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Risk factors of primary introduction of highly pathogenic and low pathogenic avian influenza virus into European poultry holdings, considering at least material contaminated by wild birds and contact with wild birds

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Abstract

Avian influenza (AI) is a constant threat to the poultry industry in Europe. Up to date assessments of the factors influencing the probability of introduction of infection into poultry holdings are required for the implementation of informed prevention and control measures. We carried out a systematic literature review to identify and synthesise the evidence on risk factors of AI introduction into European poultry holdings. A review protocol was built, which considered the electronic search strategy, criteria for relevance screening, quality assessment and data collection. A total of 941 abstracts were evaluated and 145 relevant manuscripts were selected for evaluation of the full text. This assessment resulted in the selection of 29 manuscripts for data collection and analyses. These manuscripts provided information on 54 AI introductions (outbreaks) in poultry, with 25 of those being caused by highly pathogenic AI viruses and 29 caused by low pathogenic AI viruses. The identified risk factors influencing the probability of introduction of AI in the affected poultry holdings were: the poultry species and production system, contact with wild birds or material (fomites) contaminated by wild birds, abattoirs processing and commercialising contaminated fresh meat, holdings sharing personnel or equipment, undetected circulation of avian influenza in poultry and flaws in biosecurity of the affected holdings. The main source of introduction of AI virus was considered to be contact with wild birds or contaminated material, with 42 out of the 54 studied outbreaks providing evidence to support this source of introduction. This was further supported by the higher risk of introductions reported in free range poultry holdings. In conclusion, evidence on the different risk factors for AI introduction are summarised in this SR to support decision making.

Key words: Avian influenza, poultry, risk factors, introduction risk, Europe

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Summary

A request was made to review the literature for the risk factors of primary introduction of avian influenza into European poultry holdings. The Stichting Landbouwkundig Onderzoek, Central Veterinary Institute, part of Wageningen UR (Lelystad, the Netherlands), as partner of a Consortium led by the Erasmus University Medical Centre (Rotterdam, the Netherlands), carried out this review.

A systematic (literature) review (SR) was carried out following the methodology described in the EFSA guidance for carrying out SR and the PRISMA guidelines with modifications. The objective of this SR was to identify and synthesise the evidence on risk factors of highly pathogenic avian influenza (HPAI) and low pathogenic avian influenza (LPAI) introductions into European poultry holdings, considering: at least material contaminated by wild birds and contact with wild birds.

A review protocol was followed. This protocol specified the electronic search strategies, defined the study population (poultry) and geographical limits (mainly Europe), publication type, AI virus strains and the outcome. Only manuscripts published between 01-01-2005 and 6-01-2016 were included in the SR.

A total of 941 abstracts were screened, which resulted in the selection of 141 manuscripts for full text relevance screening and quality assessment. In addition four manuscripts were identified by manual searching. Out of the 145 assessed manuscripts, 29 were selected for data collection and analysis.

Data collection retrieved information on the country where the AI outbreak took place, the year of the outbreak, the poultry production system, the virus serotype and pathotype causing the outbreak, the source of introduction and the type of evidence that led to the identification of the source of introduction.

The selected manuscripts provided information on 54 outbreaks. Eighteen outbreaks involved waterfowl (ducks, geese and farmed mallards) holdings, six holdings had mixed species including waterfowl (all holdings), chickens, turkeys or guinea fowl. Nine outbreaks involved backyard and in seven of those, waterfowl were also raised. Of the remaining outbreaks, 11 involved chicken holdings, eight turkey holdings and two outbreaks involved ornamental birds. Out of the 54 outbreaks, 29 (54%) involved free range (outdoor) holdings and 25 (46%), commercial holdings where birds were raised indoors. These observations appear to indicate that waterfowl holdings and outdoor holdings have a higher risk of introduction of AI.

One study in particular quantified the risk of introduction of AI into poultry holdings and confirmed the significant higher risk of introduction for duck and turkey holdings as well as for outdoor (free range) chicken layers.

Contact with wild birds or material contaminated with wild bird faeces was the main identified source (risk) of introduction. In 42 out the 54 outbreaks contact with wild birds was reported as the source of introduction. This was supported by: (i) the isolation of the virus strain causing the outbreak from both the affected poultry and wild birds within the same geographical and temporal window, (ii) presence of considerable numbers of wild birds in the proximity of the holding and (iii) phylogenetic inference.

Processing and commercialisation of contaminated fresh meat were the source of introduction for outbreaks (n = 3) in the UK and Germany. Abattoirs processing this meat were the source of introduction into commercial holdings which were located next to the abattoirs. These events highlight the need to assess biosecurity measures implemented in abattoirs to minimise risks of spread of infection. Additionally, commercialization of the meat originated from one of these abattoirs resulted in further two outbreaks in backyard poultry that had access to uncooked offal.

Other sources of introduction were undetected circulation of infection, in particular in backyard and rural holdings. This resulted in the spread of infection via movement of infected life birds (trade), contaminated equipment or personnel.

