

## REVIEW ARTICLE

# Prevalence and control of H7 avian influenza viruses in birds and humans

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*Received 1 September 2011; Final revision 21 November 2013; Accepted 4 December 2013; first published online 15 January 2014*

### SUMMARY

The H7 subtype HA gene has been found in combination with all nine NA subtype genes. Most exhibit low pathogenicity and only rarely high pathogenicity in poultry (and humans). During the past few years infections of poultry and humans with H7 subtypes have increased markedly. This review summarizes the emergence of avian influenza virus H7 subtypes in birds and humans, and the possibilities of its control in poultry. All H7Nx combinations were reported from wild birds, the natural reservoir of the virus. Geographically, the most prevalent subtype is H7N7, which is endemic in wild birds in Europe and was frequently reported in domestic poultry, whereas subtype H7N3 is mostly isolated from the Americas. In humans, mild to fatal infections were caused by subtypes H7N2, H7N3, H7N7 and H7N9. While infections of humans have been associated mostly with exposure to domestic poultry, infections of poultry have been linked to wild birds or live-bird markets. Generally, depopulation of infected poultry was the main control tool; however, inactivated vaccines were also used. In contrast to recent cases caused by subtype H7N9, human infections were usually self-limiting and rarely required antiviral medication. Close genetic and antigenic relatedness of H7 viruses of different origins may be helpful in development of universal vaccines and diagnostics for both animals and humans. Due to the wide spread of H7 viruses and their zoonotic importance more research is required to better understand the epidemiology, pathobiology and virulence determinants of these viruses and to develop improved control tools.

**Key words:** Avian influenza virus, control, epidemiology, pandemic, poultry, subtype H7, vaccination, wild birds, zoonotic.

### INTRODUCTION

Avian influenza virus (AIV) infections in birds are mostly of low virulence (low pathogenic; LP) which cause minimal, if any, adverse health effects. However, AIV H5 and H7 subtypes can acquire

mutations in the HA gene which increase virulence resulting in highly pathogenic (HP) viruses which can rapidly decimate poultry flocks [1]. Haemagglutinin (HA) H7 was found in combination with all nine neuraminidases (NAs) (NA1–NA9) found in AIV to produce nine different AIV H7Nx subtypes. Infections with H7 subtypes have been described in wild birds, domestic poultry and mammals including harbour seals [2, 3], swine [4], equines [5] and humans [6, 7]. While infections of humans with H7 viruses have been associated mostly with exposure to domestic

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poultry [6], infections of poultry have been mainly linked to wild birds [8–11]. Other sources for H7 viruses are live-bird markets (LBMs) particularly in the USA [12], China [13, 14], South Korea [15] and the UK [16] or backyard birds as reported in Italy [17] and the USA [18, 19].

Phylogenetic analyses of H7 viruses from birds, animals and humans indicated distinct diversion on geographical, rather than temporal or host lines. Based on the H7 HA gene, two major genetic lineages, the American and Eurasian lineages, were identified [20]. Despite regional clusters the American lineage can further be divided into North and South American sublineages, while the Eurasian lineage diversified into ten major genetic sublineages including well-defined Australian, Asian and European sublineages [21, 22]. Moreover, HPAIV H7 from different geographical locations did not cluster in a separate phylogenetic group which may support the hypothesis of their evolution from LP counterparts [22]. Interestingly, genetic exchanges between these lineages/sublineages have also been reported [22–24]. Studies have also shown that H7 subtypes isolated from different birds (wild birds *vs.* domestic poultry) across Europe and Asia or within the Americas are closely related in given regions [25, 26] with annual predominance of one or two H7 subtypes [25, 27]. Another notable feature is that the HA gene showed less heterogeneity than the genes encoding internal viral proteins, where the latter seem to be more prone to reassortment [25, 28–31], indicative of possible evolutionary selection [22, 25]. This feature, in addition to rapid replacement of circulating H7 viruses, may lead to the sudden emergence of variant viruses with efficient transmission in and among avian and mammalian species [25]. Although data are scarce, it seems that the H7 viruses of bird or human origin are not antigenically diversified as much as human seasonal influenza or recent avian H5N1 viruses [15, 25, 27, 32–34]. This minor genetic and antigenic heterogeneity between HA of H7 viruses can be helpful in developing universal diagnostics and effective vaccines for both animals and humans [27].

Since June 2012, two incidents of infections with H7 subtypes were of great concern for animal and human global health organizations. The first was the HPAIV H7N3 infection in poultry in Mexico which spilled over to two humans [35, 36]. The second was the most recent LPAIV H7N9 outbreak in China which caused the death of 43 out of 133 laboratory-confirmed cases of infected humans [37]. Therefore,

in this comprehensive review we summarize information available on the emergence of H7 virus infections in wild birds, domestic poultry and humans, particularly during the past 15 years, and summarize the possible control mechanisms in domestic poultry as a first line of defence to reduce or prevent human infections.

## PREVALENCE OF H7 SUBTYPES IN WILD BIRDS AND DOMESTIC POULTRY

### Wild birds

Wild birds are regarded as the natural reservoir for all AIVs, including H7 subtypes, which have the potential to efficiently replicate in domestic poultry [9, 15, 23, 38–44] and pose a risk for human infection [29, 44, 45] with or without prior adaptation. In the last 15 years, surveillance has shown a wide spread of multiple H7 AIV in wild birds, particularly in mallards [10, 15, 17, 21–28, 30, 31, 38, 40, 42, 44, 46–85]. Findings of several surveillance systems and the deposited genome sequences in two different influenza virus databases, GenBank and GISAID, indicated the following. (1) All H7 isolates in wild birds were of low pathogenicity. (2) All H7N subtype combinations were reported from wild birds (Table 1). (3) Geographically, the most prevalent subtype in wild birds was H7N7 (reported from 21 countries) which is also endemic in wild birds in Europe followed by H7N1 (reported from 18 countries) and H7N3 (reported from 15 countries). (4) H7N5 was the least reported subtype (two countries) and restricted to North America followed by H7N4 (three countries), H7N6 (six countries), H7N2 (seven countries), H7N8 (eight countries) and H7N9 (nine countries). (5) Compared to Europe, H7N3 has been frequently reported from the Americas. (6) All H7N subtypes were reported from the USA.

### Domestic poultry

Outbreaks of LPAIV and HPAIV of subtype H7 in domestic poultry including commercial poultry, backyard birds and birds from LBMs have been frequently reported, ranging from largely asymptomatic infections to rapidly fatal disease. Serological evidence without isolation of any H7 virus has been reported in backyard poultry in Germany in 2001 [86] and Côte d'Ivoire in 2007–2009 [87], commercial chicken flocks in Egypt in 2009–2010 [88], domestic ducks in

Table 1. Emergence of AIV subtype H7 in wild birds, domestic poultry and humans (1990–2013)

