

# Classical Swine Fever among Pig Herds and its Control in Cantho Province, Mekong Delta

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## Abstract

We collected 17 fatal pigs and 5 sera of the sows from 10 farms between July 2002 and June 2003 for laboratory diagnosis, to identify the cause of the disease by pathological, serological and virological examination. On the other hand clinical symptoms observed were compared with those of the criteria generally used to recognize the diseases by the veterinary technicians. We also investigated the farms of the diseased pigs to analyze the epidemiological data with the laboratory results, and finally discussed on how the disease can be prevented. Consequently classical swine fever (CSF) was confirmed in all the farms. The virus genome of 5'NTR identified was all of the field strain. Lack of the booster before mating (five farms), or deviation from or improper vaccine indication were traced. The definition of Dich Ta, epidemic diarrhea incurable by antibiotics, may well reflect CSF, while other clinical signs observed at the onset could be recognized also as paratyphus. Thus it was recommended to perform prompt laboratory diagnosis by direct immuno-fluorescent or -peroxidase antibody tests which would be confirmed by RT-PCR. And consistent pathological findings, depletion of lymphoid tissue which enhances the secondary infection, could be used as supportive evidence. Once accurate diagnosis were established, more cases as well as the importance of classical swine fever would be revealed which allows the stakeholders appropriate selections of the control method, such as introduction of the animals from only reliable sources, booster for the gilts and growers, prevention by unique and rigid vaccination program and prompt slaughter of the sick animal.

## Introduction

Classical swine fever (CSF) is a highly contagious viral disease of swine which has serious socio-economic consequence (Van Oirschot, 1999, OIE classification of diseases, List A).

From the pig holders interview in Tan Phu Thanh village (Kamakawa et.al, 2002), pig disease especially the death of piglets were sought to be one of the serious constraints of pig production. And most of the fatal pigs were of peracute, or febrile disease incurable by antibiotics, some of which showed epidemic feature and clinical symptoms doubtful of CSF.

In Vietnam about 18,106 cases (heads) of CSF were reported annually (Handistatus, OIE, 2000) and some case studies were undertaken to define the epidemiological role of CSF (Nguyen Thi Hanh Chi, 2000, Li Thi Kim Lan, 2001, Nguyen Tien Dung, 2002) , yet no comprehensive paper on the diagnosis of the disease was found.

In the Mekong delta, southern Vietnam, pig disease are recognized by veterinary technicians with only a few symptoms such as “Pho Thuong Han (PTH)”; literary meaning “looks like injure and cold”, “Tu Huyet Trung (THT) or Toi”; “septicemia and blood accumulation or sudden death” and “Dich Ta Heo (DT)”; “epidemic diarrhea of pig”, but named respectively as paratyphus (or Salmonellosis), haemorrhagic septicemia (or Pasteurellosis) and hog cholera (conventional name of CSF)(NAVETCO 1995). In the pig disease statistics of Cantho province (Department of animal health, Cantho province, 2000) only a small number of hog cholera cases were reported compared to other diseases such as paratyphus and haemorrhagic septicemia, which might lead to confusion that latter diseases were more important for control than CSF, without any differential diagnosis (Table1).

To identify what is the real cause of the disease having more negative impact for the pig-holders, whether CSF is underestimated or not, we collected fatal pigs between July 2002 and June 2003 for laboratory diagnosis. The sampled farms were also investigated to collect the epidemiological information. The clinical symptoms observed were compared to those recognized by veterinary technicians, and pathologically examined to find out consistent lesion. All of the samples were virologically tested utilizing genetical diagnosis; RT-PCR and confirmed both by digestion with restriction enzyme or sequence of the amplified product. Finally the disease control methods were discussed based on the test results obtained.

## Materials and methods

### **(1) Collection of the samples and epidemiological information**

Cantho province (Tinh) has about 82 wards (Phuong), town (Thi tran) and villages (Xa) which belongs to 2 cities (TP, TX) and 7 districts (Huyen) (Map of Cantho province 2001). The sample was collected between end of July 2002 and June 2003 through a veterinarian who had local veterinarians' network in 3 towns, 1 ward and 8 villages of 2 cities and 4 districts (15%(12/82)). There were 10 farms with fatal herds most of

which had difficulty in treatment with antibiotics or sulfamids, out of approximately 100 inquiries received. Total 17 fatal piglets and 18 sera (including 5 sera from the sow) were collected for laboratory examination. The farms were interviewed and/or visited twice, first at the time of outbreak and then a few months later.

