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Avian Influenza Viruses (AIVs) in poultry products: a review

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REVIEW ARTICLE

Avian influenza viruses in poultry products: a review

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Abstract

The extensive circulation of the H5N1 highly pathogenic avian influenza (HPAI) virus, and the human health threat that it poses, has raised concerns over the food safety implications of this infecting poultry. In addition, among the most important risk factors for the possible emergence of avian influenza (AI) in the European Union (EU) and United States (U.S.), the European and Food Safety Agency (EFSA) and U.S. Department of Agriculture's (USDA) Animal and Plant Health Inspection Service (APHIS), respectively, have identified legal and illegal importations of infected poultry commodities. This paper reviews existing knowledge on the presence of viable avian influenza viruses in poultry commodities.

Introduction

The occurrence of the H5N1 HPAI virus throughout Asia Africa and Europe has raised concerns about food safety issues for poultry products for human consumption and for the risk of transmission of infection to animals via swill feeding, predation and scavenging. The introduction of the disease into a naïve country through trade of poultry commodities has been reported and increased these concerns (Tumpey *et al.*, 2002).

Food-borne transmission of infection through poultry products may only occur if there is viable virus in the commodity and the concentration of the virus is sufficient to infect the given host(s) that are exposed to the source of infection. To date, there is reported evidence of the transmission of H5N1 infections to mammals through swill feeding using carcasses of infected birds. Moreover, in some cases of human infection with H5N1 virus, viral transmission was suspected to have occurred after the consumption of fresh duck blood or undercooked poultry products (Quirk, 2004; Schuettler, 2005).

Current EU legislation authorises the importation of poultry products from a specified list of countries (WHO) and defines 'poultry' as: "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". The World Organisation for Animal Health (OIE) provides a very similar definition of poultry "all domesticated birds, including backyard poultry, used for the production of meat or eggs for consumption, for the production of other commercial products, for restocking supplies of game, or for breeding these categories of birds, as well as fighting cocks used for any purpose" (OIE, 2007) and defines commodity as "animals, products of animal origin intended for human consumption, for animal feeding, for pharmaceutical or surgical use or for agriculture or industrial use, semen, embryos/ova, biological products and pathological materials". Similarly to

the EU guidelines, according to the OIE code poultry commodities may be traded provided they come from an NAI- (Notifiable Avian Influenza) free country, zone or compartment (OIE, 2007).

Here we summarise existing information on the presence of AIVs in poultry commodities according to the EU and OIE definition of poultry to aid decision makers in preventing virus spread through trade and to address food safety issues for humans and animals. In addition, areas requiring further investigation are highlighted. In tables 1 and 2 detailed information on the studies cited is presented.

Meat

Field and experimental investigations have been conducted to assess the presence of viable virus in skeletal muscle of birds. However, the majority of the literature published prior to the emergence of H5N1 has dealt with the pathological features of avian influenza infections, focusing on the histopathological lesions in several tissues, including skeletal muscle. Although a report of the detection of AIVs in poultry meat by immunohistochemical techniques was published at the end of the 1980s, only later, with the emergence of H5N1, was poultry meat perceived as a public health risk leading to targeted research in this area.

Highly pathogenicity avian influenza (HPAI). The ability to colonize chicken muscle tissue by a HPAI virus of the H5N1 subtype was assessed by Tumpey et al (Tumpey *et al.*, 2002) using a duck isolates (A/duck/Anyang/AVL-1/01) to infect 4 week old SPF chickens. Following intranasal infection with 10^6 EID₅₀/0.1ml of virus, breast muscle was found positive on day 2 and 3 pi with titres ranging from 5.3 to 5.5 log₁₀ EID₅₀/g.

Swayne and Beck (Swayne & Beck, 2005) also investigated the presence of H5 HPAI viruses in chicken meat. Three- to four-week-old specified pathogen-free (SPF) chickens, received

10^6 EID₅₀/0.1ml of the A/chicken/Pennsylvania/1370/1983 (H5N2 HPAI) oro-nasally. On day 1, 3 and 5 post infection (pi) virus was isolated from breast and thigh meat samples with average titres of $10^{2.2}$ to $10^{2.7}$ EID₅₀/g in breast meat samples and of $10^{2.6}$ to $10^{3.2}$ EID₅₀/g in thigh meat samples.