Biosecurity flaws were associated with the introduction of infection, particularly in commercial holdings that kept poultry indoors. Of note are three outbreaks in chicken breeder holdings, which are expected to have the highest level of biosecurity within the poultry industry. Contaminated fomites such as footwear worn by personnel and equipment were described as the most likely routes of introduction.

Finally, risk factors of infection (transmission) quantified during major epidemics of HPAI in Italy, the Netherlands and Romania as well as LPAI epidemics in Italy were summarised and common risk factors for the risk of introduction and transmission identified. These factors were poultry species and production system, distance to rivers and streams and poor biosecurity.

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1. Introduction

1.1. Background and Terms of Reference as provided by the requestor

This contract was awarded by EFSA to: Consortium leader Erasmus University Medical Centre (Rotterdam, the Netherlands) and in particular to partner 1 : Stichting Landbouwkundig Onderzoek, Central Veterinary Institute, part of Wageningen UR (Lelystad, the Netherlands)

Contractor/Beneficiary: EFSA

Contract title: Data collection, literature review and spatial models for virus spread in preparation to the mandate on avian influenza

Contract number: OC/EFSA/ALPHA/2015/01

ToR 3 "Assess the current situation in the EU and elsewhere as regards the risk of a possible introduction of highly pathogenic avian influenza (HPAI) (H5N8) virus and possibly other HPAI viruses to EU poultry holdings",

and;

ToR 4 "Assess the continuous risk posed by low pathogenic avian influenza (LPAI) (subtypes H5 and H7) for the introduction from the wild bird reservoir into poultry holdings taking into account risks for holdings where poultry is kept in open air runs and the suitability of surveillance and biosecurity measures aimed at protection of poultry against LPAI infection",

it is considered appropriate to review the literature (publication period 2005-2015) for the risk factors of primary introduction of HPAI and LPAI viruses of subtype H5 and H7 into European poultry holdings, considering: at least material contaminated by wild birds and contact with wild birds. In addition, the risk factors found may be checked for overlap with risk factors for between-farm transmission mentioned in the EFSA opinion of 2008 on avian influenza (http://www.efsa.europa.eu/en/efsajournal/pub/715) and in the EFSA opinion of 2006 on avian influenza (http://www.efsa.europa.eu/en/efsajournal/pub/357).

The objective of this literature review was to identify and synthesise the evidence on risk factors of HPAI and LPAI introductions into European poultry holdings, considering: at least material contaminated by wild birds and contact with wild birds.

2. Data and Methodologies

2.1. Data

Data was retrieved from peer-reviewed studies and reports. The approach to data collection followed a rigorous selection of published studies/reports following the systematic review (SR) methodology as described in the EFSA guidance for carrying out SR (EFSA, 2010) and the PRISMA guidelines with modifications, since the PRISMA statement refers mainly to intervention studies.

For this report, a study refers to a manuscript reporting primary research where risks are quantified following standard epidemiological and statistical procedures. A report refers to published peer-reviewed manuscripts where outbreaks of avian influenza (AI) in poultry are described.

2.2. Methodologies

A SR protocol was followed, which is provided as appendix 1. The following steps were followed: (i) literature search, (ii) relevance screening, (iii) quality assessment and data extraction and (iv) data

analysis and summation. Three scientists, one virologist and two epidemiologists with extensive experience in avian influenza in Europe carried out the SR.

2.2.1. Literature search

Table 1 presents a summary of the search strategies used for the literature search. Three search strategies were used targeting a very sensitive search with low specificity. The first strategy retrieved a total of 937 citations, the second 454 and the third retrieved 3. The latter specifically targeted citations on the novel (2014-2015) H5 strains (H5N8, H5N2 or novel reassortant H5N1) in the USA.

