



Surveillance of Infection with Japanese Encephalitis Virus and Hepatitis E Virus in Swine in Northern Luzon, Philippines

**Fidelino Malbas Jr., Mariko Saito, Yusuke
Sayama, Cecille Lopez, Lorna Baculanta, Hazel
Galang, Socorro Lupisan, Hitoshi Oshitani**

Background

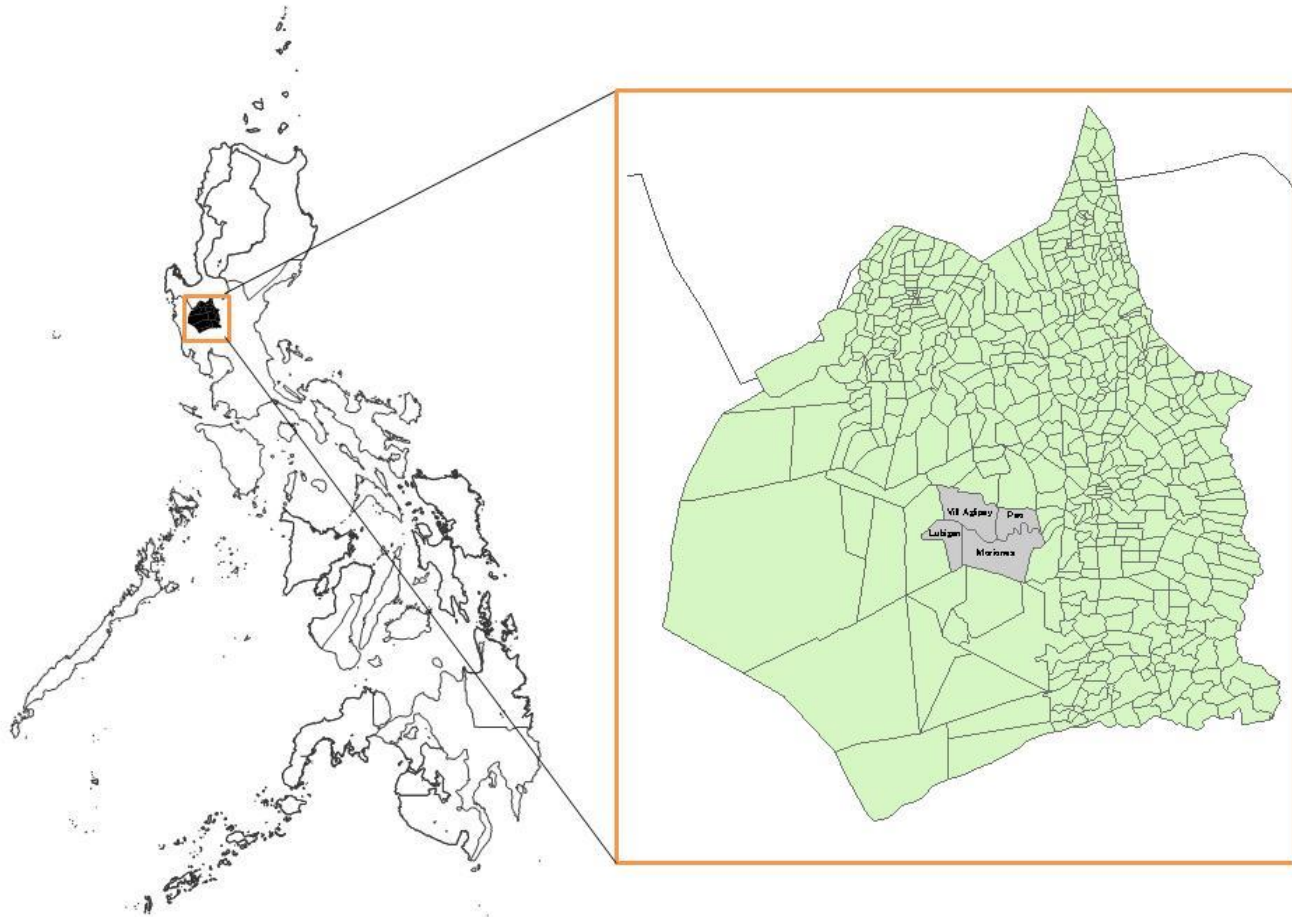
- In December 2008 Ebola-Reston virus was detected from swine farms of Region I and III. Lessons learned from the event is the importance of the continuous surveillance, and monitoring of possible disease from swine before it affects human
- The situation of the major zoonosis between swine and human, such as Japanese Encephalitis virus, Hepatitis E, influenza were not well known in the Philippines