Subtype	Country	Wild birds, year [ref.]	Domestic poultry, year [ref.]*
H7N1	Australia	2005–2008 [46]	
	Belgium	2008 [65], 2009 [42]	
	Canada	2001 [Wentworth <i>et al.</i> , unpublished data]†	2000 [98]
	China	2010 [Chai <i>et al.</i> , unpublished data]	
	Denmark	2007 [67], 2008 [66], 2009 [67]	2008 [66], 2010 [67]
	Egypt	2004 [52]	
	France	2001 [Rousset <i>et al.</i> unpublished data]	
	Georgia	2010 [53]	
	Germany	2004 [68]	
	Hong Kong	2011 [147]	
	Italy	2002 [25]	<b>1999–2000</b> , 2001 [99–104], 2008 [105]
	Japan	2012 [69]	
	Mongolia	2001 [54]	
	The Netherlands	2007 [Spiro <i>et al.</i> , unpublished data]	2011 [106]
	Singapore	1994–1995 [20]	
	South Africa		1990s, [107], 2009 [106], 2012 [106]
	Spain	2006–2009 [70]	
	Taiwan	1998–2007 [60]	
	USA	2006–2008 [63, 64]	
	H7N2	Australia	2007 [21, 78]
China			2002 [29]
Denmark		2009 [67]	
Italy		1993 [20]	
The Netherlands		2006 [28]	
South Korea		2003–2004 [59]	2009 [15], 2010–2011 [106]
Spain		2009 [79]	
UK			<b>2007</b> [111, 113, 171]
USA		2006–2008 [63, 64]	1990s–2006 [64, 90, 91], <b>2002–2003</b> [173, 174]
H7N3		Australia	
	Bolivia	2001 [24]	
	Canada	2001 [44], 2006 [48], 2007 [48], 2009–2011 [49]	<b>2004</b> [124, 175], <b>2007</b> [125]
	Chile		<b>2002</b> [126]
	China	2005 [146]	2011 [127]
	Egypt	2006 [52], 2007 [52]	
	Germany		2008 [113]
	Georgia	2010 [53]	
	Italy	2001 [25]	2002– <b>2003</b> ‡ [9], 2004 [128], 2007 [105]
	Japan	2006 [71]	
	Mexico	2012 [106]	<b>2012</b> –2013 [35, 130–134]
	The Netherlands	2000 [27]	
	Pakistan		<b>1994–1995</b> [135], <b>2000–2001</b> [137], <b>2003–2004</b> [136–138]
	Peru	2008 [72]	
	Portugal	2005–2009 [57]	
	South Korea	2006 [59], 2007 [59], 2008 [59], 2011 [15]	
	Taiwan	1998–2007 [60]	2011 [106]
	Ukraine	2010–2011 [61]	
	UAE		<b>1998</b> [139]
	UK		<b>2006</b> [113, 180]
USA	1999 [40], 2001 [73, 74], 2002 [73], 2006 [23, 31], 2007, 2008 [63, 75], 2010 [49], 2011 [49]	1999 [91, 114], 2004 [91], 2008 [64], 2011 [106]	

Table 1 (cont.)

Subtype	Country	Wild birds, year [ref.]	Domestic poultry, year [ref.]*	
H7N4	Australia		<b>1997</b> [142]	
	Canada	2007 [Wentworth <i>et al.</i> , unpublished data]		
	Italy	2004–2006 [10, 17]		
	USA	2001 [73], 2002 [73], 2006–2008 [23, 63, 64]		
H7N5	Canada	2006 [80]		
	USA	2006 [23], 2007 [63]		
H7N6	Australia	2006 [78], 2007 [21]		
	Canada	2009 [Wentworth <i>et al.</i> , unpublished data]		
	Hong Kong	2011 [146]		
	Japan		2009 [55, 143, 144]	
	South Korea		2010 [15]	
	Taiwan	1998–2007 [60]		
	Ukraine	2010–2011 [61]		
	USA	2006–2008 [64]		
	H7N7	Australia	2005–2008 [21, 46]	<b>2012–2013</b> [130]
		Belgium	2010 [42]	<b>2003</b> [145]
Bulgaria		2008 [47]		
Canada		2007 [48, 125], 2009–2011 [49]		
China		2009 [146], 2012 [Zhu, unpublished data]	2003 [25], 2013 [146]	
Czech Republic		2011 [50]		
Egypt		2004 [52], 2005 [51], 2006 [52]		
Georgia		2010 [53]		
Germany		2005–2006 [28]	2001 [147], <b>2003</b> [86], 2009 [106], 2011 [148], 2013 [106]	
Hungary		2007 [30]		
Ireland			1998 [149]	
Italy		2004, 2005 [10, 25]	2004 [17], 2006 [113], <b>2013</b> [130]	
Japan		2001–2004 [54], 2008 [55]		
Mongolia		2007 [56]		
The Netherlands			<b>2003</b> [150, 152, 181], 2006 [113], 2011 [106], 2012 [106], 2013 [106]	
Northern Ireland			1998 [154]	
North Korea			<b>2005</b> [38]	
Poland		2008 [28], 2009 [28]		
Portugal		2005–2009 [57]		
Slovenia		2009 [58]		
South Africa			1996 [20], 2010–2011 [15]	
South Korea		2007 [15], 2008 [15], 2009 [59], 2010 [59]	2010 [106]	
Spain			<b>2009–2010</b> [155]	
Sweden		2002 [27], 2003 [27], 2005 [26], 2008 [26]		
Taiwan		1998–2007 [60]		
UK			<b>2008</b> [113] 1996§ [185]	
Ukraine		2010–2011 [61]		
USA	1998–2000 [62], 2006–2008 [63, 64]	2008 [64]		
H7N8	China	2005 [146], 2009 [146]		
	Japan	1999 [81]		
	The Netherlands	2006 [Spiro <i>et al.</i> , unpublished data]		
	South Korea	2005 [38], 2008 [38]	2011 [113]	
	Spain	2004 [82]		
	Sweden	2004 [82]		
	Ukraine	2006 [83]		
	USA	2006–2008 [64], 2010–2011 [Wentworth <i>et al.</i> , unpublished data]		

Table 1 (cont.)

Subtype	Country	Wild birds, year [ref.]	Domestic poultry, year [ref.]*
H7N9	China		<b>2013</b> [158–163, 186–192]
	Czech Republic	2009 [50]	
	Egypt	2006 [52]	
	Guatemala	2008 [76]	
	Japan	2009 [69], 2011 [69]	
	Mongolia	2008 [56]	
	South Korea	2011 [15]	
	Spain	2005 [77], 2008 [50]	
	Sweden	2002 [27]	
	Taiwan		<b>2013</b> § [200]
H7Nx	USA	2000 [40], 2005 [74], 2006–2008 [64], 2011 [Wentworth <i>et al.</i> , unpublished data]	2007 [64], 2009 [156], 2011 [49]
	Denmark	2007–2010 [57]	
	Portugal	2005–2009 [57]	
	Switzerland	2006–2009 [84]	
	Zimbabwe	2007–2009 [85]	

\* Outbreaks in poultry caused by HPAIV appear in bold face and human infections are highlighted in grey.

† Unpublished data were retrieved from the public GenBank sequence database.

‡ Only seroconversion.

§ Infections were reported only in humans not in poultry.

India in 2009–2011 [89] and many bird species in USA in 1997–2011 [49, 64, 90, 91]. Prior to 1990, there were few reported outbreaks of H7 AIV in poultry: panzootic outbreaks of H7N1 in 1901–1930s [92, 93], H7N3 in 1971 and 1979–1980 and H7N9 in 1988 in the USA [20, 94], H7N3 in 1963, H7N7 in 1977 and 1979 and H7N1 in 1982 in England [20, 95], H7N7 in 1976 and 1985 in Australia [96], H7N7 in 1977 and 1987 in Germany and H7N2 in 1979 in Israel [20]. Isolation of different H7 viruses from domestic poultry since the 1990s is summarized in the following sections.

### H7N1

*Canada.* In 2000, a H7N1 virus was isolated from a turkey-breeding establishment in Canada (Ontario) with a history of decreased egg production, respiratory disorders and mortality [90, 97, 98]. The virus was classified as LP [98], the source of infection was not identified and no regulatory measures were taken [97].

*Denmark.* In April 2008, a LPAIV H7N1 was isolated from a flock of 250 domestic ducks in close proximity to wild birds kept for shooting [66]. In 2010, two flocks of mallards were infected with LPAIV H7N1 [67]. The source of infections of the three flocks was assumed to be wild birds [67].

*Italy.* During 1999–2001, LPAIV H7N1 was identified in 199 outbreaks, mostly in commercial chickens and turkeys in North-Eastern Italy [99]. The virus might have been introduced by wild birds based on its close genetic relatedness with Eurasian and South African viruses of wild-bird origin [100, 101]. In December 1999, HPAIV H7N1 emerged after mutation of LPAIV and infected over 413 poultry farms; many avian species were infected including not only turkeys and chickens but also guinea fowl, ostriches, quails, ducks, pheasants, Sakr falcon, sparrows and doves [102]. This epidemic had severe socioeconomic implications for the poultry industry in Italy due to the death of over 13 million birds, disruption of poultry marketing and interruption of poultry production before its total eradication in 2000 [103, 104]. In 2008, the isolation of two LPAI H7N1 viruses was reported from dealer/rural farms without further spread [105].

*The Netherlands.* In March 2011, an infection of a flock of layer hens with LPAIV H7N1 resulted in the destruction of over 127000 birds [106].

