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Swine Influenza

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Preface

From the first detailed clinical description of the disease in the Midwestern United States in 1918, to the isolation of the causative agent, the first of any influenza virus, in 1930 (Shope 1931) to its role in the genesis of the 2009 human pandemic (Garten et al. 2009), swine have played a central role in the ecology of influenza. Although not considered the major natural reservoir for influenza A viruses, that distinction belongs to aquatic waterfowl, swine are host to a limited but dynamic assortment of viruses (Webster et al. 1992). A number of subtypes of influenza A viruses of human and avian origin, including H1, H2, H3, H4, H5, H7, and H9, have been isolated from global swine populations (reviewed in Brockwell-Staats et al. 2009). Most of these isolations have, however, been limited in number and it is only H1 and H3 influenza viruses that are known to have formed stable lineages in swine. In this respect, swine influenza viruses (SIV) are similar to their counterparts in humans where H1 and H3 viruses have also been maintained. The nature of these H1 and H3 viruses differs between the two host populations, however, and, as discussed throughout this book, are even different in swine populations in different geographic regions of the world due to multiple introductions of avian and human influenza viruses.

The dynamic nature of SIV poses difficulties for the swine industry as a recurring respiratory disease, and also for public health as a source of zoonotic infection. Human infections with SIV have been recorded regularly since the introduction of more routine testing in humans. Many of these zoonotic events have occurred in instances where humans and swine are in close contact and have typically been dead-end events with little to no further spread in humans. The virologic features of SIV that limit their spread in humans are largely unknown, but the host range barrier between human and swine highlights the fact that adaptation of a virus in one mammalian host does not necessarily mean that it is well adapted to replication in another (Landolt et al. 2003). This observation is somewhat in conflict with earlier dogmas in influenza where it was suggested that mammalian passage of avian influenza viruses was a prerequisite for the emergence of human pandemics. Swine were often identified as this mammalian host due to a number of factors including the limited number of other described natural mammalian hosts and the

fact that swine appeared unique in having the receptors preferred by both human and avian influenza viruses (Ito et al. 1998). The observation that swine appeared uniquely susceptible to avian and human viruses and that avian viruses grew poorly in humans led to the postulation that these animals were the mixing vessel for human pandemic viruses; and for a number of years popular thinking, without much definitive proof, was that the 1957 and 1968 human pandemics likely arose in pigs (Scholtissek et al. 1978). Subsequent human infections with H5N1, H7, and H9N2 viruses with domestic poultry as the likely source and realizations that swine were not unique in their ability to harbor avian and human viruses shifted thinking toward poultry being as important as swine as reservoirs of viruses with pandemic potential. Indeed, the global spread of highly pathogenic H5N1viruses focused a lot of research effort and funding toward avian hosts at the expense of solidifying activities in swine. Although surveillance and research activities of influenza in swine continued, and to some degree increased, during the first decade of the twenty-first century, these activities were dwarfed by the efforts going on in wild and domestic poultry species. The isolation of a novel influenza virus (i.e., pandemic H1N1) from a 10-year-old boy in California in April 2009 indicated that more of the influx of resources should have been funneled into further understanding the global SIV situation. The virus from the 10-year-old was obviously of SIV ancestry, but it was different enough from any other virus characterized that its direct precursors still remain a mystery. In addition, in 2012 zoonotic transmission of SIV (both H3N2 and H1N2 subtypes) containing the matrix gene from the pandemic H1N1 virus was reported. These strains appeared to be able to spread more easily from pigs to people than other influenza viruses of swine. More than 300 people from 10 states were reported to have been infected with these new strains resulting in hospitalizations and 1 death; limited human-to-human transmission was detected (Lindstrom et al. 2012). Importantly, the main risk factor for infection was exposure to pigs, mostly in the context of agricultural fair settings.

With these events firmly at center stage, it is a good opportunity to review what we know about SIV as a disease of swine and also as a continued zoonotic threat. The 15 chapters presented in this book provide contemporary reviews of research on SIV. The book begins with a general overview of influenza viruses by Stephan Pleschka discussing the virus and its replication in detail. The history of SIV in North America, Europe, and Asia is discussed by Stacey Schultz-Cherry, Christopher Olsen, and Bernard Easterday, by Roland Zell, Christoph Scholtissek, and Stephan Ludwig, and by Huachen Zhu, Richard Webby, Tommy Lam, David Smith, Malik Peiris, and Yi Guan, respectively. As indicated in these reviews, the European, North American, and Asian SIV evolution follows different pathways. Whereas descendants of classical SIV and the novel triple reassortant viruses are found in North America, avian-like swine H1N1 viruses emerged in Europe in 1979 after an avian to swine transmission and spread to all major European pig-producing countries where they circulate with H3N2 and H1N2 reassortants. Classical swine H1N1, human-origin H3N2, avian-like H1N1 and the triple reassortant viruses all co-circulate in Asian pigs. The clinicopathological features of SIV infections in pigs are described by Bruce Janke. Macroscopic and

