Factors that trigger human infection with animal influenza virus progressing into a pandemic are poorly understood. Within a project developing an evidence-based risk assessment framework for influenza viruses in animals, we conducted a review of the literature for evidence of human infection with animal influenza viruses by diagnostic methods used. The review covering Medline, Embase, SciSearch and CabAbstracts yielded 6,955 articles, of which we retained 89; for influenza A(H5N1) and A(H7N9), the official case counts of the World Health Organization were used. An additional 30 studies were included by scanning the reference lists. Here, we present the findings for confirmed infections with virological evidence. We found reports of 1,419 naturally infected human cases, of which 648 were associated with avian influenza virus (AIV) A(H5N1), 375 with other AIV subtypes, and 396 with swine influenza virus (SIV). Human cases naturally infected with AIV spanned haemagglutinin subtypes H5, H6, H7, H9 and H10. SIV cases were associated with endemic SIV of H1 and H3 subtype descending from North American and Eurasian SIV lineages and various reassortants thereof. Direct exposure to birds or swine was the most likely source of infection for the cases with available information on exposure.

Introduction
Influenza virus type A, a member of the family Orthomyxoviridae, is an enveloped virus with a negative-sense, single-stranded RNA genome organised in eight gene segments, which encode at least eleven proteins. Antigenic and genetic diversity of two surface glycoproteins, haemagglutinin (HA) and neuraminidase (NA), is used to classify type A influenza viruses into subtypes; 18 HA and 11 NA subtypes are known to date [1-5]. Water- and shorebirds were identified as reservoirs harbouring all subtypes, except A(H17N10) and A(H18N11) of which RNA was recently detected in bats from Guatemala and Peru, respectively [2,3]. Reservoir animals typically do not display symptoms. In contrast, the diversity of influenza viruses in mammalian hosts is limited to specific subtypes. Human-adapted seasonal influenza viruses since the beginning of the 20th century have had HA subtypes H1, H2 and H3, combined with NA subtypes N1 and N2.

The segmented nature of the genome facilitates the exchange of genetic material if a host is co-infected with two genetically different type A influenza viruses. This reassortment process, also known as antigenic shift if it involves the gene segment encoding the HA, can result in the generation of viruses with surface antigens against which the human population may not have pre-existing, protective antibodies. Additional flexibility is conferred by the accumulation of mutations during replication, potentially resulting in amino acid substitutions that can affect pre-existing immunity if the HA is involved (antigenic drift), host range, virulence, and other factors [6]. If this results in sustained
human-to-human transmission of a virus against which a large proportion of the world’s human population is immunologically naïve a pandemic can develop resulting in a large number of human cases occurring simultaneously worldwide [7,8]. Such novel introductions of reassorted viruses were at the root of four influenza pandemics in the last 100 years, and claimed the lives of millions of people, namely the ‘Spanish flu’ A(H1N1) in 1918, the ‘Asian flu’ A(H2N2) in 1957, the ‘Hong Kong flu’ A(H3N2) in 1968, and the recent pandemic caused by influenza A(H1N1)pdm09 in 2009 [9,10]. Influenza A(H1N1)pdm09 has replaced previous human seasonal A(H1N1) viruses [11] and, together with A(H3N2) and influenza B viruses, has been causing seasonal influenza epidemics in humans since 2009. With the emergence of the influenza A(H3N2) pandemic in 1968, influenza A(H2N2) viruses ceased to circulate in humans, but H2 subtypes are still present in birds and were also recently isolated from diseased swine [12,13].

The factors that determine whether an animal influenza virus may acquire the ability to efficiently spread among humans are poorly understood [14]. Reassortment is not a necessary prerequisite for human infection, and there is clear documentation of direct transmission and human disease caused by animal influenza viruses, in particular avian (AIV) and swine (SIV) influenza viruses, such as AIV A(H5N1), A(H9N2) and various H7 subtypes, as well as European avian-like SIV A(H1N1) [15-22]. Early detection and in-depth investigation of such events may provide clues for (future) risk assessment of animal-to-human transmissions.

This review was conducted under the framework of the FLURISK project funded by the European Food Safety...
Table 1a
Virological evidence of human infection with avian influenza A viruses, excluding high pathogenicity A(H5N1)

| Year | Confirmed cases | Subtype | Symptom | Method | Location | Reference |
|------|-----------------|---------|---------|--------|----------|-----------|
| 1959 | 1/1             | H7N7    | Unknown | Culture in embryonated chicken eggs; subtyping with specific antisera | United States | Campbell et al. 1970 [52] |
| 1977 | 1/1             | H7N7    | Keratoconjunctivitis | Virus isolation | Melbourne, Australia | Taylor and Turner 1977 [53] |
| 1979 | 1/1             | H7N7    | Keratoconjunctivitis | Virus isolation | United States | Beare and Webster 1991 [75] |
| 1991 | 11/2, 14/3, 15/6 | H6N1, H4N8, H10N7 | Ranging from respiratory and some constitutional symptoms to no symptoms | Culture in embryonated chicken eggs | United States | Beare and Webster 1991 [75] |
| 1996 | 1/1             | LP H7N7 | Conjunctivitis | Culture on rhesus monkey kidney cells | United Kingdom | Kurtz et al. 1996 [55] |
| 1998 | 5/5             | H9N2    | Acute respiratory symptoms | Culture in embryonated chicken eggs, HI and NI assay | Shantou (n=3), Shaoguan (n=2), China | Guo et al. 1999 [70] |
| 1999 | 2/2             | H9N2    | Mild ILI | Cultured by WHO reference laboratory | Hong Kongb | Peiris et al. 1999 [16] |
| 1999 | 1/1             | H9N2    | Fever, cough, bronchitis | MDCK cell culture, HI and NI assay | Guangzhou, China | Guo et al. 2000 [71] |
| 2003 | 453/89 (including one fatality) | H7N7 | Conjunctivitis, ILI (fatality: fever, pneumonia, multi-organ failure, respiratory insufficiency) | Cell culture (used for the first 25 confirmed cases, afterwards RT-PCR used as screening method); typed and subtyped by HI assay (turkey RBC), RT-PCR | Gelderland, Nijmegen, The Netherlands | Koopmans et al. 2003; Fouchier et al. 2003; Osterhaus et al. 2003; Osterhaus et al. 2004; Fouchier et al. 2004; van der Groen et al. 2004; van Regenmortel et al. 2004 | Hout et al. 2006 [66] |
| 2004 | 2/2             | LP and HP H7N3 | Conjunctivitis, mild ILI | RT-PCR, cell culture, sequencing | British Columbia, Canada | Tweed et al. 2004 [18] |

Where more than one human case is stated, diagnostic tests were performed for all cases as stated in the Methods section, unless otherwise specified.

