CEVA HANDBOOK
OF POULTRY DISEASES

7 AVIAN INFLUENZA

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DEFINITION

Avian Influenza (AI) is caused by viruses from the family Orthomyxoviridae, whose highly pathogenic form occurs as a systemic infection with the potential for epidemic or pandemic events affecting domestic and a substantial number of wild birds and mammals. The low-pathogenic AI induces a mild form of the disease that can be accompanied by respiratory signs and a drop in egg production.

HISTORY AND SYNONYMS

Information about Avian Influenza (AI) dates back to 1878 in northern Italy, where a highly contagious disease causing high mortality in poultry, named «fowl plague», was first described. This is believed to be the first outbreak of highly pathogenicavian influenza (HPAI) (Peroncito, 1878). Over the ensuing years, descriptions of HPAI appear under the names typhus exudatious gallinarum, la peste aviaria, Brunswick disease, fowl grippe etc. (Lupiani & Reddy, 2009).

In 1981, the disease caused by the highly virulent AI viruses is officially termed «highly pathogenic avian influenza» (Bankowski, 1981). Swayne & Halvorson (2008) divide the AI history into three periods: early reports about HPAI; detection of low pathogenic avian influenza (LPAI) and identification of the AI viruses in asymptomatic wild avian reservoir hosts.

Since the first HPAI outbreak in Italy (1878), about 30 more outbreaks have been reported, half of them during the last two decades. In most cases, the outbreaks are due to mutation of LPAI viruses into highly pathogenic ones. The most important outbreaks are: in 1983, a low-pathogenic H5N2 virus mutated into a highly-pathogenic variant and caused extensive mortality in the USA (Eckoade et al.,1986); the same happened in 1994 in Mexico (Villareal, 1998); in 1999 in Italy, H7N1 mutated into a highly-pathogenic isolate and caused the death of 14 million birds (Zanella, 2003); in 2002 in Chile, the losses caused by mutation of the low-pathogenic H7N3 into high pathogenic were estimated at $31 million (Rojas et al., 2002).

In other instances, outbreaks have been caused by HPAI viruses: the 1994 H7N3 outbreak in Pakistan (Naeem, 1998); the 1997 outbreak in Hong Kong; the 2003 H7N7 outbreak in the Netherlands; the 2004 H7N3 outbreak in Canada; outbreaks between 2003 and 2006 in eastern Asia due to persistence of HPAI H5N1 (Lupiani & Reddy, 2009).

In a different domestic fowl species, LPAI forms were detected during the 1950s and 1960s (Alexander, 1987;

Senne et al., 1986) although their economic impact on the poultry industry was lower compared to that of HPAI.

The potential role of asymptomatic wild birds as reservoir hosts for AI viruses was serologically proven in different parts of the world by the end of 1960s and the early 1970s (Easterday et al., 1968; Slepuskin et al., 1972; Winkler et al., 1972). Later, AI viruses were isolated in at least 105 avian species from 26 different families (Olsen et al., 2006).

Cases of human disease caused by avian influenza viruses (AIVs) have been confirmed since 1997. Infections caused by H5N1 (in 1997) and by H9N2 (in 1999) were established in both men and chickens in Hong Kong. During the first outbreak, 6 out of the 18 hospitalized people died. In 2003, two people in Hong Kong became infected with H5N1, one of them with fatal consequences. In 2003, during an AI outbreak (H7N7) in the Netherlands, more than 80 people working in the poultry sector were infected, and one of them died (the veterinarian visiting the affected farm). In this case, human to human transmission of the disease was confirmed. In 2003, H9N2 infection was confirmed in a child from Hong Kong, who then recovered.

H9N2 is occasionally detected in men, causing flu-like disease differing from the usual symptoms of influenza in humans (H1N1 and H3N2) (Guo et al., 1999; 2001).

CHARACTERISTICS AND CLASSIFICATION OF THE PATHOGEN

AI is caused by type A influenza viruses, from the *Influenzavirus A* genus of the Orthomyxoviridae family. Influenza A viruses are classified into subtypes according to the type of two superficial protein antigens: haemagglutinin (H) and neuraminidase (N). Sixteen haemagglutinin (H1–H16) and nine neuraminidase antigens (N1–N9) are known. H1–H16 antigens are responsible for the binding of the virus to the infected cell and possess a haemagglutinating activity with two antigenic determinants: group- and species-specific. N1–N9 antigens have enzyme functions. Both proteins are main targets of the immune

response. The average size of virions is 80–120 nm. They are pleomorphic, spherical, oval or rod-shaped, with single-stranded and segmented RNA genome (Zarkov, 2003).