Epidemiological information such as course of the outbreak, population of the pigs at the time of outbreak by age and litter, morbidity (pigs showing clinical symptom (sick pigs) / pigs of the same litter or age, or pig population), mortality (pigs died or fatal / sick pigs), vaccination record of piglets and the sow (date and make of the recent vaccination), introduction of the animals, outbreak of the neighbour were interviewed.

Clinical symptoms observed were recorded and classified into the category of disease/symptoms generally recognized by the local veterinary technicians, which reflected the Vietnamese name of the disease (Table1).

Table 1 Pig disease criteria generally recognized by the local veterinary technicians and the cases reported in Cantho province (2000)

Disease or symptom in Vietnamese	Direct translation	Symptoms recognized by the vet. technicians	Disease name	Case (head)*
Pho Thuong Han	Injure and cold	Shivering, trembling, constipation, fever	Paratyphus, Salmonellosis	52,125 28%
Tu Huyet Trung (= Toi)	Septicemia and blood accumulation, sudden death	High fever, respiratory distress, multifocal haemorrhage, sudden death	Pasteurellosis, Haemorrhagic Septicemia	35,371 19%
Dich Ta Heo	Epidemic diarrhea	Diarrhea incurable by antibiotics	Hog Cholera	316 0.2%
Tieu Chay	Diarrhea	Diarrhea	Diarrhea	67,018 36%
Nhung Benh Khai	Other diseases	-	-	31,331 17%
Total				186,161 100%
Pig population				244,315

\* Source: Department of animal health, Cantho province

## (2) Laboratory tests

### *Sample preparation, serological and pathological examination*

Blood samples were collected both in coagulant-added and/or heparin Na-added aseptic tubes. Number of white blood cells was counted and sera were separated after a few hours in 4 °C, then centrifuged at 3,000 rpm 10 minutes. The sera were examined for antibody against pseudorabies (Aujeszky's disease) by Latex agglutination test (Viral Antigen, USA), porcine reproductive and respiratory

syndrome by PRRS-ELISA kit (IDEXX laboratories Inc., USA) and classical swine fever by CSF-ELISA kit (Chisso, Japan).

Seventeen fatal pigs were dissected and major organs such as tonsil, spleen, kidney, lung, liver, heart, ileum-cecum junction, lymph nodes, brain and other gross lesions were sampled for agent detection as well as for pathological examination. The former was kept in  $-80^{\circ}\text{C}$  and the latter in 10% buffered formalin. Preparation of the tissue sections was carried out by a conventional method, and observed microscopically with hematoxylin and eosin stain.

A piece of tonsil and spleen were weighed, minced and grinded by glass particles in the mortar, and dissolved in 10 fold amount of media(5% horse serum,  $60\ \mu\text{g/ml}$  Kanamycin,  $5\ \mu\text{g/ml}$  amphotericin, 5% L-glutamin, 11%  $\text{NaHCO}_2$ (pH7.1-7.4) added Eagle's MEM). Centrifuged at 3,000rpm for 5 minutes and the supernant was pre-filtrated twice then finally filtrated by  $0.45\ \mu\text{m}$  filter and stored in  $-20^{\circ}\text{C}$  or  $-80^{\circ}\text{C}$ .

