeks post challenged
Serum 3 = 6 weeks post challenged, serum 4 = 4 weeks post challenged

FIG. 3. Analysis of bovine serum titration curves uisng Bommeli kit.

	CEDI (50% competition)	UBI (end point)	Bommeli (end point)
Serum			
1	0 (0)		
2	100 (EP 2,500)	100	640
3	3000 (EP 10,000)	1000	7000
4	1000 (EP 16,000)	800	5,500

3.5. Comparison of Analytical sensitivity data on selected pig sera

Table XI shows the values obtained for the recommended dilution in test; the reciprocal of the dilution to achieve the OD designated (0.6) and the endpoint (reciprocal of the last dilution positive).

TABLE XII. TITRATIONS OF PIG SERA USING DIFFERENT KITS

	Recommended dilution for test	**0.6 OD**	**E.P. (0.2)**	**Ratio IAH/UBI at 0.6 OPD**	**Ratio IAH/UBI at EP**
IAH	OD (1/200)				
1 w post challenge	0.42	120	500	120/1 = 120	500/20 = 25
2 w post infection	1.21	550	2000	550/0 = 550	2000/40 = 50
1 w post infection	1.88	1500	6000	1500/120 = 12.5	6000/800 = 75
5 w post challenge	0.71	250	800	250/20 = 12.5	800/100 = 8
5 post challenge	0.68	250	800	250/20 = 12.5	800/100 = 8
3 w post vaccination vacc	0.24	0	240	0	240/1 = 240
UBI	OD (1/20)				
1 w post challenge	0.25	0	20		
2 w post infection	0.31	0	40		
1 w post infection	1.58	120	800		
5 w post challenge	0.67	20	100		
5 w post challenge	0.73	20	100		
3 w post vaccination vacc	0.19	0	0		

4. CONCLUSION AND DISCUSSION

The detection of antibody to NSP of FMDV using various types of kit from WRL, UBI, Bommeli, and CEDI gave a high DSp when testing non vaccinated cattle and pig sera, in the range of 99-100% and 98.7-99.5% in single vaccinated animals groups. High DSps were also found for multiply vaccinated animals (range 97.7-100%) using the early WRL and UBI kits in the face of antibodies against structural proteins as assessed from the LPBE results.

The LP ELISA titres of such sera increased generally according to the increased number of vaccinations. Linchongsubongkoch [14] reported similar results, where the NSP tests in multiple vaccinated animals gave a high specificity by NSP test but low specificity by virus infection associated antigen agar gel immunodiffusion (VIA-AGID) test. This is not surprising since VIA is a part of the vaccine and induces antibodies in a good proportion of cattle even after a single vaccination, and this percentage rises on subsequent vaccination. Tests using the same cattle and buffaloes sera showed that there was a 65% specificity and 35% false positivity using the VIA test [8]; [9]. The current work confirms that the VIA-AGID test is not appropriate for countries that use vaccination.

The measurement of antibodies to structural protein of FMDV type O, A and Asia1 by LP ELISA [4]; [6] found that a low LP ELISA titre was detected in pig sera possibly from young animals presenting maternal immunity.

The DSn studies indicated a range of 84.6–93.8% in 1 m post infected cattle, and range 73.3–82.4% at 2 m post infection. Cattle from these groups may not all have been infected (which would affect the DSn figures) as a result of lack of contact or protection

through vaccination. However, it was shown that all the field sera showing clinical signs of FMD type O, contained high titres of antibodies to FMDV type O, A and Asia1 by LPBE. Cattle were vaccinated with trivalent vaccine after an outbreak and gave titres of were 3.216 + 0.310, 2.496 + 0.415 and 2.637 + 0.314, respectively.

The NSP test proved highly effective when used to survey cattle and buffalos moving in the FMD sero surveillance buffer in the multilateral project on "Tristates Commission on the Establishment of Malaysia Thailand Myanmar Peninsular Campaign of FMD Freedom" or MTM project.

The Department of Livestock Development (DLD) has established a national plan for FMD control and eradication programme with the two main objectives, (1) to strengthen the vaccination campaign to increase herd immunity in the animal population and 2) to restrict both domestic and international animal movement.

An FMD trivalent vaccine has been used to vaccinate animal twice a year, countrywide. Serum samples are collected regionally. The LPBE is used to determine the titres of antibodies to FMDV type O, A and Asia, in order to estimate the likely herd immunity. The NSP ELISAs are also being used to differentiate between vaccinated and infected animals. Results demonstrated a low incidence of viral replication in field animals in the face of vaccination with trivalent vaccine (e.g. 1 m post vaccination where a small number of NSP positive sera were found by UBI kit in range 1.3–14.2%). This indicates that there is a low incidence of FMD outbreaks in the face of vaccination and a high herd immunity against FMDV types O, A, and Asia1 (72.6%, 70.4% and 74.5%) respectively.

The situation in pigs is interesting and there seems to be a circulation of virus in heavily vaccinated farms where there is no apparent clinical signs. The results of the trivalent protected pigs producing antibodies to NSP some weeks after challenge, without clinical signs, need further investigation.

In conclusion, the use of NSP ELISAs to differentiate between vaccinated and infected animals has become an important assay for disease control. Although there was a demonstrable variation, all the later kits give a useable DSn and Dsp for use in sero-monitoring and surveillance of FMD at national and regional level. This form of assay as a standardized test in the control and eradication of FMD in the region is necessary to meet the target of establishment of FMD free zone in the S. E. Asian countries under the OIE regulations and also would be useful in international trade of livestock in FMD free countries.

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