The spread of pathogens through trade in poultry hatching eggs: overview and recent developments

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Summary

The international trade in poultry hatching eggs could potentially facilitate the global dissemination of poultry disease. Provided the guidelines of the World Organisation for Animal Health (OIE) on breeding flock hygiene are followed, of those avian diseases currently listed by the OIE, only highly pathogenic avian influenza (HPAI), Newcastle disease (ND), and avian mycoplasmosis (caused by Mycoplasma gallisepticum or M. synoviae) should be considered likely to be spread through trade in this commodity. Furthermore, the impact of HPAI and ND on egg production and hatchability will constrain the potential for these agents to be spread by poultry hatching eggs.

Keywords


Introduction

The international trade in poultry hatching eggs, like that in poultry meat, may present an opportunity for the global spread of disease. This review is restricted to a discussion of the likelihood of avian diseases listed by the World Organisation for Animal Health (OIE) being disseminated through such trade. For further information, the reader is referred to peer-reviewed import risk analyses published by the Ministry of Agriculture and Forestry Biosecurity New Zealand that have examined the risks associated with both listed and unlisted diseases in poultry hatching eggs (67, 70) and poultry egg products (68, 69).

For disease to be spread by poultry hatching eggs, the aetiological agent must be able to infect poultry species and either disseminate to the reproductive tract and persist in traded eggs, or penetrate the eggshell and contaminate its contents after the egg has been laid.

The species susceptible to natural infection by the aetiological agents of OIE-listed avian diseases have been discussed previously (33), so this review is largely limited to the likelihood of these agents disseminating to the reproductive tract of infected poultry or penetrating the eggshell, and subsequently being found in hatching eggs.

Chapter 6.4. of the OIE Terrestrial Animal Health Code (Terrestrial Code) (112) specifies hygiene and disease security procedures for poultry breeding flocks and hatcheries, including recommendations applicable to:

- breeding establishments (Article 6.4.1.)
- hatching egg hygiene and transport (Article 6.4.2.)
- hatchery buildings (Article 6.4.3.)
- hatchery building hygiene (Article 6.4.4.)
- personnel and visitors (Article 6.4.5.)
- hygiene measures used during the handling of eggs and day-old birds (Article 6.4.6.)
- methods to sanitise hatching eggs and hatchery equipment (Article 6.4.7.).
Provided that these recommendations are followed, then only those infectious agents known to disseminate to the reproductive tract of poultry and infect egg contents should be considered likely to spread to other countries through the trade in hatching eggs.

**Avian influenza**

Low pathogenicity avian influenza (LPAI) infections of domestic poultry may result in decreased egg production, although mild to severe respiratory signs are more common, possibly accompanied by huddling, ruffled feathers, lethargy, and, occasionally, diarrhoea (97). Intratracheal inoculation of poultry with LPAI virus may result in localised infection of the respiratory tract with histological lesions and viral antigen distribution restricted to the lungs and trachea, although pancreatic necrosis has been described in turkeys (23, 71, 88, 101). Intravenous inoculation of poultry with LPAI virus results in swollen and motiled kidneys with necrosis of the renal tubules, interstitial nephritis, and high viral titres in kidney tissues (88, 92, 93, 95, 98, 99, 100, 102). This renal tropism is strain-specific and is most consistently associated with experimental intravenous inoculation studies (97). However, Alexander and Gough (3) did report the recovery of H10N4 LPAI virus from kidneys taken from hens presenting with nephropathy and visceral gout. Salpingitis, accompanied by a mild to moderate drop in egg production, has been associated with a non-pathogenic H7N2 virus (118), but there have been no reports of LPAI infection of poultry eggs.

Highly pathogenic avian influenza (HPAI) infection of poultry results in necrosis and inflammation of multiple organs, including the cloacal bursa, thymus, spleen, heart, pancreas, kidneys, brain, trachea, lungs, adrenal glands, and skeletal muscle (71, 76, 94). Histopathological lesions described include diffuse non-suppurative encephalitis, necrotising pancreatitis, and necrotising myositis of skeletal muscle (1). Viral antigen can be detected in several organs, most commonly the heart, lungs, kidneys, brain, and pancreas (71).

An epidemiological investigation into the spread of HPAI in the Netherlands in 2003 suggested that mechanical transmission on contaminated eggs and egg trays might have been a significant factor in disease dissemination (104).

