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[illegible] 2 ORF2 [illegible] ORF1 [illegible] ORF3 [illegible] DNA [illegible]

[illegible]

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A B S T R A C T

[illegible] 2 (PC[illegible] 2) [illegible] ORF1 ([illegible] ORF1) [illegible] ORF3 ([illegible] ORF3) [illegible] PC[illegible] 2 [illegible] ORF2 ([illegible] ORF2) [illegible] DNA [illegible] ORF1 [illegible] CD8^{+} [illegible] ORF1 [illegible] ORF3 [illegible] ORF2 [illegible] ORF3 [illegible] ORF1 [illegible] ORF3, [illegible] ORF1 [illegible] ORF3 [illegible] ORF2 [illegible] ORF1 [illegible] ORF3 [illegible] ORF3 [illegible] ORF2 [illegible] *in vivo.*

[illegible] 2009 [illegible]

Introduction

P[illegible] (PC[illegible]) [illegible] DNA [illegible] 17 [illegible] ([illegible] ., 1982). PC[illegible] PK-15 ([illegible] ., 1974). [illegible] PK-15-[illegible] PC[illegible], [illegible] PC[illegible] 1, [illegible] ([illegible] ., 1995; [illegible] ., 1986). [illegible] PC[illegible] 2 [illegible] 1991, [illegible] (PM[illegible]) ([illegible] ., 1998; [illegible] E[illegible], 2000; [illegible], 1997). PM[illegible] 12 [illegible] 5–30% [illegible] ([illegible], 1997). [illegible] PM[illegible], PC[illegible] 2 [illegible] (PDN[illegible]) [illegible] ([illegible] ., 2007).

[illegible] PC[illegible] 1 [illegible] PC[illegible] 2 [illegible] ([illegible] ., 1998; [illegible] ., 1997, 1998; [illegible] ., 2006). [illegible] DNA [illegible] PC[illegible] 1 [illegible] PC[illegible] 2 [illegible] 90%, [illegible] PC[illegible] 1 [illegible] PC[illegible] 2 [illegible] 68 [illegible] 76%. PC[illegible] 2 [illegible] 3 [illegible] ORF[illegible]: ORF1 [illegible], ORF2 [illegible] ORF3 [illegible] 945, 702 [illegible] 315 [illegible]. PC[illegible] 2 ORF1 [illegible] 35.7 [illegible] ([illegible]) [illegible] ([illegible] ., 1998). PC[illegible] 2 ORF2 [illegible] 27.8 [illegible] ([illegible]) [illegible] ([illegible] ., 2000; [illegible] ., 2000; [illegible] ., 2001). PC[illegible] 2 ORF3 [illegible] PC[illegible] 2 [illegible] PC[illegible] 2-[illegible] ([illegible] ., 2005).

A[illegible], PC[illegible] 2 [illegible] ([illegible] ., 2000) [illegible] *Escherichia coli* ([illegible] ., 2005) [illegible] PC[illegible] 2. [illegible] ([illegible] ., 2000) [illegible] ([illegible] ., 2004; [illegible] ., 2009) [illegible] PC[illegible] 2 ([illegible] ., 2001; [illegible] ., 2005), [illegible] ORF3 [illegible] PEP CAN [illegible] (185–211) [illegible] PC[illegible] 2 [illegible] ([illegible] ., 2000). [illegible], 2 [illegible] (81–100 [illegible] 201–220) [illegible] 1 [illegible] ORF3 [illegible] (31–50) [illegible] ([illegible] ., 2007).

PC[illegible] 2 [illegible] ([illegible] ., 2003; [illegible] .,

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E-mail address: [illegible]

0042-6822/$ – [illegible] 2009 [illegible]
[illegible]:10.1016/[illegible].2009.07.035

[illegible]

Results

In vitro expression of mammalian expression vector

[illegible]

Table 1

[illegible]

Group	I		
		CD4+ (%)	CD8+ (%)
ORF2 + CI	1.54 0.32	15.80 0.67	14.94 2.56
ORF2 + ORF1	1.38 0.13	14.00 2.87	11.58 2.41
ORF2 + ORF3	1.36 0.20	13.07 1.75	12.08 1.83
DNA	1.00 0.00	9.40 3.80	11.63 2.17

[illegible] ($P<0.05$).