Objectives

- To describe the seasonality of JEV in the blood samples of swine.
- To identify the genotype of JEV circulating in the Philippines.
- To detect the presence of other possible disease, such as Hepatitis E virus, influenza virus, Norovirus and Nipah virus by antigen/antibody detection from serum and feces from swine.

Project Sites

Municipality of San Jose, Tarlac Province, Philippines.
Where the JE outbreak was reported in 2003



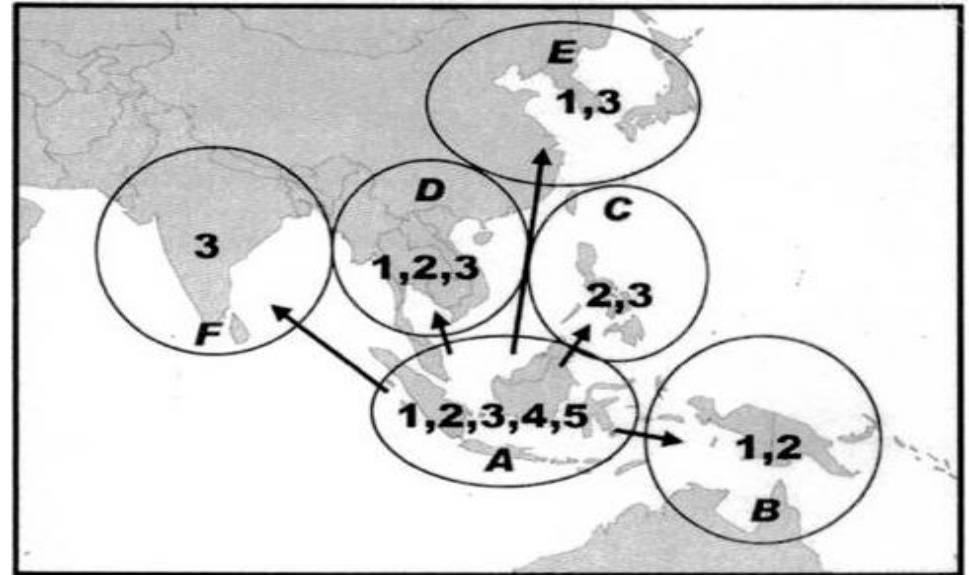
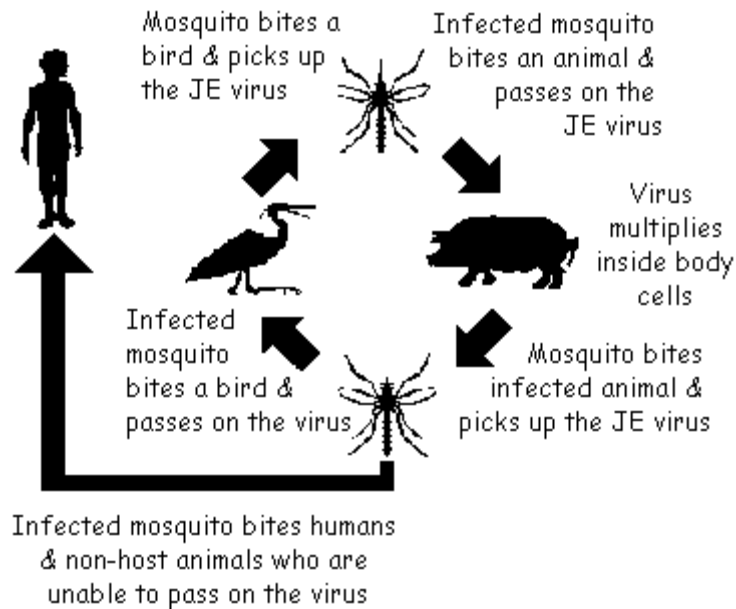
Methodology