Table 1: Details of the literature search strategies

Review question	**Database: Pubmed**	
What are the risk factors on primary introduction of HPAI and LPAI virus strains into European poultry holdings?	Justification for choosing the source:	To date pubmed is considered one of the most complete databases for scientific biomedical literature.
	Date of the search:	5-1-2016
	Date span of the search:	2005 – 2016
	Date of the latest database update included in the search:	6-1-2016
	Search strategy 1: This search strategy retrieved a total number of studies n = 937	(poultry OR chicken OR broiler OR layer OR duck OR quail OR goose OR turkey OR pheasant OR guinea fowl OR partridge OR swan OR ostrich OR pigeon) AND ("avian influenza" OR HPAI OR LPAI OR H5N1 OR H5N8 OR H7N1 OR H7N7 OR H10N7 OR H5 OR H5N2 OR H5N3 OR H7N2 OR H7N3) AND (spread OR introduction OR transmission OR "transmission pathway" OR "transmission risk*" OR pathway OR between OR movement OR risk* OR "risk factor*" OR driver* OR farm* OR holding* OR facilit* OR "secondary infection" OR "between-farm*" OR "between-flock*") AND (europe* OR EU OR Russia OR Denmark OR France OR Ukraine OR Spain OR Sweden OR Norway OR Germany OR Finland OR Poland OR Italy OR United Kingdom OR UK OR Romania OR Belarus OR Kazakhstan OR Greece OR Bulgaria OR Iceland OR Hungary OR Portugal OR Serbia OR Austria OR Czech Republic OR Ireland OR Georgia OR Lithuania OR Latvia OR Croatia OR Bosnia OR Herzegovina OR Slovakia OR Estonia OR Netherlands OR Switzerland OR Moldova OR Belgium OR Albania OR Turkey OR Macedonia OR Slovenia OR Montenegro OR Cyprus OR Luxembourg OR Andorra OR Malta OR Liechtenstein OR San Marino OR Monaco OR Vatican City) AND (("2005/01/01"[PDat] : "2015/01/31"[PDat]) AND Animals[Mesh:noexp])
	Search strategy 2 n = 454	(poultry OR chicken OR broiler OR layer OR duck OR quail OR goose OR turkey OR pheasant OR guinea fowl OR partridge OR swan OR ostrich OR pigeon) AND "avian influenza" AND (HPAI OR LPAI OR H5N1 OR H5N8 OR H7N1 OR H7N7 OR H10N7 OR H5 OR H5N2 OR H5N3 OR H7N2 OR H7N3) AND (introduction OR transmission OR entry OR "primary infection" OR "primary outbreak" OR "primary case" OR "primary source" OR "first case" OR "first outbreak") AND (europe* OR EU OR Russia OR Denmark OR France OR Ukraine OR Spain OR Sweden OR Norway OR Germany OR Finland OR Poland OR Italy OR United Kingdom OR UK OR Romania OR Belarus OR Kazakhstan OR Greece OR Bulgaria OR Iceland OR Hungary OR Portugal OR Serbia OR Austria OR Czech Republic OR Ireland OR Georgia OR Lithuania OR Latvia OR Croatia OR Bosnia OR Herzegovina OR Slovakia OR Estonia OR Netherlands OR Switzerland OR Moldova OR Belgium OR Albania OR Turkey OR Macedonia OR Slovenia OR Montenegro OR Cyprus OR Luxembourg OR Andorra OR Malta OR Liechtenstein OR San Marino OR Monaco OR Vatican City OR UK)
	Search strategy 3 n = 3	(poultry OR chicken OR broiler OR layer OR duck OR quail OR goose OR turkey OR pheasant OR guinea fowl OR partridge OR swan OR ostrich OR pigeon) AND "avian influenza" AND (novel H5N8 OR novel H5N2 OR novel assortment H5N1) AND (introduction OR transmission OR entry OR "primary infection" OR "primary outbreak" OR "primary case" OR "primary source" OR "first case" OR "first outbreak") AND (United States OR USA)

Total number of summary records retrieved after removing duplicates	941

2.2.2. Relevance screening

The purpose of the relevance screening is to rapidly remove studies not relevant to the review. Two levels of relevance screening were used. For level 1, titles and abstracts of all retrieved studies were reviewed using the following selection criteria:

- The abstract and title are written in English.
  Outcomes: Yes, No.
- The publication is primary research, a simulation model or description of outbreaks (reviews, opinion papers, questionnaires, surveys, reports which are non-scientific literature are not considered)
  Outcomes: Yes, No, Unknown, not applicable.
- Influenza A virus is the subject studied.
  Outcomes: Yes, No, Unknown.
- The study was performed in poultry.
  Outcomes: Yes, No, Unknown, not applicable.
- Geographical limits are Europe or USA.
  Outcomes: Yes, No, Unknown.
- Introduction, transmission, spread, risk factors, surveillance, outbreak, and synonymous terms are mentioned
  Outcomes: Yes, No, Unknown.

Manuscripts were excluded if they scored a "no" on at least one of the criteria. Manuscripts that scored a "unknown" or "not applicable" were still considered and evaluated during the next relevance screening.

The level 2 of the relevance screening was conducted reading the full manuscript. An Excel dataframe was created where the answers and outcomes (selected/not selected) to each of the questions answered in this screening were recorded for every manuscript screened in this level. The questions were as follow:

- All criteria of the first screening are met and score a "yes".
- The serotype of the Influenza A virus being studied is known.
- The pathogenicity of the virus (low pathogenic or highly pathogenic) is known.
- Are potential factors associated with the introduction of AI into a poultry holding reported/identified. Any of the following factors:
  a. Transport of birds (other species) between farms
  b. Production type
  c. Farm size
  d. Farm density or distance to infected farm
  e. Human contacts
  f. Waste management practices
  g. Different poultry species or other livestock species in one farm (mix holding)
  h. Direct and indirect contact with wild birds (close to waterbodies where wild birds were seen).
  i. Other potential risk factor such as: poor biosecurity, presence of pigeons, problems with control of rodents, sea level, etc is reported, 0 = otherwise.

Only manuscripts that meet all criteria are selected.

2.2.3. Quality assessment and data extraction

Quality assessment was done in order to identify key features required to assess the risk of introduction of avian influenza into poultry holdings. The key features that needed to be described in a manuscript for final inclusion and data collection were: (i) the country and the year of introduction, (ii) the avian influenza virus serotype and pathotype, (iii) the production type of the affected poultry holding(s) and (iv) the source of introduction. These features would also allow the identification of manuscripts describing the same outbreaks, so that they can be considered jointly or redundant manuscripts excluded (no additional relevant information could be obtained). Finally, data collection of selected manuscripts would also collect information on the type of evidence used to support the reported source of introduction.