The different virions possess different combinations of H and N antigens. All combinations between H1–H16 and N1–N9 antigens are possible, hence the high serotype diversity. The influenza 1 viral subtypes are classified into strains, whose description contains the type, host, place and year of first isolation, number (if available) and antigenic subtype. For example, the H5N1 virus isolated from chickens in Hong Kong in 1997 belongs to the strain A/chicken/Hong Kong/y385/97 (H5N1).

The *Influenzavirus A* genus is characterized by a high degree of antigenic variation. When the changes in surface proteins and the mutagenesis of H- and N-antigens are slow and insignificant, the event is called antigenic drift.

It is linked to the appearance of a new variant within the subtype. The reassortment (major and sudden change of surface proteins) is called antigenic shift. Due to this process of reassortment, new subtypes with different H- and N-antigens are continually emerging. The occasional appearance of new influenza viruses is due to the antigenic shift.

Such viruses are not neutralised by the immune response and could provoke influenza epidemics or pandemics.

The classification of AIVs into HPAI and LPAI viruses is based on their genetic features and the severity of disease in experimentally infected chickens. Only subtypes containing H5 and H7 are highly pathogenic. Those containing different haemagglutinins are detected only in low-pathogenic forms (Zarkov et al., 2006). Any H5 or H7 avian influenza virus isolation is notifiable to OIE.

AIVs demonstrate enzymatic, toxic, receptor and infective activity. Enzyme (transcriptase) activity catalyzes the synthesis of RNA through a RNA polymerase. The toxic viral properties are expressed by AIVs ability to induce necrobiotic pathological alterations, without previous intracellular replication of the virus. Via the haemagglutinin, the virus binds to cells, hence its receptor activity. The reproduction occurs in nuclei and the cytoplasm, beginning with binding on cell receptors and entry through fusion in the vacuole. The second phase is a transcription, occurring in the nucleus with the viral RNA

polymerase. The third phase – translation – takes place in the cytoplasm, where the structural and non-structural proteins are synthesized. During the subsequent phase, replication occurs and virions are formed, which then leave the cell. The resistance of AIVs is not high and they can be inactivated if exposed to 56°C for 15 min. Direct sunlight kills them after 40 min. Low temperatures have a conservation effect. At 4°C they are preserved for several weeks. At pH <4 and >12.7 the viruses are rapidly destroyed. After removal of the organic matter, chemical disinfectants such as quaternary ammonium salts, 5% formalin and 2% sodium hydroxide inactivate the virus (Obreshkov et al, 1978).

EPIDEMIOLOGY

LPAI viruses are widely distributed among wild and domestic fowl. Those detected among wild birds can be divided according to their origin into Eurasian or American. HPAI viruses in domestic fowl are eradicated in most developing countries.

Although many wild bird species may be infected with AIVs, waterfow from the order Anseriformes (ducks, geese, swans) and Charadriiformes (gulls and shorebirds) are the main reservoir hosts for AI. It has been established that the distribution of AIVs from these birds to domestic fowl can cause HPAI or LPAI outbreaks (Lupiani & Reddy, 2009). In wild ducks, prevailing virus subtypes are known to change on a periodical basis.

Information regarding the distribution of AIV subtypes in other bird species is limited. Isolates from caged birds usually contain H3 or H4, but highly pathogenic H5 and H7 have also been detected. In sporadic cases, AIVs have been found in pigeons.

Turkeys and other gallinaceous birds are not natural reservoir hosts for AIVs (Perdue et al., 1999; Suarez & Schultz, 2000).

AIVs are spread via faeces, saliva or nasal discharges. The faecal-oral route is the main way transmission occurs, as faeces contain large amounts of the virus. Faecal-cloacal transmission is also possible. Faecal transmission is facilitated by the

persistence of AIVs in aquatic environments over long periods of time, especially at low ambient temperatures.

The virus is spread by direct contact between infected and susceptible birds or indirectly, via aerosol route or contaminated objects (Easterday et al., 1997). AIVs are easily transferred between premises by farm per sonnel or used equipment.