The H5N3 (A/tern/South Africa/61) was used as a reference virulent strain in a study conducted by Kishida et al (Kishida *et al.*, 2004). Birds of four weeks of age received 10^7 EID₅₀/0.1ml intra nasally, blood and meat samples were collected on day 3, 7 and 14 pi. The H5N3 HPAI infected birds died within day 1 pi and virus was isolated from muscles with viral titres $> 10^4$ EID₅₀/g.

Low pathogenicity avian influenza (LPAI). Field studies have proven that a LPAI virus of H9N2 subtype could be detected in muscle of imported chicken carcasses from China to Japan (Kishida *et al.*, 2004). To assess the tissue tropism of this subtype, H9N2 strains (A/chicken/aq-Y-55/01; A/chicken/aq-Y-135/01; A/chicken/Beijing/2/97 and A/duck/Hokkaido/9/99), isolated from different sources, were tested for their ability to replicate in muscle tissues of four week old SPF chickens infected intra-nasally (10^7 EID₅₀/0.1ml). The first two viruses had been isolated from meat samples of imported carcasses, the Beijing/97 strain from diseased chickens and the Hokkaido/99 strain. The H9N2 strains isolated from meat of imported carcasses were recovered in muscle but not in blood samples of infected birds on day 3 pi only. Viral titres in meat ranged from $10^{1.6}$ to 10^2 EID₅₀/1g. No viable virus was detected in muscle tissue of birds infected with the other two H9N2 strains which had not originally been isolated from muscle tissues.

In another experiment, no LPAI virus was recovered from blood, breast and thigh meat samples by virus isolation following experimental infection of four week-old SPF chickens receiving 10^6 EID₅₀/0.1ml oro-nasally of the following viruses: A/turkey/Virginia/159512/2002 (H7N2 LPAI); A/chicken/New York/21586-8/99 (H7N2 LPAI) (Swayne & Beck, 2004).

Turkey meat. One study is available in literature on the presence of AIV in turkey meat (Toffan *et*

al., 2008). Two H7N1 viruses, HPAI (A/turkey/Italy/4580/99) and LPAI (A/turkey/Italy/3675/99), were used in this study to infect vaccinated and non-vaccinated birds with a whole inactivated bivalent vaccine (H5 and H7). Fifty-day old turkeys received 10^6 EID₅₀/0.1ml per bird. None of the samples obtained from vaccinated and challenged birds with either of the HPAI or the LPAI strains, contained viable virus. In non-vaccinated LPAI challenged turkeys no viable virus was detected in meat and expectedly, meat obtained from non-vaccinated birds challenged with the HPAI strain, contained significant amounts ($10^{4.38}$ EID₅₀/g) of virus.

Duck meat. Field and experimental investigations exist on the possibility of recovering H5N1 virus from meat of Pekin ducks (*Anas platyrhynchos*). Tumpey et al (Tumpey *et al.*, 2002) reported the isolation of H5N1 HPAI from duck meat (*Anas platyrhynchos*) during a national survey on imported meat from China to South Korea. Subsequently, four H5N1 HPAI viruses (A/duck/Anyang/AVL-1/01 isolated from duck meat; A/Environment/Hong Kong/437/99; A/chicken/Hong Kong/317.5/01 and A/chicken/Hong Kong/220/97), were tested for pathogenicity studies in two-week old Pekin ducks. Groups of eight ducks each were composed and birds infected with 10^6 EID₅₀/0.1ml. On days 2, 4, 7 and 14 pi skeletal muscle was collected from infected ducks. Only ducks infected with the A/duck/Anyang/AVL-1/01 showed presence of virus in skeletal muscle on day 2 pi with titres ranging from 10^3 to 10^4 EID₅₀/g (Tumpey *et al.*, 2002).

Li *et al.* (Li *et al.*, 2006) reported the detection of viable virus in duck muscle of birds experimentally infected with H5N1 HPAI (A/goose/Vietnam/3/2005) (10^6 EID₅₀/ml intra-nasally). Although the infected birds did not show any clinical signs, titers of 10^3 EID₅₀ per gram of meat was detected in muscles on day 3 pi.