3. Assessment/Results

3.1. Literature search and relevance screening

Citation searching ended on 6 January 2016. Three search strategies were used and after deduplication 941 citations were available for relevance screening (Table 1). A total of 141 citations passed the first level of screening and 4 additional manuscripts were identified by searching de bibliographies of the selected articles, therefore a total of 145 manuscripts passed to the second level of screening. A summary of the reasons for exclusion during the first level screening is presented as Appendix B. In addition, an excel file "SR dataset.xlsx" where detailed information of the second relevance screening for each of the 145 manuscripts screened is provided with this report.

Following the second level of screening, a total of 29 manuscripts were selected for data extraction. Excluded manuscripts (n = 116) were manuscripts describing (hereafter only a few references are given as examples, for a full overview see file "SR datasets.xlsx"): simulation models evaluating control measures (Backer et al., 2015; Dent et al., 2011; Dorigatti et al., 2010; Gonzales et al., 2014; Nickbakhsh et al., 2013; Nickbakhsh et al., 2014; Smith and Dunipace, 2011; te Beest et al., 2011), theoretical risk assessments (Edmunds et al., 2013; Hop and Saatkamp, 2010; Malladi et al., 2015; Martinez et al., 2009; Sanchez-Vizcaino et al., 2010), evaluation of control measures during epidemics (Busani et al., 2007a; Busani et al., 2007b; Mulatti et al., 2010a), quantifying or studying between or within farm transmission during epidemics (Bos et al., 2009), descriptive reviews (Artois et al., 2009; Brown et al., 2007; de Jong et al., 2009; van den Berg, 2009), quantifying risk factors for transmission or infection during epidemics (Busani et al., 2009a; Busani et al., 2009b; Mulatti et al., 2010b; Thomas et al., 2005; Ward et al., 2009a; Ypma et al., 2012), economic analysis of control measures (Boni et al., 2013; Longworth et al., 2014a, b), evaluation of surveillance data or design of surveillance programmes where some of the key features identified for selection and data collection were not provided (Comin et al., 2011; Gonzales et al., 2010; Welby et al., 2010), manuscripts describing the same outbreak as other manuscripts which were considered better suited for this study (Bouwstra et al., 2015a; Ward et al., 2008b), news releases or letters on peer-reviewed journals on the occurrence on outbreaks (Anonymous, 2007; team, 2006, 2007b) or other reasons not relevant to our study (Bertran et al., 2013; team, 2007a).

3.2. Data analysis and summation

All selected manuscripts (N = 29) provided information on 54 primary introductions or initial between farm spread following introduction when the detected index case was not the first infected farm. Data from these 54 outbreaks, which took place between 2003 and 2015 in Europe, were collated. Twenty

five of these outbreaks were caused by HPAI viruses (HPAIV) and 29 by LPAI viruses (LPAIV). In Tables 2 and 3 are summarised the countries and serotypes causing these outbreaks.

Table 2: Outbreaks of highly pathogenic avian influenza in poultry holdings in Europe

Serotype	Denmark	France	Germany	Hungary	Netherlands	Romania	Spain	UK	Total
H5N1	1	1	4	2		2		4	14
H5N8			4		5			1	10
H7N7							1		1
Total	1	1	8	2	3	2	1	5	25

Table 3: Outbreaks of low pathogenicity avian influenza in poultry holdings in Europe

	Belgium	Bulgaria	Denmark	Finland	France	Germany	Italy	UK[(a)]	Total
H3N8			1						1
H5	1								1
H5N1					1		1		2
H5N2	1	1					1		3
H5N3							1		1
H5N7			1						1
H5N8		1							1
H6N1					1				1
H6N8					1				1
H7N1			3				1		4
H7N3							2	3	5
H7N7						4	2		6
H9N1								1	1
H9N2				1					1
Total	2	2	5	1	3	4	8	4	29

(a): An H7N2 outbreak was also reported in 2007. The manuscript mentioning this outbreak described affected human cases (team, 2007a). No other manuscript could be found about this outbreak nor any DEFRA reports on the internet.

In the following sections, identified factors that influence the risk of introduction and initial spread of avian influenza (HPAIV or LPAIV) into poultry holdings in Europe will be assessed and discussed.

3.2.1. Risk of introduction attributed to the poultry production system

Poultry species and production system are considered to be factors that influence the probability of introduction of avian influenza into poultry holdings. Most of the outbreaks selected for analysis involved waterfowl (ducks, geese and farmed mallards). Eighteen out of the analysed 54 outbreaks involved waterfowl holdings, in addition six holdings had mixed species including waterfowl (all holdings), chickens, turkeys or guinea fowl (Figure 1). Three of these holdings were affected holdings in Italy, where they were categorised as rural or dealer holdings with poultry populations close to or higher than 1000 birds (Cecchinato et al., 2010). Nine outbreaks involved backyard and in seven of those, waterfowl were also raised. Of the remaining outbreaks, 11 involved chicken holdings (layers and breeders), eight turkey holdings and two outbreaks involved ornamental birds (Figure 1).