HI: haemagglutination inhibition; HP: highly pathogenic; ILI: influenza-like illness; LP: low pathogenic; MDCK: Madin Darby canine kidney; MN: microneutralisation; n.a.: not applicable; Ni: neuraminidase inhibition; PCR: polymerase chain reaction; RBC: red blood cells; RT-PCR: reverse transcription polymerase chain reaction; WHO: World Health Organization.

a In most cases the number tested reflects only the confirmed cases reported in the reviewed paper where the authors did not report on the true number of suspected cases tested.
b Hong Kong Special Administrative Region (SAR), China.
### Table 1b

Virological evidence of human infection with avian influenza A viruses, excluding high pathogenicity A(H5N1)

| Year | (Tested)/ confirmed cases | Subtype | Symptoms | Method | Patient information and nature of exposure | Location | Reference |
|------|---------------------------|---------|----------|--------|---------------------------------------------|----------|-----------|
| 2004 | 2/2                       | H10N7   | 'Illness' (not further specified) | Virus isolation | Infants with indirect contact to poultry (father of one is poultry merchant) | Egypt | Pan American Health Organization, 2004 [76] |
| 2006 | 1/1                       | LP H7N3 | Conjunctivitis | PCR (no serological confirmation reported) | Source: infected poultry | United Kingdom | Nguyen-Van-Tam et al. 2006 [57] |
| 2007 | 4/4                       | LP H7N2 | Conjunctivitis, ILI | Confirmed influenza A+ | Source: Infected poultry | United Kingdom | Editorial team, 2007 [58] |
| 2007 | 1/1                       | H9N2    | Mild ILI | Information not available | Source: probably a bird market | Hong Kongb | United States Centers for Disease Control and Prevention 2008 [67] |
| 2008, 2009 | 2/2d                 | H9N2    | ILI, vomiting, dyspnoea | Rapid test, RT-PCR; MDCK cell culture; immunofluorescence assay; sequencing | Immunocompromised persons (with and without poultry contact) | Shenzhen and Hong Kongb | Cheng et al., 2011 [68] |
| 2010 | 7/2                       | H10N7   | Conjunctivitis, rhinorrhoea, sore throat | PCR, partial sequencing of haemagglutinin genes (no virus culture); no evidence of seroconversion | Abattoir workers exposed to infected poultry | New South Wales, Australia | Arzey et al. 2012 [20] |
| 2011 | 1/1                       | H9N2e   | Fever, headache, runny nose, cough, sneezing | Partial sequencing | 51-month-old female exposed to slaughtered chickens | Bangladesh | International Centre for Diarrhoeal Disease Research, Bangladesh 2011 [69] |
| 2012 | 2/2                       | HP H7N3 | Conjunctivitis; no fever or respiratory symptoms | rtRT-PCR (n=2), culture in embryonated chicken eggs (n=3), sequencing (n=1) | 32- and 52-year-old female/male poultry worker exposed to infected poultry | Jalisco, Mexico | United States Centers for Disease Control and Prevention 2012 [59] |
| 2013 | 251/251 (including 56 fatalities) | LP H7N9 | Ranging from mild symptoms and recovery to severe respiratory symptoms and death | Virus isolation, PCR | Source: not specified | Shanghai, Beijing, Hong Kongg, Anhui, Fujian, Jiangsu, Jiangxi, Guangdong, Guizhou, Henan, Hunan, Hebei, Shandong, Zhejiang, provinces, China; two cases were imported to Taiwan from mainland China | European Centre for Disease Prevention and Control updated rapid risk assessment [23] |

Where more than one human case is stated, diagnostic tests were performed for all cases as stated in the Methods section, unless otherwise specified.

HI: haemagglutination inhibition; HP: highly pathogenic; ILI: influenza-like illness, LP: low pathogenic; MDCK: Madin Darby canine kidney; MN: microneutralisation; n.a.: not applicable; NI: neuraminidase inhibition; PCR: polymerase chain reaction; RBC: red blood cells; RT-PCR: reverse transcription polymerase chain reaction; rtRT-PCR, real-time reverse transcription polymerase chain reaction; WHO: World Health Organization.

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* In most cases the number tested reflects only the confirmed cases reported in the reviewed paper where the authors did not report on the true number of suspected cases tested.

* Hong Kong Special Administrative Region (SAR), China.

* Authors assume that influenza A-positive test suggests influenza A(H7N2) infection because of close temporal-spatial links with influenza A(H7N2)-infected poultry and low seasonal influenza activity at that time.

* One patient and her asymptomatic husband showed a titre of 80 in the microneutralisation assay, three and two weeks after onset of illness respectively.

* Belongs to G1 lineage.

* At the time of writing (31 January 2014) the number of cases was still on the increase.
| Year | (Tested)\(^a\)/confirmed cases | Subtype | Symptoms | Method | Patient information and nature of exposure | Location | Reference |
|------|---------------------------------|---------|----------|--------|------------------------------------------|----------|-----------|
| 2013 | 1/1                             | H6N1    | ILI, mild pneumonia | Virus isolation, full genome sequencing | 20-year-old female; no exposure to poultry | Taiwan   | Centers for Disease Control 2013 [79] |
| 2013 | 1/1                             | H10N8   | Severe pneumonia and death | Not specified | 73-year-old immunocompromised female with underlying illness; exposed to live poultry | Jiangxi province, China | ProMED 2013 [77] |
| 2013 | 1/1                             | H9N2    | Chest infection, low fever, chills and cough | Not specified | 86-year-old male with underlying illness; no recent poultry exposure | Shenzhen/Hong Kong\(^b\) | ProMED 2013 [72] |
| 2014 | 1/1                             | H9N2    | Illness (not further specified); recovered | Not specified | 7-year-old male with poultry exposure | Hunan, China | ProMED, 2014 [73] |
| 2014 | 1/1                             | H10N8   | Sore throat, dizziness, loss of strength, severe pneumonia\(^c\) | Not specified | 55-year-old female; visited agricultural market | Jiangxi, China | ProMED 2014 [78] |
|      | Total                           | 784/386 |           |        |                                          |          | 29        |

Where more than one human case is stated, diagnostic tests were performed for all cases as stated in the Methods section, unless otherwise specified.

HI: haemagglutination inhibition; HP: highly pathogenic; ILI: influenza-like illness, LP: low pathogenic; MDCK: Madin Darby canine kidney; MN: microneutralisation; n.a.: not applicable; NI: neuraminidase inhibition; PCR: polymerase chain reaction; RBC: red blood cells; RT-PCR: reverse transcription polymerase chain reaction; rtRT-PCR, real-time reverse transcription polymerase chain reaction; WHO: World Health Organization.

\(^a\) In most cases the number tested reflects only the confirmed cases reported in the reviewed paper where the authors did not report on the true number of suspected cases tested.

\(^b\) Hong Kong Special Administrative Region (SAR), China.

\(^c\) At the time of writing (31 January 2014) the patient was hospitalised and in critical condition.
| Year     | (Tested)/confirmed cases | Subtype | Symptoms | Method | Patient information and nature of exposure | Location | Reference |
|----------|--------------------------|---------|----------|--------|--------------------------------------------|----------|-----------|
| 1974     | 1/1 (fatal)              | swH1N1  | Pneumonia | Culture on WI-3B-, HeLa- and rhesus-monkey kidney-cell, inoculation of mice; HI assay (guinea pig RBC) | 16-year-old Hodgkin’s disease patient living on swine farm | Minnesota, United States | Smith et al. 1976 [42] |
| 1976     | 20/5 (including 1 fatality) | swH1N1  | Acute respiratory illness, pneumonia | Embryonated chicken egg culture, HA assay | Previously healthy soldiers without known exposure to swine | New Jersey, United States | Gaydos et al. 1977 [28] |
| 1976     | 1/1                      | swH1N1  | Mild ILI | Virus isolation; HI assay | 22-year-old swine worker exposed to ill, influenza-positive swine | Wisconsin, United States | United States Centers for Disease Control and Prevention 1976 [29] |
| 1976     | 1/1                      | swH1N1  | ILI      | Virus isolation; HI assay | 13-year-old boy living on swine farm | Wisconsin, United States | United States Centers for Disease Control and Prevention 1976 [29] |
| 1979/80  | 2/2                      | swH1N1  | ILI      | Virus isolation; embryo chicken egg culture; HI assay | 20-year-old fell ill after close contact with swine; 6-year-old visitor of livestock show without direct contact with swine | Texas, United States | Dacso et al. 1984 [31] |
| 1982     | 1/1 (fatal)              | swH1N1  | Pneumonia | Cynomolgus monkey kidney cells; embryonated chicken egg culture; RNA-oligonucleotide mapping | 4-year-old female leukemia patient; no known exposure to swine | Nevada, United States | Patriarca et al. 1984 [32] |
| 1983     | 3/3                      | swH1N1  | Information not available | Isolation | 65-year-old male with occupational exposure to swine; 10-year old female and 27-year-old male with unknown exposure to swine | Russia | Chuvakova et al. 1985 [27] |
| 1986     | a) 1/1, b) 2/2           | swH1N1  | a) Pneumonia, b) Mild ILI | Various cell cultures; embryonated chicken egg culture; HI and NI assay | a) 29-year-old farmer exposed to ill, influenza-infected pigs, b) 50-year-old employee exposed to ill, influenza-infected pigs, and 3-year-old with no known contact with pigs | a) The Netherlands, b) Switzerland | De Jong et al. 1988 [33] |

Where more than one human case is stated, diagnostic tests were performed for all cases as stated in the Methods section, unless otherwise specified.