microscopic lesions of SIV infection, after natural and experimental infection, are described. The use of accurate diagnostics assays for diagnosis and surveillance for SIV are summarized by Susan Detmer, Marie Gramer, Sagar Goyal, Montserrat Torremorell, and Jerry Torrison. Since our collective knowledge regarding the worldwide occurrence of influenza among swine is incomplete, this review focuses on basic laboratory assays needed for the detection of the virus and viral nucleic acids within clinical samples and for antiviral antibodies in serum samples.

The epidemiology of swine influenza worldwide is of exceptional importance with the potential of the pig acting as a "mixing vessel" where both avian and human influenza viruses can undergo genetic reassortment resulting in the creation of novel viruses. The reviews by Alessio Lorusso, Amy Vincent, Marie Gramer, Kelly Lager, and Janice Ciacci-Zanella on North American, by Ian Brown on European, and by Young-Ki Choi, Philippe Noriel Pascua, and Min-Suk Song on Asian swine influenza epidemiology shed light on how this unique ability of pigs results in ever expanding new genotypes and subtypes in pigs. Vaccination is still one of the most important and effective strategies to prevent and control influenza for both the animal and human population. The review by Kristien van Reeth and Wenjun Ma discusses the current and future options to control this economically important swine disease.

The zoonotic aspects of SIV infections are reviewed by Whitney Baker and Gregory Gray. Most of these infections have been sporadic cases with a recent increase of case reports in concert with modern pig farming and the emergence of triple reassortant SIV. The advent of pandemic H1N1 and its impact on human health is discussed by Ian York and Ruben Donis, while Julia Keenliside discusses its impact on animal populations. Hadi Yassine, Chang-Won Lee, and Yehia Saif describe another important interspecies transmission event of influenza A viruses, namely the one between swine and poultry. Swine viruses are continuously isolated from poultry species, especially turkeys, and they are causing economic losses. Finally, Elena Govorkova and Jonathan McCullers cover the critical area of approved and investigational antiviral drugs.

We would like to thank the contributors for their patience during the assembly of this volume. We hope that all readers will gain insight from these contributions that will enhance their individual research and teaching activities.

J. A. Richt
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Contents

Overview of Influenza Viruses

Stephan Pleschka

Abstract The influenza virus (IV) is still of great importance as it poses an immanent threat to humans and animals. Among the three IV-types (A, B, and C) influenza A viruses are clinically the most important being responsible for severe epidemics in humans and domestic animals. Aerosol droplets transmit the virus that causes a respiratory disease in humans that can lead to severe pneumonia and ultimately death. The high mutation rate combined with the high replication rate allows the virus to rapidly adapt to changes in the environment. Thereby, IV escape the existing immunity and become resistant to drugs targeting the virus. This causes annual epidemics and demands for new compositions of the yearly vaccines. Furthermore, due to the nature of their segmented genome, IV can recombine segments. This can eventually lead to the generation of a virus with the ability to replicate in humans and with novel antigenic properties that can be the cause of a pandemic outbreak. For its propagation the virus binds to the target cells and enters the cell to replicate its genome. Newly produced viral proteins and genomes are packaged at the cell membrane where progeny virions are released. As all viruses IV depends on cellular functions and factors for their own propagation, and therefore intensively interact with the cells. This dependency opens new possibilities for anti-viral strategies.

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1 Introduction

Influenza viruses (IVs) are a continuous and severe global threat to mankind and many animal species. The resulting disease gives rise to thousands of deaths and enormous economic losses in livestock each year. Clearly, influenza is a highly contagious, acute respiratory disease with global significance that affects all age groups and can occur repeatedly. Since waterfowl represents the natural reservoir for the etiological agent of the disease—the influenza and many other animal species can be infected, the virus cannot be eradicated. Therefore, a constant re-emergence of the disease will continue to occur (Palese and Shaw 2007; Webster 1999; Wilschut 2005; Wright et al. 2007). Epidemics appear in the human population almost annually and are due to an antigenic change of the viral surface glycoproteins (Fig. 1). Furthermore, highly pathogenic strains of influenza A virus have emerged unpredictably but repeatedly in recent history as pandemics like the "Spanish-Flu" that caused the death of 20–40 million people worldwide (Taubenberger et al. 2000; Webster 1999). The 2009 pandemic outbreak of the swine-origin IV (S-OIV, "Mexico-Flu") and its rapid spread around the world, as well as repeated human infections with highly pathogenic avian IV (HPAIV) of the H5-subtype demonstrated the imminent danger that IV continues to pose to both the human population and economically relevant animals.