### **RT-PCR to detect 5' non-translated region of the pestivirus genome and differentiation of genogroup 1 (CSFvirus) by digesting amplified product with restriction enzyme BglI**

RNA was extracted from  $200\ \mu\text{l}$ \* of pre-filtrated 10% homogenate or plasma or sera (High pure viral RNA kit, Roche Diagnostics) and finally dissolved in  $50\ \mu\text{l}$  nuclease-free redistilled water. (\* vaccine or cell cultured viral fluid in half volume).  $2.5\ \mu\text{l}$  of RNA template was mixed with  $22.5\ \mu\text{l}$  of primer mixture (consisted of  $1\ \mu\text{l}$  of each primers( $10\ \mu\text{M}$ (or  $\text{pmol}/\mu\text{l}$ );  $2\ \mu\text{l}$  of  $10\text{mM}$  dNTP;  $1.25\ \mu\text{l}$  of  $100\text{mM}$  DTT solution;  $0.5\ \mu\text{l}$  of  $5\text{U}/\mu\text{l}$  RNase Inhibitor;  $0.5\ \mu\text{l}$  of Titan enzyme mix (AMV and expand high fidelity, Roche),  $5\ \mu\text{l}$  of 5 x RT-PCR reaction buffer ( $7.5\text{mM}$   $\text{MgCl}_2$  and DMSO) (Titan one tube RT-PCR kit (Roche)) and  $11.25\ \mu\text{l}$  of nuclease-free redistilled water. The solution was processed by a thermocycler (GeneAmp PCR 2700 system, Applied biosystems) heated at  $50\ ^{\circ}\text{C}$  for 30 minutes, denatured at  $94\ ^{\circ}\text{C}$  for 2 minutes, and successive 35 cycles of denaturation at  $94\ ^{\circ}\text{C}$  for 30 seconds, annealing at  $55\ ^{\circ}\text{C}$  for 30 seconds, elongation at  $68\ ^{\circ}\text{C}$  for 45 seconds, and a final prolonged elongation at  $68^{\circ}\text{C}$  for 7 minutes, then kept at  $4^{\circ}\text{C}$ .

A primer pair targeting 5'NTR; 324 (ATG CCC T/A TA GTA GGA CTA GCA) and 326(TCA ACT CCA TGT GCC ATG TAC) were used for pestivirus genome detection (Vilcek et al.1994). The electrophoresis analysis of the PCR product (288bp) was carried out in 2 to 3 % agarose gel using 0.5 x tris-borate-EDTA buffer (TBE) ( $0.45\text{M}$ , pH8.3-8.5) with  $0.467\ \mu\text{g/ml}$  ethidium bromide at 100V 10-20 minutes, then the gel images were observed in the UV trans-illuminator and recorded by a Polaroid camera.

Amplified product was digested by restriction enzyme BglI(8U), containing  $10\ \mu\text{l}$  of amplified product in a  $20\ \mu\text{l}$  reaction mixture ( $1/10$  of  $10\text{mM}$  Tris-HCl,  $200\text{mM}$  KCl,  $0.1\text{mM}$  EDTA,  $1\text{mM}$  DTT,  $0.01\%$ BSA,  $50\%$  glycerol (pH8.0)),  $37^{\circ}\text{C}$  1 hour. Digestion into 247bp and 41bp fragment was expected for group 1(CSF virus).

### Virus detection by CSF specific fluorescent antibody

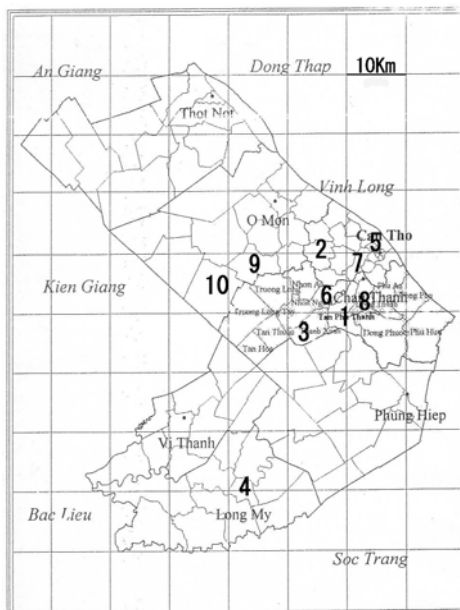
The  $10^{-1}$  homogenate of tonsil, spleen and plasma with dilution of  $10^0$ ,  $10^{-1}$ ,  $10^{-2}$ ,  $10^{-3}$ ,  $10^{-4}$  and control were injected to the CPK cell culture surface at the 2<sup>nd</sup> day, proliferated on 3 to 4 pieces of cover slips (18x6mm) in the 6-well plates ( $2.3 \times 10^{-4}$  cells/cm<sup>2</sup>). The plate was kept in 37°C for 1 hour for absorption, tilted every 15 minutes, then the surface of cell were washed with PBS(-) three times and filled with the 10% horse serum added growth medium. A cover slip was harvested after 3 to 7 days of incubation and a fluorescent antibody test against CSF virus (Kyoto Biken, Japan) was carried out for detection of the antigen. The supernatant of the culture was harvested for further passage and the cell plasma was collected after breaking the cells by freezing and melting, then centrifuged at 3000rpm 5 minutes for separation of the supernatant.