BSL: biosafety level; cl: classical swine lineage; HA: haemagglutination; HeLa: Henriette Lacks cervical cancer cells; HI: haemagglutination inhibition; ILI: Influenza-like illness; MAb: monoclonal antibodies; MDCK: Madin Darby canine kidney; MN: microneutralisation; n.a.: not applicable; NI: neuraminidase inhibition; RBC: red blood cells; RT-PCR: reverse transcriptase polymerase chain reaction; rRT-PCR: real-time reverse transcriptase polymerase chain reaction; sw: swine; SwL: swine-like; tr: triple reassortant; WI-38: human embryo lung fibroblast.

a In most cases the number tested reflects only the confirmed cases reported in the reviewed paper where the authors did not report on the true number of suspected cases tested.

b Number of humans from whom virus could be reisolated.

c Swine/Taiwan/7310/70 related to A/Hong Kong/1/68.

d Named A/Mayo Clinic/103/74, inhibited by antisera against A/swine/1976/31 and A/swine/Wisconsin/67.

e A/New Jersey/8/76.

f A/New Jersey/8/76-like influenza virus.

g A/Netherlands/386/86, A/Genova/5521/86, A/Genova/5200/86.

h Highest homology with European swine-influenza viruses.
## Table 2b

| Year     | (Tested)/confirmed cases | Subtype        | Symptoms                        | Method                                                                                     | Patient information and nature of exposure                                                                 | Location                    | Reference |
|----------|--------------------------|----------------|---------------------------------|--------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------|-----------------------------|-----------|
| 1988     | 1/1 (fatal)              | swLH1N1        | Pneumonia                       | RNA fingerprinting; partial sequencing;                                                   | 32-year-old pregnant woman exposed to pigs at county fair showing ILI                                      | Wisconsin, United States    | McKinney et al. 1990 [34] |
| 1991     | 1/1 (fatal)              | swLH1N1        | Pneumonia                       | Rhesus monkey kidney cell culture; embryonated chicken egg culture; rtRT-PCR; oligonucleotide mapping; HI and NI assay; sequencing; experimental infection of swine | 27-year-old animal caretaker exposed to swine showing respiratory symptoms                                | Maryland, United States     | Wentworth et al. 1994 [35] |
| 1992/93  | 2/2                      | swH3N2         | Mild respiratory symptoms       | Virus isolation; HI assay; sequencing                                                       | 1- and 2-year-old with no known exposure to swine                                                      | The Netherlands              | Claas et al. 1994 [41] |
| 1993     | 1/1                      | swH1N1         | Pneumonia                       | Tertiary monkey kidney cell culture; RT-PCR; immunofluorescence assay on MDCK cells; sequencing; HI assay | 5-year-old living on swine farm (health status of swine not known)                                      | The Netherlands              | Rimmelzwaan et al. 2001 [36] |
| 1994     | 2/2                      | swH1N1         | Mild ILI                        | Embryonated chicken egg culture; MDCK cell culture; HI- and NI-assay; sequencing; RT-PCR and other PCR-types; Experimental infection of swine | 39- and 30-year-old BSL3 laboratory workers exposed to influenza-infected pigs                           | Wisconsin, United States    | Wentworth et al. 1997 [37] |
| 1995     | 1/1 (fatal)              | swH1N1         | Severe pneumonia                | Virus isolation and subtyping                                                                 | 37-year-old healthy woman working on pig farm (health status of pigs unknown)                           | Minnesota, United States    | Kimura et al. 1998 [38] |
| 1999     | 1/1                      | H3N2           | Mild ILI                        | MDCK cell culture; HI and NI assay; RT-PCR; sequencing                                      | 10-month-old girl (neither she nor her family had recent contact with pigs)                             | Hong Kong                   | Gregory et al. 2001 [39] |
| 2002     | 1/1                      | H1N1           | ILI                             | MDCK cell culture; HI assay (also serologically confirmed by HI)                           | 50-year-old farmer (possibly from pigs showing respiratory symptoms)                                    | Switzerland                 | Gregory et al. 2003 [21] |
| 2004-05  | 1/1/11                   | HN8/N1         | Mild ILI                        | MDCK cell culture; HA assay (turkey RBC); HI assay; rapid tests; RT-PCR; sequencing          | 25- and 4-year-old male; neither had direct contact to pigs (incidental contact with backyard pigs could not be excluded) | Philippines, Thailand       | Komadina et al. 2007 [85] |

Where more than one human case is stated, diagnostic tests were performed for all cases as stated in the Methods section, unless otherwise specified.

BSL: biosafety level; cl: classical swine lineage; HA: haemagglutination; HeLa: Henriette Lacks cervical cancer cells; HI: haemagglutination inhibition; ILI: Influenza-like illness; MAb: monoclonal antibodies;
MDCK: Madin Darby canine kidney; MN: microneutralisation; n.a.: not applicable; NI: neuraminidase inhibition; RBC: red blood cells; RT-PCR: reverse transcriptase polymerase chain reaction; rtRT-PCR: real-time reverse transcriptase polymerase chain reaction; sw: swine; SwL: swine-like; tr: triple reassortant; WI-38: human embryo lung fibroblast.
a In most cases the number tested reflects only the confirmed cases reported in the reviewed paper where the authors did not report on the true number of suspected cases tested.

b Highest homology with European swine-influenza viruses.

H Patient's husband developed ILI symptoms one day before the patient (virus isolation not done)

i A/Maryland/12/91.
j Human-avian reassortants: A/Netherlands/5/93, A/Netherlands/35/93.
k Strain was similar to strain used in swine experiment and closely related to Sw/IN and A/WI/3523/88.
l Patient showed a titre of 160 by HI assay, the patient's mother a titre of 20, father, brother and grandparents titres of less than 10.
m Virus was closely related to viruses circulating in European pigs.

o Hong Kong Special Administrative Region (SAR), China.
p A/Switzerland/8808/02 (European avian-like lineage).
q Swine-like viruses: HA genetically similar to swine viruses circulating in swine in Asia at the time and to viruses that circulated in North America in the 1990s, whereas NA and internal genes were similar to European swine viruses.