Although proof of true vertical transmission of HPAI is limited (38), H5N2 HPAI virus has been recovered from the yolk and albumen of eggs from naturally infected chicken flocks (22), and from experimentally infected hens (14). Unpublished studies cited by Swain and Beck (96) were reported to demonstrate the presence of HPAI virus in 85% to 100% of eggs laid three to four days after experimental inoculation of poultry with HPAI virus. However, HPAI infections are lethal to the embryo and hatching of infected eggs has never been demonstrated (97).

Articles 10.4.10. and 10.4.11. of the *Terrestrial Code* recommend that hatching eggs of poultry should be derived from parent flocks that have been kept in a notifiable avian influenza-free country, zone, or compartment for at least 21 days prior to, and at the time of, the collection of the eggs (113). Given the limited evidence associating natural infection with transmission in hatching eggs, the recommendations of the *Terrestrial Code* are clearly adequate to prevent the international dissemination of avian influenza through this trade.

**Newcastle disease**

Newcastle disease (ND) is defined by the OIE as an infection of poultry caused by a virus (NDV) of avian paramyxovirus serotype 1 (APMV-1) that meets the criteria for virulence described in the *Terrestrial Code* (115). It has been suggested that the spread of ND from one bird to another occurs primarily through aerosols or large droplets, although the evidence to support this is lacking (4). During infection, large amounts of virus are excreted in the faeces and this is thought to be the main method of spread for avirulent enteric avian paramyxovirus (APMV) infections which are unable to replicate outside the intestinal tract (5).

*In situ* hybridisation studies (18), using four-week-old chickens experimentally infected with APMV-1 isolates, revealed widespread viral replication in the spleen, caecal tonsil, intestinal epithelium, myocardium, lungs, and bursa following challenge with viscerotropic velogenic strains. Neurotropic velogenic strains are associated with viral replication in the myocardium, air sacs, and central nervous system. Challenge with mesogenic viral strains is followed by viral replication in the myocardium, air sacs, and (rarely) in splenic macrophages. Lentogenic isolates result in minimal transient viral replication confined to the air sac at five days post exposure, and the myocardium at five to ten days post exposure.

Lancaster and Alexander (59) cited a study which reported that NDV was able to penetrate the cuticle and shell of eggs, and occasionally the outer shell membrane, especially in cracked eggs.

Previously, true vertical transmission of NDV was considered to be controversial (2), since experimental assessment of this route of transmission using virulent viruses is usually hampered by the cessation of egg laying.
in infected birds. However, Beard and Hanson (13) did state that NDV had been isolated from eggs laid by diseased hens, citing three reports to support this position.

Although infection with NDV usually results in the cessation of egg laying, which dramatically reduces the potential for vertical transmission, some studies have documented the recovery of virus from the eggs of infected hens (24, 77, 86), and NDV has been isolated from the albumen of eggs laid by experimentally challenged vaccinated chickens (70). Epidemiological studies of sporadic NDV outbreaks in Taipei China between 1998 and 2000 suggested that vertical transmission may have played a role in disease dissemination and artificial infection studies demonstrated successful hatching of chicken embryos infected with low titres of NDV (29). Article 10.13.8. of the Terrestrial Code recommends that poultry hatching eggs should be derived from parent flocks that have been kept in an ND-free country, zone, or compartment for at least 21 days prior to, and at the time of, the collection of the eggs (115). As with avian influenza, limited evidence associating natural infection with transmission in hatching eggs suggests that these recommendations of the OIE are clearly adequate to prevent the international dissemination of NDV.

**Infectious bursal disease**

Two serotypes of infectious bursal disease virus (IBDV) are recognised (IBDV-1 and IBDV-2) (64). Very virulent strains of IBDV-1 (vvIBDV) have also been described (30). Chickens are the only animals known to develop clinical disease and distinct lesions when exposed to IBDV (39). Serotype 1 and 2 viruses have been isolated from chickens (64). The cloacal bursa is the primary target organ and infection leads initially to cloacal oedema and hyperaemia, followed by atrophy around five days after infection. Microscopic lesions in other lymphoid tissues are described (39).