*South Africa.* In the early 1990s, sporadic outbreaks of LPAIV H7N1 were reported in ostriches, particularly in the winter season, in different provinces of South Africa inducing clinical signs ranging from no symptoms to greenish diarrhoea

and even mortality in naturally or experimentally infected ostriches [107, 108]. In all reported instances wild birds were the most likely vectors to have introduced the virus to ostriches [20, 109]. In 2009, H7N1 of low virulence was reported again in ostriches [106]. Since January 2012, nine outbreaks of LPAIV H7N1 in clinically healthy commercial ostriches in Western Cape province were reported to the OIE. The outbreak is still unresolved and the source of infection remains unknown [106].

### H7N2

*China.* In 2002, LPAIV H7N2 was isolated from chickens in a slaughterhouse in Hebei province [29]. The virus is thought to have originated from wild birds. The HA gene of that virus was closely related to a H7N1 virus isolated from an African starling in England in 1979, while the NA was similar to a human H2N2 virus. The virus was able to replicate in experimentally infected chickens and mice without prior adaptation [29].

*South Korea.* In 2009, in Jeonnam province, a LPAIV H7N2 was isolated from asymptomatic domestic ducks at a LBM which was found to be genetically closely related to viruses of wild-bird origin. Nevertheless, the source of the virus was not fully identified [15]. In 2010 and 2011 two outbreaks were reported in poultry farms resulting in the destruction of over 50000 chickens and ducks [106].

*UK.* In May 2007, LPAIV H7N2 was isolated from dead chickens on a smallholding in North Wales. The death of birds began 2 weeks after introduction of chickens purchased from a LBM [110–112]. Further spread of the virus to a poultry farm near St Helens in North-West England was reported in June 2007. All birds at the infected premises and within the 1 km surrounding zone were slaughtered [113].

*USA.* Since the early 1990s, LPAIV H7N2 has been primarily maintained within LBMs, particularly in the North-East of the USA. Infections frequently spilled over into commercial poultry holdings and the infection continued to be endemic causing severe economic losses [91, 114, 115]. Efforts to eradicate the virus from the LBM system were unsuccessful and 40–60% of LBMs in the North-East regions regularly tested positive for LPAIV H7N2 [114]. The virus was first isolated from LBMs and commercial

farms in 1993 [19, 114]. In 1996–1998 in Pennsylvania, infection of more than 2·6 million commercial birds in 47 flocks with LPAIV H7N2 induced respiratory distress, decreased egg production and increased mortality rates. The infection was linked to LBMs in New York and New Jersey [116, 117] where the virus had been frequently reported from LBM and non-LBM birds between 1995 and 2000 [118]. In 1999 in New York, LPAIV H7N2 was isolated from a flock of 40 000 quails linked to LBMs, and from chickens in Delaware [90]. In 2001, the virus was isolated from chickens in New York and Florida [90]. Moreover, in 2001–2002, the virus was isolated in Pennsylvania from two broiler-breeder and five broiler flocks with acute respiratory disorders and/or increased mortality rates. The initial infection was again linked to LBMs [119]. In 2002, the largest outbreak of LPAIV H7N2 in the USA occurred in Virginia and the surrounding states, resulting in depopulation of 4·7 million birds in 197 farms in about 4 months [64, 91, 115]. In 2003, in Connecticut and Rhode Island, two independent outbreaks of LPAIV H7N2 were confirmed in five commercial layer chicken farms showing respiratory distress and a temporary drop in egg production [91]. The virus affected over 2·9 million birds and a loss of US\$ 149 million was estimated [99]. In 2004, LPAIV H7N2 outbreaks were identified in two commercial broiler chicken flocks in Delaware and in one flock in Maryland. The former was possibly linked to LBMs in New Jersey [91]. In 2005 in New York, LPAIV H7N2 was isolated from a duck production facility [91] and in 2006 from chickens in New York and New Jersey [64]. Despite the long-term persistence of LPAIV H7N2 in LBMs in the USA, no HPAIV has evolved although genetic changes towards higher virulence were reported [114, 120–122].

### H7N3

*Australia.* During the 1990s, Australia experienced two limited HPAIV H7N3 outbreaks in commercial poultry; one in Victoria in 1992 in 12700 broiler breeders and 5700 ducks [123] and another in Queensland in 1994 in 22000 laying hens [96]. Wild birds roaming around the lake and river close to the affected farms were assumed to be the source of infections. Nevertheless, neither H7 virus nor antibodies were detected in samples obtained from wild birds in the surrounding area of the outbreaks [96].

*Canada.* From February to May 2004, in Fraser Valley, British Columbia, LPAIV H7N3 was isolated, with an increased mortality rate, from a chicken farm containing 9200 birds [124]. Adjacent to this farm HPAIV H7N3 evolved from a LPAIV and spread to 42 commercial and 11 backyard poultry flocks resulting in the destruction of about 17 million birds and total economic losses of over CAN\$ 380 million [97, 124]. In September 2007, another limited HPAI H7N3 outbreak was reported in roosters, broiler-breeders and turkey pullets in a low-density poultry producing area in Saskatchewan, Canada. Again, the prior circulation of a LPAIV H7N3 in affected premises was described as the donor for the HPAIV [125]. Epidemiological investigations suggested direct contact with wild aquatic birds or contamination of water with LPAIV H7N3 as the likely source of infection [125], which was further supported by the close identity of the genome of the viruses isolated from the domestic birds and the contemporary H7 viruses of wild-bird origin in the North American sublineage [8].

*Chile.* In April–May 2002, LPAIV and HPAIV H7N3 were simultaneously isolated from broiler-breeder chickens and turkey-breeder farms. About 617000 birds were destroyed [99, 126]. Phylogenetic analysis indicated that all gene segments belonged to the North American H7 lineage with close relatedness to wild-bird-origin viruses, whereas the PA and NP genes were most closely related to H7N7 viruses of equine origin. The HPAIV H7N3 originated from co-circulating LPAIV H7N3 [41, 126].

*China.* In 2011, in Eastern China, four LPAI H7N3 viruses were isolated from apparently healthy commercial domestic ducks in LBMs. Phylogenetic analysis indicated that those viruses belonged to the Eurasian lineage and acquired their genes from different AIV subtypes of aquatic-bird origin via reassortment [127].

*Germany.* In 2008, LPAIV H7N3 was isolated from turkeys in Germany [113].

*Italy.* In 2002–2003, poultry flocks, particularly turkey flocks, in Northern Italy were severely affected by a LPAI H7N3 epidemic [9, 128]. Culling of poultry and vaccination eradicated the disease by September 2003 [128]. Phenotypic and genetic characterization indicated that the parental virus was

derived *in toto* from wild ducks isolated in 2001 [9]. In February 2004, the virus was detected in free-range domestic ducks and geese in a backyard flock. Another wave in September to December 2004 of LPAIV H7N3 affected meat turkey farms and a quail farm in a high-density turkey population area [128, 129]. In 2007, a second, unrelated LPAIV H7N3 was identified in geese and chickens of a rural farm but the source of infection was not confirmed [105]. Furthermore, another distinct LPAI H7N3 virus infected a total of 4164 rural/hobby birds (three farms), over 52000 poultry and ornamental birds (seven dealer flocks) and 73158 commercial meat turkeys (six farms). Symptoms ranged from no signs (rural/hobby birds) to mild respiratory illness, anorexia and increased mortality rates (turkeys) [105].

*Mexico.* From June 2012 to July 2013, HPAIV H7N3 was isolated from different poultry farms and backyards in several regions in Mexico [130, 131], resulting in the culling of at least 22.4 million birds in 272 layer farms, 46 breeder farms and 202 backyard operations with a total cost of over US\$ 720 million [36, 130, 132]. Vaccination and culling of infected birds are the statutory control strategy. Although no reports regarding the source of infection were available, wild birds and/or poultry trade were assumed as a possible source of infection [132]. The virus was closely related to viruses of wild-bird origin as well as chicken and human isolates from Canada in the North American sublineage [132–134].

