Veterinary Microbiology 174 (2014) 9–15

Contents lists available at ScienceDirect

Veterinary Microbiology

journal homepage: www.elsevier.com/locate/vetmic

Temporal insight into the natural generation of a new reassortant porcine influenza virus in a swine holding

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ARTICLE INFO

Article history:
Received 4 July 2014
Received in revised form 18 August 2014
Accepted 26 August 2014

Keywords:
Swine influenza A virus
H1N1
Reassortant

ABSTRACT

The influenza A virus (IAV) subtypes H1N1, H3N2 and H1N2 are the most prevalent subtypes in swine in Italy. Reassortant influenza A viruses subtypes in swine appeared in European pig population. In particular reassortant viruses carrying genome segment from the pandemic H1N1 (H1N1pdm) are reported in many European countries, including Italy. In a 1000 sows farrow-to feeder farm, in Northern Italy, we isolated 10 IAVs from recurrent episodes of respiratory disease in 45–70 days-old pigs from September 2012 to June 2013. The antigenic and genetic characterization of the swine IAV isolates showed the contemporary circulation of H1N1 avian-like and H1N1pdm strains in the first outbreak. The analysis of the viruses isolated subsequently showed the circulation of H1N1pdm IAV and then the establishment of a new previously undescribed H1N1 reassortant strain with a pandemic derived hemagglutinin gene and all the other seven segments of swine H1N1 avian-like lineage.

1. Introduction

Swine influenza is a highly contagious acute viral disease of the respiratory tract in pigs which is distributed worldwide. The disease is economically damaging primarily due to weight loss and reduced weight gain of affected pigs but on occasions infection can be more severe, particularly if exacerbated by the presence of other pathogens or factors. Influenza A viruses (IAVs) are members of family *Orthomyxoviridae* genus *Influenzavirus* A, which are classified into 18 hemagglutinin (HA) and 11 neuraminidase (NA) based to the antigenicity of these surface glycoproteins (Tong et al., 2013). The genome of IAV is made of eight RNA segments encoding for 12 proteins, with some additional newly identified protein products (Webster et al., 1992; Wise et al., 2009; Jagger et al., 2012; Wu et al., 2014). Segmentation of the genome allows for genetic exchange between differing IAVs, when these viruses meet within a co-infected cell, the process is called reassortment or genetic shift. The term genetic drift, in contrast identifies the continuous accumulation of nucleotide substitutions over time (Scholtissek, 1995; Worobey et al., 2014). Viruses originating from these mechanisms of rapid evolution can be able to infect and spread among pigs and occasionally, may also cross the species barrier into other host populations (Olsen et al., 2006).

Nowadays, there are three principal subtypes of swine IAV circulating in the European and in the Italian pig

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http://dx.doi.org/10.1016/j.vetmic.2014.08.026
0378-1135/

population H1N1, H3N2 and H1N2. The H3N2 viruses circulate in Europe since 1984 and resulted from a genomic reassortment between human-like (hu-like) H3N2 viruses and avian-like (av-like) H1N1 IAVs. The H1N2 subtype circulating in Italy arose from a genomic reassortment between H1N1 of human origin and avian H3N2 IAV (Moreno et al., 2013). The most prevalent swine IAV subtype in Europe is the av-like H1N1 which appeared in European pigs in 1979 and harbor a whole genome of avian origin (Kyriakis et al., 2011; Brown, 2013).

The human pandemic H1N1 2009 virus (H1N1pdm), first detected in April 2009, is a novel reassortant influenza A (H1N1) virus of swine origin in which the NA and M genes are genetically related to those of the Eurasian swine lineage, while the remaining six genes are similar to those of North American triple-reassortant swine IAVs (Garten et al., 2009). This virus spread quickly on a global scale in the human population and infection in pigs, through anthropozoonotic transmission, was reported in many countries. Mild disease indistinguishable from endemic swine influenza viruses was induced in pigs (Keenliside, 2013). The H1N1pdm IAV subtype is sporadically detected in Italian pig farms since his first detection in Italy in 2009 (Moreno et al., 2010).

Reassortants between the main lineages of porcine IAV have been detected repeatedly (Moreno et al., 2009, 2011) and at present the swine H3N2, the H1N2 human derived lineage and the H1N1 av-like are established in Italian pig population as endemic strains (Kyriakis et al., 2011). Reassortant porcine IAV viruses carrying genome segment from the pandemic H1N1 are reported in many countries i.e. Germany (Starick et al., 2011, 2012), UK and Hungary (Howard et al., 2011), Korea (Kim et al., 2013) and Italy (Moreno et al., 2012).