Cap-specific cellular immune responses

[illegible]

Total IgG antibody response to PCV2 Cap protein

[illegible]

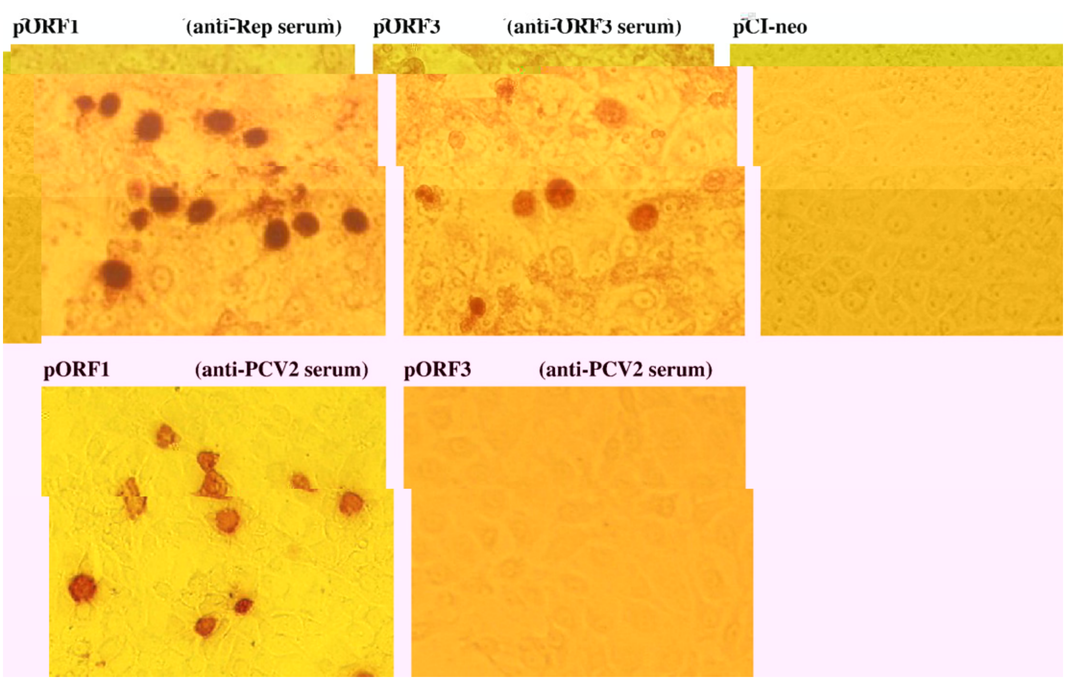

Fig. 1. [illegible] in vitro. [illegible] PK-15 [illegible] ORF1, ORF3 [illegible] IPMA. [illegible]