*Pakistan.* Pakistan experienced destructive outbreaks with AIV H7N3 in domestic poultry. The initial outbreaks of HPAIV H7N3 were reported in broiler-breeder and broiler flocks in Northern Pakistan from December 1994 to April 1995 [135]. The virus emerged from a low virulent virus after circulation of the latter in poultry over a period of time [136]. Despite vaccination and biosecurity enforcement both H7N3 pathotypes were sporadically transmitted to and among poultry in different production sectors [34, 137]. In 2000–2001, LPAIV and HPAIV H7N3 were identified in chickens in Pakistan [137]. In April 2003, LPAIV H7N3 re-emerged in Southern Pakistan causing up to 70% drop in egg production and 20% mortality in commercial poultry. Epidemiological data were lacking to determine the source of this LPAIV; however, it was genetically linked to the 1994–1995 epidemic suggesting that backyard birds were

probably the reservoir of the virus. From November 2003 to June 2004, HPAIV H7N3 emerged from LPAIV and spread widely in poultry throughout the country, whereby 522 farms were affected [136]. The initial introduction of the Pakistani H7N3 was probably by wild birds and HPAIV emerged after multi-step reassortment of wild-bird-origin AIV and Pakistani H9N2 viruses [34, 138].

*Taiwan.* In 2011, two outbreaks of LPAIV H7N3 in two duck-breeder farms were reported to the OIE. The affected birds were clinically healthy and investigation into the source of infection was inconclusive [106].

*United Arab Emirates (UAE).* HPAIV H7N3 was isolated from a peregrine falcon in 1998 in UAE which possibly, due to phylogenetic data, had acquired the infection through contact with birds in Pakistan although epidemiological data do not agree with this assumption [139].

*UK.* In April 2006, LPAIV H7N3 was confirmed in two outdoor layer chicken flocks and one housed broiler-breeder chicken flock in Norfolk, Eastern England [16, 113, 140]. The infection was believed to be introduced via wild birds which had direct contact with the free-range layer flocks. Moreover, incrimination of fox carriage of carcasses or contaminated footwear was suspected in infection of the indoor broiler-breeders [140, 141]. Over 45 000 birds in the three premises were culled [16, 141].

*USA.* In 1999, LPAIV H7N3 was isolated from ten samples in LBMs in New York [114] and from ducks in Pennsylvania [91]. In 2004, the virus was reported in poultry in New York and Massachusetts [91]. In 2008, a LPAIV H7N3 closely related to the North American H7 viruses circulating in wild birds was detected from an asymptomatic 65-week-old commercial broiler-breeder chicken flock in Arkansas [64]. The infection was controlled after culling of the flock, and cleaning and disinfection of the premises [64]. In March 2011, the isolation of LPAIV H7N3 was reported from 29-week-old turkeys in Missouri, which lead to the destruction of more than 29 000 birds [49, 106].

#### H7N4

*Australia.* In November 1997, in New South Wales a HPAIV of subtype H7N4 infected 300 000 commercial

chickens and 261 3-month-old emus with up to 90% mortality rates in chickens but neither clinical disease nor mortality in emus. Although samples obtained from wild birds in the vicinity of the infected premises were negative for AIV, the source of infection was assumed to be an infection of emus housed in open pens via direct contact with infected wild birds. In June, 1998 the state was officially declared free of HPAI. Losses were estimated to be A\$4.5 million [142].

#### H7N5

To date, neither published reports nor deposited sequences for H7N5 infections in domestic poultry exist.

#### H7N6

*Japan.* In 2009, LPAIV H7N6 was isolated from three Japanese quail (*Coturnix japonica*) farms. The infection of quails resulted in culling of infected premises and a total loss of US\$9.75 million was estimated in order to eradicate the disease. Introduction of the virus through wild birds, mainly pintails, was considered the possible source of infection [55, 143, 144].

*South Korea.* In 2010, LPAIV H7N6 was isolated from a domestic duck farm in Jeonnam province. The virus was probably a reassortant from different viruses of aquatic-bird origin [15]. A total of 23 410 birds were culled to eradicate the disease [106].

#### H7N7

*Australia.* From November 2012 to March 2013, HPAIV H7N7 outbreaks resulting in the destruction of about 50 000 commercial layer hens in New South Wales, were reported to the OIE [130]. The virus probably originated from wild birds attracted by water reservoirs in the region [130].

*Belgium.* Eight outbreaks of HPAIV H7N7 were reported in 2003 in commercial chickens and turkeys as an extension of the Dutch H7N7 outbreak (see below). The disease was eradicated within a few months due to rapid culling of infected flocks, preventive depopulation of high-risk contact flocks and enforcement of biosecurity measures. Nevertheless, the epidemic resulted in the destruction of 2.3 million birds [145].



*China.* In 2003, four LPAI H7N7 viruses were isolated from domestic ducks at Poyang Lake, Jiangxi province, China [25]. A H7N7 virus, which has a highly similar genotype to H7N9 virus and is also infectious to ferrets, was detected in chickens in Eastern China in 2013 [146].

*Germany.* Since 2000, Germany has experienced four incidents of LPAI H7N7 and one incident of HPAI H7N7 in domestic poultry. In 2001, LPAIV H7N7 was isolated from an asymptomatic small free-range mixed (chickens, turkeys, geese, ducks) household flock in Southern Germany and a total of 145 birds were culled. There was contact between this flock and wild birds, which was possibly the source of infection [147]. The second introduction of LPAIV H7N7 occurred in 2009 and resulted in the destruction of 16700 birds on one farm [106]. The third introduction occurred in May 2011 and was relatively widespread, resulting in the destruction of more than 80000 chickens, turkeys, ducks and geese from 23 commercial and backyard premises within a few days [106, 148]. Infected birds showed mild to severe respiratory manifestations, decreased egg production and elevated mortality rates; introduction of the virus through wild birds or from a contemporary outbreak in poultry in The Netherlands were the most likely scenarios [148]. The fourth introduction was reported recently in May 2013 from a free-range turkey flock and resulted to date in the destruction of ~34000 birds [106]. The only incident of HPAIV H7N7 in Germany was reported in 2003 which spread from The Netherlands to Belgium and Germany and led to the culling of 419000 birds [86]. The virus had been isolated from ducks and chickens in commercial farms.

*Ireland.* In 1998, a total of 320000 turkeys and chickens were affected by 29 outbreaks of LPAIV H7N7, 28 on turkey farms and one in a chicken farm. The disease was successfully eradicated within 7 weeks and wild aquatic birds were considered the likely source of infection [149].

*Italy.* LPAIV H7N7 was isolated from domestic backyard ducks and geese in 2004, which was closely related to a contemporary virus of wild birds [10, 17], and from commercial ducks in 2006 [113]. In mid-August, 2013 in northern and central Italy four outbreaks of HPAI H7N7 were confirmed in

commercial laying hens and fattening turkey holdings. More than 800000 birds in the infected premises were depopulated. Although not yet conclusive, prior infection with LPAIV, possibly introduced via wild birds, which has mutated into the virulent form, was suggested as the likely source of infection. Genetically, the virus is related to H7 viruses isolated from wild birds in Italy as well as to H7 which occurred in chickens in Germany and The Netherlands in 2003–2011 ([130]; G. Cattoli, unpublished data). The outbreaks are still on-going.

*The Netherlands.* From February to May 2003, a devastating HPAIV H7N7 epidemic occurred in poultry in the central part of The Netherlands and the cross-border regions of The Netherlands, Belgium and Germany. A total of 255 outbreaks were confirmed in poultry and over 30 million birds died or were culled [150–152]. The source of infections was linked to a reassortant virus originating from two independent introductions of H7N3 and H10N7 into commercial poultry from wild mallards [26, 150, 152]. In August 2006, LPAIV H7N7 was isolated from chicken breeders [113]. In 2011, two outbreaks of LPAIV H7N7 occurred in 8000 and 54000 free-range laying-chicken flocks. All birds were culled and the source of the virus was reported as unknown [106]. In August 2012, LPAIV H7N7 was detected from an asymptomatic free-range laying-hen flock with a total of ~31000 birds [106]. In March 2013, LPAIV H7N7 was detected on two poultry farms with increased mortality rates of 83000 and 23500 layer hens where some birds also showed decreased egg production. Introduction of the virus into the infected premises from wild birds was the most likely scenario [106, 153].

*Northern Ireland.* In 1998, infections of two turkey farms and one chicken farm with LPAIV H7N7 were reported. Epidemiological investigation supported the hypothesis of wild birds being the potential source of infection [154].

*North Korea.* In 2005, three HPAI H7N7 outbreaks were reported in chickens in North Korea [38]. Birds showed respiratory disorders and over 218000 chickens were culled [90, 130].

*South Africa.* In 1996, an LPAIV H7N7 was isolated from ostriches. Interestingly, the virus was genetically closely related to a Taiwanese wild duck isolate and the Italian H7N1 from poultry in 1999 [20].

