Orthomyxoviruses

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Abstract

The viruses under the family *Orthomyxoviridae* are responsible for a variety of important respiratory diseases in humans and different animal species. The seven genera under the family are the influenza viruses A, B, C and D, *Quaranjavirus*, *Thogotovirus* and *Isavirus*. Viruses are highly evolving, and the genetic reassortment among viruses is seen only within the same genus and never been reported in between viruses from different genera. Influenza A viruses (IAVs) infect humans and different animals including birds, pigs, equines, dogs, cats, whales and seals. To date, there are 18 different haemagglutinins (H1 to H18) and 11 different neuraminidases (N1 to N9) for influenza A viruses. Influenza B viruses (IBVs) are exclusively human pathogens, while influenza C virus (IVC) affects humans and pigs. Serological evidence of ICV was recently detected in camels. Influenza D virus (IDV) was reported in pigs with influenza-like symptoms. IAVs cause recurrent epidemics of varying severity in humans and different animal species due to antigenic drift, gradual accumulation of point mutations, during replication under immune pressure induced by vaccines or prior infections. Several animal species act as important mixing vessel hosts. This chapter provides information on various orthomyxoviruses emphasizing upon virus properties, strains/types, genome, host, ecology, pathobiology, diagnosis and control.

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19.1 Preamble

Members of the family *Orthomyxoviridae* are responsible for a variety of important respiratory diseases in humans and different animal species. The family of *Orthomyxoviridae* possesses a negative-sense, single-stranded RNA segmented genome. It possesses seven different genera: the influenza viruses A, B, C and D, *Quaranjavirus*, *Thogotovirus* and *Isavirus*. Viruses of the same genus can undergo genetic reassortment; however, reassortment has never been reported in between viruses from different genera. Influenza A viruses (IAVs) infect humans and different animals including birds, pigs, equines, dogs, cats, whales and seals. To date, there are 18 different haemagglutinins (H1 to H18) and 11 different neuraminidases (N1 to N9) for influenza A viruses. Influenza B viruses (IBVs) are exclusively human pathogens, while influenza C virus (IVC) affects humans and pigs. Serological evidence of ICV was recently detected in camels (Salem et al. 2017). Influenza D virus (IDV) was reported in pigs with influenza-like symptoms. The virus and/or its serological evidence was detected also in cattle, sheep, goats and dromedary camels (Su et al. 2017). The genus *Thogotovirus* contains viruses of ticks in two different species, Dhori virus and Thogoto virus. The genus *Isavirus* contains infectious salmon anaemia virus as a prototype. The genus *Quaranjavirus* included two new species, Quaranfil virus (QRFV) and Johnston Atoll virus (JAV), and a tentative member, Lake Chad virus (LKCV). Although recently recognized as orthomyxoviruses, they were detected a long time ago: QRFV in Egypt, in 1953; JAV in the North Pacific, in 1964; and LKCV in Nigeria, in 1969 (Clifford et al. 1968). QRFV was isolated from ticks (*Argas arboreus*), children with febrile disease and seabirds. It also causes a lethal respiratory disease and meningoencephalitis experimentally in mice (Baskerville and Lloyd 1976).

Johnston Atoll virus (JAV) was isolated from ticks (*Ornithodoros capensis*) collected in 1964 from a Noddy Tern (*Anous stolidus*) nest, Johnston Atoll in the central Pacific (Clifford et al. 1968). No human disease has been associated with JAV, but experimentally, it is lethal to newborn and weanling mice and to 1- to 2-day-old chicks. LKCV is also lethal to newborn mice and is shown to be antigenically related to QRFV. Wellfleet Bay virus (WFBV) was the responsible pathogen for causing cyclic mortality events since 1998, in common eiders (*Somateria mollissima*) in the United States (Allison et al. 2015). In 2010, a Cygnet River virus (CyRV) induced a fatal disease in captive Muscovy ducks (*Cairina moschata*) in South Australia. WFBV is closely related to CyRV suggesting that they may be geographic variants of the same virus (Allison et al. 2015).

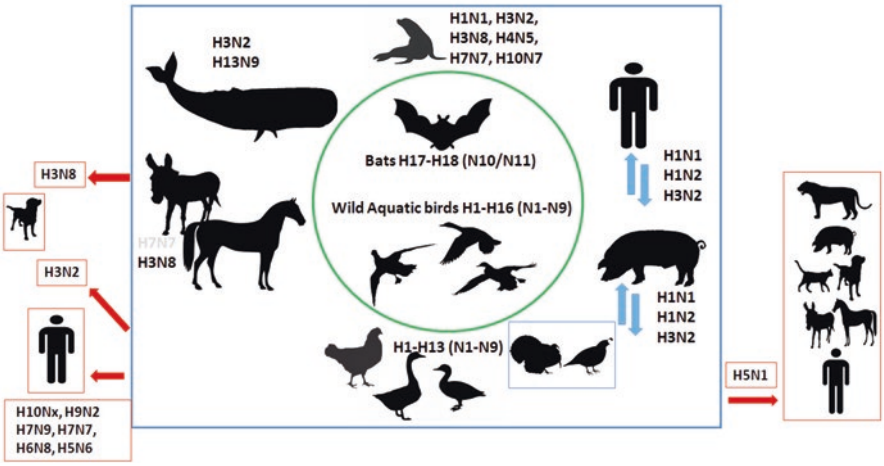

Fig. 19.1 Influenza A virus subtypes in final and reservoir hosts. Bats are reservoir hosts of H17 and H18 (N11 and N12), while wild aquatic birds are reservoir host for all other influenza subtypes. Some influenza A viruses cross species barrier and infect other species (red boxed)

IAVs cause recurrent epidemics of varying severity in humans and different animal species (Fig. 19.1) due to antigenic drift, gradual accumulation of point mutations, during replication under immune pressure induced by vaccines or prior infections. Moreover, reassortment, swapping of gene segments of two different IAVs, during replication enables continuous evolution of IAVs in nature causing devastating panzootic in different animal species and occasionally human pandemics. Domestic pigs, turkeys and quails are important hosts that could act as a mixing vessel. The influenza A viruses circulate in reservoir hosts, mainly wild aquatic birds: especially ducks, gulls and shorebirds that spread IAVs across continents with subsequent transmission to the respective final host including human and different animal species (Maclachlan et al. 2017). More recently, bats are reservoir for H17N10 and H18N11 (Maclachlan et al. 2017). In the wild bird reservoirs, IAVs mainly replicate in the intestinal epithelium resulting in an efficient faecal excretion of the virus (Maclachlan et al. 2017; Suarez and Sims 2013).

19.2 History

In September 1872, a panzootic of equine influenza was reported in Canada which was associated with a fatal epidemic in poultry in the United States between 15 November and 15 December 1872 and followed by major influenza epidemics in 1873 and 1874 (Morens and Taubenberger 2010). Major epizootics of equine influenza recurring in the United States was also recorded in 1880–1881, 1900–1901 and 1915–1916 (Morens and Taubenberger 2010), with no record of associated avian influenza outbreaks. In 1901, a filterable agent was isolated from chickens suffering

from fowl plague that was later on classified as an influenza virus of H7 subtype (Suarez and Sims 2013).