In another study carried out by Pantin Jackwood et al (Pantin-Jackwood *et al.*, 2007) four different H5N1 HPAI A/Vietnam/1203/2004; A/Thailand PB/6231/2004; A/crow/Thailand/2004 and A/Egret/HK/757.2/2002 were tested. Pekin ducks of two and five weeks of age were intra-nasally infected with 10^5 EID₅₀/ml of each virus. Tissues from infected birds were collected on day

2 pi for virus isolation and histopathological examinations. Birds were observed until day 14 pi. All viruses used in this study were able to replicate in skeletal muscle of infected birds of two weeks of age. An additional pathogenicity study was conducted in five-week-old Pekin ducks with only two viruses: A/Egret/HK/757.2/2002 and A/Thailand PB/6231/2004. Of these, only the A/Egret/HK/757.2/2002 was detected in muscle tissue by virus isolation after 2 days pi. Viral titres ranged from 10^2 to 10^4 EID₅₀/g in birds of five week of age and between 10^4 and 10^6 EID₅₀/g in the 2 week old birds. Yamamoto and colleagues (Yamamoto *et al.*, 2007) assessed the ability to colonize skeletal muscle by H5N1 HPAI virus in call ducks (*Anas platyrhyncha var domestica*), a half breed of wild and domestic duck, commonly reared for meat consumption in Japan. The virus tested was the A/chicken/Yamaguchi/7/2004 administered in four-week-old call ducks at the dose of 10^8 EID₅₀/ml. On day 4 pi virus was recovered in skeletal muscle with viral doses of 10^7 EID₅₀/g. Viral antigen was also detected by immunochemistry in two-week-old call ducks on day 3 and 5 pi.

A laboratory investigation on the efficacy of whole inactivated AI vaccine in waterfowl assessed the viral colonization in meat of experimentally infected Pekin duck (*Anas platyrhynchos*) using an H5N1 HPAI (A/duck/Vietnam/12/2007) (Beato *et al.*, 2007). Ducks were infected with 10^7 EID₅₀/0.1ml of virus oro-nasally. Viable virus was detected in 100% of meat samples collected from unvaccinated birds on days 3 and 4 pi, and in none of the samples collected from the vaccinated birds. The amount of virus present in meat collected at day 3 p.i. was $10^{1.5}$ EID₅₀/g. In positive samples collected at day 4 p.i., the amount of virus was $<10^1$ EID₅₀/g.

Eggs

The most likely route of viral infection in eggs is thought to be through the contamination of the shell by faecal material after the ovoposition. However, the presence of AIVs in the egg contents may occur by vertical transmission of the virus. In literature there are few studies aimed at assessing

the vertical transmission of AI. Samadieh and Bankowski (Samadieh & Bankowski, 1971) assessed the vertical transmissibility of AIVs in turkeys. Two viruses, for which no information on pathogenicity is reported, (T/Calif/meleagrium/64 and T/Calif/5142/66) were used to infect embryonated turkey eggs on 6, 10 and 18 days of incubation with $10^{2.25}$ ELD₅₀/0.2 ml via the allantoic cavity. Eggs inoculated on day 6 of incubation survived inoculation and were chilled on day 18 or 25 post inoculation. The embryos were positive for virus isolation and the allantoic fluid exhibited HA activity. Unexpectedly, eggs inoculated on day 10 of incubation died following inoculation. Of the four poults that survived the inoculation and hatched, two were killed for analysis on day 1 and on day 7 after hatching. Internal organs of poults were negative for virus isolation. However, birds developed low HI titres (1:8-1:16). All eggs inoculated on day 18 of incubation that died before day 25 of incubation were positive by virus isolation; virus was not isolated from poults killed on day 2 or 7 after hatching.

Virus was not recovered in fertile eggs laid by inoculated turkey hens with either of the viruses intra-nasally, intra-tracheally, intra-abdominally and inseminated with contaminated semen. However HI antibodies were detected in serum of poults hatched laid by inoculated hens with titres ranging from 1:4 to 1:16 even though no virus was isolated regardless of the route of infection.

It is worth mentioning that, although not falling in the definition of poultry, a field study showed the isolation of a H3 subtype virus in 1/61 black-headed gull embryos (*Larus ridibundus*) confirming the possibility of virus transmission by eggs. However the viral penetration of AI through the egg-shell, which may have been contaminated after the egg is formed or laid, rather than a trans-ovarial transmission, remained questionable (Gresikova *et al.*, 1979).