- Blood, nasal swab and rectal swab from each swine were randomly collected in the study sites. Total of 50 swine/sampling in every other months.
- Samples were separated within the same day of the collection and kept in the freezer.
- RNA were extracted at RITM and tried to detect influenza virus (nasal), Japanese encephalitis virus (serum), Hepatitis E virus (serum, rectal swab) by RT-PCR
- Anti JEV/ anti HEV IgG and IgM were detected by ELISA (NIID protocol)

Collected samples (By geographic distribution)

	July 2010	September 2010	December 2010	January 2011	Total
Bgy. Pao	13	13	13	14	26
Bgy. Villa Aglipay	12	13	13	11	25
Bgy. Moriones-San Juan	12	13	8	0	25
Bgy. Moriones- Centro	0	12	15	12	12
Bgy. Lubigan	10	0	0	13	10
TOTAL	47	51	49	50	197

Japanese Encephalitis



By Solomon T, J Virol 2003

- Mosquitoes borne disease
- Mainly distributed in Asian country.
- Previously reported genotype 2 & 3 in the Philippines

Initial Results

-Japanese Encephalitis

*JEV RNA were detected from serum

Age (Month)	July				September			
	No. of swine	IgM No. (%)	IgG No. (%)	JEV RNA*	No. of swine	IgM No. (%)	IgG No. (%)	JEV RNA*
2	5	2 (40)	5 (100)	0	0	0	0	0
3	21	5 (24)	11 (52)	1	Genotype 3			0
4	5	1 (20)	3 (60)	0				0
5	2	1 (50)	1 (50)	0	25	1 (4)	19 (76)	0
6	4	4 (100)	4 (100)	0	0	0	0	0
7	2	0	1 (50)	0	5	0	5 (100)	0
8	4	3 (75)	3 (75)	0	2	0	2 (100)	0
9<	4	0	4 (100)	0	4	0	4 (100)	0
Total	47	16 (34.0)	32 (68.0)	1 (2%)	51	2 (4.0)	32 (63.0)	0

Initial Results

-Japanese Encephalitis

**JEV RNA were detected from
nasal swab

Age (Month)	December				January				
	No. of swine	IgM No. (%)	IgG No. (%)	JEV RNA**	No. of swine	IgM No. (%)	IgG No. (%)	JEV RNA*	
2	2	0	2(100)	0	5	0(0)	5(100)	0	
3	7	1(14.3)	3(42.9)	0	16	2(12.5)	11(68.8)	0	
4	19	2(9.5)	10(52.6)	1	Genotype 3			2(66.7)	0
5	7	0	3(42.9)	0	12	4(41.7)	4(41.7)	0	
6	6	0	4(66.7)	0	12	1(8.3)	11(91.7)	0	
7	1	0	1(100)	0	2	1(50)	1(50)	0	
8	7	1(14.3)	6(85.7)	0	0	-	-	-	
9<	0	-	-	-	0	-	-	-	
Total	49	4(8.2)	29(59.2)	1	50	9(18.0)	34(68.0)	0	

Summary

- Approx. 65 % of swine showed anti JEV IgG positive throughout the period. Positive rate of Anti-JEV IgM were highest in July.
- Only 2 samples has been confirmed by sequencing and those were belong to genotype 3.
- There are some other positive samples in RT-PCR. Confirmatory test will be needed.