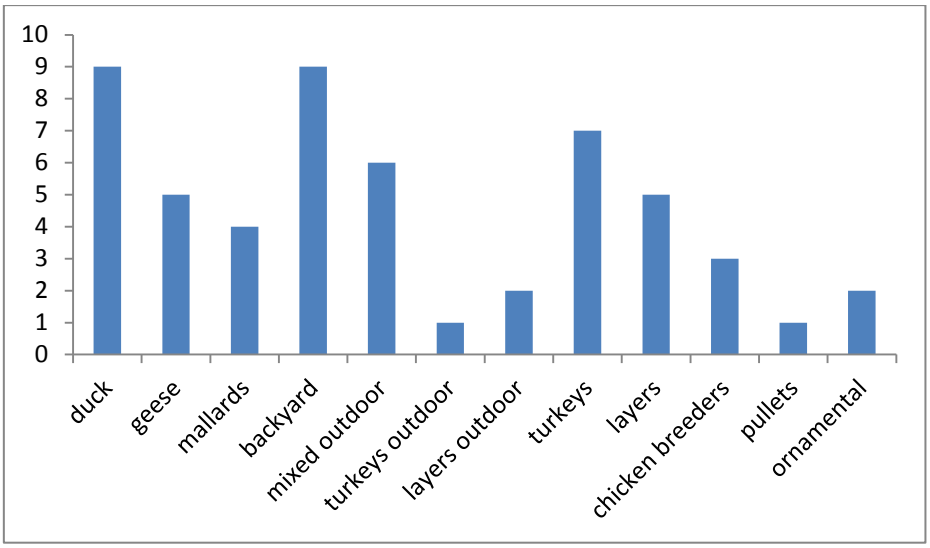

Figure 1: Number of analysed outbreaks of avian influenza (LPAI and HPAI) distributed by poultry production system.

Out of the 54 outbreaks, 29 (54%) involved free range (outdoor) holdings and 25 (46%) involved commercial holdings where birds were raised indoors. These indoor holdings were duck holdings (n=9), turkey holdings (n =7), layer holdings (n = 5) and chicken breeder holdings (n = 3) (Figure 1). Of note are the three outbreaks in chicken breeder holdings, where biosecurity is expected to be high in relation to other production systems. These data do not provide a strong indication of free-range (outdoor) farming as an indirect risk for introduction of avian influenza, in particular for Gallinaceous (turkeys and chickens) species. It does however indicate that there is a risk of infection associated with species susceptibility. With waterfowl and turkeys appearing to have a higher risk of becoming infected with avian influenza than chickens.

A study in the Netherlands (Gonzales et al., 2013) made a formal quantification of the relative risk (RR) of introduction of avian influenza (LPAI) into different production systems using active surveillance data. The study showed that there is a significant higher risk of LPAI introductions in duck, turkey and outdoor (free range) layer holdings compared to layer holdings that keep the layers indoors (reference category). With the exception of broilers, no significant differences were observed between chicken holdings that keep poultry indoors (Table 4).

The higher frequency of outbreaks observed in waterfowl and turkeys holdings than that observed in chicken holdings, in the data analysed in this review, is in agreement with the higher RR estimated by Gonzales et al (Gonzales et al., 2013). This higher risk could be related to the higher susceptibility of waterfowl (Mundt et al., 2009) and turkey (Tumpey et al., 2004) to infections with avian influenza virus than chickens. This susceptibility is relevant when we consider that outbreaks in duck and turkey holdings were mainly in holdings that kept these poultry indoors. In case of farmed waterfowl, species affinity with wild waterfowl might also play a role in the risk of introduction. This is also valid for the virus, since no interspecies adaptation might be required when the virus is transmitted from wild birds to farmed waterfowl.

Outdoor farming is also considered a risk factor for introduction, since it will facilitate direct contact with wild birds or with an environment contaminated by wild birds. This risk was confirmed when comparing the risk of introduction into outdoor layer holdings with indoor layer holdings (Table 4).

Table 4: Relative risk, with accompanying 95% confidence limits, of introduction of low pathogenic avian influenza virus infection into poultry holdings[(a)].

Poultry production system	Relative Risk		
	Mean	UCL[(b)]	LCL[(b)]
Broiler breeders	0.3	0.0	2.4
Pullets	0.7	0.1	5.7
Layer indoors	1[(c)]		
Layer outdoors	11.1	4.9	25.2
Turkeys	7.7	2.0	29.3
Duck meat	12.8	1.6	103.6
Duck breeders	24.5	6.4	94.1
Broilers	0.0[(d)]		

(a): Source: Gonzales et al (Gonzales et al., 2013)
(b): LCL = lower confidence limit, UCL = upper confidence limit.
(c): Layers indoor were the reference category for the estimation of relative risks
(d): No introduction in broiler holdings was observed during the study period: 2007 to 2010.