Chicken eggs: HPAI virus. The first report of the presence of avian influenza viruses in chicken eggs is dated 1985. The presence of an H5N2 was detected in yolk, albumen and on the shell surface of eggs collected during the 1984 HPAI outbreak in Pennsylvania and Virginia from commercial layers and broiler flocks (Cappucci *et al.*, 1985). The egg shell, albumen and yolk were

sampled from eggs obtained from commercial layers, broiler breeders and turkey breeder flocks. Most of the eggs sampled were of market quality with a low percentage (10%) with lesions referable to AI infection. AIV was recovered from the yolk, albumen and shell surface of chicken eggs only. In general virus recovery was higher from albumen than from yolk or albumen and yolk together. In addition virus was recovered at a lower rate from eggs laid from chicken flocks not showing clinical signs.

Chicken eggs: LPAI virus. Internal contents (yolk, albumen and vitelline membrane) of eggs laid on day 3 and 5 pi by experimentally infected intra-tracheally and intravenously, SPF laying hens with a H4N8 (A/chicken/Alabama/7395/75) subtype were found negative (Shalaby, Slemons, & Swayne, 1994). Rather unexpectedly, AIV was recovered from the ovarian and oviduct tissue of hens infected by both routes of infection.

Turkey eggs. There is only one report on the presence of AIV in turkey eggs. Narayan et al (Narayan *et al.*, 1969) reported the isolation of the H5N9 HPAI strain (A/turkey/Ontario/7732/66) in eggs laid by turkeys exposed to experimental infected turkeys. Virus was detected in the yolk after disinfection of the shell.

During the outbreak caused by the H5N2 HPAI strain in the US, a survey on eggs collected in affected turkey breeder flocks was carried out (Cappucci *et al.*, 1985). No virus was recovered from turkeys eggs (Cappucci *et al.*, 1985).

Waterfowl eggs. A field investigation conducted at border crossings between China and Vietnam resulted in the isolation of an H5N1 HPAI virus from shell washes of duck and goose eggs in China (Li *et al.*, 2006). Three out thirty samples of egg shell washes were found positive by RRT-PCR from both duck and geese eggs and subsequently confirmed by virus isolation.

Quail eggs. The only available study indicating viral infection in quail eggs is from Promkuntod *et al.* (Promkuntod *et al.*, 2006). In this study the presence of AIV was investigated in the albumen, allantoic fluid and oviduct of naturally infected Japanese quails (*Coturnix coturnix japonica*) in Thailand. The viral titres ranged from $10^{4.6}$ to $10^{6.2}$ ELD₅₀/ml in the internal content of eggs (mixture of albumen and allantoic fluid).

Feathers

Very little data is available on the presence on AIVs in feathers. This aspect of the disease has become an issue for trade purposes related to feathers used for duvets and clothing.

The presence of AIVs in feathers is most likely to be related to contamination through faecal material and contaminated dust, although H5N1 (A/chicken/Hong Kong/220/1997, HPAI) virus have been detected on day 1 pi in the feather follicle epithelium of chickens, Japanese quails, turkeys and Guinea fowl infected intranasally with a viral inoculum containing $10^{5.8}$ to $10^{6.2}$ EID₅₀/0.1ml (Perkins & Swayne, 2001).

A recent study by Yamamoto *et al.* (Yamamoto *et al.*, 2007) investigated the presence of the A/chicken/Yamaguchi/7/2004 (H5N1 HPAI) in feathers of call ducks (*Anas platyrhyncha* var *domestica*) by histopathological examination. Feathers, especially those still developing, of call ducks infected with a H5N1 presented necrosis of the epithelium observed from the epidermal

The identification of AIV of the H5N1 subtype by immunochemistry in feathers and skin of domestic ducks (*Anas platyrhyncha* var *domestica*) and geese (*Anser cygnoides* var. *domestica*) of four weeks of age has been recently reported (Yamamoto *et al.*, 2008). Two different HPAI viruses were used A/chicken/Yamaguchi/7/2004 and A/chicken/Miyazaki/K11/2007 at the dose of 10^8 EID₅₀/0.1ml administered intra nasally.