Hepatitis E

	A	B	C	D	E
Source of virus	feces	blood/ blood-derived body fluids	blood/ blood-derived body fluids	blood/ blood-derived body fluids	feces
Route of transmission	fecal-oral	percutaneous permucosal	percutaneous permucosal	percutaneous permucosal	fecal-oral
Chronic infection	no	yes	yes	yes	No/Yes
Prevention	pre/post- exposure immunization	pre/post- exposure immunization	blood donor screening; risk behavior modification	pre/post- exposure immunization; risk behavior modification	ensure safe drinking water

Initial Results

-Hepatitis E

*HEV RNA were detected from rectal swab

Age (Month)	July				September			
	No. of swine	IgM No. (%)	IgG No. (%)	HEV RNA*	No. of swine	IgM No. (%)	IgG No. (%)	HEV RNA*
2	5	0	1 (20)	0	0	0	0	0
3	21	4 (19)	11 (52)	0	10	0	0	0
4	5	0	2 (40)	0	5	0	1 (20)	1
5	2	0	1 (50)	0	25	2 (8)	9 (36)	4
6	4	0	1 (25)	0	0	0	0	0
7	2	0	1 (50)	0	5	0	0	0
8	4	0	3 (75)	0	2	0	2 (100)	1
9<	4	0	3 (75)	0	4	0	0	0
Total	47	4 (9)	23 (49)	0	51	2 (4)	12 (24)	6 (12%)