Swine IAV was first isolated from pigs in 1930, 15 years after the greatest 1918 Spanish human flu pandemic. These early viruses have been proven to be H1N1, and it has been circulated in swine for about 80 years without great antigenic changes (Maclachlan et al. 2017). Influenza vaccines are used in humans since the 1940s and in horses 20 years later (Daly et al. 2011). In poultry, blanket vaccination strategy against HPAIV was applied in the 1990s during the Mexican H5N2 outbreaks.

19.3 Virus Properties

19.3.1 Morphology

Virions are spherical or pleomorphic with helical symmetry with a size range of 80–120 nm in diameter. Filamentous forms of the virions were also detected.

19.3.2 Viral Genome

Orthomyxoviruses possess 6–8 negative-sense, single-stranded RNA segments. Influenza virus types A and B and *Isavirus* contain eight RNA segments; influenza C and influenza D, seven RNA segments; and *Quaranjavirus* and *Thogotovirus*, six RNA segments. The segment length ranges from 736 to 2396 nt, and the total genome size ranges from 10.0 to 14.6 kb. Distinguishing features and conservative terminal sequence are presented in Tables 19.1 and 19.2. The RNA segment contains complementary sequences with promoter activity.

19.3.3 Proteins

Viral proteins of orthomyxoviruses include (i) replication proteins (RNA-dependent RNA polymerase: RNdRp) including PA, PB1 and PB2; (ii) internal core protein (nucleoprotein [NP]), which is associated with each RNA segment forming ribonucleoprotein (RNP); (iii) surface type I membrane glycoproteins (haemagglutinin [HA, HE {HEF} or GP]) that are involved in attachment, fusion and neutralization; and (iv) a non-glycosylated matrix protein (M1 or M). M2 and BM2 function as proton-selective ion channels in mammalian cells, acidifying the virion interior that is important for uncoating and fusion and equilibrating the intraluminal pH of the trans-Golgi apparatus

with that of the cytoplasm. The ion channel activity of only the former is inhibited by anti-influenza A drugs, amantadine and rimantadine: (v) orthomyxoviruses may code for two non-structural proteins (NS1, NS2 [NEP]). Virion enzymes (variously represented and reported among genera) include a transcriptase (PB1 in

Table 19.1 The genomic segment organization of different genera of *Orthomyxoviridae*

	Influenza A	Influenza B	*Isavirus*	Influenza C	Influenza D	*Quaranjavirus*	*Thogotovirus*
Seg. no.	8	8	8	7	7	6	6
Seg. 1	PB2	PB2	P[a]	PB2	PB2	PB2	PB2
Seg. 2	PB1	PB1	P[a]	PB1	PB1	PB1	PB1
Seg. 3	PA	PA	NP	PA	P3	PA	PA
Seg. 4	HA	HA	P[a]	HE[b]	HE	Unknown	GP[d]
Seg. 5	NP	NP	F	NP	NP	GP	NP
Seg. 6	NA	NA	HE	M1/CM2	P42	Unknown	M and ML
Seg. 7	M1 and M2	M1 and M2	NS[c]	NS1/NEP [NS2]	NS1/NEP [NS2]	–	Unknown
Seg. 8	NS1 and NEP (NS2)	NS1 and NEP (NS2)	M1 and RNA binding protein	–	–	–	–

[a]The segments 1, 2 and 4 encode the P proteins based on limited homologies to other RdRp.
[b]Receptor binding and fusion activities. It also functions as the receptor-destroying enzyme, 9-0-acetylneuraminyl esterase.
[c]Interferon antagonistic function (34.2 kDa) and a protein of unknown function (17.6 kDa).
[d]It is unrelated to any influenza virus protein but shows amino acid sequence similarity with the glycoprotein gp64 of baculoviruses.

Table 19.2 Terminal sequences of different genera of *Orthomyxoviridae*

Genus	Terminal sequence
Influenza A	5′-AGUAGAAACAAGG and 3′-UCG(U/C)UUUCGUCC
Influenza B	5′-AGUAG(A/U)AACAA and 3′-UCGUCUUCGC’
Influenza C	5′-AGCAG(U/G)AGCAAG and 3′-UCGUCUUCGUC
Influenza D	NA[a]
Isavirus	5′-AGUAAAAA(A/U) and 3′-UCG(U/A)UUCUA
Quaranjavirus	5′-AGCAAUCACAA and 3′-UCGUUAGUGU(A/U)(A/G)
Thogotovirus	5′-AGAGA(U/A)AUCAA(G/A)GC and 3′-UCGUUUUUGU(C/U)CG (segments 1–5) or 3′-UCACCUUUGUCCG (segment 6)

[a]NA [not identified]: To date, no available sequences for the gene termini of influenza D viruses.

influenza viruses A, B and C and thogotoviruses), an endonuclease (PA in influenza viruses A, B, C) and a receptor-destroying enzyme (neuraminidase [NA] for influenza A and influenza B viruses or 9-0-acetylneuraminyl esterase in the case of the influenza C virus HE [HEF] protein).

19.3.3.1 PB2

In addition to its role in viral RNA transcription/replication, PB2 plays a role in host range restriction. Amino acid substitution at amino acid residue number 627 from glutamic acid (found in avian isolates) to lysine (found in human influenza viruses) confers efficient replication in mice and humans. However, viruses without Glu 627 Lys mutation were detected in both severe and fatal human cases (Shaw et al. 2002).

19.3.3.2 PB1

PB1 is required for the initiation and elongation of the viral RNA. A second open reading frame PB1-F2 polypeptide is present in some influenza A viruses (Chen et al. 2001).

19.3.3.3 PA

PA is an important component for the polymerase complex and is needed for viral RNA replication by acting as an elongation factor or through facilitating the binding of PB1 to viral RNA and in the transcription process. It possesses a serine protease that supports efficient viral growth but not in cell culture (Fodor et al. 2003).

19.3.3.4 Haemagglutinin

HA is responsible for the attachment and penetration of viruses into cells. HA (HA0) is cleaved by cellular proteases into HA1 and HA2 subunits. The signal sequence at the N-terminal part of the protein is removed. HA cleavage is essential to expose the N-terminal part of the HA2 (hydrophobic terminus), which is responsible for fusion of the viral envelope and the endosomal membrane. The protease cleavability of the HA is affected by the number of basic amino acids at the cleavage site and the presence of the carbohydrate. The highly pathogenic (HP) avian influenza viruses (AIVs), members of influenza A viruses, have multibasic cleavage site motifs, whereas low pathogenic (LP) AIVs possess monobasic cleavage site motifs

(basic amino acids are arginine “R”, lysine “K” and rarely histidine “H”). The presence of carbohydrate adjacent to the cleavage site may sterically hinder the access of proteases to the cleavage site. However, the insertion of two basic amino acids in the cleavage site restores the HA cleavability probably due to the formation of a loop structure which is accessible to the cellular proteases. The amino acid downstream of the cleavage site (the amino terminal residue of the HA2) also affects HA cleavage (Horimoto and Kawaoka 1995).