### Sequence of the RT-PCR amplified product

The PCR products were purified (QIA quick PCR purification or gel extraction kit, QIAGEN), then amplified again by the same primer ( $0.16 \mu\text{M}$ ) with dye terminator (Cycle sequencing kit. Big Dye Terminator v1.1, ABI PRISM, 96°C2min, 25cycles of 96°C10sec.55°C5sec, 60°C4min, 4°C) then purified by spin column to remove the dye (Centri sep. Princeton separations), and finally sequenced (310 Genetic Analyzer, ABI prism). Two hundred forty nucleotides were phylogenetically analyzed (Genetyx-win software. SDC) and compared with those of other pestivirus group (bovine viral diarrhea and border disease virus) and CSFV of Japanese origin (GPE-, ALD).

## Results

### (1) Overview of the sampled farms



The farms belonged to Cantho province, mainly in the outskirts of Cantho city, two in O Mon district (9,10) and a farm in Long My (4), in 50km radius from the city center. (Figure1, Table2)

Seven farms were small holders keeping 1 to 5 sows, and three medium sized farms raising more than 5 sows and/or producing 100 pigs per year were included in the study. Four farms were introducing growers from outside for finishing (case7-10) and the latter 3 just (re-)started pig raising due to the favorite sales price of pig.

### (2) Description of the outbreaks and recent vaccination history (Table2)

Many young pigs and some sows were seriously affected by the disease. Some cases ended only in a few days, and some extended over two months with intermittent

death. Mortality of the herd ranged between 7 % (case7) to more than 90 % (case 2).

Sow's parity of the sick piglets was  $1.50 \pm 0.40$  (n=14) . Nine sows out of 14 were of 1<sup>st</sup> parity. In general sows were vaccinated simultaneously with piglets, which was a few weeks before pregnancy (within 6 months before delivery). Five farms didn't give any booster of CSF vaccine for the sow/gilt of affected litter before mating (case2, 3, 6, 8, 9), a farm (case1) didn't vaccinate promptly to the sow and piglets as usual, and three farms had regular vaccine program (case 4, 5 and 7).

Two cases (9,10) had preceding disease outbreak of the introduced herd of unknown source, that were just bought from the middleman selling piglets on boat.

### **(3) Clinical symptoms and pathological observations**

Frequently observed clinical symptoms were those recognized as PTH or Paratyphus (①~④) and DT(⑨) or hog cholera (Table3), and some farms had symptoms which falls in that of THT (⑤~⑧). Leucopenia was seen in 5 farms out of 6. Acute or chronic enteritis, pneumonia and petechiae, ecchymoses in lymph nodes, kidney, lung or bladder were observed in many pigs. Proliferation of reticular cells, hystiocytic hyperplasia and encephalitis was observed in some pigs. Consistent microscopic lesion was depletion of the lymphoid tissue in spleen, lymph nodes or intestine, and more features of various secondary infections were observed in some pigs.

### **(2) Serological and virological test results (Table4)**

CSF virus genome of 5'NTR was identified in all the cases by RT-PCR and the amplified product was confirmed by both digestion with restriction enzyme and sequence, and the live virus was detected in three cases (5, 7 and 9). By phylogenetic tree analysis, 5'NTR (240nt) of four vaccine (NAVETCO, Pest-vac, Coglapest and Pestiffa) viruses and the field strains were divided into different clusters.