r Both isolates tested negative against human strains.
s To determine whether isolates belong to influenza A or B.
# Table 2c

| Year | (Tested)/confirmed cases | Subtype | Symptoms | Method | Patient information and nature of exposure | Location | Reference |
|------|--------------------------|---------|----------|--------|-------------------------------------------|----------|-----------|
| 2005 | 1/1                      | trH1N1  | Sore throat, runny/stuffed nose, cough, fever | Cell culture, RT-PCR, sequencing | 50-year-old swine farm resident exposed to ill swine (not influenza confirmed) | Iowa, United States | Gray et al. 2007 [92] |
| 2005 | 1/1                      | trH1N1  | Acute mild respiratory illness; no fever | MDCK cell culture; RT-PCR; sequencing | Vaccinated one month before illness; assisted in butchering swine; | Wisconsin, United States | Newman et al. 2008 [89] |
| 2005 | 1/1                      | trH3N2  | ILI | Culture on primary rhesus monkey kidney cells; sequencing | Previously healthy swine farmer exposed to influenza-positive pigs | Canada | Olsen et al. 2006 [40] |
| 2006 | 1/1                      | swH3N2  | ILI | HI assay (guinea pig RBC); sequencing | 7-month-old child living on communal farm; no direct exposure to animals, seropositive swine found on farm | Canada | Robinson et al. 2007 [96] |
| 2007 | 1/1                      | trH3N2  | Parotitis, nasal congestion; no fever, cough or pharyngitis | Virus isolation; HI assay (turkey RBC); sequencing | 6-year-old boy living on swine farm (no illness in swine observed) | Canada | Bastien et al. 2009 [95] |
| 2007 | 26/2                     | swH3N1  | ILI | Virus isolation; sequencing | People exposed to ill swine at county fair | Ohio, United States | Vincent et al. 2009 [87]; Yassine et al. 2009 [88] |
| 2005–09 | a) 10/10 b) 1/1          | a) sw trH1N1 b) trH1N2 | Ranging from mild ILI to pneumonia | Rapid point of care test (n=8); Virus culture (n=7); rt RT-PCR (n=6); HI assay; complete genome pyrosequencing | Seven males (age: 16 months–36 years) our females (age: 4–48 years); exposure ranging from unknown contact, close proximity and direct contact with swine (some pigs were ill) | Wisconsin, Missouri, Iowa (n=3), Ohio (n=2), Illinois, Michigan, Minnesota and Texas, United State | Shinde et al. 2009 [93] |

Where more than one human case is stated, diagnostic tests were performed for all cases as stated in the Methods section, unless otherwise specified.

BSL: biosafety level; cl: classical swine lineage; HA: haemagglutination; HeLa: Henriette Lacks cervical cancer cells; HI: haemagglutination inhibition; ILI: Influenza-like illness; MAb: monoclonal antibodies; MDCK: Madin Darby canine kidney; MN: microneutralisation; n.a.: not applicable; Ni: neuraminidase inhibition; RBC: red blood cells; RT-PCR: reverse transcriptase polymerase chain reaction; rRT-PCR: real-time reverse transcriptase polymerase chain reaction; sw: swine; SwL: swine-like; tr: triple reassortant; WI-38: human embryo lung fibroblast.

* In most cases the number tested reflects only the confirmed cases reported in the reviewed paper where the authors did not report on the true number of suspected cases tested.
* Hs HA, Ns NA, M, NP, NS genes descended from classical swine-, PB1 from human- and PA and PB2 from avian influenza virus lineages
* Patient showed a two-fold titre increase in MN assay (not in HI assay) and four family members and the patient’s brother-in-law were seronegative by MN and HI assay.
* Predominant genotype of subtype H3N1 in North American pigs.
* Same genotype as human/classical swine/avian reassortant that emerged in 1998 in North America.
* Four of seven household members of the patient and four of 46 other residents of the farm showed serological evidence of infection.
* Household members were only serologically screened.
* Virus from at least two individuals was isolated and sequenced and turned out to be nearly identical to the swH3N1 isolated from the ill swine; not done for all human cases.
* HA related to H1y cluster (H1N2-like) of contemporary H3-SIV, NA related to swine N1 phylogenetic cluster and internal genes were from triple-reassortant SIV lineage and group with cluster IV of A(H3N2) viruses.
* HsN1: HA, NA, NP, NS, M (classical swine, North-American lineage), PB2, PA (avian, North American lineage), PB1 (human seasonal H3N2).
* HsN2: HA (human seasonal H3N3), NP, M, NS (classical swine, North-American lineage), PB2, PA (avian, North-American lineage), PB1, NA (human seasonal H3N2).
### Table 2D

Virological evidence of human infection with swine influenza A viruses

| Year   | (Tested)/confirmed cases | Subtype       | Symptoms                          | Method                                    | Patient information and nature of exposure                                                                 | Location                        | Reference               |
|--------|--------------------------|---------------|-----------------------------------|-------------------------------------------|-----------------------------------------------------------------------------------------------------------|--------------------------------|------------------------|
| 2008   | 1/1^dd                   | SwL trH1N1^ee| ILI, vomiting, diarrhoea          | Rapid test; culture; rRT-PCR; sequencing | 19-year-old male exposed to healthy appearing pigs (no physical contact)                                      | South Dakota, United States    | Dawood et al. 2011 [90]|
| 2008   | 1/1                      | swH1N1^ff     | ILI                               | MDCK cell culture; immunofluorescence using MAb; PCR; partial sequencing | 50-year-old-female with direct contact to asymptomatic pigs                                              | Spain                         | Adiego Sancho et al. 2009 [22] |
| 2009   | 1/1^es                   | swH3N2        | Fever, cough, sore throat         | Rapid test; tRT-PCR; sequencing           | 12-year-old boy (touched healthy appearing swine at a county fair, pigs were seropositive)               | Kansas, United States         | Cox et al. 2011 [97]    |
| 2009   | 3/3^gg                   | sw trH1N1^ii  | ILI                               | MDCK cell culture; tRT-PCR; HI assay (turkey RBC)^jj, sequencing | Swine workers (one was immunized in 2008, other two never received an influenza vaccination)^kk           | Saskatchewan, Canada          | Bastien et al. 2010 [91]|
| 2009–11| 3/3                      | swH1N1^ll     | Not specified                     | Not specified                            | Three adult, male swine workers exposed to ill, influenza-confirmed pigs                                 | Switzerland                   | European Centre for Disease Prevention and Control 2012 [86] |
| Aug-Dec| 12/12^mm                 | H3N2v^nn      | ILI, vomiting, diarrhoea          | Rapid tests; tRT-PCR; sequencing          | 50% exposed and 50% not exposed to swine; (11 children, one adult male); Most attending agricultural fair | Hawaii, Illinois, Indiana, Iowa, Maine, Maryland, Michigan, Minnesota, Ohio, Pennsylvania, Utah, West Virginia, Wisconsin, United States | United States Centers for Disease Control and Prevention 2012 [99],2013 [25] |
| 2012   | 309/309                  |               |                                   |                                            |                                                                                                          |                               |                        |
| 2013   | 19/19^mm                 |               |                                   |                                            |                                                                                                          |                               |                        |
| Total  | 455/401                  |               |                                   |                                            |                                                                                                          |                               |                        |

Where more than one human case is stated, diagnostic tests were performed for all cases as stated in the Methods section, unless otherwise specified.

BSL: biosafety level; cl: classical swine lineage; HA: haemagglutination; HeLa: Henriette Lacks cervical cancer cells; HI: haemagglutination inhibition; ILI: Influenza-like illness; MAb: monoclonal antibodies; MDCK: Madin Darby canine kidney; MN: microneutralisation; n.a.: not applicable; N: neuraminidase inhibition; RBC: red blood cells; RT-PCR: reverse transcriptase polymerase chain reaction; tRT-PCR: real-time reverse transcriptase polymerase chain reaction; sw: swine; SwL: swine-like; tr: triple reassortant; WI-38: human embryo lung fibroblast.