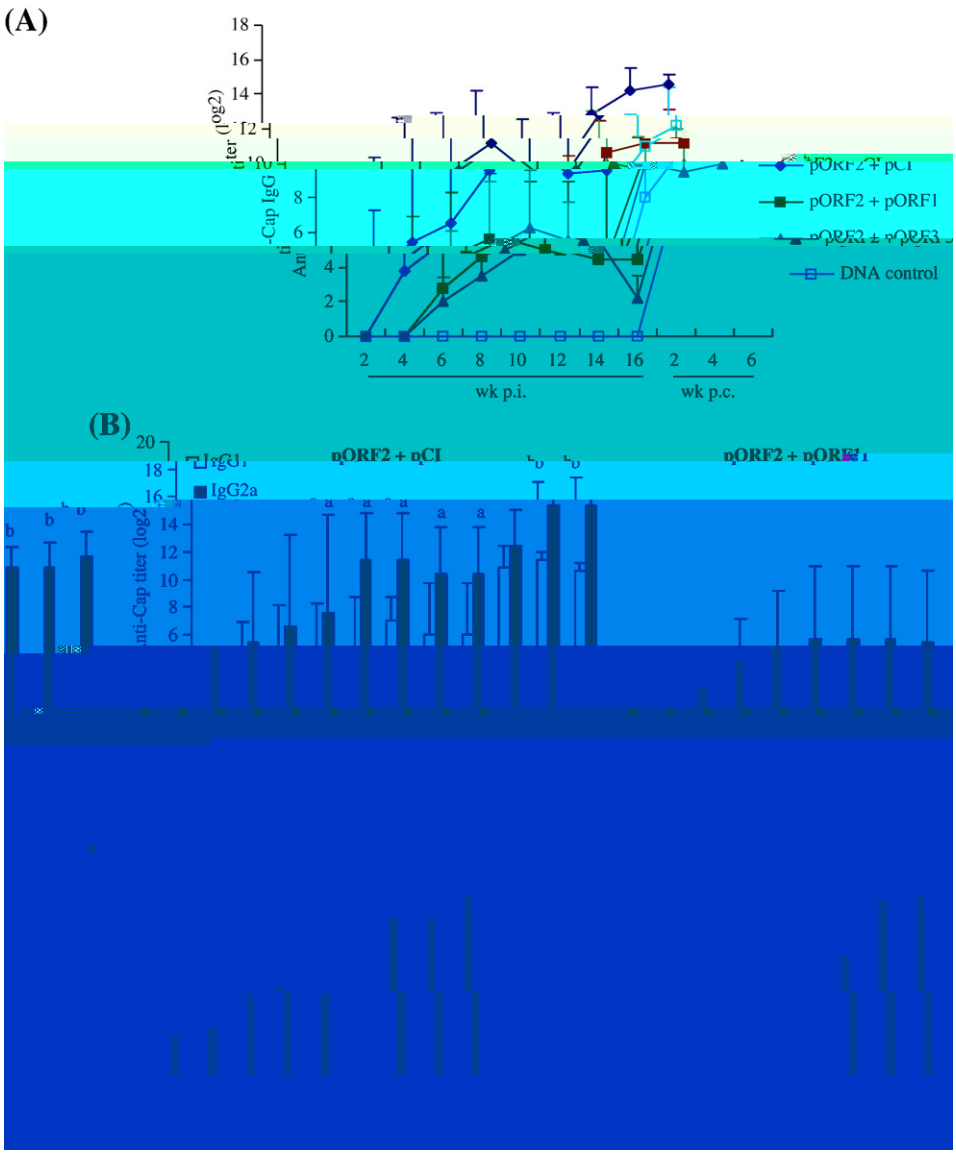

Fig. 2. K e 5 o 5 6 I G (A) I G 6 e (B) 5C 6 5 o 5 e . o . . e I G, I G1 I G2 5 e e e e 5ELI A. E o 5 e 5 e o 5 o (*n*=5). e e 5 6 ' oe o 6 fi 5 e I G 6 e 5 e o *P*<0.05 *P*<0.01, e e 5 e 55 e 5 e o . M e e e e 516 ee .

o 2 (10 ee .) 6.3 3.4 o 2 (12 ee .), e e 5 e (F . 2A). 5 5 5 , 5 e C - e fi I G o ORF2 + CI e e fi 5 e 5 5 5 ORF2 + ORF1 (5 12–16 ee) ORF2 + ORF3 (5 10–12 ee) o (*P*<0.05). e e 5 e 5 5 5 e o 5 5 o ORF1 o ORF3 5 6 5 e C - e fi 5 o e o e 6 5 e ORF2 .

IgG isotype profiles against PCV2 Cap protein

e e 5 5 o e 5 e 5 e o 6 e - e e ( ) e 5 e o e ; I G2 o e e o 1-e 5 5 o , e e 2-e 5 5 o e e 5 e o 5 o o I G1 e e I G2 (Mo Co , 1989). o e 5 6 e 5 5 e 5 e o e e o e , e e e 5 e I G 6 e - die 5C 6 . I e e , 6 e e e e e I G2 5 6 - 5 I G1, 5 fi 5 e e e ORF2 + CL (58–16 ee .) ORF2 + ORF3 (56–10 14 ee .) o (*P*<0.05) (F . 2B), e 5 1 e e o e 5 e o - o e - o . F o o e e , 5 6 o I G1 I G2 e 5 o e e 1 o , 5 5 e 6 die - die e e 5 o 5 5 o 5 o e e . I 6 e 5 , o 5 C I G1 5 o e e e 6 6 ORF2 + ORF1 6 e e e 6 6 5 e PC 2 e ; o e e , 5 e o - fi 5 e - e e e 5 e e I G1 I G2 5 6 - e e e o - e e o e 5 - o . O e , o 5 e 5 5 5 e ORF1 ORF3 o 6 5 e 5 o o 1 e o e e ORF2.