Antibody against CSF virus was not detected in the vaccinated herd of case 4, 5 and 7. And case 9 had maternal antibody. Antibody against pseudorabies and PRRS was neither detected in the sow or piglets (case1~9).

Table2 Overview of the sampled farms, population in the outbreak and the recent vaccination

Farm	1	2	3	4	5	6	7	8	9	10
District (Huyen)	Chau Thanh A	TP. Can Tho	Chau Thanh A	Long My	TP.Can Tho	Chau Thanh A	TP. Can Tho	Chau Thanh B	O Mon	O Mon
Village(Xa) or Ward (Phuong)	Tan Phu Thanh	Giai Xuan	Thanh Xuan	Long My	Xuan Khanh	Nhon Nghia	An Binh	Dong Thanh	Truong Thanh	Truong Xuan
Distance from city centre (km)	<20	<20	<30	<50	<10	<30	<10	<20	<30	<30
Pig farm type	Breeder(B)	B	B	B	B*1	B	B + Finishing (F)*2	B+F	B+F	B+F
Pig farm size*3	Medium	Small	Small	Small	Medium	Small	Medium	Small	Small	Small
Pig affected*6	①sow,②weanling	suckling	suckling	weanling*8	①suckling and weanling ②suckling	suckling	weanling	①finishing ②weanling ③sow	suckling*9	grower
Onset age parity)	①(3),②50(2,3)	13 (1)	15,23(1,2)	36 (2)	①7,40 (1) ②26 (1)	9 (1)	47,51 (1,3)	①180,②40,50(1,1),③(1)	7 (1)	①>55, ②70
Affected litter (or Head)	①(1), ②2	1	2	1*8	1	1	2	①1,②2,③(1)	1	2
Onset date	7/5/02	9/9/02	9/23/02	9/29/02	9/9/02	12/18/02	1/10/03	1/20/03	5/16/03	4/2/03
End date	8/7/02	10/02	n.a.	11/7/02	11/24/02	12/20/02	1/14/03	4/10/03	5/23/03	6/20/03
Sampled date	8/5/02	9/16/02	9/30/02	11/7/02	11/24/02	12/20/02	1/14/03	2/25/03	5/17/03	6/10/03
Duration:days	33	30	≥7	39	76	2	4	81	7	64
Pig population before the outbreak										
Total (a)	39	12	20	31	24	17	96	27	25	16
Piglet(1a)	31	11	18	29	18	15	23	20	13	3
Grower(2a)	0	0	0	0	0	0	60	5	11	12
Breeder(3a)	8	1	2	2	6	2	13	2	1	1
Fatal pigs (b)	①1, ②18	11	12	10	18	4	7	22	13	11
Mortality(b/a)	49%	92%	≥60%	32%	75%	24%	7%	81%	52%	69%
Piglet(b/1a)	58%	100%	≥67%	34%	100%	27%	30%	95%	100%	0
Grower(b/2a)	—	—	—	—	—	—	0	40%	0	92%
Breeder(b/3a)	13%	0	0	0	0	0	0	50%	0	0
Last vaccination; Age (days old)	no	no	no	35	①no, 33,②21	no	22	①③n.a.,②no	no	n.a.
Last vaccination of the sow: months pre-mating	2	>6	>6	1	1	>6	1	>6	>6	n.a.
CSF Vaccine type*4	NAV	no	no	Cog	PesV	NAV	NAV	n.a.*5	Pestiffa*7	n.a.*7

\*1 Only breeding. Other breeders: breed and finish some \*2 Introducing growers from outside to finish \*3 Small: ≤5sows or 50 grower/finishing; medium: 6-49 sows or 51-490 grower/finishing \*4 NAV: NAVETCO (domestic lapinised vaccine), Cog: Coglapest (Hungary), PesV: Pest-Vac (Brasil), Pestiffa (France), n.a.: not available \*5 Injected Coglapest 7days after the onset (2/17/03) to 20piglets & the sow \*6 Only fatal cases \*7 Other pigs of the same farm had been vaccinated by NAV vaccine \*8 8/8/02 another sow had fatal disease (not included) \*9 Two weeks ago neighbour slaughtered 5 growers introduced from outside. One remaining grower was tested(9).