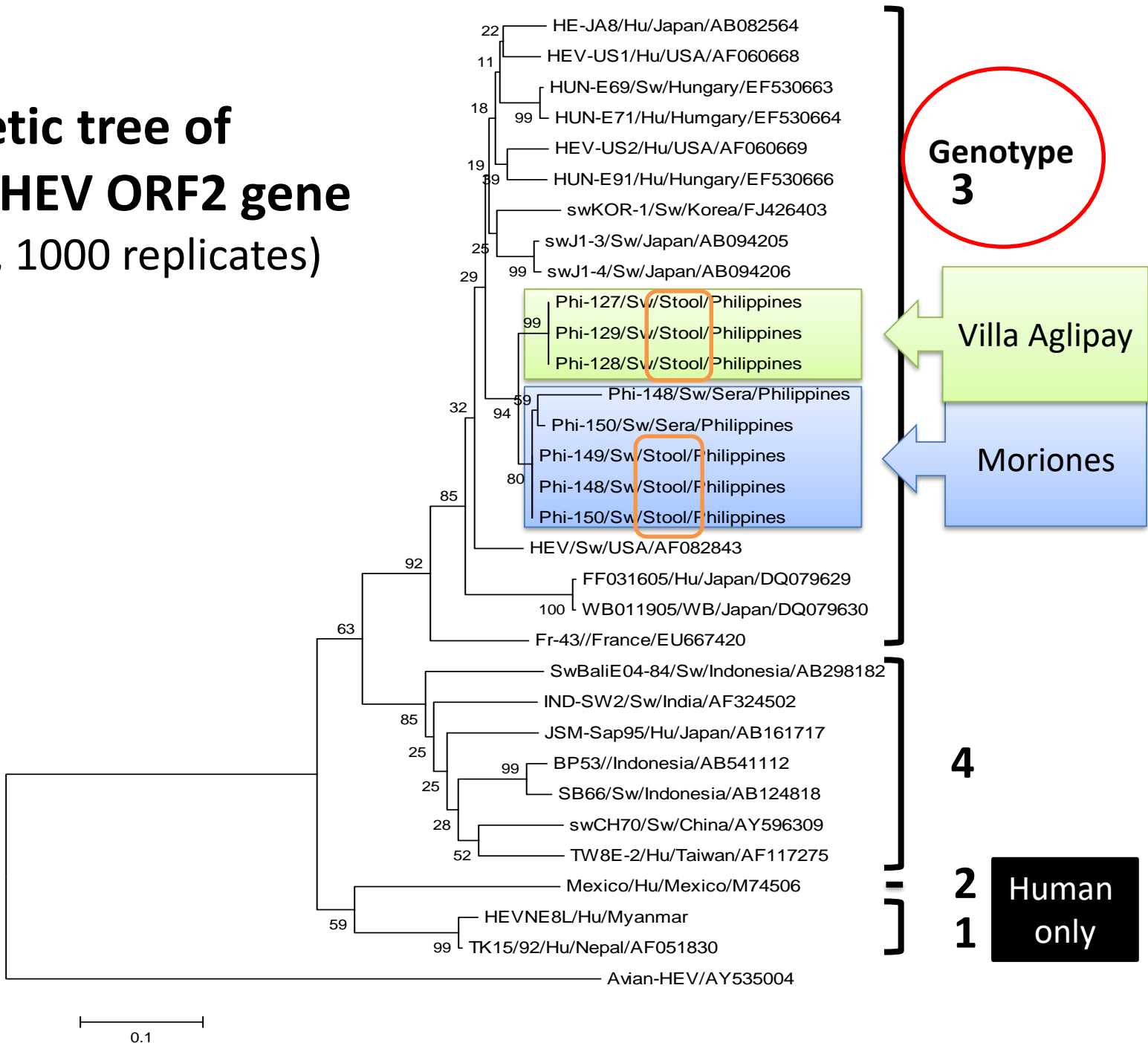
Initial Results

-Hepatitis E

*HEV RNA were detected from rectal swab

Age (Month)	December				January			
	No. of swine	IgM No. (%)	IgG No. (%)	HEV RNA*	No. of swine	IgM No. (%)	IgG No. (%)	HEV RNA*
2	2	0	1(50)	ND	5	0	2(40)	ND
3	7	1(14.3)	4(57.1)	ND	16	0	4(25)	ND
4	19	1(5.3)	3(15.8)	ND	3	0	2(66.7)	ND
5	7	3(42.9)	3(42.9)	ND	12	0	8(66.7)	ND
6	6	0	1(16.7)	ND	12	0	3(25)	ND
7	1	0	0	ND	2	0	0	ND
8	7	1(14.3)	3(42.9)	ND	0	-	-	ND
9<	0	-	-	ND	0	-	-	ND
Total	49	6(12.2)	15(30.6)		50	0	19(38)	

Phylogenetic tree of
354 bp of HEV ORF2 gene
(NJ method, 1000 replicates)



Summary

- 25-50% of swine showed anti HEV IgG positive. Low percentage of anti HEV IgM through the period and December was the highest (12%).
- HEV were detected from 6 rectal swab samples and 2 serum samples and all of them were clustered into genotype 3.

SURVILLANCE OF INFECTION WITH JAPANESE ENCEPHALITIS VIRUS AND OTHER VIRAL AGENTS OF ZOONOTIC DISEASES IN SWINE IN NORTHERN LUZON, PHILIPPINES

Fedelino Malbas Jr.¹, Mariko Saito², Yusuke Sayama³, Cecille Lopez³, Lorna Baculanta¹, Wilfredo Aure¹, Estrella Cruz¹⁽⁺⁾, Hazel Galang¹, Socorro Lupisan⁴, Remigio Olveda³, Hitoshi Oshitani²

1 Research Institute for Tropical Medicine
 3 Tarlac Provincial Hospital

2 Tohoku University Graduate School of Medicine
 4 Tarlac Provincial Veterinary Office

BACKGROUND

- In December 2008 Ebola – Reston virus was detected from the swine farm of Region I & II. Lessons learned from the event is the importance of continuous surveillance and monitoring of possible disease from swine before it affects human.
- The situation of the major zoonotic diseases between swine and humans, such as Japanese Encephalitis virus, Hepatitis E virus, Influenza were not well known in the Philippines.

OBJECTIVES

- To describe the seasonality of JEV in the blood samples of swine.
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- To detect the presence of other possible pathogens, such as Hepatitis E virus, influenza virus, Norovirus and Nipah virus by antigen/antibody detection from serum and feces from swine.

METHODOLOGY

Blood, nasal swab and rectal swab from randomly samples swine were collected in the study sites. Total of 50 swine/sampling in July, September, December and January 2011.

Samples were separated within the same day of the collection and kept in the freezer.

RNA was extracted at RTM, influenza virus (nasal), Japanese encephalitis virus (serum), Hepatitis E virus (serum, rectal swab) were detected by RT-PCR.