19.3.3.5 Receptor Binding

Influenza A and B viruses bind to α2,6-sialyllactose, N-acetylneuraminic acid, α2,6-galactose-(NeuAca2,6Galb1,4Glc) and α2,3-sialyllactose-(NeuAca2,3Galb1,4Glc). The majority of avian and equine influenza A viruses bind the NeuAca2,3Gal, whereas human and swine influenza viruses bind the NeuAca2,6Gal. Swine tracheal epithelium harbours both types of sialyloligosaccharides: hence, pigs are potential mixing vessel for both influenza viruses. Human viruses infect preferentially nonciliated cells with SAα2,6Gal sialyloligosaccharides, while avian viruses infect ciliated cells with SAα2,3Gal sialyloligosaccharides. Duck intestinal epithelium and equine tracheal epithelium possess SAca2,3Gal but not SAa2,6Gal. α2,3-N-Glycolyl sialic acid (NeuGca2,3Gal) is prevalent in the equine tracheal and duck epithelium supporting the replication of influenza A with specificity to this type of receptor but not allowing replication of viruses that bind to N-acetyl sialic acid (Matrosovich et al. 2004; Ito et al. 1998).

The amino acids of HA at position 226 and 228 (H3 numbering) are determinants for receptor-binding specificity. Substitution of Gln to Leu 226 and Gly to Ser 228 changes the receptor-binding specificity from avian to human receptor binding. Amino acid substitution at amino acid residue numbers 136, 190, 195 and 225 (H3 numbering) also alters the binding affinity in a variable degree (Martin et al. 1998). Human influenza viruses grown in mammalian cell culture contain the same HA amino acid sequences of those initially isolated from humans; however, receptor-binding variant mutants can be selected during propagation in chicken eggs (Mochalova et al. 2003).

19.3.3.6 NP

The viral RNA wraps the NP protein and the RNA-binding region of the NP of influenza A virus is located between amino acid residues 91 to 188. NP is important for viral RNA synthesis and some critical mutations in NP lead to defects in RNA replication. NP is presumed to be an important determinant of host range restriction (Maclachlan et al. 2017).

19.3.3.7 NA

The NA protein of influenza A viruses is the second major glycoproteins on the virus surface. The NA is a receptor-destroying enzyme that is essential for both virus entry and release from infected cells. The balance between the HA and NA functions is critical for influenza virus replication. The NA plays a role in host range restriction, and the NA substrate corresponds to the preferential receptor

recognition by the HA molecule and is determined by the NA amino acid at position 275 (Kobasa et al. 1999). Currently, there are eleven types of NA among the influenza A viruses. With the exception of N10–N11, the nine NA subtypes are separated into two main groups based on the structure and the phylogenetic analysis. Group 1 NA included N1, N4, N5 and N8, while group 2 included N2–N3, N6–N7 and N9. NA is an attractive target for anti-influenza drugs due to its role in virus release from infected cells. In humans, oseltamivir and zanamivir are active against both group 1 and group 2 NA as well as influenza B NA. Meanwhile, laninamivir is another long-acting NA inhibitor including oseltamivir-resistant viruses in adults. Peramivir is approved in Japan for use in patients above 1 month of age (Gaymard et al. 2016).

19.3.3.8 M1

M1 is a type-specific antigen, determines the virus morphology of influenza viruses and is a determinant of virus budding and assembly. It is also required for nuclear export of viral RNP complexes. M1 acts as a molecular switch that inhibits RNP transcription activity and initiates the final step of virus assembly (Rossman and Lamb 2011).

19.3.3.9 M2

M2 functions as a pH-activated ion channel that permits protons to enter the virion during uncoating and that modulates the pH of intracellular compartments, an essential function for the prevention of acid-induced conformational changes of intracellularly cleaved HAs in the trans-Golgi network. The activity of the M2 ion channel is targeted by amantadine and rimantadine hydrochloride. Drug-resistant mutations include mutations in amino acid residue numbers 27, 30, 31 and 34. The M2 ectodomain may play a role in virion incorporation (Rossman and Lamb 2011).

19.3.3.10 Other M Gene Products of Type B and C Viruses

BM2

BM2 is encoded by the M gene of type B virus (Horvath et al. 1990). It possesses equivalent function of the type A M2 protein ion channel activity. It also prevents HA from being subjected to low-pH-induced conformation during transport to the cell surface (Horvath et al. 1990).

NS1

NS1 inhibits of interferon (IFN) response to ensure efficient viral replication. It also induces apoptosis in infected cells with other viral proteins such as NA. NS1 inhibit mRNA splicing and the nuclear export of cellular mRNA, to increase the viral mRNA synthesis (Marc 2014).

NS2(NEP)

NS2 is referred to as nuclear export protein (NEP). It contains a nuclear export signal (NES) and interacts with the cellular nuclear export factor that mediates export of proteins containing NESs. It also connects the cellular export machinery with viral RNPs through M1 (Akarsu et al. 2003).

HEF

The HEF protein of influenza C virus is post-translationally cleaved into two HEF1 and HEF2 subunits. The head of the HEF contains the receptor binding site. Two regions located under the receptor-binding site in HEF1 form the esterase domain. The HEF facilitates the binding of influenza C virus to the cell receptor, an oligosaccharide with a terminal 9-O-acetyl-N-acetylneuraminic acid. Unlike the NA, HEF does not catalyse the cleavage of the linkage between sialic acid and the adjacent sugar residue, but cleave of the 9-O-acetyl group of 9-O-acetyl-N-acetylneuraminic acid. It also possesses fusion activity. Influenza C virus uses the acetylesterase activity for cell entry (Strobl and Vlasak 1993).

19.4 Avian Influenza

19.4.1 Introduction

Avian influenza is a highly infectious virus disease of poultry with potential zoonotic importance. It also affects pet, zoo and wild birds. In poultry, the LP AIVs induce a mild or subclinical infection including diarrhoea and drops in egg production in layers. The HP AIVs induce severe respiratory disorders, diarrhoea and up to 100% mortalities in domestic birds. HPAI is usually associated with H5 and H7 influenza A virus subtypes, although LPAI of the same subtypes was recorded in birds (Suarez and Sims 2013). Avian influenza viruses pose potential zoonotic importance, and many human cases were infected with different avian influenza subtypes: H10N8, H10N7, H9N2, H7N7, H7N9, H6N8, H5N6 and H5N1, among others. The latter subtype induces very high case fatality with a potential of being a pandemic strain with all catastrophic consequences.

19.4.2 History

HPAI was recognized in 1878 in Italy and was confused with fowl cholera (Suarez and Sims 2013). Although it is a filterable agent, it was detected in 1901 by Centanni and Savonuzzi; however, avian influenza was identified as an influenza virus in 1955. By the mid-twentieth century, HPAI had been diagnosed in most parts of the world. H7N1 and H7N7 subtypes were found to be the causative agents of 1901–1950s' HPAI outbreaks. In 1959 in Scotland and in 1961 in South Africa, H5N1 and H5N3 induced outbreaks in chickens, respectively (Suarez and Sims 2013).

