MOLECULAR DETECTION AND SUBTYPING OF INFLUENZA A VIRUS FROM SWINE NASAL SWAB: 2009 NATIONAL SWINE SURVEILLANCE

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ABSTRACT. A total of 6768 swine nasal swab samples were tested for the detection and subtyping of influenza A virus by conventional reverse transcription polymerase chain reaction (RT-PCR), Real-time TaqMan RT-PCR and multiplex RT-PCR assays during 2009 national swine surveillance of H1N1 in the country. The primer sets targeted for M and NP gene for detection of influenza A, HA and NA gene for subtyping of the influenza viruses. Eleven samples were positive for influenza A by conventional RT-PCR but only one sample was positive by Real-time TaqMan RT-PCR. Subtyping of the virus revealed presence of H1 subtype of classical swine influenza virus but no detectable amplified band of N1 or N2 gene was observed.

Keywords: nasal swabs, Influenza A virus, conventional RT-PCR, real time TaqMan RT-PCR, multiplex RT-PCR

INTRODUCTION

Swine Influenza Virus (SIV) is the cause of an infectious respiratory disease of swine. SIV infection in pigs occurs throughout the world. SIV was isolated in Sarawak in 1984 (KT Lim *et al*., 1985). Antibodies against SIV, H1N1 and H3N2 subtypes have also been reported in pigs in Malaysia (R Suriya *et al*., 2008). The emergence of a novel H1N1 influenza A virus of animal origin with transmissibility from human to human poses pandemic concern. Influenza A pandemic (H1N1) 2009 virus is a novel highly transmissible agent that contains a unique combination of gene segments from different swine lineage and therefore, the influenza virus infection in pigs is considered an important public health concern (Tim Pasma *et al*., 2010)

Howden KJ *et al.* reported that the pandemic (H1N1) 2009 virus was isolated from a swine herd in Alberta in May 2009. In response to the emergency situation, the DVS Malaysia has conducted a surveillance programme for swine influenza in pig farms started in May 2009. The main objective of the surveillance is to determine the presence of influenza A/H1N1 viruses (pH1N1) in Malaysian pig population.

TABLE 1: Primer description and assays

Set	Detection	Assay	Target gene	Primer description
1	influenza A virus	conventional RT-PCR	M, NP	Primer sequences from avian, swine and pandemic H1N1 influenza A viruses (by WHO collaborating Centre for influenza at CDC, Atlanta, US). Amplification fragment of 510 bp for M and 329 bp for NP
2	influenza A virus	real-time TaqMan RT-PCR assay	M	Primer/ probe (by AAHL, CSIRO) to detect viruses from different species, including avian, equine and/ or other species, **excluding** pandemic H1N1 2009 influenza virus
3	influenza A virus	real-time TaqMan RT-PCR assay	M	Primer/ probe (by AAHL, CSIRO) to detect viruses from pandemic H1N1 2009 and swine influenza A virus (**including** strains of classic swine influenza)
4	Subtyping of influenza A virus	Multiplex RT-PCR assay	HA, NA	Two primer sets of H1 and H3 and two primer sets of N1 and N2 (YK Choi *et al.*)

MATERIALS & METHODS

Samples

A total of 6768 nasal swab samples (every 10 nasal swabs were placed in a tube of 2 ml viral transport medium) from various pig farms in the country were submitted to VRI between May to December 2009. The nasal swabs were centrifuged at 3000 rpm for 20 minutes and the supernatants were used for RNA extraction. A reference strain of swine influenza virus H1N1 (A/ Swine/Kuching/3966/84) was used in the RT-PCR assays.

Primers, viral RNA extraction and assays

Viral RNA was extracted using TRI reagent from 250 µl volume of nasal swab homogenate. The conventional RT-PCR assay for detection of influenza A viruses was performed using *AccessQuick™ RT-PCR System (Promega)* using primers set 1 (Table 1). To distinguish both classical and pandemic 2009 H1N1 viruses, the RNA was subjected to real time Taqman RT-PCR assay using primers/ probe of set 2 and set 3. For HA and NA subtype differentiation, four primer sets of H1, H3, N1 and N2 (primer set 4) were used to amplify 1006 bp, 663 bp, 754 bp and 502 bp of HA and NA genes of each subtype respectively. Subtyping of the viruses was determined by multiplex RT-PCR assay.

RESULTS

488 bp and 510 bp PCR products were amplified from eleven nasal swab samples using the conventional RT-PCR assay. Upon real time TaqMan RT-PCR assay, only one nasal swab sample showed specific

TABLE 2: Detection and subtyping of influenza A virus from swine nasal swab samples

Sample type	Sample no.	Influenza A by conventional RT-PCR assay	Influenza A by realtime Taqman RT-PCR assay		Subtype of HA and NA gene by multiplex RT-PCR assay			
			Excluding 2009 pH1N1	Including 2009 pH1N1	H1	H3	N1	N2
Nasal swabs	6768	11	1	0	1	0	0	0

amplification with threshold (Ct) value of 21.1. The remainder 10 samples had cycle threshold value greater than 37 and were classified as indeterminate results after 3 repetitive assays. Real time TaqMan RT-PCR assay on the positive sample using primer set 3, indicated that the detected virus was not of pandemic 2009 H1N1.

Multiplex RT-PCR amplification with H1 and H3 primer sets, revealed of H1 subtype of classical swine influenza virus but no detectable amplified product of N1 or N2 gene was observed. Summary of the results were shown in Table 2.

DISCUSSION

Various primers have been used to detect and distinguish both classical and pandemic 2009 H1N1 viruses for this study. The first isolation of swine influenza H1N1 subtype in Malaysia was made from a pig in 1984 (K.T. Lim *et al*., 1985). Since then, no other report on the detection of swine influenza H1 is available in Malaysia although the detection of H1N1 subtype has been reported from pigs in different countries including Indonesia Thailand and US (Y.K. Choi *et al*., 2002).

It is essential to conduct laboratory tests according to international standards (OIE/WHO) for accurate and rapid detection. Molecular method was chosen for this surveillance programme because it is rapid, sensitive and specific compared to virus isolation. The results showed indeterminate results and it could be due to very low copy of viruses. To increase chances of virus isolation we need to collect samples from acute respiratory disease of pigs with typical clinical signs such as fever, coughing, discharge from the nose or eyes, sneezing and breathing difficulties.

A rapid method for detection and subtyping is necessary to obtain detailed information on the prevalence of different subtypes of influenza A virus for tracking prevalent strains in local swine populations.

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