Table3 Clinical symptoms and pathological observation

Farm	1	2	3	4	5	6	7	8	9	10
<b>Recognised clinical symptoms</b>										
Pho Thuong Han (PTH)										
① Shivering, trembling,	+	+	+	+	+	+		+		+
② Constipation	+	+		+	+			+	+	+
③ Mild fever (<42), anorexia	+	+	+	+	+		+	+	(+)	+
④ Cyanosis in extremities							+			
Tu Huyet Trung (THT)										
⑤ High fever ( $\geq 42$ )										
⑥ Respiratory distress						+			+	
⑦ Quick death						+			+	
⑧ Subcutaneous haemorrhage	+			+	+			+		+
Dich Ta Heo (DT)										
⑨ Diarrhea incurable by antibiotics	+	+	+	+	+		+	+/-		
Others										
⑩ Eye discharge or swollen eyelid						+		+/-		+/-
⑪ Emaciation	+			+				+		
Sampled number (12 pigs)	1	1	n.a.	n.a.	n.a.	n.a.	1	4	2	3
White blood cell (/ $\mu$ l)	5500	6200	n.a.	n.a.	n.a.	n.a.	21000	3275 $\pm$ 441	7950 $\pm$ 3430	6533 $\pm$ 2100
<b>Gross lesion (17 pigs)</b>	1	1	2	1	3	1	1	4	2	1
① Ulcer, erosion in ileocaecal junction			+/-	+			+	+		+
② Catarrhal enteritis	+	+								
③ Abdominal dropsy	+			+						+
④ Pneumonia		+	+		+		+	+/-	+	+
⑤ Petechiae and ecchymoses in lymph nodes, kidney, lung etc.	+	+	+	+	+	+		+		
⑥ Spleen infarction			+		+/-			+/-		
⑦ Swollen spleen					+/-			+/-		+
⑧ Nephritis							+			
<b>Microscopic lesion (11 pigs)</b>	n.t.	n.t.	n.t.	1	3	n.t.	n.t.	4	2	1
① Depletion of lymphoid tissue	n.t.	n.t.	n.t.	+	+	n.t.	n.t.	+	+	+
② Perivascular cuffing	n.t.	n.t.	n.t.	-	+	n.t.	n.t.	n.t.	-	n.t.
③ Other inflammatory lesions	n.t.	n.t.	n.t.	-	Sp <sup>*1</sup>	n.t.	n.t.	Pn <sup>*2</sup> +/-	Int pn <sup>*3</sup>	Toxo <sup>*4</sup>

\*1 Spleen, \*2 pneumonia(broncho- and interstitial), \*3 Interstitial pneumonia, \*4Toxoplasma infection(int. pneumonia, necrotic fibrinous hepatitis, necrotic lymph nodes), +/- : some pigs, (+): only sow

Table 4 Serological and virological test results against CSF virus

Farm	1	1	2	3	3	4	5 <sup>*1</sup>	5	5	5	5	6	6	7	8	8	8	8	8	9 <sup>*2</sup>	9	9	9	10	
Age: days (parity)	60	60	20	15	30	75	(1)	46	46	49	(1)	10	(1)	55	55	55	55	65	(1)	70	7	8	(1)	75	
Days after onset	10	10	7	<3	7	39	76	19	19	22	50	3	3	4	15	15	15	15	15	>16	1	2	2	5	
CSF vaccination																									
Type	NAV	n.a.	n.a.			Cog								NAV	NAV					Cog	n.a.		Pestiffa	n.a.	
Sow's booster pre-mating (months)	2	>6	>6			1								>6	1					>6	n.a.		>6	n.a.	
Days post injection	—	—	—			40	50	24	24	27	23	—	>300	22						8	n.a.	—	—	>210	n.a.
CSF-ELISA Ab	—	—	—	n.a.	n.a.	—	+	—	+ <sup>d</sup>	—	+	n.a.	+	—	—	+	+	+	+	+ <sup>d</sup>	+	+	+	n.t.	
RT-PCR (5'NTR) & sequence																									
Tonsil	+rs	n.a.	+rs	+rs	+r	+rs	n.a.	+rs	+rs	+ <sup>w</sup> r	n.a.	—	n.a.	+s	+rs	+rs	+rs	+rs	+rs	n.a.	n.a.	+ <sup>w</sup> s	+ <sup>w</sup> r	n.a.	n.t.
Spleen	+	n.a.	+	—	+	+	n.a.	+	+	+ <sup>w</sup> s	n.a.	+rs	n.a.	+rs	+	+	+	+	n.a.	n.a.	+ <sup>w</sup>	+ <sup>w</sup>	n.a.	n.t.	
Serum	—	+	n.a.	n.a.	n.a.	n.t.	+ <sup>w</sup>	n.t.	n.t.	n.t.	—	n.a.	—	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	—	Plas. +s	n.t.	n.t.	—	Plas. +s
Direct fluorescent antibody test (cell)	Spl. retest	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	Spl. +	n.t.	n.t.	n.t.	n.t.	Spl. +	n.t.	Ton. retest	n.t.	n.t.	n.t.	Plas. +	n.t.	n.t.	n.t.	n.t.	