A certain host-specific adaptation of AIVs does exist, although a cross-species transmission is also encountered, especially among closely related host species as chickens, turkeys, guinea-fowl and quails. Data for vertical transmission are not available. AIVs are lethal for embryos, and therefore hatching of contaminated eggs is not possible (Swayne & Halvorson, 2008).

The transmission of some AIVs from birds to mammals is possible through direct or indirect contact. Humans may become infected by contact with sick or dead birds, but also by swimming in contaminated water. This is best known for the highly pathogenic Asian H5N1 subtype. Sporadic HPAI H5N1 infection in domestic cats, tigers, leopards and other mammals has been reported. In most instances, a close contact or consumption of infected birds was present (Swayne & Halvorson, 2008).

Several cases of human infection were mentioned in the "History and synonyms" section (see above).

Primary sources of infection for commercial poultry flocks include other domestic fowl, migrating waterfowl and other wild birds, domestic pigs and birds kept as pets (Alexander, 1982; 1993). Once infected, the flock is a potential source of the virus for its entire life. In wild waterfowl, AIVs are maintained by passage between susceptible birds all year round, with a peak during the autumn migration among young birds. Upon arrival, they infect vulnerable birds in their new location, which then develop an infection cycle of their own. This way, local birds contribute to AIVs propagation during the winter and to re-infection of migratory waterfowl during the spring migration (Stallknecht et al., 1990; Hanson et al., 2000).

LPAI is characterized by high morbidity and low mortality, usually < 5% unless complications with secondary pathogens or infection in very young birds are present. In HPAI outbreaks both morbidity and mortality rates are very high: 50–89% on average, and up to 100% in some instances (Swayne & Halvorson, 2008).

CLINICAL SIGNS AND PATHOLOGY

The incubation period varies from one day to 12 weeks. After infection with the highly pathogenic H5N1 strain, the first symptoms appear after 2 to 5 days.
The virus dose, route of exposure, the host species and the possibility of detecting clinical signs are all relevant for determination of the incubation period. It should be taken into consideration that many infections with LPAI viruses remain asymptomatic regardless of the age and the species of birds (Swayne & Halvorson, 2008).
The clinical signs of disease depend on the AIV pathotype (low or highly pathogenic). Other factors such as the environment, acquired immunity and concurrent infections will also influence the variability of symptoms (Easterday, 1997).

1/ Clinical signs and pathology after infection with LPAI viruses

In laying birds, a drop in egg production and hatchability is often reported. General signs of discomfort such as depression, lethargy, ruffled feathers, decreased feed and water intake are also present.

Experimental studies with the low pathogenic H6N2, detected for the first time in Bulgaria, demonstrated clinical signs such as stunted growth, impaired feathering, moderate septicaemic lesions and immuno-suppressive lesions (Zarkov & Dinev, 2008).

Fig.1

Marked stunted growth in a 21-day-old broiler chicken (right) after intratracheal inoculation with H6N2 compared to its hatchmate from the control group (left).

Fig.2

Stunted growth and impaired feathering in mallard ducks, after experimental intravenous infection with H6N2 LPAI virus (B) compared to a control duckling (A).

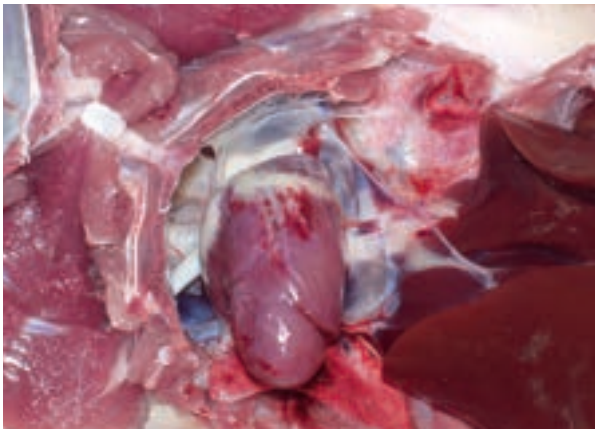

Fig.3

Epicardial haemorrhages both in the apex and the base of the heart in a 14-day-old turkey poult, 14 days after intravenous inoculation with H6N2.