Although Sivanandan et al. (91) reported bursal necrosis and atrophy in specific-pathogen-free (SPF) chickens experimentally infected with an IBDV-2 isolate, Ismail et al. (51) found that five different IBDV-2 isolates — including the isolate used by Sivanandan et al. (91) — caused no gross or microscopic lesions in SPF chickens and had no significant impact on bursa-to-body-weight ratio when compared to uninfected controls. There is no evidence to suggest that either IBDV-1 or IBDV-2 is transmitted through the egg (39).

**Turkey rhinotracheitis**

The main sites of turkey rhinotracheitis virus (TRTV) replication are the epithelial cells of the turbinates and the lungs (66). An early study of experimentally infected 30-week-old turkeys demonstrated virus localisation in the turbinates and trachea, while the lungs, air sacs, spleen, ovary, liver, kidneys, and hypothalamus all tested negative for the virus (52). Similarly, Pedersen et al. (75) detected virus in the turbinates, sinus, trachea, and lungs of experimentally infected four-week-old poults and found that turbinate tissues were significantly more productive sources of virus and viral RNA than either lung or tracheal specimens.

There is no evidence that TRTV is transmitted in eggs (43, 46) and Khera and Jones (55) were unable to show viral replication in the oviducts of experimentally infected chicks or poults. However, Jones et al. (52) demonstrated short-lived viral replication in the oviduct of turkeys nine days after experimental challenge.

The most compelling evidence for the vertical transmission of avian pneumovirus is the study by Shin et al. (89) in neonatal turkeys, originating from infected breeders, in which products testing positive by polymerase chain reaction were detected in egg contents and newly hatched chicks. However, the validity of these results has been questioned as live virus was not isolated and these results have not been confirmed by other researchers (70).

From the available evidence, the spread of TRTV through international trade in poultry hatching eggs should be considered unlikely.

**Marek’s disease**

Marek’s disease virus (MDV) replicates in feather follicle epithelial cells (19), and virus associated with feathers and dander is infectious (15, 20, 21). Marek’s disease virus is cell-associated in tumours and in all body organs, except in the feather follicle (83). Clean poultry hatching eggs should not be considered a vehicle for the transmission of MDV.

**Avian infectious bronchitis**

Avian infectious bronchitis virus (IBV) multiplies primarily in the respiratory tract. Following experimental exposure to IBV in aerosols, the concentration of virus is greatest in the trachea, lungs, and air sacs, with lesser amounts recovered from the kidneys, pancreas, spleen, liver, and bursa of Fabricius (50). Nephropathic strains of IBV are also recognised (34) and these may cause significant mortality without respiratory lesions (119). Strains of IBV can also replicate in many parts of the alimentary tract without associated enteric disease (6, 26, 53). Prolonged
virus excretion from infected birds has been described and both the kidney and the caecal tonsil have been suggested as sites of persistent IBV infection, although the kidney is considered to be more likely (35).

Cavanagh and Naqi (28) stated that there were reports of virus isolations from eggs up to 43 days after recovery from infection, even though chickens have been hatched from infected flocks and raised free of IBV. However, this statement on IBV transmission in the egg has been removed from the latest (12th) edition of this text (27). Furthermore, this unreferenced statement by Cavanagh and Naqi (28) is the only reference that could be found to transmission of this virus in the egg (70). It would be reasonable to conclude that there is insufficient evidence to suggest that poultry hatching eggs could be a vehicle for the transmission of IBV.

### Avian infectious laryngotracheitis

Infectious laryngotracheitis virus (ILTV) can be recovered from the tracheal tissues and tracheal secretions for six to eight days after infection (11, 82) and may also spread to the trigeminal ganglia (11, 49), which are considered to be the main sites of latency for this virus (108). Transmission of the virus contained in the interior or exterior of poultry eggs has not been demonstrated (47).

### Duck virus hepatitis

Transmission of duck virus hepatitis (DVH) infection can occur via aerosols or oral inoculation (48, 80). Clinical signs develop rapidly after infection and death may occur within three to four days. Morbidity can reach 100%, with 95% mortality in ducklings less than one week old. Once ducklings reach five weeks, morbidity and mortality may be low or negligible (110).

Gross lesions associated with this disease are consistently seen in the liver, possibly accompanied by congestion in the spleen or kidneys. Histopathological lesions are confined to the liver (40). Egg transmission presumably does not occur, since ducklings hatched on infected premises can be moved directly from the incubators to clean premises to reduce the losses from DVH (10, 110).

