

4. RESULTS

The study was conducted to compare analytical sensitivity of three diagnostic methods for avian influenza virus isolation and detection of avian influenza virus of subtype H5N1 with different methods and samples .

4.1 The sensitivity among three diagnostic methods

4.1.1 The result of virus isolation by using embryonated egg. For the cloacal swabs sample, at the concentration 10^0 - 10^{-3} all samples were positive result. At the concentration 10^{-9} , all samples were negative. For lung organs sample the concentration 10^0 - 10^{-3} all were positive samples. At the concentration 10^{-8} all samples were negative. (Table.2)

4.1.2 The result of virus isolation by using MDCK cells. For the cloacal swabs sample at the concentration 10^0 - 10^{-3} all samples were positive result. At the concentration 10^{-8} all samples were negative. For lung organs sample at the concentration 10^0 - 10^{-3} were positive all samples. At the concentration 10^{-8} all samples were negative. (Table.3)

4.1.3 The result of virus isolation by using RT-PCR. For the cloacal swabs sample at the concentration 10^0 - 10^{-1} all samples were positive result. At the concentration 10^{-4} all samples were negative. For lung organs sample the concentration 10^0 - 10^{-1} were positive all samples . At the concentration 10^{-3} all samples were negative. (Table.4)

Table 2. The result of virus isolation by using embryonated eggs

Method	Concentration Of virus	Cloacal swabs replication		Lung organs replication	
		+	-	+	-
Egg	10^0	10	0	10	0
	10^{-1}	10	0	10	0
	10^{-2}	10	0	10	0
	10^{-3}	10	0	10	0
	10^{-4}	8	2	9	1
	10^{-5}	7	3	7	3
	10^{-6}	7	3	6	4
	10^{-7}	6	4	5	5
	10^{-8}	4	6	0	10
	10^{-9}	0	10	0	10

- = negative result

+ = positive result

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Table 3. The result of virus isolation by using MDCK cells

Method	Concentration Of virus	Cloacal swabs replication		Lung organs replication	
		+	-	+	-
MDCK	10 ⁰	10	0	10	0
	10 ⁻¹	10	0	10	0
	10 ⁻²	10	0	10	0
	10 ⁻³	10	0	10	0
	10 ⁻⁴	9	1	9	1
	10 ⁻⁵	9	1	7	3
	10 ⁻⁶	7	3	6	4
	10 ⁻⁷	5	5	4	6
	10 ⁻⁸	0	10	0	10
	10 ⁻⁹	1	9	0	10

- = negative result

+ = positive result

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Table 4. The result of virus isolation by using RT-PCR

Method	Concentration Of virus.	Cloacal swabs replication		Lung organs replication	
		+	-	+	-
RT-PCR	10^0	10	0	10	0
	10^{-1}	10	0	10	0
	10^{-2}	9	1	9	1
	10^{-3}	2	8	0	10
	10^{-4}	0	10	0	10
	10^{-5}	0	10	0	10
	10^{-6}	0	10	0	10
	10^{-7}	0	10	0	10
	10^{-8}	0	10	0	10
	10^{-9}	0	10	0	10

- = negative result

+ = positive result

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4.2 Minimum detectable virus concentration

Using Kaplan-Meier survival estimates for

4.2.1 Minimum detectable virus concentration between diagnostic method (Table5)

4.2.1.1 Minimum detectable virus concentration by using embryonated eggs was 10^{-7} (HA).

4.2.1.2 Minimum detectable virus concentration by using MDCK cell was 10^{-6} (HA).

4.2.1.3 Minimum detectable virus concentration by using RT-PCR was 10^{-2} (HA).

4.2.2 Minimum detectable virus concentration of sample type (Table6)

4.2.2.1 Minimum detectable virus concentration using cloacal swab was 10^{-5} (HA)

4.2.2.2 Minimum detectable virus concentration using lung organs was 10^{-4} (HA)

Table 5. Detected proportions of samples in ten-fold concentrations in each method

Method	Conc. 10^{-x}	No. of positive samples	No. not positive	Proportion detected	Standard Error	
egg	3	20	3	0.85	0.0798	
	4	17	3	0.70	0.1025	
	5	14	1	0.65	0.1067	
	6	13	2	0.55	0.1112	
	7	11	7	0.20	0.0894	
	8	4	4	0.00	.	
	mdck	3	20	2	0.90	0.0671
		4	18	2	0.80	0.0894
5		16	3	0.65	0.1067	
6		13	4	0.45	0.1112	
7		9	8	0.05	0.0487	
9		1	1	0.00	.	
rtPCR		1	20	2	0.90	0.0671
		2	18	16	0.10	0.0671
		3	2	2	0.00	.