We describe the antigenic and genetic characterization of H1N1 IAVs strains detected repeatedly in 45–70 day-old pigs in an Italian 1000-sow farrow-to feeder located in Northern Italy. Five recurrent respiratory outbreaks were observed from September 2012 to June 2013 in the farm, PRRS infection was controlled by sow and piglet vaccination while no IAV vaccination was performed. Laboratory investigations highlighted active PRRSv circulation and the presence of concomitant bacterial infections (*Streptococcus suis* type 2 and *Haemophilus parasuis*). Further virological investigations permitted to detect IAV circulation; 10 H1N1 IAVs belonging to H1N1pdm and H1N1 av-like lineage were isolated. Co-circulation of these strain lead to the generation of a novel H1N1pdm reassortant (Chiapponi et al., 2013).

2. Methods

2.1. Virus detection and antigenic characterization

During five respiratory outbreaks occurred from September 2012 to June 2013, nasal swabs from animals with respiratory clinical signs were sampled and tested as pool of five for the influenza A virus using a real-time RT-PCR (Brookes et al., 2010).

Swabs from RT-PCR positive pools were inoculated onto MDCK and CACO-2 cells and into 11-day-old SPF chicken embryonated eggs (Chiapponi et al., 2010) in order to isolate the virus. The cell-culture supernatant and allantoic fluid were tested by hemagglutination assay (HA) with chicken erythrocytes, using the standard procedure (OIE, 2010). To reveal the presence of IAV, a double-antibody sandwich ELISA was used (Siebinga and de Boer, 1988). The positive samples were tested using Multiplex RT-PCR and real-time RT-PCR for virus subtyping (CDC, 2009; Chiapponi et al., 2012). The antigenic properties of the viruses were determined using hemagglutination-inhibition (HI) tests performed according to standard procedures (OIE, 2010) using a panel of hyperimmune swine and chicken antisera raised against A/swine/Italy/284922/2009 H1N2 (swine), A/swine/Italy/1521/1998 H1N2 (chicken), A/swine/Scotland/410440/1994 H1N2 (swine), A/swine/Finistere/2899/1982 H1N1 (swine), A/swine/Cotè d'Amor/0388/2009 H1N1 (swine), A/swine/Flanders/1998 H3N2 (swine), A/swine/Italy/290271/2009 H1N1pdm (swine).

The sera were produced in pigs or chickens and the HI titer against the homologous viruses was between 1016 and 2560. Swine sera were treated with receptor-destroying enzyme to remove non-specific inhibitors of hemagglutination and absorbed onto chicken erythrocytes to remove agglutination factors. The viruses were tested in triplicate against twofold serum dilutions, starting at a dilution of 1:20. HI tests were performed with four hemagglutinating units (HAU) of virus and 0.5% chicken red blood cells. Titers were expressed as geometrical means of the reciprocal of the highest dilution inhibiting four HAU (OIE, 2010).

2.2. Genome sequencing and analysis

Viral RNA was extracted from cell cultured virus or nasal swab and amplification of full genome segments was obtained as described previously (Lycett et al., 2012). A sequencing library of the purified amplicons was prepared with NEXTERA-XT kit and sequenced on a MiSeq sequencer using a Miseq Reagent Kit v2 in a 250-cycle paired-end run (Illumina Inc., San Diego, CA, USA). Sequencing reads were de-novo assembled by Seqman NGen DNASTAR application (version 11.2.1).

Full genome sequences of all the strains isolated in the study, of the strain A/California/04/2009 H1N1pdm and of the Italian isolate A/swine/Italy/55230/2012 H1N1 av-like, were concatenated and aligned by ClustalW using MEGA5 (Tamura et al., 2011). Recombination analysis was performed by RDP, MaxChi, Bootscan, in the RDP3 software package (Martin et al., 2010) on the alignments of the genomes concatenated (13133 nucleotides).

Blast analysis of individual segments was performed at http://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=-blastn&PAGE_TYPE=BlastSearch&LINK_LOC=blasthome.

Gene sequences from the outbreaks strains and H1N1 reference sequences retrieved from Genbank were aligned with ClustalW using MEGA5. The phylogeny of individual segments was reconstructed. Phylogenetic trees were inferred with the maximum likelihood (ML) method implemented in IQ-TREE package 0.9.6 (Minh et al., 2013). The robustness of the ML trees was statically evaluated by bootstrap analysis with 1000 bootstrap