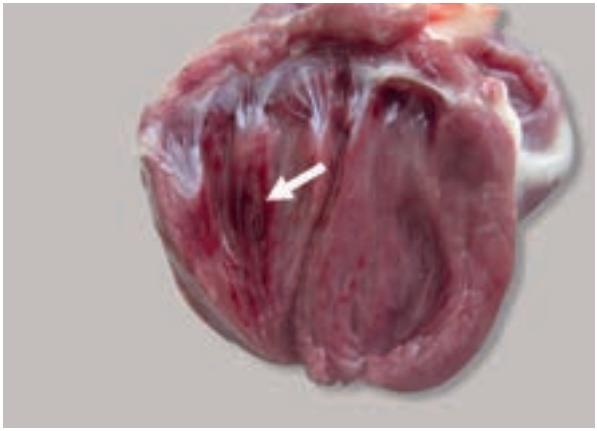

Fig.4

Haemorrhages in the myocardium and endocardium of a mallard duckling 14 days after intravenous inoculation with H6N2.

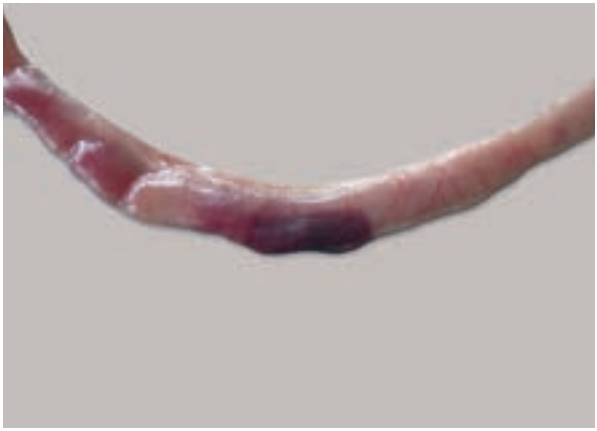

Fig.5

Massive haemorrhages in the Payer's plaques of small intestinal mucosa 14 days after intratracheal inoculation with H6N2.

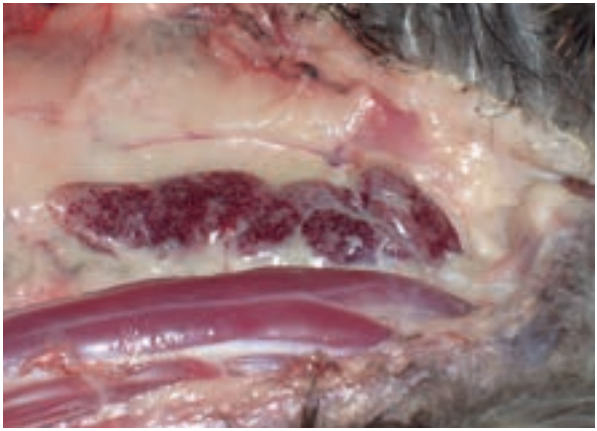

Fig.6

Multiple petechial haemorrhages in all thymus lobes in a turkey poult 8 days after intratracheal inoculation with H6N2. This is a typical lesion, always present in different fowl species (chickens, turkeys, ducks) infected via different routes (intravenous, intratracheal or oral) with H6N2.

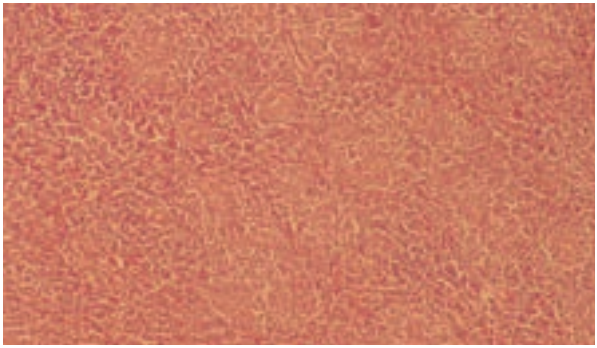

Fig.7

Marked atrophy of the white pulp of the spleen in a chicken, 14 days after texposure to H6N2.