In 2010–2011, LPAI H7N7 viruses were isolated from six epidemiologically linked duck farms [15].

*South Korea.* In 2010, two independent outbreaks of LPAIV H7N7 occurred in subclinically infected domestic ducks in seven farms that were epidemiologically linked and resulted in the destruction of ~105 000 birds [106].

*Spain.* From October 2009 to January 2010, an HPAI H7N7 outbreak was reported for the first time in commercial poultry in Spain and no links to other poultry holdings were observed [155]. The source of infection was not recognized. The outbreak resulted in the destruction of ~278 000 birds [130].

*UK.* In 2008, a single outbreak of HPAI H7N7 was reported in a free-range chicken-layer farm in North Oxfordshire, England, resulting in the destruction of 25 000 birds [113, 130, 156]. Extensive surveillance did not elucidate the definite source of infection but incrimination of a semi-captive population of wild mallards kept for shooting close to the infected premises was speculated [113, 157].

*USA.* In 2008, several LPAI H7N7 viruses were isolated from chickens and turkeys in California, North Carolina and Pennsylvania [64].

#### H7N8

*South Korea.* In 2011, LPAIV H7N8 was isolated from a subclinically infected commercial meat duck in the South-Western part of the Republic of Korea during routine surveillance. A total of 19 200 ducklings in four farms were destroyed. The source of the virus was not detected [113].

#### H7N9

*China.* Emergence of H7N9 in mainland China was first reported in Shanghai on 19 February 2013. The virus was detected in LBMs in several cities/provinces in the Eastern region of China as well as in Beijing in the North [158, 159]. Infected poultry showed no clinical illness and this outbreak might have passed without much attention; however, the virus jumped to humans causing severe even fatal infections (see below). More than 561 000 birds in LBMs were culled. The lack of clinical signs in poultry is a real challenge to controlling the disease among China's six billion domestic birds [160].

To date, the virus has been isolated from chickens, ducks and a pigeon in LBMs, whereas samples obtained from geese, pigs or wild birds have tested negative [159, 161, 162]. Experimental studies showed that the virus can transmit from chicken-to-chicken and in ducks but with a lower transmission rate in the latter [162]. Surveillance and phylogenetic analysis indicated that related H7 and N9 AIVs have jumped from wild birds to domestic waterfowl and then to chickens, which subsequently reassorted with the enzootic H9N2 viruses in chickens to generate the current form of H7N9 [14, 146, 163]. Closure of LBMs was effective in limiting the spread of infections in poultry and humans [164–166].

*USA.* In June 2007, LPAIV H7N9 was isolated from asymptotically infected turkeys on a multi-age turkey operation in Nebraska during routine slaughter surveillance and a total of 144 000 birds were slaughtered [64, 106]. In 2009, two LPAI H7N9 outbreaks were identified in 20 000 commercial broiler breeders with a history of 10–20% decrease in egg production in Kentucky [156] and 160 000 commercial poultry in Minnesota [49, 106]. In April and July 2011, three and five isolates were obtained from geese and guinea fowl from Nebraska and from turkeys in Minnesota, respectively [49].

### EMERGENCE OF H7 SUBTYPES IN HUMANS

Unlike wild birds and domestic poultry, H7 viruses occasionally infect humans causing mild, if any, clinical manifestations, mainly conjunctivitis and/or influenza-like illness (ILI). Infection of humans with H7 viruses was first recorded for HPAIV H7N7 in the USA in 1959 [167], HPAIV H7N7 in Australia in 1977 [168] and LPAIV H7N7 from seals to humans in the USA in 1978–1979 [2, 169]. Since the 1990s, reports of human infections with H7 viruses have markedly increased. Humans acquire H7 viruses primarily through direct exposure of the mucous membranes (mainly the eye) to infectious secretions and excretions from infected poultry, or contaminated products. Among infected persons, it is mainly veterinarians, poultry workers and those involved in the culling of infected poultry that are most frequently infected [170]. Infections were caused by LPAIV

H7N2, H7N3, H7N7 and H7N9, and by HPAIV H7N3 and H7N7 as discussed below.

### H7N2

*UK.* After the outbreak of LPAIV H7N2 in poultry in 2007 a total of four human infections out of 14 suspected cases were confirmed; two in Wales and two in North-East England. All cases had been in close contact with infected poultry and exhibited conjunctivitis and respiratory disorders [111, 171]. Three of the cases were hospitalized, antiviral medication was given and all four recovered [110, 111, 172].

*USA.* Two occurrences of H7N2 infections were reported in the USA. The first was in 2002 when a person involved in culling of infected H7N2 turkeys and chickens in Virginia developed ILI and exhibited anti-H7N2 antibodies [173]. The second case was in November 2003 in New York, where LPAIV H7N2 was isolated from an immunocompromised 48-year-old man. The patient denied any contact with live poultry and the source of infection remains unknown. The patient was hospitalized with fever and upper as well as lower respiratory tract illness. Both cases recovered fully from their respiratory illnesses [174].

### H7N3

*Canada.* In 2004, two poultry workers were infected during an outbreak of LPAIV/HPAIV H7N3 in British Columbia. LPAIV H7N3 was isolated from one poultry worker with unilateral conjunctivitis and coryza, and HPAIV H7N3 was isolated from a second poultry worker with conjunctivitis and headache. Both patients fully recovered after oseltamivir treatment [175]. Interestingly, no antibodies were detected in serum samples collected from either patient several weeks after recovery [175, 176]. Similarly, no seroconversion was observed after an experimental infection of ferrets with HPAIV H7N3, although the virus did transmit to in-contact ferrets [177].

*Italy.* During the outbreak of LPAI H7N3 in Italy in 2003 a total of seven (3·8%) out of 185 poultry workers exposed to turkeys and chickens exhibited antibodies against H7N3 AIV. Only one patient had

a history of conjunctivitis, while the others were asymptomatic and no virus could be isolated [178].

*Mexico.* In July 2012, during the most recent HPAI H7N3 outbreak in poultry in Jalisco, two poultry workers developed conjunctivitis after exposure to infected poultry and were confirmed to be infected with HPAIV H7N3. The first patient was a 32-year-old female who had collected eggs from an infected poultry farm. The second patient was a 52-year-old male who worked at the same farm. Both patients were hospitalized, treated symptomatically and recovered fully [35]. After experimental infection of mice and ferrets the Mexican HPAIV H7N3 showed enhanced virulence and efficient replication in both animal models. Furthermore, the virus was able to transmit efficiently to in-contact ferrets [179].

*UK.* In 2006, during the outbreak in poultry in Norfolk, a poultry worker from the affected farm exhibited conjunctivitis and infection by H7N3 AIV was confirmed. Treatment of the patient with oseltamivir was immediately initiated. An additional ~100 people received oseltamivir as a prophylactic course and seasonal influenza vaccine, and of these five poultry workers presented conjunctivitis and/or ILI. All of them tested negative for influenza virus [180].

### H7N7

*The Netherlands.* Between 1 March and 16 May 2003, during the HPAI H7N7 outbreak in The Netherlands, Belgium and Germany, 86 people working with chickens were infected as well as three of their family members. This outbreak represents the first non-H5N1 avian influenza outbreak in humans. Infected persons experienced mild to moderate conjunctivitis and/or ILI, except a 57-year-old veterinarian with some degree of reduced immunity, who died from pneumonia and acute respiratory distress syndrome [152, 181]. He acquired the infection through visiting many farms with infected birds and developed conjunctivitis 30 h after his last visit [181]. Serological investigation showed that at least 1000 persons had acquired a subclinical infection with this virus [182, 183]. Personal protection, vaccination with seasonal human influenza virus vaccines and oseltamivir administration were successfully used to combat the infection [181, 184].

*UK.* In 1996, a LPAIV H7N7 was isolated from a 43-year-old housewife. The woman was hospitalized with conjunctivitis, which subsided after 4 days. No seroconversion was reported. She acquired the infection through penetration of a piece of straw in her eye during cleaning out her poultry shed, which contained 26 pet ducks of various breeds. The poultry shed was located next to a small lake where the pet ducks mingled freely with wild mallards and geese. Nonetheless, no virus was isolated nor any clinical signs observed from the pet and wild ducks 1 month before the onset of her illness [185].