### Fowl cholera

All bird species are thought to be susceptible to infection with *Pasteurella multocida*, with the organism being found in the nasal clefts of infected birds (45, 78, 79). The spread of *P. multocida* within a flock occurs through contaminated nasal, oral, and conjunctival excretions (45), with transmission via the mucous membranes of the pharynx and upper respiratory tract.

Transmission of the organism through the egg seldom, if ever, occurs. Glisson *et al.* (45) cited a study of more than 2,000 eggs from chickens with chronic fowl cholera that showed no evidence of *P. multocida*. There is no current evidence to justify the imposition of sanitary measures on poultry hatching eggs to control the spread of fowl cholera.

### Pullorum disease and fowl typhoid

Pullorum disease and fowl typhoid are systemic infections and *Salmonella Gallinarum-Pullorum* can be recovered from most internal organs of infected chickens, including the liver, spleen, caeca, lungs, heart, ventriculus, pancreas, yolk sac, synovial fluid, and reproductive organs (90). Up to 33% of the eggs laid by an infected hen can be contaminated with *S. Gallinarum-Pullorum* (90).

Chronic infection localised in the ovary may result in contamination of the ovum after ovulation (12, 16), although the primary mode of vertical transmission of *S. Gallinarum-Pullorum* is considered to be localisation of the organism to the ovules before ovulation (17, 90).

In addition, faecal contamination of eggs is recognised as leading to contamination of the egg contents, following rapid penetration of the cuticle, shell, and shell membranes by *Salmonella* spp. (107).

Chapter 6.4. of the Terrestrial Code provides guidance on the bacteriological monitoring of poultry breeding flocks and hatcheries for *Salmonella* spp. and other standards relating to egg hygiene and sanitisation (112). Providing that these recommendations are followed, no further sanitary measures should be necessary to ensure against the spread of *S. Pullorum-Gallinarum* in poultry hatching eggs.

### Avian mycoplasmosis

*(Mycoplasma gallisepticum)*

*Mycoplasma gallisepticum* can be recovered from the oviduct and cloaca of infected birds, as well as from suspensions of tracheal or air sac exudates, turbinates, lungs, or sinus exudate (7, 37, 65, 72). Although
M. gallisepticum can be found predominantly in respiratory tissues, more virulent strains are recognised as disseminating more widely after infection (31, 103).

Horizontal transmission of Mycoplasma spp. occurs either through aerosol or infectious droplet transmission, resulting in localised infection of the upper respiratory tract or conjunctiva, or through venereal transmission (32, 58, 61).

Mycoplasma gallisepticum has been recovered from the phallus or semen in male turkeys (116) and in chicken semen (117). Salpingitis in chickens due to M. gallisepticum infection has been described (36, 81). Natural vertical transmission of M. gallisepticum is recognised and the recovery of the organism from the eggs of infected poultry has been described by several authors (44, 62, 73, 85, 87).

Levisohn and Kleven (60) have previously stated that, since M. gallisepticum is transmitted in embryonated (fertile) eggs, buyers or importers of day-old breeder replacement chicks or hatching eggs should be provided with an assurance that the flocks from which the eggs originated are free of infection, to prevent dissemination of the disease. Similarly, Article 10.5.5. of the Terrestrial Code recommends that, in addition to compliance with hygiene and disease security standards, hatching eggs of chickens and turkeys should come from establishments free from avian mycoplasmosis (114).

Avian mycoplasmosis (Mycoplasma synoviae)

Birds infected with Mycoplasma synoviae typically develop synovitis of the tendon sheaths, joints, and keel bursa, which may progress to a caseous exudate extending from tendon sheaths and joints into muscle and air sacs (56, 57). Airsacculitis may be seen in the respiratory form of the disease (41, 84). Mycoplasma synoviae can be recovered from these lesions in the early stages of disease but viable organisms may no longer be present once chronic disease has developed. Birds remain carriers for the rest of their lives (57). Following experimental infection, M. synoviae can be recovered from the trachea and sinus and, although gross lesions may be seen in other organs (liver, spleen, and kidneys), the organism is only consistently recovered from these sites after intravenous inoculation (42, 54).

Vertical transmission of M. synoviae has been documented, with the organism recovered from embryos and hatched chicks from both naturally and experimentally infected birds (25, 106), with egg transmission peaking four to six weeks after infection (57).