The skin, including numerous feathers, was removed from the head, neck, back, shoulder,

abdomen, thigh, and tail for histopathological examination. For virus isolation the skin from the neck was sampled. All skin samples obtained on day 3 and 5 pi from geese infected with the Miyazaki strain were positive by virus isolation with titers ranging from $10^{4.4}$ and $10^{2.5}$ EID₅₀/g. With the Yamaguchi strain only one skin sample from ducks were positive on day 3 pi ($10^{3.5}$ EID₅₀/g) and one skin sample from geese was positive on day 5 pi ($10^{4.5}$ EID₅₀/g). Moreover the detection of H5N1 HPAI in feathers by virus isolation was reported during the 2007 H5N1 epidemic in UK in a turkey farm (Slomka, 2008).

Other potential commodities

Eating habits may vary widely from country to country and, as a result, the variety of poultry products for consumption that may be traded across borders, either legally or illegally, will reflect this difference. This has important bearing not only in the context of animal health, but also human health, especially in those countries where raw poultry products are consumed.

Liver. The presence of viable virus of liver of infected Pekin ducks was investigated by Beato *et al.* (Beato *et al.*, 2007). Ducks were infected with an H5N1 HPAI (A/chicken/Vietnam/12/2005) oronasally with 0.1ml of viral suspension containing 10^7 EID₅₀. Liver samples collected on day 3 and 5 pi resulted positive by virus isolation.

Blood of chickens: HPAI virus. A significant amount (titres higher more than 4 log₁₀ EID₅₀) of A/tern/South Africa/61 virus (H5N3 subtype) was recovered in blood of infected birds on day 1 pi by Kishida *et al.* (Kishida *et al.*, 2004). Chickens of four weeks old received 10^7 EID₅₀/0.1ml intranasally. Swayne and Beck (Swayne & Beck, 2004) detected viraemia from day 1 to day 3 pi. in four-week-old SPF chickens infected with the H5N2 HPAI strain (A/chicken/Pennsylvania/1370/1983) with 10^6 EID₅₀.

Blood of chickens: LPAI virus. Interestingly Kishida et al (Kishida *et al.*, 2004) reported the isolation of LPAI viruses in blood only when chickens were co-infected with *Staphylococcus aureus* and A/chicken/aq-Y-55/01 (H9N2); or A/chicken/Beijing/2/97 (H9N2).

None of LPAI viruses tested (A/turkey/Virginia/159512/2002 (H7N2 LPAI); A/chicken/New York/21586-8/99 (H7N2 LPAI)) were recovered from blood samples by virus isolation after experimental infection of four week-old SPF chickens receiving 10^6 EID₅₀/0.1ml oronasally (Swayne & Beck, 2004).

Blood of turkeys:HPAI virus. Narayan and colleagues (Narayan *et al.*, 1969) detected viraemia in 24-week-old turkeys infected with a HPAI isolate H5N9 subtype (A/turkey/Ontario/7732/66) intranasally with 0.5 ml containing $10^{8.7}$ EID₅₀. Virus was detected at 16 and 24 hours pi with titres ranging from $10^{2.7}$ to $10^{3.7}$ EID₅₀/ml. The same strain was not recovered from blood of six-month-old ducks and geese and seven-month-old pigeons receiving 10^8 EID₅₀ intravenously.

The only available data on the presence of LPAI virus in blood of turkeys is reported by Toffan et al (Toffan *et al.*, 2008) who investigated the occurrence of viraemia in non vaccinated and vaccinated challenged turkeys with H7 subtype viruses. None of the vaccinated birds challenged with LPAI or HPAI viruses showed viraemia by RRT-PCR or virus isolation. All non-vaccinated infected turkeys with HPAI strain showed viraemia on day 3 pi with viral titres in blood ranging from 10^1 to $10^{5.8}$ EID₅₀/0.1ml. Interestingly, on day 2 pi two non-vaccinated birds challenged with LPAI virus, showed viraemia by RRT-PCR and only one sample was confirmed by virus isolation.

Blood of ducks: HPAI virus. Following infection with 10^7 EID₅₀/0.1ml of H5N1 HPAI (A/duck/Vietnam/12/2005) in vaccinated and non-vaccinated Pekin ducks of seven weeks of age, the occurrence of viraemia was demonstrated (Beato *et al.*, 2007). Only in non-vaccinated infected birds was viable virus was detected on day 3 and 4 pi in sacrificed and dead birds, respectively.