^a In most cases the number tested reflects only the confirmed cases reported in the reviewed paper where the authors did not report on the true number of suspected cases tested.

^dd Contacts of the patient and people exposed to swine during the event were serologically screened.

^ee Distinct from A(H1N1)pdm09 and similar to triple-reassortant swine viruses circulating in the United States shortly before.

^ff Phylogenetically close to A/Switzerland/8808/02 (European avian-like lineage) [19].

^gg In addition, 27 of 34 visitors of the county fair participated in a survey: none reported ILI.

^hh No household members were ill at the time.

^ii Distinct from A(H1N1)pdm09; NS, NP, M, PA, PB1 and PB2 were similar to a North-American swine triple reassortant and HA and NA were most similar to A/Brisbane/59/2007 (H1N1)-like viruses.

^jj For antigenic characterisation.

^kk Mild respiratory illness was present in less than 1% of the swine herd the workers were exposed to (no confirmation whether it was due to influenza A infection).

^mm Influenza A H3N2 variant (comprises genes from avian, swine and human origin) with M gene derived from A(H1N1)pdm09.

^nn Case count as available on 31 January 2014.
Authority (EFSA). The main objective of FLURISK is the development of an evidence-based influenza risk assessment framework (IRAF) to assess the potential of animal influenza viruses to cross the species barrier and cause sustained infections in humans. The work presented here aims at describing available evidence for animal-to-human influenza virus transmissions.

**Methods**

**Search strategy**

We performed a literature search using Medline, Embase, SciSearch and CabAbstracts. Search terms included ‘influenza’, ‘influenza virus’, ‘animals’, ‘swine’, ‘birds’, ‘poultry’, ‘wild bird’, ‘water bird’, ‘waterfowl’, ‘goose’, ‘duck’, ‘chicken’, ‘turkey’, ‘environment’, ‘animal-to-human’, ‘transmission-to-humans’, ‘interspecies transmission’, ‘human’, ‘case’, ‘seroprevalence’, ‘serosurveillance’, ‘prevalence’, ‘incidence’, ‘risk factor’, ‘exposure’ and various subtypes of influenza virus; the terms were used alone or in combinations using Boolean operators. Full search details are available from the corresponding author on request. Only articles published in English were included and the search covered all years available in the respective databases, Medline from 1946, Embase from 1947, SciSearch from 1980, CabAbstracts from 1973, all up to February 2012. The search algorithm automatically discarded duplicates. Newly published evidence that came to our attention between February 2012 and January 2014 was also included. Case counts of avian influenza A(H7N9) and A(H5N1) cases were updated on 31 January 2014 based on the latest figures reported by the European Centre for Disease Prevention and Control (ECDC) [23] and by the World Health Organization (WHO) [24]. Case counts of human infections with swine influenza variant A(H3N2)v were retrieved on 31 January 2014 from the website of the United States (US) Centers for Disease Control and Prevention (CDC) as posted on 18 October 2013 [25]. Grey literature was searched in a non-systematic way.

**Inclusion and exclusion criteria**

Included were papers indicating evidence of human infection with animal influenza viruses (selection criterion 1, Figure 1). Two investigators first screened all papers by title and, when necessary, by abstract. All articles meeting this first criterion were reviewed for details of the methods used to diagnose the infection. Experimental and observational studies describing human infection with animal influenza viruses other than influenza A(H5N1) were included. Articles solely describing human infection with A(H5N1) were excluded, and for influenza A(H5N1) and A(H7N9), the official WHO and ECDC statistics from notifications under the International Health Regulations were used for completeness. Commentaries, reviews, articles dealing with influenza in animals only, studies solely assessing human-to-human transmission of an animal influenza virus (i.e. most of the literature on influenza A(H1N1)pdm09), and articles referring to study subjects described in prior original publications were excluded (selection criterion 2, Figure 1). Studies based on serological evidence only were excluded to ensure high specificity of the findings. Papers were screened for information on the time period when the study was conducted, total number of people sampled, patient information, nature of exposure (e.g. occupational, recreational), possible exposure to diseased animals, influenza virus subtypes included in testing, number of virologically confirmed cases, information on vaccination history if stated, methods used for confirmation, geographic region and study design. Available data were extracted and summarised in tables. Grey literature such as ProMED, and reference lists from articles were screened for possible additional relevant papers. Virus detection by culture or (real-time) reverse transcription polymerase chain reaction (rtRT-PCR) and sequencing was considered to be definitive proof of infection, listed as virological evidence (Tables 1 and 2).

**Search output and article selection**

The initial search yielded 6,955 articles, with 4,905 articles resulting from the Medline search, and the others from additional searches (Figure 1). Search outputs from Embase, SciSearch and CabAbstracts yielded 631, 553 and 866 references, respectively. After screening of titles, abstracts and application of the second selection criterion, a total of 89 publications were selected. The majority of these would also have been identified solely through the Medline search.

Thirty additional studies were retrieved through scanning of reference lists of articles identified via the literature review, were retrieved from grey literature or came to our attention after February 2012. Of these 119, 59 publications and reports described virological evidence for infection of humans and met all other inclusion criteria; 60 papers provided some evidence for human infection, but only based on antibody testing and will therefore be described elsewhere. Seven publications containing both serological and virological evidence were counted once in the total reference count but were included twice in the subdivision according to type of evidence in Figure 1.

Most studies discussed in a review of case reports of SIV infections in humans by Myers et al. [26] were also identified in our literature search. For completeness of the human case count, virologically confirmed civilian (n=23) and military cases of Fort Dix (n=5), although discussed in detail in the review by Myers et al. [26], were also included in the current review [21,27-42] (Table 2). For two virus-confirmed cases from the review by Myers et al. [26], the reference could not be retrieved or did not provide full confirmation; these cases were therefore excluded from our listing in Table 2 [43,44].
Results

The evidence for virologically confirmed infections of humans with avian or SIV is listed in Tables 1, 2 and 3. The exposure status of infected patients is summarised in Table 4. A total of 386 cases of human infection with non-A(H5N1) AIV were described, of which 375 were caused by natural infection and 11 were infected experimentally (Table 1).

Regarding human infections with SIV, a total of 401 naturally (n=396) and experimentally (n=5) infected cases were detected in the published and grey literature in English. This included, three virologically confirmed SIV A(H1N1) cases originally published in Russian by Chuvakova et al. [27] because they were listed in the review by Myers et al. [26]. The majority of cases (n=340) were naturally infected by a SIV variant A(H3N2)v [25]. Recognised in US swine in 2010, this variant combines seven genes from the contemporary North-American A(H3N2) SIV lineage and has acquired the M gene of the A(H1N1)pdm09 virus [25]. The remaining 56 naturally infected, virologically confirmed human cases were caused by different circulating SIV or SIV reassortants (Table 2). Five persons were experimentally infected with SIV [45]. The majority of AIV- and SIV-infected patients had been exposed to animals (Table 4).