## H7N9

*China.* Between January and November 2012, no evidence of H7N9 in Eastern China was obtained after serological investigation of 1544 serum samples from poultry workers and other occupational groups [186]. From 19 February to 12 July 2013, a total of 43 human fatalities out of 132 infected cases were reported [37]. As of 30 April 2013 preliminary epidemiological investigations including the first 122 patients indicated several points [37, 159, 187]. (1) The majority of human infections were detected between 19 March and 24 April. (2) Infections were reported in ten regions of Eastern China and from Beijing in the North. (3) The incubation period was generally less than 1 week and the course of infection took about 2 weeks. (4) Infected humans exhibited ILI and the majority (>70%) of confirmed cases were severely or critically ill suffering from rapid progressive respiratory disorders and fatal outcomes. (5) The age of the patients ranged from 2 to 91 years with an average of 61.5 years. (6) Over 69% of the patients were male with regional differences probably due to variable contact with poultry. (7) About 84% were urban residents. (8) LBMs were the main source of infection; however, poultry farms or backyard holdings should not be overlooked [159, 188, 189]. (9) Of the confirmed cases four persons were poultry workers and 77% had a history of exposure to live birds, particularly chickens. (10) Although the majority of close contacts were negative for A/H7N9, human-to-human transmission of H7N9 virus in two family clusters was suspected [158]. (11) Patients commonly suffered from chronic underlying conditions [159]. (12) The virus was found to be resistant to amantadine but sensitive to oseltamivir [190–192]. (13) No vaccine has yet been launched.

It should be noted that this avian-origin H7N9 virus has an unusual affinity to attach to human receptors in the upper and lower respiratory tract similar to human-adapted seasonal H3N2 influenza viruses [193]. Moreover, the virus grew well in the respiratory tract of experimentally infected ferrets, mice and pigs causing fatal pneumonia and was transmitted to in-contact animals [194–199].

*Taiwan.* The first case of H7N9 in Taiwan was confirmed on 3 April 2013 [200]. The patient was 53 years old and had returned recently from Jiangsu province, China. The man had a history of chronic hepatitis B virus infection with no history of contact with poultry. Three contact persons were negative for H7N9. The patient recovered after intensive treatment with oseltamivir and peramivir [201, 202].

## CONTROL OF H7 IN POULTRY

Classical eradication of avian influenza in poultry was based on enforcement of biosecurity measures, surveillance and culling of infected birds and/or those in a quarantine zone. Nevertheless, the costs of mass depopulation of poultry are unbearable, particularly in developing countries with an under-resourced poultry industry infrastructure [203]. Since the late 1990s, due to the continuing and widespread outbreaks of LPAIV, the use of vaccines for the control of H5 and H7 infections has been approved to control the disease in poultry and to prevent possible mutations to HPAIV [204].

### Vaccination

In the context of H7 viruses, several vaccines have been developed but only a few have been evaluated and used in the field to combat both LP and HP H7 outbreaks [30, 99]. Poultry was vaccinated using inactivated vaccines in Italy against HPAIV H7N1 in 2000–2002 and LPAIV H7N3 in 2002–2004 [129], in Pakistan against HPAIV H7N3 in 1995–2004 [136, 137], in USA, Connecticut against LPAIV H7N2 in 2003 [99] and, most recently, against the on-going H7N3 outbreak in Mexico since 2012 [36, 132]. A bivalent H7/H9 oil-based inactivated vaccine was used in Pakistan [99] and a corresponding H5/H7 vaccine was used in Italy [129]. A number of outbreaks of H7N1 and H7N3 HPAIV were also reported in vaccinated commercial turkeys and chickens, as the vaccines were not capable of completely

preventing infection [136, 205]. Research trials for vaccination of chickens (and turkeys) against H7 viruses are summarized in Supplementary Table S1 (available online).

#### *Conventional inactivated vaccines*

Inactivated virus preparations are the most widely used type of vaccine to protect different poultry species against virulent or avirulent AIV strains. However, the delayed onset of a solid protection [2–3 weeks post-vaccination (p.v.)], the short lifespan of broilers, the need of individual administration by parenteral routes, local reactions to the vaccines (and/or formalin content) and high production costs are the main disadvantages of inactivated vaccines [206]. Importantly, the use of conventional homologous inactivated vaccines can interfere with the serological surveillance in naive poultry due to the induction of undistinguishable AIV-specific antibodies. Furthermore, high antibody titres after vaccination can mask an active infection with any field viruses [207].

A number of experimental studies have been conducted to develop and evaluate H7 vaccines in different laboratories with variable standards, vaccines and results (Supplementary Table S1). Chickens that received a primary vaccination with an inactivated LPAIV H7N1 at 3 days or 3 weeks of age, or received another dose 4 weeks post-primary vaccination were fully protected from morbidity, mortality and virus replication after challenge with HPAIV H7N1 at 4 weeks or 20 weeks p.v. [208]. The maximum level of antibody response was obtained 3–4 weeks after the primary immunization [208]. Most recently, several H7N3 inactivated vaccines given at different ages (1 day, 3, 7 or 22 weeks) protected chickens against the new Mexican HPAIV H7N3 at 3 weeks p.v. [36, 209], while H7N2 vaccine induced 90% protection [36]. In another study, vaccination of 3-week-old chickens with inactivated forms of four antigenically different H7N3 and one H7N7 HPAIV induced a broad and variable humoral immune response at 3 weeks p.v. When challenged with two antigenically distinct Pakistani HPAIV H7N3, vaccinees were clinically protected and excreted only low amounts of virus from the respiratory tract [210]. An intramuscular immunization of 3-week-old chickens with HPAIV H7N7 vaccines with varying antigen content conferred protection against challenge with a homologous HPAIV H7N7 at 2 weeks

p.v. [211]. In another study, 6-week-old chickens were vaccinated with commercially available H7N1 and H7N3 vaccines and challenge infection was performed 1–2 weeks later with HPAIV H7N7 [212]. All vaccinated birds were clinically protected, but virus shedding and transmission to unvaccinated contact chickens were remarkably lower in H7N1-vaccinated birds [212]. Vaccination of chickens and turkeys with bivalent H5/H7 vaccines at 19 and 40 days of life protected against LPAIV and HPAIV H7N1 at 10 days p.v. [213]. In that experiment HPAIV, but not LPAIV, was isolated from lungs and no virus was isolated from muscles in any of the vaccinated birds [213]. Vaccination of 8- and 30-day-old turkeys with LPAIV H7N1 prevented clinical disease and viral shedding after challenge with heterologous LPAIV H7N3 at 71 days [214]. Similarly, vaccinating turkeys once or twice with H7N1 protected the birds from clinical signs, mortality, totally blocked viral shedding and prevented transmission to in-contact turkeys after challenge with HPAIV H7N7 [215]. Moreover, a single vaccination with a H7N2 vaccine protected chickens and turkeys against challenge with the homologous LPAIV H7N2 [216].

To overcome low antigenic identity mainly of HA between vaccines and field viruses, reverse genetics (rg) were increasingly used to develop matching viruses for use in inactivated forms for vaccination. Chickens, that were immunized twice at ages 2 and 4 weeks with a whole H7N2 or a reassortant rgH7N8 virus vaccine were fully protected after infection with H7N2 virus at 2 weeks p.v. [217]. Similarly, an inactivated rg-LPAIV H7N7 vaccine containing the PB2, PB1, PA, HA, NA and NS genes from a wild duck H7N7 virus and the NP and M genes from a rapidly growing H9N2 virus [54] induced peak serum HI titre between 3 and 6 weeks p.v. No clinical signs were observed in chickens challenged 10–21 days p.v. with HPAIV H7N1 and viral excretion was significantly reduced compared to the control group [54]. In another study [218] an HPAIV H7N1 was attenuated by altering the caspase cleavage motifs of the NP and M2 proteins. An intramuscular immunization of 11-day-old chickens with this virus protected the chickens against challenge with a lethal dose of wild-type virus at day 21 p.v. Taken together, the results obtained in these studies may indicate that protection afforded by H7 vaccines is less affected by antigenic variation as reported for H5N1 viruses recently described in China and Egypt [219, 220].