Although there are no recommendations in the current Terrestrial Code regarding M. synoviae, it would be prudent for buyers or importers of day-old breeder replacement chicks or hatching eggs to require assurance that the flocks from which the eggs originated are free from M. synoviae to prevent dissemination of the disease, consistent with the advice from Levisohn and Kleven regarding M. gallisepticum (60).

Avian chlamydiosis

Large numbers of chlamydiae can be found in the respiratory tract exudate and faeces of infected birds (8). Following experimental inoculation of turkeys with four strains of chlamydiae, primary replication was found to occur throughout the respiratory tract after two to seven days, with subsequent replication occurring throughout the intestinal tract, especially in the jejunum, caecum, and colon (105). An earlier study (74) quantified the tissue distribution of Chlamydothila psittaci in turkeys after aerosol exposure and found that the organism multiplied primarily in the lungs, air sac system, and pericardium. However, infectivity was also detected in other tissues (including the kidneys) and in the muscle tissue after 120 h. For diagnostic purposes, the best tissues from which to recover the organism are the air sacs, spleen, pericardium, heart, liver, and kidney (9).

Recovery of C. psittaci from the eggs of turkey breeding flocks has been described, with 89% of embryos from 12 flocks giving a positive result for chlamydiae using a direct immunofluorescence technique (63). Chlamydothila psittaci was also reported to have been isolated from a single embryonated chicken egg out of a total of 500 commercial hatching eggs examined (109).

Although vertical transmission of C. psittaci has been described in poultry species, the limited reports of its recovery from hatching eggs suggest that the frequency of this is low (9). Since avian chlamydiosis is considered to have a worldwide distribution (9), and very few countries claim to be free of it based on the results of targeted surveillance, it would be difficult to justify the imposition of sanitary measures against C. psittaci on imported hatching eggs.

Conclusions

Provided that OIE guidelines on hygiene and disease security procedures for poultry breeding flocks and hatcheries are followed (112), there are only a limited number of OIE-listed diseases whose causative agent should be considered likely to be present in poultry
hatching eggs. HPAI virus, NDV, *M. gallisepticum*, and *M. synoviae* should be considered the most significant pathogens that might reasonably be expected to be spread through international trade. However, infections with HPAI or NDV may result in the cessation of egg laying or embryo death, which would have a significant impact on the potential for these diseases to be spread through trade in hatching eggs.

According to OIE risk analysis methodology (111), the overall risk estimation for a commodity reflects the likelihood of entry (those issues discussed in this article), together with the likelihood of exposure and consequences of exposure. When assessing whether or not sanitary measures are justified for any disease agent likely to be associated with poultry hatching eggs, the factors specific to the importing country that might influence exposure and consequences must also be considered. For poultry hatching eggs, those factors may include:

- any quarantine procedures used for hatching imported eggs
- biosecurity practices in the domestic commercial poultry industry
- the ability of wild bird species to act as vectors of individual diseases
- the extent of backyard poultry flocks and their likely contact with commercial units
- the national presence of (or freedom from) diseases in avian species.

La propagation des agents pathogènes au travers des échanges d’œufs à couver : état de la situation et évolutions récentes

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**Résumé**
Les échanges internationaux d’œufs à couver représentent une source potentielle de propagation des maladies aviaires dans le monde. Néanmoins, dès lors que les lignes directrices de l’Organisation mondiale de la santé animale (OIE) relatives à l’hygiène des élevages de volailles reproductrices sont correctement appliquées, les seules maladies aviaires de la liste de l’OIE qui présentent un risque de propagation par le commerce de ces marchandises sont l’influenza aviaire faiblement pathogène (IAFP), la maladie de Newcastle et les mycoplasmoses aviaires à *Mycoplasma gallisepticum* et à *M. synoviae*. De surcroît, l’impact de l’IAFP et de la maladie de Newcastle sur la production d’œufs et l’éclosivité limite le risque de dissémination de ces agents pathogènes par l’intermédiaire des œufs à couver.