Conc. 10^{-x} = virus concentration in each dilution .

Table 6. Detected proportions of samples in ten-fold concentrations in each sample type

Samples	Conc. 10 ^{-x}	No. of positive samples	No. not positive	Proportion detected	Standard Error
cloacal	1	30	1	0.9667	0.0328
	2	29	7	0.7333	0.0807
	3	22	5	0.5667	0.0905
	4	17	1	0.5333	0.0911
	5	16	2	0.4667	0.0911
	6	14	3	0.3667	0.0880
	7	11	7	0.1333	0.0680
	8	4	4	0.0333	0.0328
	9	1	1	0.0000	.
lung					
	1	30	1	0.9667	0.0328
	2	29	9	0.6667	0.0861
	3	20	2	0.6000	0.0894
	4	18	4	0.4667	0.0911
	5	14	2	0.4000	0.0894
	6	12	3	0.3000	0.0837
7	9	0	0.0000	.	

Conc.10^{-x} = virus concentration in each dilution .

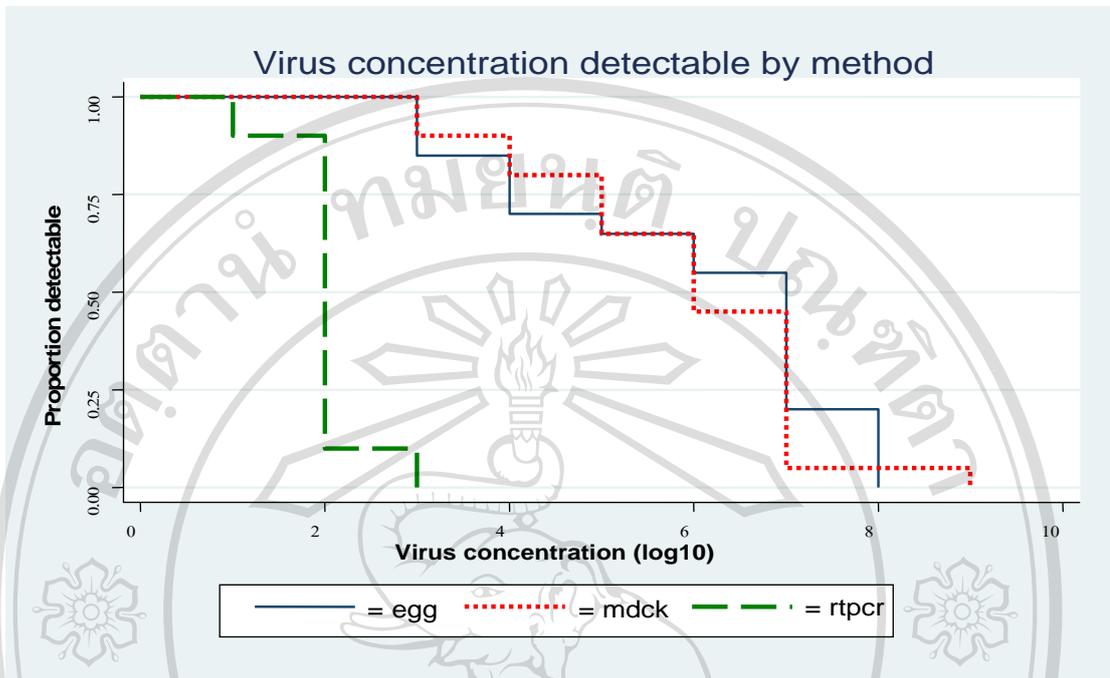


Figure 8: Comparison of the concentration between three diagnostic methods; using Kaplan-Meier survival estimates