2/ Clinical signs and pathology after infection with HPAI viruses

In chickens, poults and other gallinaceous bird species, the clinical signs after HPAI infection are a reflexion of viral replication and damage of numerous visceral organs as well as of cardiovascular and nervous systems. Respiratory signs such as rales or nervous disturbances (torticollis, paresis, paralysis), as well as a sharp increase in mortality (Swayne & Halvorson, 2008; Van Riel et al., 2009), may be observed. In peracute cases, macroscopic lesions are absent.

After the HPAI subtype HH7N7 outbreak in the Netherlands in 2003, the main observed macroscopic lesions were subcutaneous oedema in the region of the head, the comb and the wattles due to accumulation of gelatinous matter. Multiple haemorrhages on the cloacal surface and enlarged spleens were also observed. Dominating internal lesions were peritonitis, tracheitis, pulmonary oedema and pneumonias, petechial haemorrhages of the proventriculus (Van Riel et al., 2009; Armin et al., 2004).

HPAI subtype HH5N1 infections result in appearance of haemorrhages on serous and mucous coats of visceral organs: spleen, heart, pancreas, and sometimes, in the liver and kidneys. In lungs, congestion, oedema and interstitial pneumonia are observed (Swayne & Halvorson, 2008). Another common finding is subcutaneous haemorrhaging, to a variable extent.

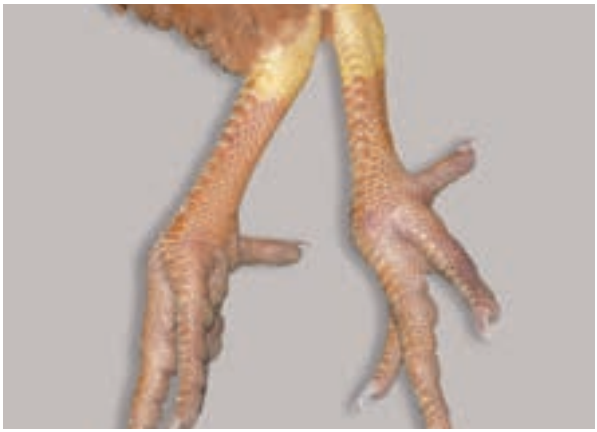

Fig.8
Diffuse subcutaneous haemorrhages of leg shanks.

Fig.9
Subcutaneous haemorrhage in the sternum region.

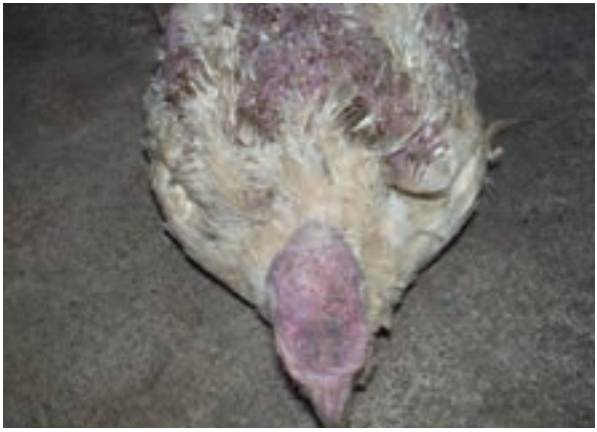

Fig.10

Depression, loss of feathers, necroses and haemorrhages in the subcutis in the region of the back in a turkey secondary to inflammatory oedema.

DIAGNOSIS

Various techniques can be used to diagnose AI, including isolation of the virus. The most commonly used specimens for virus detection are cloacal, tracheal or oropharyngeal swabs taken from live birds. From carcasses, visceral organ samples (heart, liver, spleen, lungs, trachea etc.) or swab samples from intestinal and tracheal contents may be collected. Swab tissue samples should be placed into a sterile transport medium containing antibiotics, or in sterile containers. If samples are to be analysed within 48 h, they can be stored at 4°C, other wise storage at -70°C is recommended. For serology, blood samples are used.

In order to isolate the AIV, 9-11 day old chick embryos are inoculated via the allantoic cavity. The presence of the virus is demonstrated by haemagglutination or agar gel immunodiffusion (AGID) tests. The determination of the subtype is conducted with a monospecific antiserum. The differentiation between LPAI and HPAI viruses is achieved by virulence tests in vulnerable birds after evaluation of the intravenous pathogenicity index or through determining the aa sequence at the cleavage site of the HA: if multiple basic aa sequence: HPAIV isolate.