LPAI was first reported in Germany (Dinter strain), in chickens in 1949, that was later identified as A/chicken/Germany/49 (H10N7) in 1960. LPAI viruses from domestic ducks with respiratory distress were isolated between 1953 and 1963 in Canada, Czechoslovakia, England and Ukraine. The LPAI was associated with mild respiratory disease and drops in egg production in turkeys in Canada and the United States. LPAI H5 subtype was isolated in Canada and the United States in 1966 and

1968, respectively. In 1971, a LPAI H7N3 subtype was isolated from turkeys with mild respiratory distress and diarrhoea (Suarez and Sims 2013).

During 1972, AIVs were isolated from migratory birds. Although AIVs isolated from wild aquatic birds have low pathogenicity to poultry, a few HPAIVs have been isolated from wild birds: H5N1, H5N3, H5N8 and single isolations of H7N1 (A/finch/Germany/72), H7N7 (A/gull/Germany/79) and H7N3 (A/Peregrine Falcon/UAE/2384/98) (Suarez and Sims 2013).

19.4.3 Virus Strains and Genotypes

All IAVs can infect birds except H17N10 and H18N11 that have been isolated from bats. AIVs that infect birds have two main genetic lineages: Asian lineage and North American lineage. Such lineages are further classified into sublineages and clades within each main subtype. AIVs are also classified according to their virulence in birds and their molecular characteristics into highly pathogenic avian influenza (HPAI) and low pathogenicity avian influenza (LPAI). Classical disease in poultry is usually represented by infections with AIV subtypes H5, H7 and H9 associated with N1–N9. In contrast to H5 and H7 that contain both HPAI and LPAI, all H9 viruses identified worldwide in wild birds and poultry are LPAI viruses.

In areas where LPAI is endemic or when AIV vaccination is used in the control policy, genetic variants emerge due to genetic drift which could be due to immunological pressure from vaccine or endemic infections by the circulating field strains. Antigenic shift due to reassortment could also occur and reassortment was reported for the Hong Kong H5N1.

Amino acid substitutions in different genes of H5N1 and H7N1 were associated with airborne transmission in ferrets (Sutton et al. 2014).

19.4.4 Ecology

Healthy wild aquatic birds, mainly in the orders Anseriformes and Charadriiformes, were confirmed as asymptomatic reservoirs of AIVs. In wild waterfowl, AIVs are maintained by passage in susceptible birds throughout the year, especially in juvenile birds prior to fall migration. Such migratory birds infect susceptible resident waterfowl. Wild birds may play an essential role in initial introduction of AIVs in terrestrial poultry (Olsen et al. 2006).

There are five man-made ecosystems including (i) village, backyard and hobby flocks, (ii) range-raised poultry, (iii) intensive commercial poultry, (iv) live poultry markets (LPM) and (v) bird collection and trading systems. In both developed and developing countries, rural and village poultry as well as LPM possess a high rate of infection (Suarez and Sims 2013).

Since 2003, H5N1 HPAI that began in Southeast Asia has become endemic in many countries. During 2010–2016, H5 subtype (H5N1, H5N2, H5N8, H5N6, H5N9, H5N5, H5N3) was the major circulating subtype, followed by H7 subtype (H7N9,

H7N7, H7N3, H7N2, H7N1, H7N6) and then H9 subtype (H9N2, H9N1). Other subtypes include H3N8, H3N2, H10N?, H1N2, H4N6, H10N7, H1N1 and H11N9. Interestingly, considerable percentages of wild bird isolates were due to HPAI.

19.4.5 Geographical Distribution

Avian influenza is a common disease that is widespread worldwide.

19.4.6 Host

AIV was isolated from more than 100 species of birds representing 13 different orders; however, the actual number could be much greater. AIV affects different domestic birds. AIVs cross species barrier and infect mink, seals and whales causing epidemics of respiratory distress. H5N1 HPAI virus causes sporadic infection in tigers, leopards, cats, Owston's palm civets, a stone martin and pigs as well as equines. In addition, it causes a highly fatal disease in humans. Experimentally, H5N1 can infect pigs, ferrets, rats, rabbits, guinea pigs, mice, cats, mink and nonhuman primates (Suarez and Sims 2013; Yee et al. 2009).

19.4.7 Transmission

Sources of AIV infection in commercial poultry flocks include infected domestic and confined birds, migratory waterfowl and other wild birds, as well as domestic pigs or pet birds. AIV transmission occurs by horizontal route of transmission by both direct and indirect contact through aerosol droplets or exposure to virus-contaminated fomites, people (e.g. contaminated shoes and clothing) and equipment shared in production, or live-bird marketing. Eggshell surface and the internal contents of the eggs are potential source of HPAI virus. Transmission could also occur by airborne dissemination in short distances. Swine are potential source of transmission of swine influenza viruses (H1 and H3) in turkeys. AIV is excreted for up to 36 days in chickens, 22 days in turkeys, 17 days in ducks and 56 days in pheasants (Suarez and Sims 2013).

19.4.8 Clinical Signs

Most infections by LPAI viruses in wild birds produce no clinical signs. In broiler-type chickens and turkeys, LPAI leads to mild to severe respiratory signs, while layers and breeders exhibit decreased egg production (Suarez and Sims 2013).

In wild birds and domestic ducks, most HPAI viruses produce mild clinical signs. In the last decade, HPAI H5 subtype resulted in sudden death without apparent clinical signs (e.g. neurological signs, depression, anorexia).

In turkeys, chickens and other poultry, HPAI induces severe highly fatal disease up to 100% mortality. Mortalities may appear in the absence of any clinical signs or gross lesion in the peracute form of the disease. The acute form of the disease causes cyanosis and oedema of the head, comb, wattle or snood in turkeys and reddish-blue discoloration of the shanks and feet. Diarrhoea may also be a common sign (Suarez and Sims 2013). Torticollis and opisthotonos as well as other nervous manifestations may appear in ducks and geese and birds recovering from the peracute form of the disease (Suarez and Sims 2013).

19.4.9 Post-mortem Lesions

19.4.9.1 Low Pathogenic Avian Influenza in Birds

Low pathogenic avian influenza virus causes congestion and catarrhal to serofibrinous inflammation of the trachea and sinuses. The tracheal mucosae are oedematous with congestion. Haemorrhagic ovary and the presence of yolk in the abdominal cavity may be observed in the reproductive tract of laying hens. Airsacculitis, peritonitis, visceral urate deposition and nephritis may be found in some birds.