Inactivation of AIV-infected commodities

Only a limited number of studies have investigated protocols to treat and thus inactivate AIVs present in raw products from infected poultry.

Heat inactivation of AIVs in eggs products. Recently, studies on heat inactivation using pasteurization protocols for egg products have been carried out on LPAI and HPAI viruses (Swayne & Beck, 2004). The effect of low and high temperature industrial pasteurization was investigated in five different egg products. In this study the heat resistance of two strains: an H7N2 LPAI (A/chicken/New York/13142-5/94) and an H5N2 HPAI (A/chicken/Pennsylvania/1370/83) exposed to 55°C, 57°C, 59°C, 61°C or 63°C in a water bath or in a thermocycler was evaluated when diluted in homogenized whole egg, liquid egg white, 10% salted egg yolk and dried egg white to achieve a final concentration of $10^{5.5}$ EID₅₀/ml per product. Data reported in that study showed the inefficacy of heat treatment methods (water bath and thermocycler) at 55°C to completely inactivate the viruses, while attempts with higher temperatures were successful. A positive correlation was found between the time required for inactivation of the viruses and the type of egg product in which it was contained. Viruses in 10% salt yolk had the most rapid and complete inactivation. In contrast, the HPAI virus in dried egg whites was the most resistant to inactivation. However, the time to inactivate the H5N2 HPAI (A/chicken/Pennsylvania/1370/83) virus from $10^{4.9}$ EID₅₀/ml to 10^0 EID₅₀/ml was less than is used in industry standard pasteurization protocol for in homogenized

whole egg, liquid egg white and 10% salt yolk (Swayne & Beck, 2004).

Heat and pressure inactivation of AIVs in meat. The effect of heat treatment on HPAI virus (A/chicken/Korea/ES/2003, H5N1 subtype) in chicken meat has also been investigated (Swayne, 2006). Thigh and breast chicken meat, from experimentally infected animals, was examined for virus infectivity after exposure at 30°C, 40°C, 50°C, 60°C and 70°C and after treatment at 70°C for 1, 5, 10, 30, and 60 seconds, using the heating block of a thermocycler as the inactivation method. The initial titres of infected thigh and breast meat with the H5N1 strain were $10^{6.8}$ and $10^{5.6}$ EID₅₀/gr respectively. After exposure at 30°C, 40°C and 50°C the titre in both types of meat sample remained unchanged. Complete inactivation was only reached after exposure at 70°C (1 second) and at 70°C for 5 seconds in the breast and thigh meat respectively (Swayne, 2006).

Isbarn et al (Isbarn *et al.*, 2007) reported the heat inactivation of the H7N7 HPAI virus (A/FPV/Bratislava/79) in chicken meat. The thermal inactivation of the H7N7 virus was investigated from 50 to 65°C. A virus suspension containing 10^7 to 10^8 PFU/ml was mixed to chicken meat and exposed at the above temperatures. At 50°C the virus decreased 1 log₁₀ in titre in 10 minutes, and an exposure of 90 seconds at 63°C decreased the viral titre of 4 log₁₀. In the same study the effect of pressure in inactivating AIV in chicken meat was also investigated. A pressure range from 0.1 to 500 Mpa (1bar = 0.1MPa) and the target temperature from 0 to 60°C were applied to the same mixture of chicken meat and viral suspension used for the heat inactivation experiment. Data of this study showed the sensitivity of the H7N7 strain to pressure even at low temperatures. The viral titre was reduced 5 log₁₀ in chicken meat when a pressure of 500MPa for 25 seconds was applied. Moreover viral titre in chicken meat was decreased 1.5 log₁₀ when exposed at 400 MPa for 1 minute.

Conclusions

Data collected in this review indicate that AIVs may be recovered from a variety of poultry products.. However, the presence of AIVs in poultry products is influenced by the characteristics of the viral strain, particularly its pathogenicity and thus the ability to cause systemic infection. As a consequence the host also influences the likelihood of the virus being present. Data are still fragmentary and further studies should be carried out in a more extensive and coordinated manner in order to establish proper risk assessments on the spread of infection to a given area or/and host by poultry products.