RT-PCR and RRT-PCR tests are preferred for detection of RNA or viral proteins from AIVs in field diagnostics (Spackman et al., 2002; Akey, 2003; Xie et al., 2005). These tests can also distinguish some subtypes. ELISA tests have been developed for detection of antibodies fighting against AIVs (Meulemans et al., 1987; Beck & Swayne, 1998). To determine the localisation of the viral antigen in tissue samples, monoclonal or polyclonal antibodies are used in immunofluorescence or immunoperoxidase tests (Skeles et al., 1984; Slemons & Swayne, 1990).

DIFFERENTIAL DIAGNOSIS

In terms of differential diagnosis, HPAI should be distinguished from Newcastle disease, acute fowl cholera, some intoxications etc. LPAI should be differentiated from some respiratory illnesses: infectious bronchitis, avian pneumovirus, and other paramyxovirus and mycoplasma infections.

PREVENTION AND CONTROL OF AI

The prevention strategy for AI consists of several measures: control at the sources of infection, border control, local control, and preparedness for action in case of emerging infection (Leong et al., 2008).

The measures to prevent possible sources of infection include, most notably, control of imported foodstuffs (poultry meat, eggs, live birds). Imports must come exclusively from AI-free zones. Disease-free zones should be clearly defined and the movement of birds and poultry products should be certified to guarantee their safety. All farms within the AI-free zones should be accredited for their respective type of production and should implement strict biosecurity measures. Vaccinations for AI in disease-free zones are prohibited as they may mask outbreaks of disease. An action plan in case of HPAI outbreaks must be drawn up.

Border control includes inspection of all imported birds and products of avian origin by the relevant control organisations.

Local measures of control include bio-security, biosegregation, vaccination, elimination of backyard poultry, performance of AI surveys and educational measures.

Biosecurity practices are the most important with regard to prevention and control of AI (Alexander, 2000). A key priority is to maintain the segregation between poultry farms and migrating wildbirds, especially waterfowl. Contacts between poultry farms, poultrys laughterhouses and zoos should also be avoided. To this end, all farms and slaughterhouses within the prescribed perimeter should be protected. Disinfection of personnel and transportation vehicles should be applied as strictly as possible. Access to premises should be restricted.

Biosegregation measures consist of maintaining separate zones for egg production and egg storage, using separate vehicles for transportation of eggs, one-day-old chicks, manure etc., and no movement of workers or equipment between farms.

After detection of AI, the most important measures include eradication of infected flocks, their eggs and litter in order to prevent further spread. Backyard poultry must also be eliminated; live birds markets, presenting a real danger for AI spread, should be closed.

Vaccination

When AI outbreaks occur in a densely populated region, the implementation of strict biosecurity measures may not prevent the rapid spread of infection. In such cases, vaccination could help to limit the spread of the disease. The principal aim of vaccination is to prevent or reduce clinical infectious disease (Suarez et al., 2006). In addition to controlling the disease, vaccination fulfils two other important functions. If vaccinated birds are infected, the spread of the virus in the environment is reduced (Swayne et al., 2000; 2003) and as a result, viral agents may be more rapidly eliminated. Furthermore, vaccination increases the resistance to infection by increasing the minimum viral dose needed for infection of the bird. The simultaneous restriction of virus shedding and higher resistance to infection increase the chances of interrupting the infection cycle (Leong et al., 2006).

Vaccines

The vaccines available so far are conventional and recombinant. Conventional vaccines include inactivated homologous and inactivated heterologous vaccines. The former are prepared as autogenous vaccines, i.e. contain the same field AIV strain. Such vaccines have been used during AI outbreaks in Pakistan and Mexico (Swayne & Suarez, 2000). Inactivated heterologous vaccines use a viral strain from the same H type as the field virus, but with a heterologous neuraminidase. The use of homologous vaccines renders impossible the differentiation between vaccinal and field strains, while with heterologous vaccines the antibodies working against the neuraminidase induced by the field virus can be used as a marker of natural infection (Capua & Marangon, 2000).

Several recombinant fowlpox viruses expressing the H5 antigen have been developed, one of them being licensed and used in Mexico during a vaccination campaign against the LPAI H5N2.

More recently, HVT expressing H5 antigen has yielded promising results. The infectious laryngotracheitis virus was also used to deliver H5 or H7 antigens (Luschow et al., 2001).