+r: digested by restriction enzyme BglI, +s: confirmed by sequence; +<sup>d</sup>: suspect; +<sup>w</sup>: weak positive; Plas.: plasma; Spl: spleen; Ton: tonsil \*1 Sow of the 1st case (⊙) \*2 Grower of the neighbour

## Discussion

We sampled the fatal pigs from 10 farms and all herds were diagnosed as CSF. Most of the clinical symptom observed, which was the criteria for recognition of the diseases by local veterinary technicians, fell into the category of DT, namely CSF, as well as PTH: paratyphus, which implied under-estimation of CSF in the field. Since 10 samples were collected from the inquiries mainly received from the local veterinary technicians, rather severe cases may have included in the sample population. But at least the proportion of CSF incidence seemed to be higher than recognized (Table1).

By gross examination some typical lesion of CSF such as petechial haemorrhage in kidney, spleen infarction and button ulcer in large intestine or ileo-caecal ulcer were consistently observed in only 3 farms (3, 5 and 8). Not much typical lesions were found in other farms, especially in neonates (6, 9), of which took acute form. Although the number of samples for microscopic examination was limited, lymphocyte depletion was rather easily observed in all 5 cases, including case 9, which could be a criterion for supportive diagnosis.

The mortality of the farm was high where no vaccination for CSF was performed (Farm 2, 8), however it varied in the farms with vaccinated herds. More than half of the sick litters were from the first parity, which might suggest failure of vaccination program in the first year of life. Since simultaneous vaccination of piglets and the sow was the routine, booster of the introduced growers and gilts before mating tended to be set aside. Even the most prevailing NAVETCO vaccine didn't indicate clearly the necessity of booster for the first year of life in the brochure (NAVETCO, 1995), besides no indication accompanied each product.

Table 5 Classical swine fever vaccines available in Mekong delta and their indications

Product name	Strain	Vaccination program			Immunity/ Protection year	Producer	Price (2003) VND / 10 dose
		Piglet	Sow	Boar			
Vac-xin Dong Kho DICH TA HEO	C: Chinese	1st: 15-30days	2nd: 30-45days	Minimum 2 weeks before mating or 2/3 of pregnancy (84 days)	Twice a year	1	NAVETCO, Vietnam 4,000
Coglapest	Thiverval	One injection without antibody: Immunity in 10 to 12 days, peak between 30 to 35th days			4	CEVA, Hungary	26,500
Pest-vac	C	non vaccinated sow (nvs): 14 days	vaccinated sow (vs): 60days	70-90days of pregnancy	Once a year	n.a.	Fort-Dodge, Brasil 26,500
Pestiffa	C (& Lyon?)	nvs: 7 days	vs: 7wks (low risk), 30days (endemic)	1st: one month before service, twice a year	Twice a year	2	Merial, France 62,000