Human infections with avian influenza viruses

Infections with highly pathogenic avian influenza virus A(H5N1)

To date, highly pathogenic avian influenza (HPAI) A(H5N1) viruses are the most frequently diagnosed zoonotic influenza virus infections related to avian exposure [46], although this picture may change in the near future given the recent upsurge in low pathogenic avian influenza (LPAI) A(H7N9) cases. The HPAI A(H5N1) viruses first attracted major attention in the scientific community in 1996, when a large number of domestic waterfowl died in the course of an A(H5N1) outbreak in Guangdong province in southern China. In 1997, HPAI A(H5N1) resurfaced in Hong Kong SAR, China (in the following referred to as Hong Kong); it caused a massive die-off in poultry and crossed the species barrier for the first time, infecting 18 humans, of whom six died [47,48]. From mid-2003 to March 2004, HPAI A(H5N1) spread to seven south-east Asian countries with outbreaks in poultry and waterfowl, and the first confirmed human cases, were reported in Thailand and Vietnam in 2004 [49]. In 2005, HPAI A(H5N1) accounted

Table 3

Humans naturally infected with avian influenza virus subtypes other than A(H5N1) and swine influenza virus subtypes, 1959–2014 (n=771)

| Source | Number of human cases infected with | Avian | Swine |
|--------|------------------------------------|-------|-------|
|        |                                   | subtype | subtype |
|        |                                   | H6N1   | H7N2   | HP     | H7N3   | LP     | H7N7   | LP     | H7N9   | H9N2   | H10N7 | H10N8 | Total |
| Avian  |                                   | 1      | 4      | 3      | 2      | 89     | 4      | 251    | 15     | 4      | 2     |       | 375   |
| Swine  |                                   | 47     | 2      | 7      | 340    |        |        |        |        |        |       |       | 396   |

HP: highly pathogenic; LP: low pathogenic.

* as of 27 January 2014 [23].

Table 4

Exposure status of patients infected with avian influenza virus, excluding 251 A(H7N9) and including 11 experimentally infected cases, and with swine influenza virus, 1959–2014 (n=536)

| Source | Number of cases |
|--------|-----------------|
|        | Exposed | Not exposed | Exposure status unknown | Likely exposed (H3N2v) | Other* | Total |
| Avian  | 114     | 3        | 5         | n.a.   | 13     | 135   |
| Swine  | 45      | 20       | 2         | 328    | 6      | 401   |

n.a.: not applicable.

* Experimental (avian n=11, swine n=5) or laboratory exposure (avian n=2), or human-to-human transmission (swine n=1).
for the death of a large number of migratory waterfowl at Qinghai lake, China. Shortly after this event, the virus rapidly spread to other Asian countries, Africa, Europe, the Middle East, Mongolia and Russia [50]. Over time, the viruses evolved into multiple lineages, some of which persisted and have become endemic in China, Bangladesh, Egypt, India, Indonesia and Vietnam [51].

As of 10 December 2013, the WHO has listed 648 HPAI A(H5N1) infected cases from 15 countries, confirmed according to WHO criteria and covering a time span of 10 years [24]. In total, 59% of the reported cases died [24]. Indonesia, Egypt and Vietnam reported 195, 173 and 125 cases, respectively, accounting for about 75% of the total influenza A(H5N1) human case count. These three countries also reported the majority of fatalities [24].

Infections with H7 subtype avian influenza viruses

In total, we identified 353 human cases with virologically confirmed H7 infection (Table 1). The majority of these cases (n=251) were reported in China, followed by 95 cases in Europe, six in North America and one in Australia [17,18,23,52–59]. In China, all cases were caused by the recently emerged subtype A(H7N9) [23]. Of the remaining 102 cases, 93 cases had influenza A(H7N7), five had influenza A(H7N3) and four influenza A(H7N2) (Table 1). The first two human cases infected with influenza A(H7N7) were reported in 1959 and 1977. One of these patients had keratoconjunctivitis, thought to be caused by the AIV infection [52,53]. This predilection for the ocular mucosa was confirmed when a person involved in an experimental infection of a seal with an avian-like influenza A(H7N7) developed conjunctivitis, and virus was cultured from a conjunctival swab [54,60]. In the United Kingdom (UK) in 1996, LPAI A(H7N7) virus infection was associated with mild conjunctivitis in a woman who cleaned a duck house and mentioned getting a piece of straw in her eye [55].

Among European cases, 89 humans were infected in the course of a large outbreak with HPAI A(H7N7) in poultry in the Netherlands in 2003 [17]. In contrast to the severe consequences in poultry, only mild symptoms were seen in 88 of the infected people. There was one exception, a veterinarian who died of acute respiratory distress syndrome and multiple organ failure. This person had contracted a virus with several mutations, including a known virulence marker in PB2 [56]. Most of these mutations had accumulated during circulation of the virus in poultry, showing that the public health risk may change over the course of an outbreak [61]. In February 2004, a mixed LPAI and HPAI A(H7N3) virus outbreak was reported in poultry in British Columbia, Canada [18]. Enhanced surveillance for influenza-like illness (ILI) and conjunctivitis in the course of this outbreak led to the identification of two poultry workers showing symptoms of unilateral conjunctivitis. Neither had used the recommended goggles or taken prophylactic oseltamivir. Interestingly, both virus types led to human infection: the isolate cultured from the first worker had the LPAI phenotype, whereas the strain retrieved from the second worker was classified as HPAI [19,62,63]. In 2006 and 2012, LPAI A(H7N3) was associated with one patient in the UK and HPAI A(H7N3) with two patients in Mexico. In both instances, exposure to infected poultry was documented and all patients presented with conjunctivitis [57,59]. Finally, LPAI A(H7N2) was reported as the infectious agent causing mild influenza-like symptoms and conjunctivitis in four cases in the UK in 2007 [58].

The assumption that LPAI influenza viruses were mostly associated with mild disease was challenged with the emergence of influenza A(H7N9) viruses in March 2013, when China notified the WHO of three cases infected with LPAI A(H7N9) who were severely ill and eventually died [64]. During the first wave of infections from February to May 2013, 133 human cases were reported and an additional two cases in July and August [23]. Phylogenetic studies concluded that all genes of this newly detected virus were of avian origin [64]. In October 2013, the second wave started and was still ongoing at the time of writing (31 January 2014) [23]. Between February 2012 and 27 January 2014, a total of 251 influenza A(H7N9) cases were reported, 56 of whom died [23]. Infections occurred in Anhui (n=4), Beijing (n=3), Fujian (n=15), Guangdong (n=32), Guizhou (n=1), Hebei (n=1), Henan (n=4), Hong Kong (n=3), Hunan (n=4), Jiangsu (n=31), Jiangxi (n=5), Shandong (n=2), Shanghai (n=42) and Zhejiang (n=102). Two cases were imported from mainland China into Taiwan [23]. In response to these events, China culled thousands of birds and closed several poultry markets [65], although only 39 of 48,000 samples representing 1,000 poultry markets tested positive. Most human cases had a history of exposure to birds or live bird markets [23]. As of 31 January 2014, no conclusive evidence of human-to-human transmission has been reported and the ecology of the viruses remains to be resolved.

Infections with H9 subtype avian influenza viruses

In total, we detected 15 human cases infected with AIV A(H9N2) (Table 1, Table 3). Since the mid-1990s, influenza viruses of the H9 subtype have established stable lineages in poultry in Asia and have occasionally infected humans and swine (Table 1) [16,66-69]. As of 31 January 2014, human A(H9N2) cases have only been detected in Asia, particularly in China. Six cases were identified via the literature search [16,66-68]. Of those, three reported poultry exposure and all presented with mild ILI (Table 1). Reviews conducted by Peiris [46] and Cheng et al. [68] identified six additional human infections in China reported in the Chinese literature [70,71]. Three additional cases from Bangladesh, Hunan and Shenzhen, two with and one without poultry exposure, complete the total count of fifteen human cases caused by AIV A(H9N2) [69,72,73] (Table 3). Infections with AIV A(H9N2) viruses gained public health interest when researchers found that strains circulating in Asian poultry had a receptor specificity similar to...
human influenza A viruses, which is considered one of the essential features of a human-to-human transmissible virus [74]. So far, however, no sustained human-to-human transmission of A(H9N2) influenza viruses has been reported.