2 The Virus and Its Replication

2.1 The Virion

IVs belong to the family of the *Orthomyxoviridae* and possess a segmented, single-stranded RNA-genome with negative orientation. IVs are divided into three types, A, B, and C based on the genetic and antigenic differences. They infect mammals and birds. Among the three types, influenza A viruses are clinically the most important pathogens and have been responsible for severe epidemics in humans and domestic animals in the past. Thus the focus of this chapter will be on type-A influenza viruses. A detailed description of the viral proteins and the replication

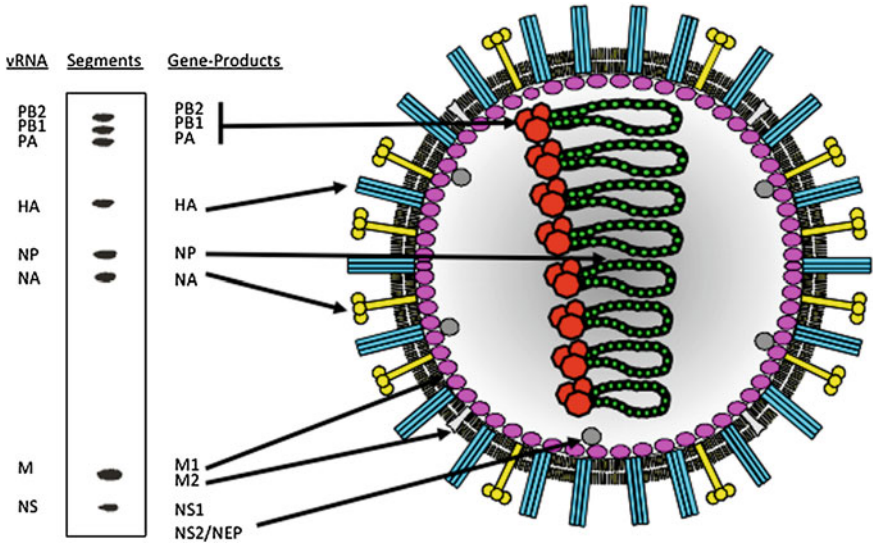

Fig. 1 The influenza A virus particle. Schematic representation of the spherical influenza A virus particle that has a diameter of about 100 nm. The eight viral RNA segments were separated by urea-polyacrylamide gel electrophoresis and visualized by silver staining (*left*). The corresponding gene products and their presumed location in the virus particle are indicated (*right*). PB1-F2 and NS1 are not a structural part of the mature virion. For details see text

cycle of influenza A viruses can be found elsewhere (Ludwig et al. 1999; Palese and Shaw 2007; Wright et al. 2007). Therefore, only an overview on these topics is given without referring to individual references.

The influenza A virus particle is composed of a lipid envelope derived from the host cell and of 9 or 10 structural virus proteins (Fig. 1 and Table 1). The components of the RNA-dependent RNA-polymerase complex (RdRp), PB2, PB1, and PA are associated with the ribonucleoprotein complex (RNP) and are encoded by the vRNA segments 1–3. The PB1 segment of many, but not all, influenza A virus strains also contains a +1-reading frame encoding the recently discovered PB1-F2 protein (Chen et al. 2001).

The viral surface glycoproteins hemagglutinin (HA) and neuraminidase (NA) are expressed from vRNA segments 4 and 6, respectively. The nucleoprotein (NP), the major component of the RNPs, is encoded by segment 5 and associates with the vRNA segments. Each of the two smallest vRNA segments code for two proteins. The matrix protein (M1) is co-linear translated from the mRNA of segment 7 and forms an inner layer within the virion. A spliced version of the mRNA gives rise to a third viral transmembrane component, the M2 protein, which functions as a pH-dependent ion channel. Employing a similar coding strategy, segment 8 harbors the sequence information for the nonstructural NS1 protein and the nuclear export protein (NEP). NEP is a minor component of the virion and is found associated with the M1 protein.

Table 1 Influenza A Virus Genome (strain A/PR/8/34)

Segment	vRNA	Protein	AA	Function(s)
1	2,341	PB2	759	Cap-binding subunit of the viral RdRp; cap-binding
2	2,341	PB1	757	Central location of the polymerase domain of the viral RdRp
		PB1-F2	87–91	Pro-apoptotic activity
3	2,233	PA	716	Cap-snatching endonuclease subunit of the viral RdRp
4	1,778	HA	566	Surface glycoprotein; receptor binding, membrane fusion
5	1,565	NP	498	Nucleoprotein; encapsidation of viral genomic and anti-genomic RNA
6	1,413	NA	454	Surface glycoprotein; receptor destroying Neuraminidase activity
7	1,027	M1	252	Matrixprotein
		M2	97	Ion channel activity, protecting HA conformation
8	890	NS1	230	Regulation of viral RdRp activity Interferon antagonist; Enhancer of viral mRNA translation; inhibition of (i) pre-mRNA splicing, (ii) cellular mRNA-polyadenylation, (iii) PKR activity,
		NEP	121	Nuclear export factor

Table 1 summarizes details of the genome segments, the encoded viral proteins and their respective function.