For the regularly vaccinated herd (4, 5 and 7) vaccine break was suspected in the beginning but the sequence of 5'NTR showed different clusters for vaccine strains and field samples that might

suggest different origin. Since no antibody was detected, vaccination failure was conceived due to early injection in the presence of maternal antibody and the successive depletion. Coglapest vaccine used in case 4 had no indication of injection programme. Pest-vac indicated booster for pregnant sows, a practice hardly adoptable by the farmers in the region. Pestiffa, a French product which contributed to the eradication of CSF in Europe ([hnos.abreu.com/Doc/pestiffa.htm](http://hnos.abreu.com/Doc/pestiffa.htm) 6/30/2003), had reasonable indication but 15 times more expensive than the local NAVETCO product. A safe and effective vaccine which has simple programme and clear indication should be adopted and diffused. And the key would be the farmers' perception to realize the importance of CSF and its prevention.

For the diagnosis of CSF, identification of the agent by direct immunofluorescence test of frozen sections of organs from affected pigs (OIE Manual, 2000) is the quickest first choice. It may cost a few more days while where the cryostat is not available, virus detection in infected cell culture by direct immunofluorescence or immunoperoxidase and confirmation by both RT-PCR and digestion by restriction enzyme BgII would be an alternative. Accurate diagnosis of the disease would change the current recognition of the veterinary technicians in which no differential diagnosis was made, and more underestimated CSF cases due to its inclusion in other categories (paratyphus, HS, diarrhea, general fever) would become apparent. Once CSF is diagnosed, slaughter of the affected herd would prevent dissemination of the disease, reduce the cost of treatment and extended loss due to its prognosis of fatality. Consequently more awareness of the farmers and local vets be created, which ensures better health control by strict vaccination practice, disease understanding and selection of the growers for introduction.

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## TÓM TẮT

Chúng tôi đã thu nhập 17 heo bệnh nặng cùng 5 mẫu huyết thanh của heo nái từ 10 trại heo vào giữa tháng 6 và 7 năm 2002, cho việc chuẩn đoán tại phòng thí nghiệm nhằm xác định nguyên nhân gây bệnh bằng việc kiểm tra tổ chức học, huyết thanh học và virus học. Mặt khác, các triệu chứng đã quan sát so sánh với những triệu chứng đặc trưng thông thường được sử dụng để phân biệt bởi các thú y viên. Chúng tôi đồng thời cũng điều tra những trại có heo bệnh để phân tích số liệu dịch tễ với kết quả phòng thí nghiệm và đi đến thảo luận bằng cách nào để ngăn chặn bệnh. Kết quả bệnh dịch tả heo được xác định có ở tất cả các trại. Tất cả các virus được phân lập từ heo mắc bệnh đều có kiểu gen 5'NTR. Đã phát hiện không tiêm phòng lập lại trước khi phối giống (5 trại) hay việc tiêm phòng không hiệu quả do không tuân thủ đúng theo hướng dẫn của nhà sản xuất. Định nghĩa bệnh dịch tả heo: là một bệnh tiêu chảy nặng, lây lan nhiều, không thể trị khỏi bằng kháng sinh. Nó là một trong những triệu chứng của bệnh dịch tả heo, trong khi dấu hiệu lâm sàng quan sát lúc mới phát hiện thì giống như bệnh Phó Thương Hàn. Do đó, chúng ta nên chuẩn đoán bằng các phương pháp chuẩn đoán phòng thí nghiệm như: miễn dịch huỳnh quang, kiểm tra kháng thể bằng Peroxidase, có thể khẳng định bằng phương pháp KT-PCR. Ngoài ra, biểu hiện bệnh cũng được ghi nhận có sự giảm mô hạch lambda. Vì thế dẫn đến các bệnh nhiễm trùng kế phát. Ngay khi phương pháp chuẩn đoán được xây dựng, những trường hợp bệnh dịch tả lợn được phát hiện nhiều hơn nhằm giúp cho người chăn nuôi lựa chọn phương pháp thích hợp trong việc điều khiển bệnh. Như nhập gia súc từ những nguồn đáng tin cậy, tiêm phòng lập lại cho heo hậu bị và heo lứa, ngăn chặn bằng một loại vaccin và chương trình tiêm phòng chắc chắn, loại thải sớm heo mắc bệnh.