Total IgG and isotype profiles to PCV2 Rep and ORF3 proteins

e 5 6 I G 6 e die PC 2 R e o ORF3 6 e e e 6 6 5 e o e o . A o F . 3A, o e e o 5 R I G e e e 6 6 e o ORF2 + ORF1 o , 5 e 5 6 - o 6.0 3.3 o 2 5 16 ee . F o o e e , o 5 o e o e e R - e fi I G, 5 e 5 6 - o 6.8 2.7 54 ee o 5 e e (. .). e 5 -

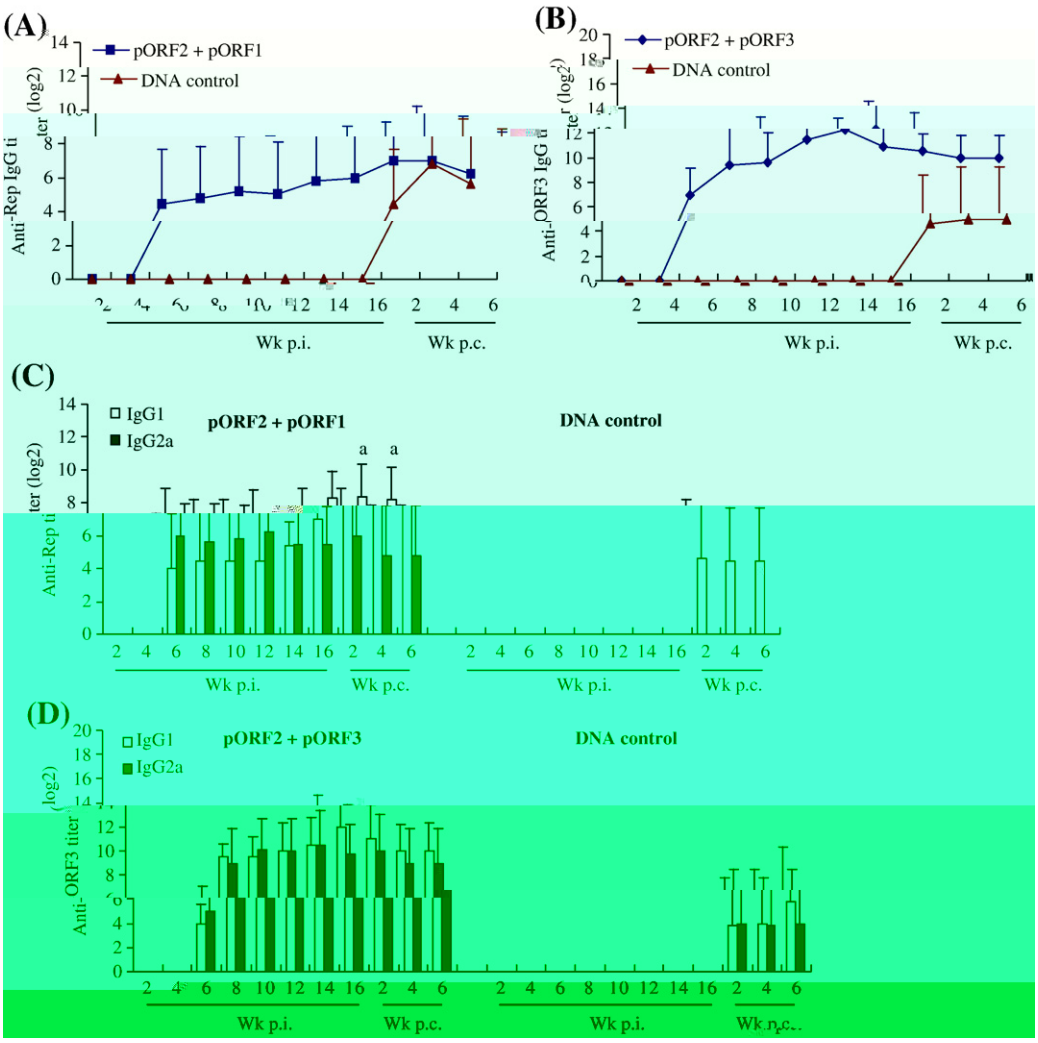

Fig. 3. K e 5 o 5 o e o e 5oPC 2 R _ ORF3 - 6 e e 5 _ e o 5 o . e 5 6 I G e e _ _ 5 R (A) ORF3 (B) - 6 _ 5 e I G 6 e e fi o R (C) ORF3 (D) - 6 _ e o o e o . e I G, I G1 I G2 5 - e e e e 5ELI A. E o _ - o 5 e _ 5 _ - e _ 5 o (*n*=5). e e 5 - ' ' o e o 6 _ fi 5 e - I G 6 e (*P*<0.05) 55 e _ e 5 e _ o . M e e e _ e _ 516 e e .