**Mots-clés**
Descripción general y evolución reciente de la diseminación de patógenos por el comercio de huevos para incubar de aves de corral

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Resumen
El comercio internacional de huevos de aves de corral para incubar podría, en potencia, facilitar la diseminación planetaria de enfermedades aviares. No obstante, a condición de que se respeten las directrices de la Organización Mundial de Sanidad Animal (OIE) en materia de higiene de bandadas reproductoras, hay sólo unas pocas enfermedades, de las que actualmente figuran en la lista de la OIE, cuya propagación a través del comercio de huevos se debería considerar probable: la influenza aviar altamente patógena (IAAP), la enfermedad de Newcastle (EN) y las infecciones por *Mycoplasma gallisepticum* y *M. synoviae*. Además, los efectos de la IAAP y la EN sobre la producción y la incubabilidad de los huevos restringirán las posibilidades de diseminación de esos agentes patógenos a través de los huevos para incubar.

Palabras clave

References


specific-pathogen-free chickens inoculated with a
waterfowl-origin type A influenza virus. *Avian Dis.*, 

kidney as a potential site of avian influenza virus persistence
in chickens *Avian Dis.*, 36 (4), 937-944.

pathology of intravenously inoculated wild duck- and
turkey-origin type A influenza viruses in chickens. *Avian Dis.*, 
39 (1), 74-84.

Immunohistochemical demonstration of influenza A
nucleoprotein in lungs of turkeys with natural and
experimental influenza respiratory disease. *Avian Pathol.*, 
21 (4), 547-577.

(1994). – Acute renal failure as the cause of death in
chickens following intravenous inoculation with avian
influenza virus A/Chicken/Alabama/7399/75 (H4N8). *Avian

The production of cerebral polyarthritis by *Mycoplasma
gallisepticum* in turkeys; the neurotoxic property of the

104. Thomas M.E., Bouma A., Ekker H.M., Fonken A.J.M.,
introduction of high pathogenicity avian influenza virus
into poultry farms during the epidemic in the Netherlands

105. Vanrompay D., Mast J., Ducatelle R., Haesebrouck F &
pathogenesis of infections in avian serovars A, B and D.

of meat-type hens exposed to *Mycoplasma synoviae*. *Poultr. Sci.*, 
55 (1), 268-273.

The penetration patterns of *Salmonella Typhimurium*
through the outer structures of chicken eggs. *Avian Dis.*, 
12 (3), 445-466.

108. Williams R.A., Bennett M., Bradbury J.M., Gaskell R.M.,
of latency of infectious laryngotracheitis virus using the

Isolation of *Chlamydia psittaci* from chicken egg: evidence of

poultry (Y.M. Saif, ed.), 12th Ed. Blackwell Publishing,
Ames, Iowa, 373-384.

Chapter 2.1. Import risk analysis. In Terrestrial Animal
Health Code, 18th Ed. OIE, Paris. Available at: www.oie.int/index.php?id=169&L=0&htmfile=chapitre_1.2.1.htm
(accessed on 28 April 2010).

Chapter 6.4. Hygiene and disease security procedures in
poultry breeding flocks and hatcheries. In Terrestrial Animal
Health Code, 18th Ed. OIE, Paris. Available at: www.oie.int/
index.php?id=169&L=0&htmfile=chapitre_1.6.4.htm
(accessed on 21 June 2010).

Chapter 10.4. Avian influenca. In Terrestrial Animal
Health Code, 18th Ed. OIE, Paris. Available at: www.oie.int/
index.php?id=169&L=0&htmfile=chapitre_1.10.4.htm
(accessed on 28 April 2010).

Chapter 10.5. Avian mycoplasmosis (*Mycoplasma
gallisepticum*). In Terrestrial Animal Health Code, 18th Ed.
OIE, Paris. Available at: www.oie.int/index.php?id=169&L=0&htmfile=chapitre_1.10.5.htm
(accessed on 21 June 2010).

Chapter 10.13. Newcastle disease In Terrestrial Animal
Health Code, 18th Ed.OIE, Paris. Available at: www.oie.int/
index.php?id=169&L=0&htmfile=chapitre_1.10.13.htm
(accessed on 28 April 2010).

persistence of avian mycoplasma in the genital system of


Characteristics of H7N2 (nonpathogenic) avian influenza
virus infections in commercial layers, in Pennsylvania,

119. Ziegler A.F., Davison S., Dunn P.A., Schneider A.,
Davison S., Miller P.G., Lu H., Weinstock D., Salem M.,
infectious bronchitis in Pennsylvania chickens