Anti JEV anti HEV IgG and IgM were detected by ELISA (IND protocol)

FIELD SURVEILLANCE ACTIVITY AND SWINE SAMPLES COLLECTION



Initial Results -Japanese Encephalitis

Age (Months)	July 2010				September 2010			
	No. of swine	IgM	IgG	JEV RNA**	No. of swine	IgM	IgG	JEV RNA**
2	5	0	0	0	0	0	0	0
3	21	0	0	0	0	0	0	0
4	5	0	0	0	0	0	0	0
5	2	0	0	0	0	0	0	0
6	4	0	0	0	0	0	0	0
7	2	0	0	0	0	0	0	0
8	4	0	0	0	0	0	0	0
9	4	0	0	0	0	0	0	0
Total	47	0	0	0	0	0	0	0

Initial Results -Hepatitis E

Age (Months)	July 2010				September 2010			
	No. of swine	IgM	IgG	HEV RNA**	No. of swine	IgM	IgG	HEV RNA**
2	5	0	0	0	0	0	0	0
3	21	0	0	0	0	0	0	0
4	5	0	0	0	0	0	0	0
5	2	0	0	0	0	0	0	0
6	4	0	0	0	0	0	0	0
7	2	0	0	0	0	0	0	0
8	4	0	0	0	0	0	0	0
9	4	0	0	0	0	0	0	0
Total	47	0	0	0	0	0	0	0

Municipality of San Jose, Tarlac Province, Philippines
 where the JE outbreak was reported in 2003.



Mosquitos Collection:
 May 2009 – March 2011
 (monthly collection in 2009
 and every month in 2010)

Swine serum collection:
 July 2010 – March 2011
 (every other month)

COLLECTED SAMPLES (BY GEOGRAPHIC DISTRIBUTION)

	July 2010	September 2010	December 2010	January 2011	Total
Ege Pan	13	13	13	14	26
Ege Villa Aguirre	12	13	13	13	25
Ege Villavieja San Juan	12	13	8	0	25
Ege Moriones Centro	0	12	15	12	12
Ege Lubigan	10	0	0	13	10
TOTAL	47	32	49	56	197

Initial Results -Japanese Encephalitis

**JEV RNA was detected from nasal swab.

Age (Months)	December 2010				January 2011			
	No. of swine	IgM	IgG	JEV RNA**	No. of swine	IgM	IgG	JEV RNA**
2	2	0	0	0	5	0	0	0
3	7	0	0	0	0	0	0	0
4	19	0	0	0	0	0	0	0
5	3	0	0	0	12	0	0	0
6	6	0	0	0	12	0	0	0
7	1	0	0	0	2	0	0	0
8	7	0	0	0	0	0	0	0
9	0	0	0	0	0	0	0	0
Total	45	0	0	0	30	0	0	0

Initial Results -Hepatitis E

Age (Months)	December 2010				January 2011			
	No. of swine	IgM	IgG	HEV RNA**	No. of swine	IgM	IgG	HEV RNA**
2	2	0	0	0	5	0	0	0
3	7	0	0	0	0	0	0	0
4	19	0	0	0	0	0	0	0
5	3	0	0	0	12	0	0	0
6	6	0	0	0	12	0	0	0
7	1	0	0	0	2	0	0	0
8	7	0	0	0	0	0	0	0
9	0	0	0	0	0	0	0	0
Total	45	0	0	0	30	0	0	0

CONCLUSION

- Approx. 65 % of swine showed anti JEV IgG positive throughout the study period. Positivity rate for Anti-JEV IgM was highest in July 2011.
- Only 2 samples were confirmed by sequencing and these belong to genotype 3.
- There are some other positive samples in RT-PCR. Confirmatory tests will be needed.
- 25-50% of swine were anti HEV IgG positive.
- Low percentage of anti HEV IgM throughout the study period and highest (12%) was observed in December 2010.
- HEV was detected from 6 rectal swab samples and 2 serum samples and all of them clustered into genotype 3.

ACKNOWLEDGEMENT

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Thank you