*Attenuated live vaccines*

In contrast to the situation in humans, the use of attenuated live influenza vaccines in poultry is not recommended by the OIE or the Food and Agriculture Organization of the United Nations (FAO) due to the potential risk of reassortment or mutations generating HPAIV [221], although experimental research showed the effectiveness of live attenuated vaccines to protect poultry against H7 viruses. Intranasal inoculation with LPAIV H7N2 not only clinically protected 1-day-old chickens against lethal infection with HPAIV H7N1 at age 15 days but also decreased viral shedding [79]. Moreover, intratracheal infection of 4-week-old chickens with LPAIV H7N3 conferred full protection against HPAIV H7N7 at 4 weeks p.v. and interrupted virus transmission [222]. Furthermore, *in ovo* vaccination with live attenuated vaccines can induce humoral- and cellular-mediated immune responses, which is advantageous compared to inactivated vaccines, where humoral immune response is induced. In a study conducted by Cai and co-workers [223] the proteolytic cleavage site from an AIV H6 was inserted into the HA of a H7 LPAIV, which improved its *in vitro* replication. Birds that were vaccinated with the rgH7 virus vaccine by the *in ovo* route had reduced viral titres from trachea and excretion of the virus was not detected in cloacal swabs at 2 or 6 weeks p.v. [223]. Altogether, the effectiveness of live attenuated vaccines and the risk of a possible reassortment with field viruses should be extensively studied and weighed against rapid onset of protection in the face of an outbreak, particularly to protect valuable birds.

*Recombinant viral-vectored vaccines*

The use of viruses as a vector for the HA (and NA) gene(s) is an alternative approach to deliver the main immunogenic determinant(s) of influenza virus in a form of live vaccine. The use of such recombinant viruses can provide a tool for differentiating AIV-infected from vaccinated animals (DIVA) (e.g. through detection of anti-NP antibodies) and, using suitable viral vectors, can be applied by mass vaccination (e.g. spray, drinking water, etc.). A major challenge for the use of recombinant avian influenza vaccines in commercial poultry is the existence of immunity against the vector virus due to previous infection or vaccination (or maternal immunity), which may interfere with stimulation of the immune system after vaccination with the recombinant vaccines.

Although H5-expressing viral-vectored vaccines have already been used in the field to control HPAI H5 outbreaks in poultry (i.e. in Mexico, China, Egypt), so far there is no record for H7, although several effective viral-vectored vaccines against this subtype have been developed (Supplementary Table S1).

*Fowl pox virus (FPV)*

Chickens vaccinated via the wing web route at age 7 days with FPV expressing H7 HA from an Australian chicken-origin virus or North-American seal-origin virus (Supplementary Table S1) survived an infection with Australian HPAIV H7N7 at 21 days p.v., whereas ~77% and ~87% of chickens vaccinated via the wing web or subcutaneously, respectively, at age 2 days with the Australian virus survived the infection at age 12 days [224]. Interestingly, a recombinant FPV co-expressing the H5 and N1 genes protected chickens clinically against an intramuscular infection with HPAIV H7N1 but did not prevent cloacal excretion of the challenge virus [225]. Further, FPV expressing H7 HA from a Eurasian H7N3 virus via the wing web or subcutaneously protected 90% of chickens from infection with Eurasian HPAIV H7N3 [206], but failed to induce protection against the Italian HPAIV H7N1 or against strains from American or Australian lineages [206, 226]. In a recent study, rFPV vaccine carrying the H7 gene from a North American LPAIV H7N2 delivered subcutaneously to 1-day-old layer chickens protected birds against an intranasal inoculation of the recent Mexican HPAIV H7N3 3 weeks later [209]. A few vaccinated chickens excreted the virus 4 days post-infection but a booster dose with an inactivated HPAIV H7N3 vaccine at age 3 weeks blocked any viral excretion [209]. FPV expressing the H5, H7 HA and chicken interleukin-18 genes was able to induce high levels of humoral- and cellular-mediated immune response and increased the body weight gain of experimentally infected chickens; however, a challenge experiment against H7 viruses was lacking [227]. Generally, individual subcutaneous (wing web route) injection of FPV recombinant vaccines, the limited host range of FPV (chickens only) and prior anti-FPV antibodies as observed in H5 experiments are the main limitations of its use in the field [228].

*Adenovirus (Ad-5)*

A recombinant, non-replicating H7 HA human adenovirus serotype 5 (Ad5)-vectored vaccine was

developed [229, 230]. An intramuscular injection of chickens with this vaccine at age 4 weeks elicited high antibody titres and all chickens were protected after challenge with a heterologous H7N3 HPAIV at 8 weeks p.v. *In ovo* vaccination with monovalent adenovirus-H7 or bivalent adenovirus H7-H5 HA recombinant vaccines induced high antibody titres between 25 and 45 days after hatch [229, 231]. Administration of the bivalent vaccine by a mass vaccination route by course spray to 1-day-old chickens induced local IgA antibodies in tears but no detectable serum antibodies [231]. Importantly, the induction of anti-H7 antibodies was not impaired even in the presence of anti-Ad5 antibodies [229, 231].

#### *Newcastle disease virus (NDV)*

The use of NDV as a vector for generation of recombinant vaccines against AIV has been frequently reported. In contrast to H5-expressing NDV-vectored vaccines, only three studies evaluated the development of NDV as vector for the expression of H7 HA. Swayne and colleagues [232] constructed a recombinant NDV H7-expressing HA of a North American H7N2 virus. Chickens that were vaccinated once or twice mounted a weak and moderate serological response, respectively. Moreover, only 10% of once-vaccinated and 40% of twice-vaccinated chickens were protected from HPAIV H7N7 challenge. In a further study, improvement of recombinant NDV H7 was described [233] and a single eye-drop immunization of chickens with this modified vaccine induced 90% protection against intranasal infection with a HPAIV H7N7 at 2 weeks p.v. In the third study a recombinant NDV expressing HA from a Eurasian HPAIV H7N1 was developed. A single oculonasal immunization of 3-week-old chickens was sufficient to protect all birds from a high lethal dose of homologous HPAIV H7N1 at 3 weeks p.v.; however, limited replication of challenge virus was observed [234].

#### *Herpes virus*

A single vaccination of chickens with a recombinant infectious laryngotracheitis (ILT) virus carrying H7 HA from an Italian HPAIV H7N1 afforded protection against challenge with a lethal dose of homologous HPAIV H7N1 without showing serious disease, and reduced the excretion of the virus compared to the control group [235]. Generation of a Herpesvirus of Turkeys (HVT) recombinant expressing HA of a HPAIV H7N1 has also been described. Vaccination

of 1-day-old chickens induced a considerable humoral antibody response at 6 weeks p.v. Five out of seven chickens infected with homologous HPAIV H7N1 remained healthy and had significantly reduced buccal and/or cloacal excretion of the virus after challenge [236].

#### *Vesicular stomatitis virus (VSV)*

After two intramuscular immunizations of chickens with non-transmissible VSV expressing HA of a HPAIV H7N1, the birds showed only minimal signs of disease after a heterologous virus infection, had reduced viral shedding and no pathological lesions were observed [237]. Serum samples collected from the boosted chickens also showed significant humoral antibody titres against two distantly related H7N1 and H7N7 viruses. Similarly, vaccination with a VSV recombinant expressing the HA antigen, and additionally the NP antigen, did not improve the protection level [237].

#### *Retrovirus*

An early study conducted by Hunt *et al.* [222] described the development of a recombinant vaccine expressing HA from seal-origin H7N7 AIV in a Rous sarcoma virus-derived vector, a replication-competent avian leukosis virus. Despite low humoral and neutralizing antibody titres post-intramuscular vaccination of 4-week-old chickens, birds were protected against intranasal inoculation with a chicken-origin HPAIV H7N7 [222].

#### *Baculovirus*

A recombinant baculovirus expressing H7 HA from a North American H7N2 virus was successfully constructed [238]. The vaccine induced considerable antibody titres after a single immunization of 2-day-old White Rock chickens. Challenge of vaccinated birds was done at 40 days p.v. using two different HPAI H7N7 viruses that belong to the Eurasian and North American lineages at 60 years apart. H7 (or H5-H7) expressing baculovirus vaccines induced 100% protection of vaccinated birds against LP H7N1 and HP H7N7 challenge. Viral excretion was significantly reduced 3 days post-infection and correlated negatively with increasing antigen content of the vaccine.