The higher number of outbreaks in outdoor holdings (including backyard) than in indoor holdings observed in the data analysed in this review adds evidence that confirms the risk outdoor holdings have for introduction of avian influenza.

3.2.2. Risk of introduction associated with contact with wild birds

Data on the potential source of introduction as reported by the authors of each of the selected manuscripts as well as the evidence provided to support the reported source was collected. Most of the primary introductions into poultry holdings (42 out of 54) were attributed to contact with wild birds or material contaminated by wild birds. This attribution was done following epidemiological investigations that excluded the presence of undetected infected poultry holdings as the source of the detected outbreak. For 14 of the reported outbreaks, the virus serotype causing the outbreak was isolated from both the affected poultry and wild birds present in the same region around the same time, with the virus isolates showing high genetic homology (Bouwstra et al., 2015b; Cecchinato et al., 2008; Gall-Recule et al., 2008; Handberg et al., 2010; Lindh et al., 2014; Terregino et al., 2007; Therkildsen et al., 2011). In addition to the phylogenetic data, presence of wild birds in the proximity of the effected holdings was also reported as further evidence for some of the analysed outbreaks (Bouwstra et al., 2015b; Gall-Recule et al., 2008; Therkildsen et al., 2011). Other sources of evidence presented to support the incrimination of contact with wild birds as the source of introduction were: proximity (epidemiological evidence) (Cecchinato et al., 2010; Cherbonnel et al., 2007; Conraths et al., 2016; Iglesias et al., 2010; Manvell et al., 2008; Marche et al., 2014; Probst et al., 2012; Ward et al., 2008a; Ward et al., 2009b) or phylogenetic inference, where virus was only isolated from the affected poultry holdings and compared with sequences reported in databases such as GenBank (Alexander et al., 2010; Bragstad et al., 2007; Bragstad et al., 2005; Corrand et al., 2012; Handberg et al., 2010; Hanna et al., 2015; Marche et al., 2014; Marinova-Petkova et al., 2016; Parker et al., 2014; Reid et al., 2011; Starick et al., 2008; Szeleczky et al., 2009; Terregino et al., 2007) (Table 5).

Introductions of AIV into outdoor poultry holdings can be reasonably associated with contact with wild birds, however it is more difficult to explain this association when poultry are raised indoors. Many of the outbreaks involved commercial poultry kept indoors, and the epidemiological and genetic evidence indicated wild birds as the source of the virus (Bouwstra et al., 2015b; Conraths et al., 2016; Gall-Recule et al., 2008; Parker et al., 2012). Presence of faecal droppings on the ground of affected premises was reported, and it has been speculated that the route of virus entry into the poultry house was via materials such as footwear, equipment or beddings contaminated with wild bird faeces (Conraths et al., 2016; Parker et al., 2012).

The studies assessed here, in particular those where the virus causing the outbreaks were recovered from both poultry and wild birds around the same geographical and temporal window, clearly show

that contact with wild birds or material contaminated by wild birds is the main risk factor for introduction of avian influenza into poultry holdings in Europe. This was further supported by the higher risk of introduction shown for holdings that keep poultry outdoor than those that keep them indoors.

Table 5: Potential sources of introduction of avian influenza virus into poultry holdings in Europe. The identified sources and the evidence presented to support the source of introduction in the selected manuscripts for data collection and analysis is summarised

Source of introduction			Number of outbreaks
	Primary evidence[a]	***Secondary evidence***	
Wild birds			**42**
	Virus Isolated in both poultry and wild birds[b]		14
		Proximity	*8*
	Proximity[c]		14
		Phylogenetic inference	*3*
	Phylogenetic inference[d]		14
Abattoir – contaminated meat			**3**
	Processing infected meat		3
		Virus isolated in both meat and infected poultry	*2*
		Imported meat	*1*
Ownership			**5**
Scavenging and presence of infected backyard flocks			**2**
	Phylogenetic inference		1
		Presence of scavengers	*1*
Infected life birds			**2**
	Import of ornamental infected animals		1
	Trade		1

(a): Primary evidence is the main evidence presented to support the identified sources of introduction (risk factors). Secondary evidence is supporting/additional evidence presented by the authors.
(b): The virus serotype causing the outbreak was isolated from both the affected poultry and wild birds present in the same region around the same time, with the virus isolates showing high genetic homology.
(c): Proximity refers to the presence of wild birds within the poultry holding or in the vicinity of the holding. It also refers to the location of the holding close to wetlands populated with wild birds.
(d): Phylogenetic inference, refers to the isolation of the virus from the affected poultry holdings only and then comparisons were done with sequences reported in databases such as GenBank.

3.2.3. Risk of introduction associated with movement of live birds or contact with infected fomites

Contaminated poultry meat was the source of introduction of HPAIV into a turkey holding in the UK (Irvine et al., 2007) and a duck farm in Germany (Harder et al., 2009). In both countries, the affected holdings also had an abattoir within their premises, where contaminated imported meat was either processed (the UK) or infected ducks from an undetected infected farm were slaughtered (Germany). Introduction of infection from the abattoirs to the poultry holdings was likely via contaminated fomites, which highlighted flaws in biosecurity in the effected holdings. The contaminated meat from the abattoir in Germany was distributed to different supermarkets within the country and resulted in two further outbreaks in backyard poultry which had access to uncooked offal from the contaminated duck meat purchased at the supermarket (Harder et al., 2009) (Table 5).