ORF3 I G e e _ 5 e e 5 e e 9.4 12.3 o 2 8 5 o 16 e e . ORF2 + ORF3 _ 6 e , e e 5 - _ o _ 4.6–5.0 o 2 e e e 6 6 o 5 o e 52 5 o 6 e e . . (F . 3B). A 5 o 6 e _ e e e 5 5 e e 5 o 5 e 5 - R e I G1 ORF2 + ORF1 - o e e fi 5 e - 5 _ I G2 54 5 o 6 e e o o _ e e , o fi 5 e - e e e 5 e e I G1 I G2 _ 5 R e o ORF3 - 6 e e o e - e , e 5 _ o - _ e e o e 5 o R e o ORF3 o o 5 o 6 - o (F . 3C–D).

Table 2
N 5 _ 5 o 5 - o 5 e e 5 e e 5 e o 5 o .

G o	N o o e 5 N 5 o / o e 6 (e N 5 - D) 5 e e .		
	2	4	6
ORF2 + CI	3/5 (320 320)	5/5 (456 527)	5/5 (648 630)
ORF2 + ORF1	0/5 (0)	3/5 (12 11)	3/5 (48 44)
ORF2 + ORF3	0/5 (0)	0/5 (0)	0/5 (0)
DNA o 5 o	0/5 (0)	1/5 (4 9)	2/5 (8 11)

- N 5 - e e e _ 5 e _ e - 5 o e _ 70% e 5 o fl o e e 5 o e . M e 5 N 5 - ≥20 e e o e e o 5 e . "0" _ e e _ 5 o"<20".
· D e e 5 e - 5 5 o e e e 5 fi 5 e e e 5 e e - o o e e . (*P*<0.05).

VN antibody responses to PCV2

e _ 5 o o e e - 5 o e 5 e PC 2 e 5 o _ e 6 6 N _ - e 5 5 e ELI A 6 5 P o 5 o - _ e e , 5 e e e o e 6 5 e e 5 - _ 5 o e _ - o (_ 5 6 o). A 6 - _ e e 5 5 e PC 2 5 - H 0201 _ 5 16 e e . , 5 o _ _ e 5 N _ 5 o e o e e o e - e ORF2 + CI - o , e e 6 e 5 _ 5 6 e e . . (_ e 2). I o 5 5 , e ORF2 + ORF1, ORF2 + ORF3 _ o 5 o - o e 6 e e _ N _ 5 o e o e . _ 5 5 5 _ , ORF2 + CI - o o e fi 5 e - N 5 6 - 5 _ ORF2 + ORF1 _ ORF2 + ORF3 - o 5 - o o 5 5 e e 5 e o 5 - _ e e e - o (*P*<0.05) (_ e 2). e e e 5 e 5 5 _ 5 5 e o e e o ORF1 o ORF3 _ e _ 5 6 _ 6 5 e ORF2- e e _ N _ 5 o e o e o o 5 PC 2 e 5 o .

Protection from PCV2 challenge

N o - o e o e e o e - e 5 e _ e e o 5 o - o . M - o o e o e 6 o 5 e e 5 o _ fi 5 - 5 o o 5 o 6 e e e o (e e 5 ., 2008). I 5 e _ e e o 5 o - o , o _ o _ o e e e o 5 e e e _ 56 e e . . (F . 4).

A o 5e DNA , e o e 5 ORF2 + CI 5e oe 5 e o e e (14.6 6.4%) o e fi 5 e e e 5 5e e e o 5 o e (38.7 5.9%) (P<0.01) (F . 4). H o e e , ORF1 ORF2 + ORF3 e o 5 5 5e e e o 5 o o . F 5e , e 5 5e PC 2 DNA e o e e -5 e PCR. F o o PC 2- o 5 o e ORF2 + ORF3 o 5 o o e e o 5 e o PC 2 DNA 5 2, 4, 6 ee . . E e e 5e ORF2 + CI ORF2 + ORF1 o e e o 5 e o PC 2 DNA 5 o o 5 5e o 5 e e

o fi 5 e e e IL-12 e e NF-α e e e (A e 5 ., 2008). e e IL-12 e e 55 e e o e 5 1 e e o e, e IL-12 5 o 55 o e 5 e 5 e e 5 5 o e (e e e 5 ., 1993). F 5 e, 5 e e e o fl 5 5 o NF-α, e 5 (9/15) o e e ORF3 o e 5 e o e 5 (A e 5 ., 2008) e o 5 5 e 5 e o ORF3 in vivo, o 5 5 e e fi 5 o e e ORF2 + ORF3 o .