From field and experimental studies conducted to assess viraemia in birds infected with HPAI and LPAI strains, it appears that both pathotypes can be isolated from blood. These findings overrule the general consensus that LPAI viruses cause localised infection replicating in the respiratory and digestive tracts only. Based on these data it may be inferred that muscle colonisation by LPAI virus may occur in poultry species under certain circumstances. With reference to poultry meat, the only available field evidence on the isolation of a LPAI virus is reported by Kishida *et al* (Kishida *et al.*, 2004). However only four studies are presently available on the detection of viruses in meat of chickens and turkeys, and no data are available for other species. The need to investigate the ability of LPAI viruses to colonize muscles tissues of other avian species included in the definition of poultry that may play an important role in the economy of a given country is crucial in making proper risk assessments for trade purposes.

HPAI virus strains are able to colonize poultry meat (Beato *et al.*, 2007; Pantin-Jackwood *et al.*, 2007) and thus infection with these viruses poses a risk of spread and transmission through this commodity (Swayne & Beck, 2005).

A larger set of references is available on the presence of the H5N1 HPAI in skeletal muscles of poultry species especially ducks (Beato *et al.*, 2007; Pantin-Jackwood *et al.*, 2007; Tumpey *et al.*, 2002). It is well documented that some of the H5N1 HPAI strains can be detected at high titres in

muscle tissues of infected chickens and ducks. Interestingly Pantin-Jackwood et al (Pantin-Jackwood *et al.*, 2007) highlighted that the presence of H5N1 HPAI virus in skeletal muscle is not related to the ability to cause disease in waterfowl thus the absence of clinical signs does not exclude the recovery of viruses in muscle tissues. In general, the rates of detection of HPAI viruses in poultry meat is higher at the early stages of infection. In all cases, the systemic nature of HPAI viruses, suggests that given infection, commodities have a greater risk of containing viable virus than with LPAI.

Two studies are available on the efficacy of vaccination in preventing viraemia and viral colonisation of meat in ducks and turkeys (Beato *et al.*, 2007; Toffan *et al.*, 2008). This topic is of great importance as vaccination has been introduced as one of the control strategies for AI. Moreover a large debate exists on the possibility of introduction of AI infection in a country/area by movements of live birds and/or poultry products from vaccinated flocks. The results of these two experiments indicate that no virus can be recovered from meat or viscera of properly vaccinated birds.

With regards to eggs, HPAI but not LPAI viruses were detected in the internal contents of eggs as well as from swabs or washes of the egg shell of chickens, ducks and quails (Cappucci *et al.*, 1985; Li *et al.*, 2006; Promkuntod *et al.*, 2006). The means by which AIVs can be present in eggs is either through contamination with infected faeces and/or by vertical transmission. It should be noted that only a limited number of observations have been carried out to investigate possibility of vertical transmission of AIVs.

With reference to other commodities, untreated blood, viscera and feathers have also proven to harbour virus in certain instances.

Although only limited studies have been published, it is reassuring that heat (Swayne, 2006) and heat and pressure treatment (Isbarn *et al.*, 2007) have been able to inactivate to acceptable levels any viable viruses in selected commodities. Further studies are necessary to assess the resistance of AI viruses in other commodities – for which no data currently exists.

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Table 1. Summary of data available in literature on the presence of HPAI viruses in poultry commodities