19.4.9.2 Highly Pathogenic Avian Influenza in Birds

Birds that die from the peracute form of the disease may have no lesions. In the acute form in chicken and turkeys, oedema and cyanosis of the head, wattle and comb are common symptoms. Petechiae on the viscera, epicardium and sometimes the muscles especially the pectoral muscles, necrotic lesions and congestions of the pancreas, spleen and heart as well as hepatosplenomegaly with parenchymal mottling were observed. Haemorrhagic enteritis and haemorrhages in the mucosa of the proventriculus, ventriculus and Peyer's patches and/or atrophied thymus and bursa can be seen in some birds.

In ostriches, there are oedema of head and neck, severe haemorrhagic enteritis, airsacculitis, hepatosplenomegaly, peritonitis, renal congestion and enlarged and firm pancreas (Suarez and Sims 2013).

19.4.10 Diagnosis

19.4.10.1 Clinical Diagnosis

High mortality rate; cyanosis and oedema of the head, comb, wattle or snood in turkey; and reddish-blue discoloration of the shanks and feet are highly suggestive of HPAI.

19.4.10.2 Samples

Tracheal, oropharyngeal or cloacal swabs from live or dead birds placed in a sterile virus transport medium containing high levels of antibiotics can be used for virus isolation or detection. Specimens from the lungs, liver and spleen, as well as

secretions and/or excretions from respiratory and intestinal tracts, are also useful (Suarez and Sims 2013).

19.4.10.3 Laboratory Tests

The direct detection of influenza A nucleoprotein in avian specimens and allantoic fluid of inoculated ECE using antigen capture immunoassays is a sensitive method for rapid detection of AIV infection but less efficient than virus isolation (Suarez and Sims 2013). Detection of the M, HA and NA genes using real-time RT-PCR is commonly used for the diagnosis of field cases. For virus isolation, the sample is inoculated in the allantoic cavity of 9–11 chicken embryos. Several egg passages may be required to isolate LPAIVs, while HPAIVs kill the embryos within 48 h. The virus detection of the chilled allantoic fluid is indicated by the presence of haemagglutinating activity using chicken erythrocytes. Newcastle disease virus (NDV) should be first excluded using specific antibodies against NDV in haemagglutination inhibition assay. If negative, then AIV detection should be screened using (monoclonal) antibodies against the type-specific nucleoprotein or matrix protein viral antigens by rapid chromatographic strips or other commercial antigen capture immunoassays (Suarez and Sims 2013).

19.4.10.4 Serology

ELISA assays have been developed to detect antibodies to AIVs.

19.4.11 Control

Control of AIV depends on the subtype, the public health importance, the country's economic status, and the epidemiologic nature of the disease (e.g. HPAI or LPAI, endemic or sporadic infections). A proper control programme for HPAI should include education and awareness, biosecurity, regular influenza surveillance and depopulation of infected poultry. In optimum conditions, HPAI outbreaks can be eradicated within 6 months to a year by traditional stamping-out programmes. In suboptimum conditions, especially in areas with high poultry production at the village or rural level, management of the disease to a low infection rate has been a realistic option. In some countries, live poultry markets, rural poultry, mixed poultry population and mixed animals including pig and poultry raising pose a serious risk for the introduction of influenza to commercial poultry. In HPAI, control procedures are accomplished by depopulation and disposal of dead birds, eggs and manure by composting, incineration or hygienic burial (Suarez and Sims 2013).

19.4.12 Vaccination

Vaccines are used in endemic areas to provide protection from LPAI and HPAI viruses. Inactivated whole AIV vaccines or reverse genetic-generated vaccine

strains, followed by chemical inactivation and oil emulsification, are commonly used. Moreover, chickens can be immunized successfully by the in ovo administration of inactivated oil emulsion vaccine. Different chimeric vaccines including fowl pox-AI haemagglutinin (H5) recombinant vaccine (rFP-AI-H5), NDV-AI-H5 and avian influenza–Marek's disease vaccine are commercially available for use in birds. Vaccination does not guarantee that the flocks are free from influenza and vaccinated birds must be monitored for the presence of AI virus until slaughtered (Suarez and Sims 2013).

19.5 Swine Influenza Virus

19.5.1 Introduction

Swine influenza virus (SIV) is a highly contagious mild swine viral disease caused by influenza A virus, mainly H1N1, H1N2 and H3N2 subtypes. Although the disease shows 100% morbidity, infected pigs might not show signs of disease manifestation and infection. SIV infections are manifested as acute respiratory disease characterized by fever, inactivity, decreased food intake, respiratory distress, coughing, sneezing, conjunctivitis and nasal discharge. The disease is zoonotic and can be transmitted to humans who come in contact to infected pigs (Vincent et al. 2014).

19.5.2 History

Swine influenza (SI) was first reported as an epizootic of respiratory disease in pigs in the Midwestern United States in 1918. It coincided with the incidence of human major influenza pandemic: the Spanish flu. The first SIV was isolated from pigs in 1930, 3 years before the isolation of swine H1N1 in humans.

19.5.3 Virus Strains and Genotypes

H1 and H3 subtypes mainly affect pigs, in addition to sporadic infections with other subtypes including: H2, H4, H5 and H9. Two distinct variants of the H1N1 swine influenza virus exist: the avian variant in Europe and the other variant in United States, similar to the original virus strain. Other strains that infect swine include human H3N2 strains in China, Europe and North America and H1N2 (triple reassortants) (Fig. 19.2). In China, three types of H3N2 were reported in swine: human-like H3N2 virus, double reassortants and triple reassortants. Infection of pigs with avian H5N1 and H9N2 types were also recorded in China (Vincent et al. 2014).

19.5.4 Ecology

There is a strong evidence that the 1930 swine strain was antigenically related to the 1918 pandemic influenza strain (reviewed by Stuart-Harris et al. (1985)). The American swine influenza A subtype H1N1 viruses spread to Europe in 1976 and have been replaced by swine H1N1 of avian origin in 1979 (Pensaert et al. 1981).

Pigs constitute a mixing vessel in which different influenza viruses can reassort. This is related to the fact that pigs are susceptible to infection by swine, avian and human influenza viruses and possess both avian-type and human-type sialic acid receptors, with subsequent possibility of emergence of pandemic influenza virus strains. In Europe, avian–swine influenza reassortants were first detected in 1979 and human–avian H3N2 viruses emerged between 1983 and 1985 and then repeatedly detected in pigs (Webster et al. 1992). Human H3N2 influenza infected the North American swine herds around 1995 and a double-reassortant swine H1N2 arisen that possessed PB1 from human H3N2. The double reassortants further acquired PA and PB2 avian internal protein genes. The triple reassortant H1N2 spread widely in the pig population. Pandemic *pdm09*H1N1 acquired the HA and NA genes from Eurasian avian-like swine H1N1 and other genes from the triple reassortant swine H1N2. It contains HA, NP and NS from classical swine H1N1, PB2 and PA from avian source, and PB1 from a human seasonal H3N2 (Fig. 19.2) (Garten et al. 2009; Vincent et al. 2014).