2.2 The Influenza Virus Replication Cycle: Viral Proteins and Their Function

2.2.1 Adsorption and Entry

The viral replication cycle is initiated by the binding of the HA to sialic-acid (neuraminic acid) containing cellular membrane resident molecules that act as receptors determinants. For example, it was shown that the epidermal growth factor receptor (EGFR) promotes uptake of IV into host cells (Eierhoff et al. 2010). Subsequently, the virus particle is taken up via endocytosis (Fig. 2) [For references: (Palese and Shaw 2007; Wright et al. 2007)]. Due to the different preferences for specific receptor determinants on the target cells of birds and humans, HA is regarded as a possible restriction factor. HAs of avian viruses bind to Sia2-3Gal-terminated sialylglycoconjugates, whereas those of human IV display a Sia2-6Gal-containing receptor-binding specificity [Reviewed in (Paulson 1985) see also (Connor et al. 1994)]. Nevertheless, it was recently shown that a strictly avian H7-type HPAIV carrying the NS segment of a H5-type HPAIV could

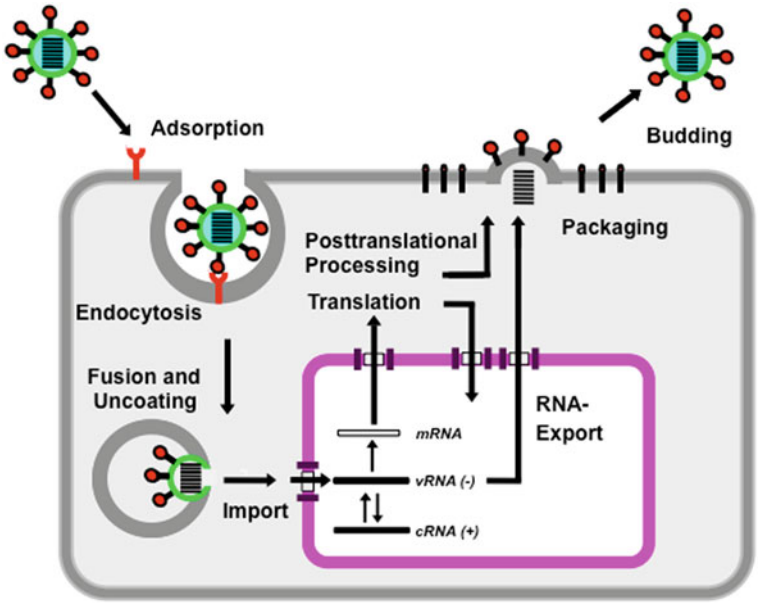

Fig. 2 The influenza viruses replication cycle. The virion attaches to the cellular receptor determinant. The receptor-bound particle enters the cell via endocytosis. After fusion of the viral and the endosomal membrane the viral genome is released into the cytoplasm. The RNPs are transported into the nucleus where replication and transcription of the viral RNA segments occur. The mRNAs are exported into the cytoplasm and are translated into viral proteins. The viral glycoproteins enter the exocytotic transport pathway to the cell surface. Replicative viral proteins enter the nucleus to amplify the viral genome. In the late stage of the infection cycle newly synthesized RNPs are exported from the nucleus and are assembled into progeny virions that bud from the cell surface

acquire the ability to replicate more efficiently in mammalian cell culture, and in contrast to the wild type was able to infect mice causing disease and death (Ma et al. 2010). Furthermore, additional NS reassortants displayed altered propagation ability of the H7-type HPAIV (Wang et al. 2010). Taken together, these results shed further light on the importance of the NS segment for viral replication, molecular pathogenicity and host range, as well as the possible consequences of a reassortment between naturally occurring H7 and H5 type HPAIVs. This indicates that the receptor HA-specificity, although important is not the sole host range and tropism determining factor.

The HA has to undergo a multitude of maturation steps, which are completely dependent on interactions with the protein processing machinery of the infected cell. To gain insight into intra-cellular post-translational processing and transport of glycoproteins, the HA has long been used as a model protein; and HA is probably the best analyzed virus component. A great amount of data has accumulated on the maturation and function of the HA during the viral replication cycle [For overviews: (Ludwig et al. 1999; Palese and Shaw 2007; Wright et al. 2007)].