Commodity	Species	Strains	Experimental (E) or Infecting dose		Titres (log ₁₀ EID ₅₀ /g) detected in commodity	Reference (First author)
			Natural (N) Infection	(EID ₅₀ log10)		
Meat	chickens	A/duck/Anyang/AVL-1/01 (H5N1)		6/0.1ml	5.3 to 5.5	Tumpey, Kishida, Swayne.
		A/chicken/Pennsylvania/1370/1983 (H5N2)	E	6/0.1ml	2.2 to 3.2	
		A/tern/South Africa/61 (H5N3)		7/0.1ml	> 4	
	turkeys	A/turkey/Italy/4580/99 (H7N1)	E	7/0.1ml	4.38	Toffan Tumpey, Li, Pantin- Jackwood
	ducks	A/duck/Anyang/AVL-1/01 (H5N1)	E and N	6/0.1ml	3 to 4	
		A/goose/Vietnam/3/2005 (H5N1)	E	6/0.1ml	3	
		A/Vietnam/1203/2004,	E	5/0.1ml	4 to 6 (2-week-old birds)	
		A/ThailandPB/6231/2004,			2 to 4 (5-week-old birds)	
		A/crow/Thailand/2004,			7	
		A/Egret/HK/757.2/2002 (H5N1)			1.5	
	A/Egret/HK/757.2/2002 (H5N1)	E	8/0.1ml			
		E	7/0.1ml			
		A/chicken/Yamaguchi/7/2004 (H5N1)				
		A/duck/Vietnam/12/2007 (H5N1)				
Eggs	turkeys	A/turkey/Ontario/7732/66 (H5N9)	E	not reported	not reported	Cappucci, Narayan
		H5N2 (Virginia/1985)	N	not reported	not reported	
	chickens	H5N2 (Virginia/1985)	N	not reported	not reported	Cappucci Li
ducks and geese	H5N1 (strain not reported)	N	Not reported	not reported		
	quail	H5N1 (strain not reported)	N	Not reported	4.6 to 6.2	Promkuntod
Feathers	chickens, turkeys, quail, guinea ducks	A/chicken/Yamaguchi/7/2004,	E	5.8-6.2/0.1ml	Not investigated	Perkins
		A/chicken/Miyazaki/K11/2007,				
		A/chicken/Hong Kong/220/1997				
	ducks	A/chicken/Yamaguchi/7/2004,	E	8/0.1ml	Not investigated	Yamamoto Slomka
turkeys	H5N1 (strain not reported)	N	Not available	Not investigated		
Liver	ducks	A/chicken/Vietnam/12/2005 (H5N1)	E	7/0.1ml	Not reported	Beato

Blood	chickens	A/tern/South Africa/61 (H5N3)	E	7/0.1ml	4	Kishida, Swayne
		A/chicken/Pennsylvania/1370/1983 (H5N2)		6/0.1ml	Not reported	
	pigeons, turkeys	A/turkey/Ontario/7732/66 (H5N9)	E	8/0.5ml	Not recovered	Narayan Toffan
		A/turkey/Italy/4580/99 (H7N1)	E	6/0.1ml	1 to 5.8	
		A/turkey/Ontario/7732/66 (H5N9)		8.7/0.5ml	2.7 to 3.7	
	ducks	A/chicken/Vietnam/12/2005 (H5N1)	E	7/0.1ml	Not reported	Beato, Narayan
A/turkey/Ontario/7732/66 (H5N9)		E	8/0.5ml	Not recovered		
Skin	ducks	A/chicken/Yamaguchi/7/2004,	E	8/0.1ml	2.5 to 4.4	Yamamoto
		A/chicken/Yamaguchi/7/2004	E	8/0.1ml	3.5	
	geese	A/chicken/Miyazaki/K11/2007			4.5	Yamamoto
			E			

Table 2. Summary of data available in literature on the presence of LPAI viruses in poultry commodities

Commodity	Species	Strains	Natural (N) or Experimental (E) infection	Infecting dose (EID ₅₀ log10)	Titres (log ₁₀ EID ₅₀ /g)	Reference
Meat	chickens	A/chicken/aq-Y-55/01 (H9N2);	E	7/0.1ml	1.6 to 2	Kishida
		A/chicken/aq-Y-135/01(H9N2)	E	7/0.1ml	1.6 to 2	
	turkeys	A/turkey/Italy/3675/99 (H7N1)	E	6/0.1ml	No infectious virus detected	Toffan
	A/turkey/Virginia/159512/2002 (H7N2)	E	6 ₀ /0.1ml			
	A/chicken/New York/21586-8/99 (H7N2)	E	6/0.1ml			
Eggs	Ducks	No data available				
	turkeys	A/turkey/California/meleagrium/64; A/turkey/California/5142/66	E	2.25/0.2ml	No infectious virus detected	Shalaby
Feathers	chickens	A/chicken/Alabama/7395/75 (H4N8)	N	Not reported	No infectious virus detected	Shalaby
	ducks and geese			Not available		
	avian species			Not available		
Liver	avian species			Not available		
Blood	chickens	A/chicken/aq-Y-55/01 (H9N2) A/chicken/Beijing/2/97 (H9N2).	E (co infection with <i>Staphylococcus aureus</i>)	6/0.1ml 6/0.1ml	Not reported	Kishida, Swayne
	turkeys	A/turkey/Italy/3675/99 (H7N1)	E	6/0.1ml	<1	Toffan
	ducks			No data available		