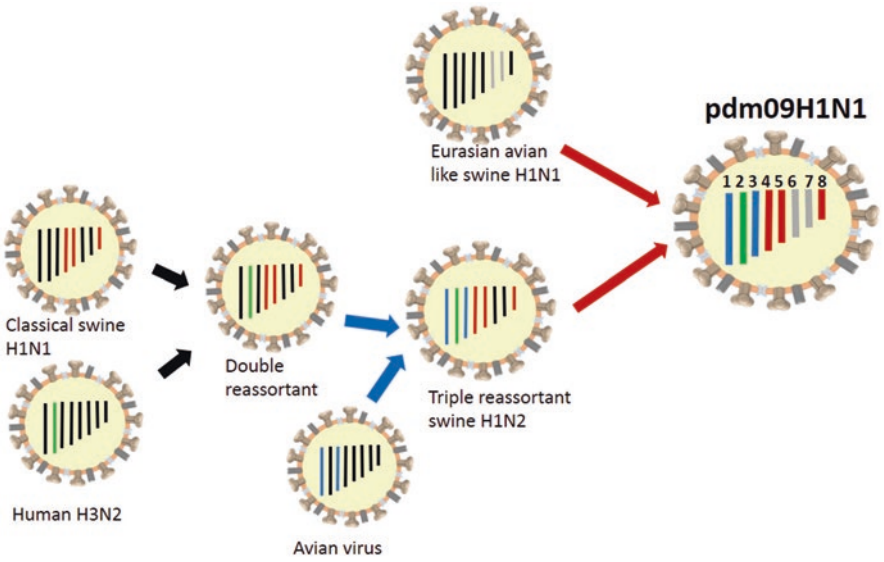

Fig. 19.2 Emergence of *pdm09*H1N1 due to the reassortment of influenza viruses

19.5.5 Geographical Distribution

Swine influenza is the most common disease in Europe, parts of Asia, North and South America and some parts of Africa.

19.5.6 Host

Swine influenza strains are enzootic in pig populations in many parts of the world and have also infected turkeys in the United States (Vincent et al. 2014; Suarez and Sims 2013).

19.5.7 Transmission

SIVs spread among pigs by direct and indirect contact to infected animals, contaminated equipment and utensils from infected pig herd.

19.5.8 Clinical Signs

The disease appears abruptly after an incubation period of 1–3 days. Most animals show subclinical infection. Clinically infected pigs exhibit fever, inappetence, huddling, weight loss, coughing, sneezing and nasal discharge; however, severe bronchopneumonia was also recorded in some animals (Janke 2014).

19.5.9 Post-mortem Lesions

Lungs of infected pigs showed a purple-red, multifocal to coalescing consolidation. The lesion usually appears in the cranio-ventral portions of the lung (Janke 2014).

19.5.10 Diagnosis

19.5.10.1 Clinical Diagnosis

Swine influenza is characterized by sudden onset of respiratory disease that may be misdiagnosed with other diseases including *Mycoplasma hyopneumoniae* and *Actinobacillus pleuropneumoniae* (Janke 2014).

19.5.10.2 Samples

Nasal swabs or oral fluids can be collected from living animals. Post-mortem samples include nasal turbinates, tonsil, trachea or lung.

19.5.10.3 Laboratory Tests

SIVs can be isolated in the allantoic sac of embryonated chicken eggs. MDCK cells are the most commonly used cell line for isolation, propagation and titration of SIV, a trypsin-containing overlay. Although they have not been explored for primary isolation, newborn swine kidney (NSK), newborn pig trachea (NPTr), porcine intestinal epithelial cell line (SD-PJEC) and bone marrow support SIV replication (Janke 2014; Khatri and Saif 2011).

For routine diagnosis, the real-time RT-PCR test has replaced virus isolation; however, virus isolation is still used to increase the initial virus titer in the original sample for genetic analyses. Identification of the virus genotype is conducted by real-time RT-PCR tests for both HA and NA genes. The results are confirmed by sequence analysis of both genes, or with specific monoclonal antibodies. The virus can be detected in tissue samples by immunofluorescence or by immunohistochemistry.

19.5.10.4 Serology

Among the serologic tests available, indirect fluorescent antibody test (IFAT), serum neutralization (SN), haemagglutination inhibition (HI) and ELISA are currently being used for swine. The most commonly used are the HI and ELISA (Janke 2014).

19.5.11 Control

Swine influenza is controlled by strict biosecurity measures and vaccination. All-in–all-out policy is used in many commercial swine producers. Biosecurity may be sufficient to minimize or exclude influenza virus infection.

19.5.12 Vaccination

Inactivated influenza vaccines are used to protect the pregnant sow and her suckling piglets or during the grow/finish phase of production. They are also used to reduce the zoonotic transmission of the disease to humans. Inactivated SIV vaccine includes two or more representatives of H1 and H3 which provide efficient homologous but only partial protection against heterologous SIV (Rahn et al. 2015).

19.6 Equine Influenza Virus

19.6.1 Introduction

Equine influenza virus (EIV) is a highly contagious rarely fatal respiratory disease of equines. EIV is associated with two subtypes of influenza A viruses: H3N8 and H7N7. H3N8 virus is currently associated with equine influenza worldwide. H7N7

virus was a potential cause of EIV with H3N8 viruses in horses for many years; the former virus has not been detected in equines for more than 25 years (Webster et al. 1992). Avian IAV subtype H5N1 was isolated from a respiratory disease in donkeys in Egypt (Abdel-Moneim et al. 2010). H3N8 infection of naïve horse population may be severe, or fatal, especially in the young or debilitated animals. EIV links to sialic acid receptor with α2,3-galactose linkage similar to AIVs.

19.6.2 History

An influenza-like epizootic in horses was reported in 1872 in the United States (Webster et al. 1992). This outbreak was speculated to be an evidence of cross-species barrier of avian influenza. The first serological evidence of EIV was reported in 1955 in Sweden, while the first isolation of the EIV from horses occurred in Czechoslovakia in 1956 that was subsequently demonstrated to be a H7N7 (A/equine/Prague/1/56). EIV subtype H3N8 (A/equine/Miami/I/63) was first reported in Florida in animals recently imported from Argentina. This virus is widespread globally and reached Europe in 1965. The first EIV oil-adjuvant vaccine was developed around 1965.

Both H7N7 (A/equi-1) and H3N8 (A/equi-2) were the only detected subtypes reported in equines. Currently, only H3N8 is detected among equines, while H7N7 viruses were not detected since the late 1970s (Webster 1993); however, the antibody to H7N7 has been detected in unvaccinated horses suggesting that the virus may still be circulating.

19.6.3 Virus Strains and Genotypes

EIVs, like other influenza A viruses, are subjected to antigenic drift, although in a rate lower than avian and human influenza viruses. In the late 1980s, European and American lineages evolved (Daly et al. 1996). Two clades were further emerged from the American lineage. Clade 1 (Florida clade I) EIV strains are widespread in American continents and also in Africa, Asia and Australia as well as Europe (Cullinane and Newton 2013). On the other hand, clade II EIV strains (Florida clade II) are responsible for EI infections in Europe and Asia. Currently, EIV vaccine contains one strain from each clade (Cullinane and Newton 2013).