Other sources of introduction into commercial poultry holdings were the presence of undetected circulation in backyard flocks (Cecchinato et al., 2008) and scavenging (DEFRA, 2006; Manvell et al., 2008). The former resulted in trade of infected live animals within rural (mixed outdoor) holdings or sharing of personnel or contaminated equipment between holdings under the same ownership (Table

5). In case of the latter, it was speculated that scavenging foxes brought infected carcasses to the premises of a broiler breeder holding, contaminating the environment and virus was later introduced into the poultry house via contaminated footwear worn by staff or egg collection teams (biosecurity flaw) (DEFRA, 2006; Manvell et al., 2008).

Finally, imported infected ornamental birds were detected in a quarantine facility in the UK (Alexander et al., 2010). Whether this type of birds would represent a risk for commercial holdings is unclear, however scenarios where ornamental birds could be taken to households which also keep backyard poultry or the farmers themselves purchasing these birds as pets cannot be excluded (Table 5).

3.2.4. Flaws in biosecurity increases the risk of introductions

It is noteworthy considering the outbreaks that took place in chicken breeder holdings (n = 3) (Manvell et al., 2008; Parker et al., 2012; Probst et al., 2012). These holdings are expected the have the highest level of biosecurity and still virus introduction took place. The route of introduction of the virus into the poultry houses was considered to take place via footwear or equipment contaminated with wild bird faeces (Conraths et al., 2016; Manvell et al., 2008). This route of entry, contaminated fomites, could also explain the routes of entry into the affected indoor holdings analysed in this review (Figure 1). Holdings with same ownership shared personnel and equipment and these were the most likely routes of transmission among these farms (Table 5). In summary, flaws in biosecurity increased the risk of introduction of infection in these commercial holdings.

Finally, the outbreaks involving the abattoirs in the UK and Germany, highlight the need to improve biosecurity measures in abattoirs to prevent contamination of their surrounding environment and therefore the risk of transmission to poultry is minimised.

3.2.5. Common risk factors for the risk of primary introduction and between-farm transmission

Risk factors for infection (transmission) were assessed during large epidemics of HPAI or LPAI in Italy (Busani et al., 2009b), the Netherlands (Thomas et al., 2005) and Romania (Ward et al., 2008a). A summary of the risk factors identified during those epidemics is presented in Table 6.

Although some risk factors might be specific to the conditions of the affected countries, some of the factors summarised in Table 6, clearly overlap with the risk factors identified here for both LPAI and HPAI introductions into poultry. Other factors associated with transmission were also mentioned during outbreak investigations (Cecchinato et al., 2010; Probst et al., 2012) and they also appear to be associated with the risk of introduction. The following are identified factors that may influence both the probability of introduction of AIV into a poultry holding and the probability of transmission from an infected holding towards other holdings:

1) The poultry species and production system. Turkeys and layer chickens appear to represent a relative higher risk for both introduction and transmission of AI. Waterfowl were not discussed in the analysis summarised in Table 6 which might be due to the structure of the poultry sector in those countries. However it has often been reported that ducks might not show clear clinical signs following infection with AIV, which can result in undetected infection and further spread as seen in the outbreaks originated at the slaughter house in Germany (Harder et al., 2009) and the recent HPAI subtype H5 outbreaks in France in 2016.
2) Environmental factors such as short distance of the holding to rivers or streams were associated with higher risk of infection in Romania (Table 6). Close distance to rivers or waterbodies could also be an indirect indicator of presence of wild birds and risk of introduction.

3) The biosecurity standards.- Low levels of biosecurity in the farm lead to between farm transmission following introductions. This was for example observed in farms having the same ownership and shared personnel and equipment between them (Alexander et al., 2010; Cecchinato et al., 2010; Manvell et al., 2008; Probst et al., 2012). The level of biosecurity is related with the poultry management system with biosecurity perceived to be lower in outdoor and backyard holdings than in indoor holdings. Therefore outdoor holdings, which have a high risk of introduction, may also represent a risk for between-farm transmission. Although this association was not significant for free range and indoor chicken holdings during the HPAI epidemic the Netherlands (Table 6).