Infections with other avian influenza virus subtypes
Experimental inoculation of human volunteers with influenza strains A(H4N8), A(H6N1) or A(H10N7) resulted in mild clinical symptoms and virus shedding in eleven volunteers [75]. In 2004, the National Influenza Center in Egypt and the WHO Influenza Collaborating Centre in the UK announced the isolation of influenza A(H3N7) virus from two children presenting with fever and cough in Egypt [76]. In Australia, virus of the same subtype could be detected by PCR in two abattoir workers with conjunctivitis who were exposed to infected poultry [20] (Table 3). In December 2013 and January 2014, human infection with A(H10N8) virus was reported for the first time in Jiangxi province, China [77,78]. Both patients were female and had visited a poultry and an agricultural market, respectively, before onset of illness. One of them was immunocompromised and had died whereas the other case was still in critical condition as of 31 January 2014. In 2013, CDC Taiwan reported a human case of AIV A(H6N1) infection causing mild pneumonia, although an avian source could not be identified [79,80] (Table 3).

Human infection with swine influenza viruses
An overview of all studies describing virologically confirmed human SIV cases is given in Table 2. In total, we identified 396 SIV-confirmed patients who were naturally infected (401 including experimental infections) (Table 2). Beare et al. [45] successfully recovered SIV from five of 20 human volunteers after experimental infection: of seven volunteers infected with SIV A(H3N2) related to A/Hong Kong/1/68, three tested virus-positive), and of 13 infected with a classical swine A(H1N1) virus strain, two tested positive. Of the naturally infected cases, 47 were infected with SIV A(H1N1), two with SIV A(H3N2), seven with SIV A(H3N2) and 340 with SIV A(H3N2)v (Table 2, Figure 2). The majority of these cases were reported in North America, 11 in Europe and six in Asia. One of the six Asian cases was infected with SIV A(H3N2) from the European lineage (Figure 2) [39]. SIV epidemiology differs between continents and was extensively reviewed for North America, Europe and Asia [81-84]. In addition to the studies discussed in the review by Myers et al. [26] we identified fourteen studies and reports describing a further 28 human SIV cases; 368 when taking into account 340 cases with SIV A(H3N2)v infection. Details on these studies are described in more detail in the following sections grouped by continent.

Infections with swine influenza A H1 subtype viruses
Asia: A 25 year-old male from the Philippines and a four year-old male from Thailand were infected with swine-like A(H1N2) and A(H1N1), respectively [85]. The isolated viruses carried HA genes most closely related to classical swine viruses circulating in Asia and North America. NA genes were most similar to circulating European SIV. Both cases showed mild ILL and neither of them had direct contact with swine, although occasional contact with backyard swine could not be ruled out.

Europe: In Spain, a 50 year-old woman developed ILL after having been closely exposed to swine on a family farm [22]. No symptoms in swine were observed and sequencing of the isolate revealed that it was closely related to avian-like SIV A(H1N1) circulating in swine in western Europe. Three cases from Switzerland were detected who had worked with influenza-confirmed swine [86].

North America: For the US, 19 confirmed cases of SIV A(H1N1) infection were described in the published literature. This number could possibly be higher because Vincent et al. [87] reported on 26 human cases presenting with ILL after exposure to ill swine on a county fair in Ohio. The authors described that isolation and sequencing was performed for at least two of the human cases. Since the exact number of virologically confirmed cases was not given, we only added the two confirmed patients to the overall SIV A(H1N1) count (Table 3). Sequences from swine and human isolates from this outbreak were identical and were similar to triple–reassortant (tr) viruses currently circulating in swine herds in the US [87,88]. Another triple–reassortant A(H1N1) SIV was detected in a 17 year-old male from Wisconsin who assisted in butchering healthy appearing swine [89]. The patient presented with acute, mild respiratory illness without fever. Similarly, Dawood et al. [90] reported infection with trSIV A(H1N1) in a 19 year-old asthmatic male who visited a swine show in South Dakota. Symptoms included fever, ILL, vomiting and diarrhoea. No respiratory illness was observed in swine at this event. A trSIV A(H1N1) was also detected in three infected swine workers in Saskatchewan, Canada [91]. Household members did not report any signs of disease. Mild respiratory illness was reported in less than 1% of the swine; however, no confirmatory test had been conducted in ill swine. Unlike trSIV identified earlier in North America, this isolate contained an HA and a NA belonging to the A/Brisbane/58/2007 A(H1N1) lineage, whereas the remaining genes were derived from trSIV A(H1N2) viruses circulating in North America since 1998. Gray et al. [92] found another trSIV A(H1N1) in the course of a prospective survey, which was isolated from an ill swine farmer exposed to swine showing respiratory symptoms. Routine national influenza surveillance reported another 10 human cases infected with trSIV A(H1N1), distinct from A(H1N1)pdm09, and one case caused by trSIV A(H1N2). The majority of those twelve patients stated exposure to swine prior to disease onset and all made a full recovery [93].

The CDC reported additional human infections with SIV-variant viruses of subtype A(H1N2)v and A(H1N1) identified in the US since 2005 [94]. These figures have
Figure 2
Timeline of emergence of swine influenza virus lineages circulating in Europe and North America indicating natural human infections from swine

HA: haemagglutinin; NA: neuraminidase; SIV: swine influenza virus; v: variant; hu: human.

Years on main arrow denote human influenza pandemics: A(H1N1) in 1918, A(H2N2) in 1957, A(H3N2) in 1968 and A(H1N1)pdm09 in 2009. Pictograms denote the origin of viral genes. Items in grey font indicate that the virus did not establish itself in the swine population and therefore did not circulate any further. Boxed items indicate infection in humans. Double-ended arrows indicate that four viruses were introduced in 1998 in the swine population.

Superscript letters refer to the genome segment constellation:
a Classical SIV A(H3N2) constitutes a reassortant between human A/BM/1918 and unknown virus.
b Recent phylogenetic evidence suggests that classical SIVA(H1N1) may have been present in Europe already in the 1930s and not around 1950 as previously assumed [122].
c Classical SIV A(H1N1) was re-introduced into Europe via Italy in 1976 and circulated in European countries until it was replaced by avian-like SIV(H1N1) in 1979 (was not replaced in England) [122].
d HA and NA from human HongKong/68-like virus, remaining genes from European avian-like swine A(H3N2). e HA from human A(H3N1) (England/80-like), NA from European reassortant swine A(H3N2), remaining genes from European avian-like swine A(H3N1).
fi HA, NA of human A(H3N2) origin, remaining genes from classical swine A(H3N1) and avian influenza origin.
fi HA, NA from classical SIV A(H3N1), remaining genes from triple-reassortant or swine A(H3N2).
fi HA, NA from seasonal human influenza viruses, remaining genes from triple-reassortant swine A(H3N2).
fi A(H3N2)variant: M gene from A(H1N1)pdm09, remaining genes from triple-reassortant swine A(H3N2). N2 antigenically different from triple-reassortant A(H3N2) from 1998.
fi Including five virus-confirmed human cases reported from Fort Dix outbreak among soldiers in New Jersey, United States in 1976 [28].
fi Nine human cases could not be assigned: Six of them were infected with reassortants that did not group with current swine lineages. Two of the six cases were from the Philippines and Thailand, infected with A(H3N2) and A(H4N2) bearing HA from the North American lineage from the 1990s and NA from European swine influenza lineages [85]. Three of the six cases were from Canada, infected with an A(H1N5) reassortant with HA and NA genes resembling those of A/Brisbane/59/2007(H5N1)-like viruses and internal genes (NS, NP, M, PA, PB1, and PB2) descending from a contemporary North American SIV A(H3N2) triple reassortant [91]. The last of the six cases was infected with triple-reassortant A(H1N5) with HA, PA, PB1, PB2, NP, M, NS from North American triple-reassortant SIV A(H1N1) lineage and NA from North American, classical swine A(H1N1) [92,123]. The remaining three cases infected with SIV A(H1N1) were described in Russia by Chuvakova et al. [27] for which no further isolate characterisation was given in the abstract of the paper.