19.6.4 Ecology

EIV strains are probably evolved from AIV, but constitute independent lineages with little evidence of reassortment between EIV and AIV.

H7N7 viruses between 1973 and 1977 were reassortants carrying H3N8 internal-protein-encoding genes except the M gene (Murcia et al. 2011). EIV H3N8 subtype arose probably from AIV in the early 1950s (Murcia et al. 2011). The H3N8 strain

possessed the PB2 and matrix proteins from North American avian strains (Gorman et al. 1990b; Ito et al. 1991), while PB1, PA, HA and NP from other influenza A viruses (Gorman et al. 1990a; Kawaoka et al. 1989; Okazaki et al. 1989). Equine NS was found to be subtype specific (Nakajima et al. 1990), as the NS segments of the H3N8 viruses were close to each other but not to H7N7 viruses.

19.6.5 Geographical Distribution

H3N8 EIV is not a seasonal disease and it is enzootic in Europe, North and South America and Asia. It is also present in India and Africa. Australia and Hong Kong, as well as New Zealand, Japan and South Africa, are now thought to be EIV-free (Cullinane and Newton 2013).

19.6.6 Host

EIV causes a respiratory disease of horses. EIV H3N8 subtype cross species to canine in 2004 causing an outbreak of canine influenza virus (CIV) in the United States (Crawford et al. 2005). Subsequently, CIV evolved as a distinct cluster of the H3N8 EIV. However, there is no current evidence of transmission of equine influenza virus from dogs to horses. Cats also showed clinical disease signs after experimental infection of EIV H3N8 subtype (Su et al. 2014). EIVs have also been isolated from swine. H3N8 EIV strains are not considered to be human pathogens (Cullinane et al. 2010).

19.6.7 Transmission

The virus is highly contagious and transmitted directly by the respiratory route through direct contact and indirectly thorough personnel, fomites and vehicles. International traveling of horses for breeding or race purposes plays an important role in the introduction of EIV strains into different countries (Cullinane and Newton 2013).

19.6.8 Clinical Signs

The clinical signs include fever (peak 42 °C), a serous to mucopurulent nasal discharge, dry cough, depression, anorexia, limb oedema and enlarged mandibular lymph nodes. Broncho-interstitial pneumonia was recorded in young foals with no maternal immunity against EIV with subsequent hypoxia and acidosis. Although not a common sign, enteritis was frequently reported in 1989 avian H3N8 epidemic in China. Anaemia, leukopaenia and lymphopaenia were recorded (Daly et al. 2011). In vaccinated exposed populations, the spread of disease is not rapid and the

clinical signs are less severe and may be limited to suboptimal performance in well-vaccinated horses, and many horses may be subclinically infected (Daly et al. 2011; Elton and Bryant 2011).

19.6.9 Post-mortem Lesions

Equine influenza infections rarely result in fatal consequences. Post-mortem finding may include bronchiolitis, peribronchiolitis and subacute interstitial pneumonia (Elton and Bryant 2011).

19.6.10 Diagnosis

19.6.10.1 Clinical Diagnosis

Rapid spread of clinical signs especially cough is suggestive of EIV in unvaccinated horses; however, in vaccinated populations clinical signs are not inconclusive (Elton and Bryant 2011).

19.6.10.2 Samples

Nasopharyngeal swabs should be collected from acute cases. Virus shedding may persist for up 7–10 days in non-vaccinated horses but 1–2 days in immune horses (Elton and Bryant 2011).

19.6.10.3 Laboratory Tests

Antigen capture ELISAs and real-time RT-PCR are used for the diagnosis of EIV. EIV-specific ELISA and human influenza ELISA kits have been used in the diagnosis of equine influenza. These kits detect the nucleoprotein which is highly conserved among influenza viruses. However, RT-PCR was found to be more sensitive than AC-ELISA. Although virus isolation is less sensitive than both ELISA and RT-PCR, it is necessary for virus characterization and strain surveillance. EIV is isolated in ECE and less frequently in MDCK (Cullinane and Newton 2013; Elton and Bryant 2011).

19.6.10.4 Serology

HI is used for testing the seroconversion against EIV. Pretreatment of sera with receptor-destroying enzymes (RDE), periodate and trypsin–periodate is used to remove nonspecific inhibitors (Subbarao et al. 1992). Virus antigen is treated with Tween-80/ether (John and Fulginiti 1966) to increase the HI sensitivity. The single radial haemolysis (SRH) assay is more reproducible than the HI test (Mumford 2000). It was estimated that SRH titers of 120–154 mm2 is needed for complete protection from homologous virus challenge and 200 mm2 for heterologous protection (Newton et al. 2006). An EIV nucleoprotein ELISA was used to differentiate horses vaccinated with a canary pox recombinant vaccine from horses that had been exposed to virus by natural infection (Garner et al. 2011).

19.6.11 Control

International movement of horses is considered one of the key factors responsible for the spread of EIV. Australia and New Zealand are free from equine influenza due to routine vaccination of imported horses and adequate quarantine periods to prevent the introduction of EIV. Some countries adopt vaccination policy to indigenous equine populations to reduce the infection of EIV (Daly et al. 2011). In countries where equine influenza virus is endemic, the economic losses due to influenza can be minimized by vaccination of highly mobile horses. In EIV-enzootic countries, surveillance is limited, vaccination is seldom required, and importation policies regarding EIV are less rigorous. The OIE recommends vaccination 21–90 days prior to shipment. Horses should be screened for EIV by RT-PCR in the quarantine. All-in–all-out quarantine should be implemented.

19.6.12 Vaccination

EIV vaccines are inactivated vaccines. Other licensed vaccines include ISCOM, MLV and recombinant canarypox. For protective antibody titer, revaccination every 4 and 6 months in young horses (Newton et al. 2000). Biannual boosters are recommended for racehorses aged 2 years and older. In older horses that received multiple vaccinations, annual booster could be satisfactory. Mismatching between the vaccine and field strains leads to subclinical infection in vaccinated horses. Equine influenza vaccines are reviewed and updated annually (Cullinane et al. 2010). Since 2010, it is recommended that international vaccines should contain a clade 1 and clade 2 virus of the Florida sublineage especially for racehorses that are frequently transported from place to place.

19.7 Infectious Salmon Anaemia Virus (ISAV)

19.7.1 Introduction

Infectious salmon anaemia virus (ISAV) is the causative agent of infectious salmon anaemia (ISA), one of the most important diseases of farmed Atlantic salmon. Virulent strains may be transmitted back to the wild population. Although the disease affects farmed Atlantic salmon held in or exposed to seawater, evidence of infection in the freshwater stage has been reported. The disease is contagious and manifested by severe anaemia and multi-organ haemorrhages with an initial low mortality rate (less than 1%); however, cumulative mortalities may reach 90%. The disease leads to severe economic losses that in certain areas result in $4.8–5.5 million annual losses (New Brunswick) and millions of fish culled to control the disease (Kibenge et al. 2004).