I5 e e o 5 5 PC 2 e e e e 5 e 5 o o 5 o e e o e e e PC 2 e e. C e 5 5 e e e e e 5 e 5 e e o e , PC 2 e e e e o e 5 o PC 2 e e PC 1–2 C e e e o (O e e 5 ., 2007). A o 5 o o 5 , 5 e ORF3 e e 5 e fi 5 5 e e e e e 5 5 e C e 5 e 5 PC 2 e 5 o . H o e e , 5 o e o o 5 5 5 ORF3 e o e e e e o 5 5 e e e o ORF1, 2 3 e 5 5 e e 5 5 o e 5 o , e 5 e 5 o . e e e , 5 e e 5 e 5 o 5 e 5 o e 55 e e e e e 5 o ORF3 e o e e .

O e , 5 e e e 5 5 , e e 5 5 e fl e e o 5 e ORF1 ORF3 o 5 e o e 5 o 5 e ORF2 o 5 e 5 e - e fi e e e o e e e 5 e e fi . e e o 5 5 e e e e e 5 o e o e ORF3 o 5 e o e 5 o C e DNA 5 o . o e 5 o o e o 5 o 5 e e 5 o 5 e o e 5 o PC 2 5 , o e e .

Materials and methods

Cells, virus, proteins, antisera and mice

A PC - e e PK-15 e e 5 e e e 5 e (MEM, G q G L , N) e e 5 10% e 5 5 e 5 o e e (FB , G o). e e 5 PC 2 H 0201 o o 5 5 o PM (o e 5 ., 2006) e e PK-15 e 15 5 e . PC 2 C e e e e e e o (L e 5 ., 2007; o e 5 ., 2005). PC 2 ORF3 e e e e e E. coli BL21 e o 5 GE -4 -1 e 5 o (A e , B AB, , e e) e 5 PC 2 ORF3 (e 5). e 5-PC 2 e e o e 5 e e e 5 e 5 PC 2 H 0201. M o e 5- 5 5-ORF3 e e e o 5 e 5 5 e o e e e ORF3 , e e 5 e . E e BALB/ e e e e o o 5 e (C e e A e o e e , C) e 5 o 5 e 5 o e e e 5 e 5 5 o e (F o 5 e 5 C L5 ., o , C).

Construction and preparation of the DNA vaccines

PC 2 ORF1 ORF3 e e o 5 e e o DNA o H 0201 (G B e o A 188355) 5 e e fi e o o : o (5′-A AACGCG CA GCCCAGCAAGAAG-3′) e e e e (5′-GCGG CGACGAC CAG AA A CA A GG-3′) o ORF1 o (5′- AAG CGACC AC GA GGAG G GG-3′) e e e e (5′-A AACGCG A GG AACCA CCCAC-3′) o ORF3. e PCR o 5 e e o e 5 o e 5 o CI-e o (P o e , M o , I) 5 o e e 5 e e e o e 5 o ORF1 ORF3, e e 5 e . o e e 5 e e e o o ORF3 in vitro, PC - e e PK-15 e e e 5 e 5 e e o 5 L e 5 e 5 (I 5 o e , C , CA). A548 o 5 5 e 5 o , e e e fi e e e o e o o e (IPMA) (o e 5 ., 2005) 5 e e PC 2- o 5 e e , o 5 e o e 5- o 5 5-ORF3 e 5 e 5 o e . o 5 5 e 5 o e e PC 2 ORF2 (ORF2) o 5 o fi e e o (e e 5 ., 2008). F , 5 e ORF1, ORF3 ORF2 e e e e G 5 o (Q e , e , CA) e DNA e .

Experimental design and samples collection

A 5 e e 58 e e e e e o e o e 5 5 e e 5 100 μ o e 5 o 100 μ PB e e 4. M e e e e 3 o e o e 5 2- e e . M e e e 5 o o 200 μ CI-e o (CI) 100 μ PB e e e 5 e DNA o 5 o . F e e o e e e 5 e o fl o 5 o e 5 (FCM) o o e 5 o (LPA) 58 e e . A516 e e ., fi e e o e o e e e e 5 e 5 o e 5 0.2 o PC 2 o ($10^{4.75}$ CID_{50}/0.1). C o e 5 o e e e o e o 6 e e 5 e e e e 5 e e e e o e 5 o o 5 o 5 e e 5 o o 5 e e o 5 o e 5 o / o e e 5 o .