The result from the graph; for the virus isolation and identification by using RT-PCR (green line) the result showed that markedly decrease of the detectable level of virus concentration which were detected at 10^{-3} . The result of the virus isolation and identification by using embryonated eggs (blue line) showed that the detectable level of virus concentration at 10^{-8} and this result has a slightly difference from the virus isolation and identification by using MDCK cells at 10^{-9} .at the last dilution (red lin

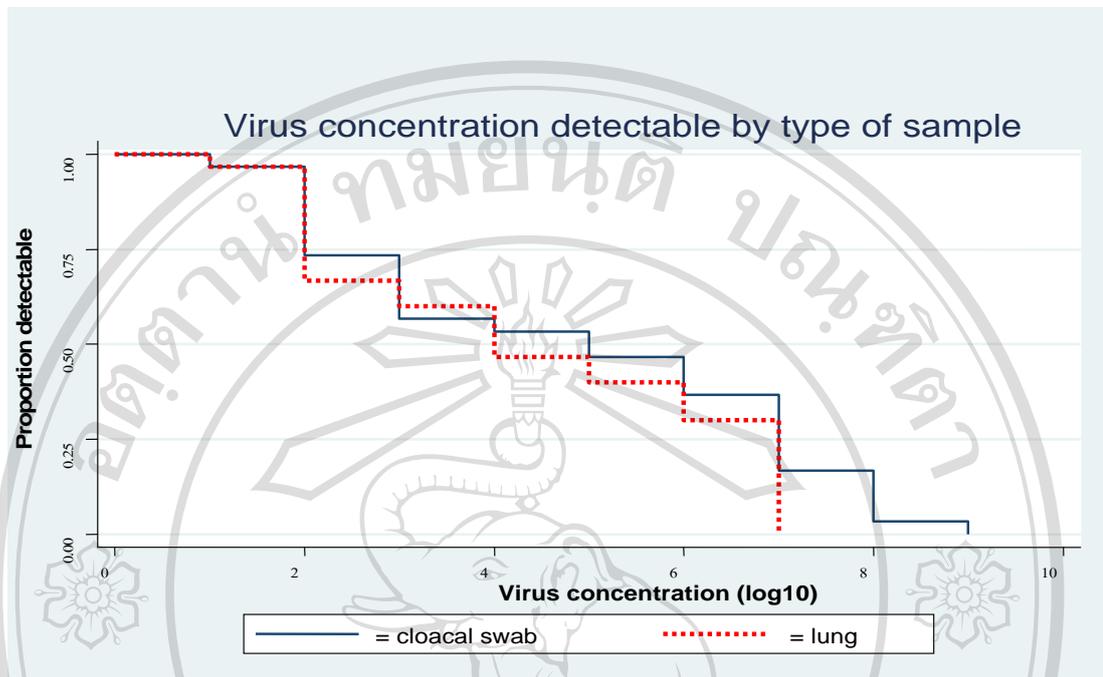


Figure 9: Comparison of the concentration between two types of samples; using Kaplan-Meier survival estimates

The virus isolation and identification from cloacal swabs (blue line) and from lung organs (red line) showed that the detectable level of virus concentration were detected at 10^{-9} and 10^{-7} at the last dilution respectively (Figure 9)

4.3 Comparison of detectability level between methods

4.3.1 Comparison among difference methods

4.3.1.1 Virus isolation by using embryonated egg and MDCK cell: the detectability level between embryonated eggs and MDCK cell was statistically not significant difference . (p=0.594)

4.3.1.2 Virus isolation by using embryonated egg and RT-PCR: the detectability level between embryonated egg and RT-PCR was statistically significant difference (p=0.001)

4.3.1.3 Virus isolation by using MDCK cell and RT-PCR: the detectability level between MDCK cell and RT-PCR was statistically significant difference. (p=0.001)

4.3.1.4 Virus isolation by using embryonated eggs, MDCK cell and RT-PCR: the detectability level among 3 methods was statistically significant difference. (p=0.001)

4.3.2 Comparison between types of sample (cloacal swab and lung organs)

The detectability level between 2 types of sample (cloacal swabs and lung organs) was statistically not significant difference. (p=0.219)