19.7.2 History

The disease was first described in Norway in 1984, and it was initially named haemorrhagic kidney syndrome. ISAV was then detected in Canada, Scotland and Chile in the 1990s. The Chilean virus was assumed to be introduced during the initial importation of salmon to America from Europe (Cottet et al. 2011; Kibenge et al. 2004).

19.7.3 Virus Properties and Classification

ISAV possesses the haemagglutinin esterase (HE) and fusion protein (F). HE is responsible for receptor-binding and receptor-destroying activities. It agglutinates the RBCs of several fish species. The virus replicates in endothelial cells and leukocytes. Nucleotide sequences of all eight ISAV genome segments have been described. The genome encodes at least 10 proteins (Table 19.1). Segments 1, 2 and 4 encode the viral polymerases: PB1, PB2 and PA, respectively. Segment 3 encodes the nucleoprotein (NP), 68 kDa. Segments 5 and 6 encode fusion (F) protein (50 kDa) and HE (the 42 kDa) responsible for receptor-binding and receptor-destroying activities. Segment 7 encodes a non-structural protein (non-spliced mRNA) that interferes with the interferon type 1 response and another not yet characterized protein (spliced mRNA). Segment 8 encodes a 22-kDa matrix protein and an RNA-binding structural protein (26 kDa) with interferon antagonistic activities (Cottet et al. 2011; Kibenge et al. 2004).

19.7.4 Virus Strains and Genotypes

There are two major lineages of ISAV – genotype I (the European genotype) and genotype II (the North American genotype) – based on the sequence variation of segments 2, 6 and 8. Various clades occur within these genotypes. The European isolates are divided into three subgroups: G1–G3 based on the sequence variation of the extracellular region of haemagglutinin. Within these two major groups, viruses with deletions in the high polymorphic region (HPR) of the haemagglutinin esterase (HE) appear to be more virulent and can be isolated in cell culture. The HPR is characterized by the presence of gaps instead of single nucleotide mutations. The viruses that cause disease outbreaks in farmed fish have deletions in HRP in comparison to the putative ancestral variant with a longer HPR (HPR0). Those viruses are classified to more than 28 different HPRs (e.g., HPR1, HPR2, HPR3). HPR and F protein are responsible for the viral virulence. ISAV is subjected to reassortment, and a four-reassortment ISAV related to the European clade was reported in outbreaks in Chile (Cottet et al. 2011; Kibenge et al. 2004).

19.7.5 Geographical Distribution

ISAV genotype I (the European genotype) is detected in Norway, Scotland, Faroe Islands and Chile, while genotype II (the North American genotype) is detected in Canada and the United States. Both Norway and Chile constitute 33 and 31% of the total salmon production farms worldwide, respectively (Cottet et al. 2011; Kibenge et al. 2004).

19.7.6 Host

ISAV induces a disease with variable mortalities in rainbow trout. ISAV also experimentally infects brown trout and herring (*Clupea harengus*). It was isolated from diseased farmed Pacific coho salmon in Chile in 1999 (Kibenge et al. 2004).

19.7.7 Reservoirs

Several species of salmonids (brown trout, sea trout, rainbow trout, steelhead trout, chum salmon, Chinook salmon, coho salmon and Arctic char) can carry virulent ISA viruses asymptomatically. Conversion of these non-/low pathogenic strains to pathogenic or virulent strains was detected after small genetic changes (Kibenge et al. 2004).

19.7.8 Transmission

The virus is excreted from infected fish into the water from skin, mucus, faeces and urine. The virus enters the fish through the gills and skin lesions; however, transmission by coprophagy is also proposed. ISAV spreads by water-borne transmission. ISAV may be transmitted vertically. The sea louse (*Lepeophtheirus salmonis*) is a possible vector for ISAV. ISAV is detected by RT-PCR in water samples 1.5 km away from infected areas (Kibenge et al. 2004). The virus is stable at pH 5.7–9.0. The virus replicates in the salmon cell lines SHK-1, TO and ASK, with a replication optimum of 10–15 °C. Some strains also replicate in the CHSE-214 cell line. ISAV possesses a unique gene organization.

19.7.9 Clinical Signs

The clinical signs appear after 2–4-week incubation period. Prior to an outbreak, mortality slightly increased over a period of 1–3 weeks. Signs include lethargy, anaemia, pale gills, leukopaenia, ascites, exophthalmia, dark skin and increased mortality. Haemorrhages in the anterior chamber of the eye and jaundice on the ventral portion of the body with yellowing of the base of the fins and on the abdomen were also observed (Thorud and Djupvik 1988).

19.7.10 Post-mortem Lesions

Yellow- or blood-tinged fluid in the peritoneal and pericardial cavities were detected, as well as petechiae on the eye, internal organs, visceral fat and skeletal muscles, together with enlarged and congested spleen and liver. The liver may be dark brown or black or covered with a thin layer of fibrin in some cases. The kidney may be swollen and dark. The gastrointestinal tract may also be congested. Hydropericardium and severe myocarditis are observed in some outbreaks (Godoy et al. 2008).

19.7.11 Diagnosis

19.7.11.1 Clinical Diagnosis

ISA is suspected in farmed Atlantic salmon with signs of anaemia and increased mortality. Haematocrit less than 10% is indicative of the disease. ISAV is confirmed in case of the presence of typical clinical signs.

19.7.11.2 Samples

Heart and mid-kidney are collected for virus isolation and/or RT-PCR. The detection of ISAV from gills or gill mucus by RT-PCR should be avoided to exclude the possibility of presence of the virus as a contaminant rather than a primary infection.

19.7.11.3 Laboratory Tests

Outbreaks of infectious salmon anaemia can be diagnosed by virus isolation, detection of antigens and RT-PCR. Avirulent virus strains can usually be detected only by RT-PCR. ISAV can be isolated in Atlantic salmon head kidney (SHK-1) or Atlantic salmon head kidney leukocyte (ASK), Chinook salmon embryo (CHSE-214), salmonid cell culture (TO) or epithelioma papulosum cyprinid (EPC) (Kibenge et al. 2004). Virus identification can be confirmed by RT-PCR, immunofluorescence and haemadsorption. Immunochromatographic strips can also be used in some countries.

19.7.11.4 Serology

ELISA can be used as a supplemental test to other assays.

19.7.12 Control

ISA is among the notifiable diseases. A single year-class fish should be stocked together. Boats and equipment as well as nets should be cleaned and disinfected regularly. Divers should disinfect their gear before and after diving and between cages. Youngest fish cages should be dived first and then cages with the older fish cages. Proper hygienic decontamination of waste water and dead fish should be

observed. Sea lice should be controlled, and stress should be minimized. ISAV can be inactivated by sodium hypochlorite, chloramine-T, chlorine dioxide, iodophors, sodium hydroxide, formic acid, formaldehyde and potassium peroxymonosulfate (Torgersen 1998).

19.7.13 Vaccination

Commercial inactivated ISA vaccine although prohibited in the European Union is available in some countries including Canada. The vaccines do not provide complete virus clearance and the fish may become carriers (Kibenge et al. 2004).

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