Lymphocyte proliferation assay (LPA)

C - e fi o o e 5 e e o e o 5 e e o o 5 e e e e M 5 e e e e o (o e 5 ., 2005). I e , e e e e e 5 e o e o 5 e e 5 8 e e . 5 o e e - e e o (4 10^6 e /) RPMI 1640 o 5 5% FB (RPMI1640-FB) 5 o e o 96- e 5 e 5100 μ / e . e o 5 o e e e e o 5 o . e PC 2 C RPMI1640-FB 5 e e 5 fi o e 5 5 o o 1 μ / (100 μ / e) 5 o 5 5 e e o . e e 5 e 537 C 5% CO_2 o 48 . e 5 μ o 3-(4,5- e 5 5 o-2-)-2,5- e - 5 5 o o e (M , , 5 L o , MO) (5 /) e 5 o e e o 4 . e e e e 5 e e 100 μ o e (10% D , 0.01 o/L HC) 5 o e e . A 20 o

Table 4

o e e e 5 5 e 5 e 5 .

G o	M o e o	I 5 o (0, 2, 4)		LPA FCM	A 5 o e 5 o	P 5 o
ORF2 + CI	10	ORF2	CI-e o	M o e e o e e	ELI A N e e e o e	M e e e e e 516 .,
ORF2 + ORF1	10	ORF2	ORF1	e e 58 .	52-	5 o o e e e e
ORF2 + ORF3	10	ORF2	ORF3			
DNA o 5 o	15	CI-e o				

E 5 e 5 e 5 e 5 e .
M o e 5 100 μ o e 100 μ o e .
M o e 5 200 μ o CI-e o 100 μ o e .

[illegible] (OD) [illegible] 570 [illegible]. [illegible] LPA [illegible] (I) [illegible]: I = (OD [illegible] − OD [illegible]) / (OD DNA [illegible] − OD [illegible]).

Flow cytometric analysis (FCM)

[illegible] 50 µl PB (10^6 [illegible]) [illegible] 50 µl [illegible] (1:200) FI C- [illegible] CD4 (L3 4) [illegible] R-PE- [illegible] CD8α (L -2) [illegible] (BD B[illegible], M[illegible], CA) [illegible] 0.5 µ [illegible] 20 [illegible] BDL R [illegible] (BD B[illegible]).

ELISA

[illegible] I G, I G1 [illegible] I G2 [illegible] PC 2 [illegible] ORF3 [illegible] ELI A. [illegible] (N [illegible], R[illegible]) [illegible] 100 µ [illegible] (1 µ / [illegible]; 2 µ / [illegible] ORF3) [illegible] 0.05 M [illegible]–HC [illegible] (H8.5) [illegible] 4 C [illegible] PB [illegible] 5% [illegible], 100 µ [illegible] 2- [illegible] (1:64) [illegible] 37 C [illegible] 60 [illegible] (HRP)- [illegible] I G, I G1, [illegible] I G2 [illegible] (1:6000; [illegible]) [illegible] 450 [illegible] OD [illegible].

Virus neutralization test

[illegible] (N) [illegible] 56 C [illegible] 30 [illegible] 10^5.0 CID50/0.1 [illegible] PC 2 H 0201 [illegible] 2- [illegible] (1:20 [illegible] 1:20,480) [illegible] 37 C [illegible] 1 [illegible] 96- [illegible] PK-15 [illegible] 10 µ [illegible] 1:10, [illegible] 2 [illegible] 48 [illegible] 37 C. F [illegible], 96- [illegible] ([illegible] ., 2006). [illegible] ≥70% [illegible].

Pathology and viremia evaluations

A56 [illegible] (e [illegible] ., 2008). [illegible] PCR [illegible]. PC 2 [illegible].

Statistical analysis

[illegible] (ANO A) [illegible] P [illegible] 12.0. [illegible] P [illegible] 0.05.

Acknowledgments

[illegible] N [illegible] F [illegible] C [illegible] (30625030, 30700025, 30370052) [illegible] P [illegible] (2008C22041, 2003C12012), C [illegible] D. P [illegible] G [illegible], [illegible] D [illegible] I [illegible].

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