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Human infections with swine influenza A H3 subtype viruses

North America: In our search we detected two cases of trSIV A(H3N2) from Canada [95,96] and one from Kansas, US [97] infected before 2011. In Canada, a six-year-old boy who lived on a swine farm presented with parotitis, nasal congestion, cough and pharyngitis, but had no fever. The swine appeared clinically healthy [95]. No swine exposure was reported in the second Canadian patient, a seven month-old child, who lived on a community farm and showed ILI symptoms [96]. Similarly, the third case from Kansas presented with ILI and is likely to have contracted trSIV A(H3N2) from swine he was exposed to at a county fair [97]. It is assumed that swine harboured the virus; PCR results performed on swine samples were negative but sera showed raised titres against SIV A(H3N2) indicating prior infection.

Between July and August 2011, a SIV variant, which accounted for the majority of reported human SIV A(H3N2) cases, appeared in the US, possibly reflecting enhanced surveillance activities in the country. This SIV A(H3N2) variant, A(H3N2)v, was first found in two children presenting with fever and respiratory signs [98,99]. Sequencing showed that this variant contained seven genes derived from the contemporary trSIV A(H3N2), circulating in the US swine population since 1998, as well as the M, gene from the A(H1N1)pdm09 virus. Since its first occurrence in 2011, A(H3N2)v has been detected in 340 humans according to data as of 18 October 2013 [25]. Since July 2012, 17 patients have been hospitalised and one patient has died due to SIV A(H3N2)v infection. Most cases have reported prolonged exposure to swine before getting ill.

Human infections following exposure to other animals

We have identified three studies describing human susceptibility to equine influenza virus, demonstrated by experimental infection with equine subtype A(H3N8) [100-102]. The literature search did not reveal evidence of humans naturally infected with equine influenza virus.

Discussion

Here we present a review of the literature for studies presenting any evidence for human infection with animal influenza viruses. Virological techniques, e.g. virus culture, PCR and sequencing provide more solid evidence of infection, whereas serological methods can help reaching a diagnosis after the virus has been cleared from the body. Virus isolation is still the gold standard in detecting AIV infection. Human cases with virological evidence identified by PCR only should be interpreted with caution as detection of viral RNA without additional serological evidence (seroconversion, more than fourfold rise in the titre of paired samples) does not necessarily imply infection, although current diagnostic methods heavily rely on case identification by PCR [103,104]. Serological results can be misleading because of the existence of cross-reactive antibodies and thus provide less solid evidence than direct detection of the infecting virus itself [105]. Therefore, we limited the current paper to studies providing virological evidence only.

Most evidence of human infection with AIV is associated with subtypes H5 and H7. Both subtypes can be linked to devastating outbreaks in poultry with high mortality, if transition from a low to a highly pathogenic state occurs [106]. Pathogenicity shown in poultry clearly does not reflect disease severity in humans: before the emergence in LPAl A(H7N9) as a cause of severe human illness, LPAl and HPAl viruses of subtype H7 had in the majority of cases been associated with mild eye infections or ILI [107]. The LPAl A(H7N9) infections diagnosed to date, however, have been unusually severe [23,64]. In addition to the severity of illness associated with these and also A(H5N1) viruses, the widespread circulation of different lineages, made possible by mutations and reassortments, justifies enhanced surveillance activities, given that few genetic changes may lead to a human-to-human transmissible virus [108,109].

Nevertheless, the evidence from experimental infections and anecdotal natural infections shows that other AIV may infect humans as well. There is insufficient systematic surveillance data to address the question whether the identified human cases reflect the level of virus circulation among wild or domestic birds, or whether certain subtypes infect humans preferentially.

Regarding SIV, there is ample evidence of human infection with A(H3N2), A(H1N1) and A(H1N2) subtypes, as well as reassortants derived from these endemic SIV lineages. Nevertheless, one has to be aware that the true number of human SIV cases is probably higher than reported since clinical symptoms of SIV are indistinguishable from seasonal influenza [86]. Whereas recent human cases in Europe were detected almost accidentally, the larger number of cases reported in the US since 2005, especially for influenza A(H3N2)v since 2011, may be the result of increased surveillance activities [86,110]. Swine were assumed to play an important role as intermediate hosts or ‘mixing vessels’ for strains of human, avian and swine origin, because they possess avian and human influenza-specific receptors in the tracheal epithelium [111]. However, recent research showed that the distribution of sialic acid receptors in the porcine respiratory tract is similar to that in humans, leading to the conclusion that humans are equally likely to constitute ‘mixing vessels’ [112,113]. The fact that influenza viruses can circulate unnoticed in swine populations [114] warrants close surveillance in this animal species as well. Co-circulation of different influenza virus strains in swine may facilitate the generation of new variants that could potentially
pose a threat for public health [115]. For instance, it is assumed that influenza A(H1N1)pdm09 was present in swine herds for months before it emerged as a pandemic strain in humans [116]. Conversely, Nelson et al. [117] reported at least 49 transmission events of influenza A(H1N1)pdm09 from humans to swine between 2009 and 2011, as well as at least 23 separate introductions of human seasonal influenza into swine since 1990.

Although there is some evidence for infection in swine with non-H1 and non-H3 subtype viruses (H9N2, H4N6) [115,118], we found no case reports describing human infection with these influenza A virus subtypes after swine exposure. Since most human infection events are associated with swine exposure, awareness of risk factors and personal protective equipment is paramount to limiting the chance of infection and preventing people working with or recreationally exposed to swine (including children) from becoming ‘bridging links’ between swine and community contacts and vice versa [92,119].

There are a few limitations to this review. The language restriction set to papers published in English only and the unsystematic search of the grey literature probably lead to the omission of additional documented human infections with animal influenza virus. Although this limitation may affect the total count of human cases, the aim of this review was to identify animal influenza subtypes, which crossed the species barrier to humans, and to our knowledge, all relevant subtypes were covered by this review.

Conclusions
There is evidence of infection of humans with animal influenza viruses belonging to various subtypes. All reported SIV cases have been exclusively associated with subtypes H1 and H3, and most AIV cases were caused by subtypes H5 and H7. Whether this reflects the prevalence of these viruses in birds kept or sold for consumption or a preferential ability to transmit to swine (including children) from becoming ‘bridging links’ between swine and community contacts and vice versa [92,119].

There are few limitations to this review. The language restriction set to papers published in English only and the unsystematic search of the grey literature probably lead to the omission of additional documented human infections with animal influenza virus. Although this limitation may affect the total count of human cases, the aim of this review was to identify animal influenza subtypes, which crossed the species barrier to humans, and to our knowledge, all relevant subtypes were covered by this review.

The circulations of viruses with yet unknown public health risks.

Note:
Numbers on influenza H5N1 and H7N9 are as of 31 January 2014.

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Authors’ contributions
GF and MK drafted the analysis plan. GF performed the study of the literature and drafted the manuscript. MK, AM and EdB contributed to the analysis of the data and critically reviewed the manuscript. AM performed the final edit of the manuscript before submission, including an update of counts and addition of most recent evidence. All authors were involved in discussion of parts of the analyses that were presented during consortium meetings and critically reviewed drafts of the manuscript.

Conflict of interest
None declared.

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