Table 6: Identified significant risk factors for infection during epidemics of highly pathogenic avian influenza (HPAI) in Italy (H7N1 in 2001), the Netherlands (H7N7 in 2003) and Rumania (H5N1 in 2005-2006) as well as low pathogenic avian influenza in Italy (H5 and H7 serotypes)

Country	Risk Factor	HPAI		LPAI	
		Hazard ratio	**95% CI**	**Hazard ratio**	**95% CI**
Italy[(a)]	**Production system**				
	Broilers	1			
	Layers	6.83	4.61 - 10.11		
	Turkeys	11.1	7.66 - 16.11		
	Breeders (turkeys and chickens)	6.23	3.81 - 10.20		
	Others species	1.48	0.87 - 2.51		
	Farm size				
	< 10000	1		1	
	30000	1.42	1.03 - 1.94	1.44	1.11 - 1.87
	50000	2.54	1.74 - 3.70	2.24	1.46 - 3.43
	> 50000	3.27	2.25 - 4.74	4.5	2.03 - 9.94
	Distance in meters from nearest outbreak				
	> 4500	1		1	
	1500	4.55	3.15 - 6.56	4.09	3.12 - 5.36
	3000	3.29	2.36 - 4.59	4.73	2.95 - 7.59
	4500	3.03	2.21 - 4.15	2.82	1.4 - 5.70
		Odd ratio	**95% CI**		
Netherlands[(b)]	**Production system**				
	Layers vs Broilers	2.05	1.29 - 3.27		
	Free range chickens vs indoor	1.28	0.84 - 1.95		
Romania[(c)]	**Anthropogenic and environmental**				
	Road within 5 Km	5.27	1.21 – 22.9		
	River or stream within 5 Km	1.97	1.06 - 3.72		

(a): HPAI estimates adapted from Busani et al (2009b) and LPAI estimates from Busani et al (2009a). Factors related with geographical location (e.g Region, altitute) are not included.
(b): Data from Thomas et al (2005). Only chicken holdings were included in that study. Odd ratios in the table were adjusted for farm size. The authors categorised layers as "layer finishers" .
(c): Data taken from Ward et al (2008a). Only results from the multivariable analysis which included all epidemic phases in 2005 – 2006 were taken for this table. The epidemiological unit for these studies were villages.

4. Conclusions

This study reviewed and summarised the evidence on the factors influencing the risk of introduction of avian influenza into poultry holdings in Europe. Collected evidence indicates that the main risk for introduction is contact with wild birds or fomites contaminated with wild bird faeces. Additional factors influencing the risk of introduction were: (i) poultry species, with waterfowl species and turkey having a high risk for infection, (ii) poultry production system, with outdoor (free range) holdings having a higher risk of introduction than indoor holdings, (iii) processing and commercialisation of contaminated fresh meat, (iv) movement, including import, of infected poultry or ornamental birds and (v) biosecurity flaws, which allowed the introduction of AIV into poultry holdings via contaminated fomites. Some of the identified factors, such as poultry species and production system and levels of biosecurity, may also be associated with the risk of between-farm transmission following introductions.

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Abbreviations

AI	Avian influenza
AIV	Avian influenza virus
HPAI	Highly pathogenic avian influenza
LCL	Lower confidence limit
LPAI	Low pathogenic avian influenza
RR	Relative risk
SR	Systematic review
UCL	Upper confidence limit

Appendix A – Summary of the systematic review protocol

Review question:	What are the risk factors on primary introduction of HPAI and LPAI virus strains into European poultry holdings?
Years	2005-2016
Language	English
Publication type	• Peer-reviewed papers, including original research papers (including models), reviews and outbreak reports • Grey literature can be included but only in the event of data gaps within the primary literature
	Comment/Explanation: • For reviews the original paper(s) referenced should be screened, if the reference was published 2005-2016. Papers in the review published before 2005 are not to be considered • MS/country reports will be excluded as these are considered in a different part of the opinion
Geographical limits	• Only papers considering European countries will be included, with the exception for papers discussing the recent (2014-2015) H5 strains (novel H5N8, novel H5N2, novel reassortant H5N1) in the USA
Population	• Any species or breed of commercial poultry will be included in the literature search
	Comment/Explanation: • 'poultry' means all birds that are reared or kept in captivity for the production of meat or eggs for consumption, the production of other products, for restocking supplies of game birds or for the purposes of any breeding programme for the production of these categories of birds, including chickens, duck, quail, goose, turkey, pheasant, guinea fowl, partridge, swans, ostrich and pigeon • 'commercial poultry' means poultry kept for commercial purposes • All ages, any breed and both genders will be included
AI strains	European HPAI AND LPAI strains (2005-2015): • HPAI viruses: H5N1, H5N8, H7N1, H7N7 • LPAI viruses: H10N7, H5, H5N1, H5N2, H5N3, H7N1, H7N2, H7N3, H7N7 and others
Outcome	Primary introductions (or first detection) of avian influenza virus into poultry holdings

Appendix B – Summary of excluded manuscripts in the first level of relevance screening

Reasons for exclusion of manuscripts during the first level of relevance screening. This screening was done on the titles and abstracts of retrieved citations post deduplication.

Reasons for exclusion	Number of studies excluded
Not in poultry: mostly humans and other mammals	148
Manuscripts on: diagnostics, immunology, pathology, transmission experiments (laboratory) in poultry	203
Not in Europe or USA	187
Wild birds: mostly pathology studies and surveillance	75
Descriptive reviews	47
Reports/letters, opinions: not scientific	40
Other language: Russian, French, Spanish, German, Dutch, Chinese, Polish	53
Survival, behavioural studies (humans), economic analysis	39
Avian influenza was not the subject of the study	9
Total	**801**