### DNA vaccines

In two early studies [239, 240] two immunizations of immunocompetent chickens at ages 3 and 7 weeks by intramuscular, subcutaneous and intraperitoneal routes with H7-expressing plasmid DNA from seal HPAIV H7N7 gave protection from an intranasal lethal dose of HPAIV H7N7. In another study, chickens were vaccinated twice at 3-week intervals, with plasmids containing the H7 genes from Australian or European HPAI H7N7 viruses. The chickens were fully protected against challenge with HPAIV H7N7 3 weeks after the second vaccination. However, vaccination with a plasmid containing the NP gene from a H5N1 virus was not effective in protecting chickens against that H7N7 virus [241]. Another experiment showed that a single- or two-dose vaccination of 3-week-old chickens with a H7 HA-expressing plasmid was able to afford clinical protection and reduce viral shedding after challenge with homologous HPAIV H7N1 [242]. In two different experiments chickens received two immunizations of DNA vaccines encoding the H7 gene alone or in combination with the M gene from an Italian LPAIV H7N1, which had induced high humoral antibody titres at 3 weeks p.v. After infections with different LPAI H7N1 viruses (Supplementary Table S1) all birds remained healthy and excreted no or low virus quantities [243, 244].

### Therapy

The generation of rapid protection, suitability for mass administration (e.g. via feed or drinking water), and suitability for all types of birds and against all types of AIV are desirable features of therapeutics for the control of AIV. However, due to several limitations concerning availability, applicability, costs, efficacy and health hazards the use of antivirals has not been regularly or widely applied for combating avian influenza in commercial poultry [245] and only limited experimental research on this subject is available. It was found that oseltamivir was non-toxic for chicken embryos and was able to stop HPAIV H7N1 from replicating in inoculated eggs [246]. The most available antiviral drug is amantadine which was able to reduce the replication of three different H7 viruses *in vitro* if added to the cell culture 1 h before infection [247]. Moreover, sensitivity of HPAIV A/chicken/Germany/34 H7N1 to amantadine has been frequently studied in earlier publications [247–249]. Similar results were obtained using 3-deazaadenosine (3DA-Ado) and

1-(5-isoquinolinesulphonyl)-2-methylpiperazine against HPAIV H7N1 [249]. Moreover, amantadine and green tea extract protected chicken embryos from a H7N3 infection [250]. Inhibition of HPAIV H7N7 *in vitro* was also obtained after exposure to Echinaforce<sup>®</sup> (extract of *Echinacea purpurea* herb and roots) [251]. On the other hand, genetic analysis conducted by Ilyushina *et al.* [252] indicated that amantadine-resistance markers in H7 viruses existed only in the North American lineage between 2000 and 2004 including H7N2 of chicken origin. Moreover, in absence of any antiviral drug application amantadine-resistance markers existed in H7N2 viruses in LBMs in the USA [12] and in a wild-bird-origin H7 virus in Europe [25]. Selection of amantadine-resistance variants preclude the large-scale application in domestic poultry; however, it might be valuable to protect zoo birds or unique birds in the face of an outbreak [245].

### GENERAL REMARKS AND PERSPECTIVES

AIV of the H7 subtype is prevalent in wild birds and domestic poultry and can accidentally infect humans. Surveillance of wild birds may be helpful to assess the risk for spillover into domestic poultry. The H7N7 subtype is endemic in Europe and these viruses were frequently reported in domestic poultry where ten European countries reported H7N7 outbreaks, of which seven reported the HP phenotype. In contrast, H7N3 was predominant in wild birds and domestic poultry in the Americas. Ecological and genetic traits enabling establishment of these viruses in wild birds and favouring transmission to domesticated poultry need further investigation. In domestic poultry, HPAIV was restricted to subtypes H7N1 (Italy), H7N3 (Australia, Canada, Chile, Mexico, Pakistan, UAE), H7N4 (Australia) and H7N7 (Australia, Belgium, Italy, Germany, The Netherlands, North Korea, Spain, UK). All but four outbreaks in poultry were successfully eradicated within a few months; LPAIV H7N1 in South Africa, HPAIV H7N3 in Mexico, HPAIV H7N7 in Italy and LPAIV H7N9 in China are still on-going. Apart from the Dutch HPAI H7N7 outbreak in 2003, which extended to Germany and Belgium, no other H7 outbreak in domestic poultry was confirmed to be transboundary. Generally, it has been noted that there is a shortage in complete genome characterization of viruses isolated from wild birds and domestic poultry,



particularly the LP pathotypes, and greater efforts are required on this issue.

Five of the eight episodes of AIV infections in humans were caused by subtype H7N7. On the other hand, no evidence of human infection with H7N1 in Italy in the devastating epidemic of 1999–2001 was obtained. Whether the pre-circulation of H1N1 viruses in humans can protect against or mask an infection with H7N1, or whether the latter has less zoonotic potential compared to H7N7 virus remains to be elucidated. Lack of seroconversion in H7-infected persons raises a concern on the actual number of infected humans and also questions the sensitivity of current diagnostics [253]. It should be noted that the prophylactic use of oseltamivir was found to reduce the seroprevalence of H7 antibodies in professionals exposed to infected poultry significantly [253, 254]. Moreover, asymptomatic self-limiting infections with LPAIV H7 may indicate unnoticed widespread infections in humans and may favour reassortment with human influenza viruses (i.e. H1N1). Therefore, we suggest more in-depth research to investigate the gene constellation/mutations required for an effective establishment or the transmission of H7 viruses in/among humans or after reassortment with H1N1 viruses as recently discovered for H5N1 [255, 256]. In humans, high and low pathogenicity of AIV should be defined. It is not necessarily deducible that HPAIV of the H7 subtype in poultry is also of high virulence in humans. In The Netherlands HPAIV H7N7 was highly pathogenic in poultry with only one fatal human case. Furthermore, other reported human cases infected with HPAIV H7N7 (or H7N3 in Mexico) exhibited self-limiting disease and rarely required hospitalization. In contrast, infection with LPAIV H7N9 caused the death of a significant number of humans in China within few weeks, although it induced no clinical signs in poultry, turning humans to sentinels for AIV infection in chickens. Together, infections with LPAIV should not be neglected because in poultry: (1) it is more prevalent than HPAIV, (2) it induces health disturbance and production disorders *per se* or in the case of secondary infection, (3) it has the potential to mutate into highly pathogenic forms and decimate poultry flocks within few days, and (4) in humans, it can cause fatal infection or (5) can silently circulate and be a potential source for reassortment with human influenza viruses.

To reduce the risk of human infections by domestic poultry, infection in birds must be rapidly eliminated.

Prevention of contact of backyard or commercial poultry with wild birds, continuous surveillance of LBMs and vaccination of poultry against H7 are effective tools to control the infection in domestic poultry. The limited genetic and antigenic variation of H7 viruses from different species and different times can help in production of a universal vaccine; e.g. a seal-origin H7 virus protected chickens from HPAIV H7 infections and a recombinant baculovirus H7-vectored vaccine protected chickens against a virus from 60 years ago (Supplementary Table S1). It seems also that compared to H5, H7 vaccines are less affected in their efficacy in case of a lower HA protein identity to the challenge virus; a minimal identity of 84% was capable of providing 100% protection (Supplementary Table S1). Nevertheless, all vaccine studies reviewed herein were conducted in the absence of maternal immunity, which has been found to interfere with an active vaccination of chickens at an early age. Moreover, long-term use of the vaccines will generate immune-escape variant strains and none of these studies was conducted with *in vitro* generated antigenic-drift variants. Additionally, most of these studies were conducted in chickens, only rarely in turkeys, and not at all in other birds. A therapeutic approach to control H7 viruses may be useful as an ancillary tool for rapid protection regardless of the virus subtype or bird species. However, most of the therapeutic studies are historic and were conducted on old isolates and more research on this topic is required.

In conclusion, H7 AIV is of great importance for both the poultry industry and human health. Many questions and few answers are available on the epidemiology, pathobiology and virus evolution and more research is required to improve our current understanding in order to effectively control infection in poultry and reduce or prevent the potential of a pandemic occurring.

#### SUPPLEMENTARY MATERIAL

For supplementary material accompanying this paper visit <http://dx.doi.org/10.1017/S0950268813003324>.

#### DECLARATION OF INTEREST

None.

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