In general, the subcutaneous application of inactivated vaccines is advised. Chickens may also be vaccinated in ovo with an inactivated oil emulsion vaccine (Stone et al., 1997).

REFERENCES

- Akey, B.L., 2003. Low pathogenicity H7N2 avian influenza outbreak in Virginia during 2002. Avian Dis., 47, 1099-1103.
- Alexander, D.J., 1982. Avian influenza. Recent Developments. Vet Bull., 52, 341-359.
- Alexander, D.J., 1987. Criteria for the definition of pathogenicity of avian influenza viruses. In Proceedings of the Second International Symposium on Avian Influenza, B.C. Easterday, (ed.) U.S. Animal Health Association, Richmond, Virginia, 228-245.
- Alexander, D.J., 1993. Orthomyxovirus infections. In Virus Infections of Birds, J.B. McFerran and M.S. McNulty, (ads.). Elsevier Science, London. 287-316.
- Alexander, D.J., 2000. A review of avian influenza in different bird species. Vet Microl., 74, 3-13.
- Bankowski, R.A., 1981. Introduction and objectives of the symposium. In: Proceedings of the First International Symposium on Avian Influenza, R.A. Bankowski, (ed.). U.S. Animal Health Association, Richmond, Virginia, VII-XIV.
- Beck J.R. & D.E. Swayne, 1998. Evaluation of ELISA for avian influenza serologic and diagnostic programs: Comparison with agar gel precipitin and hemagglutination inhibition tests. In Proceedings of the Fourth International Symposium on Avian Influenza, D.E. Swayne & R.D. Slemons, (eds.). U.S., Animal Health Association, Richmond, Virginia. 297-304.
- Easterday, B.C., D.O. Trainer, B. Tumova & H.G. Pereira, 1968. Evidence of infection with influenza viruses in migratory waterfowl. Nature, 219, 523-524.
- Easterday, B.C., V.S. Hinshaw & D.A. Halvorson, 1997. Influenza. In: Diseases of Poultry, 10 ed. B.W. Calnek, H.J. Barnes, C.W. Beard, L.R. McDougald and Y.M. Saif (eds.), Iowa State University Press, Ames, Iowa, 583-605.
- Elbers, A.R.W., B. Kamps & G. Koch, 2004. Performance of gross lesions at postmortem for the detection of outbreaks during the avian influenza A virus (H7N7) epidemic in The Netherlands in 2003. Avian Pathology, 33, 4, 418-422.
- Eskoade, R.J. & L.A. Silverman-Bachin, 1986. Avian Influenza in Pennsylvania the Beginning. In: Proceedings of the Second International Symposium on Avian Influenza, 22-32.
- Guo, Y., J. Dong, M. Wang, Y. Zhang, J. Guo & K. Wu, 2001. Characterization of hemagglutinin gene of influenza A virus subtype H9N2. Chin Med J., (Engl), 114, 76-79.
- Guo, Y., J. Li, X. Cheng, M. Wang, Y. Zhou, C.Li. et al., 1999. Discovery of men infected by avian influenza A (H9N2) virus. Chin J Exp Clin Virol., 13, 105-108.
- Hanson, B.A., D.E. Stallknecht, D.E. Swayne, L.A. Lewis & D.A. Senne, 2003. Avian influenza viruses in Minnesota ducks during 1998-2000. Avian Dis., 47, 867-871.
- Leong, H.K., C.S. Goh, S.T. Chew, C.W. Lim, Y.N. Lin, S.F. Chang, H.H. Yap & S.B. Chua, 2008. Prevention and Control of Avian Influenza in Singapore. Ann Acad Med Singapore, 37, 504-509.
- Lupiani, B. & S.M. Reddy, 2009. The history of avian influenza. Comparative Immunology, Microbiology and Infectious Diseases, 32, 311-323.
- Luschow, D., Werner, O., Mettenleiter, T.C., et al., 2001. Protection of chickens from lethal avian influenza A virus infection by live-virus vaccination with infectious laryngotracheitis virus recombinants expressind the hemagglutinin (H5) gene. Vaccine, 19, 30, 4249-4259.
- Meulemans, G., M.C. Carlier, M. Gonze & P. Petit, 1987. Comparison of hemagglutination-inhibition, agar gel precipitin, and enzyme-linked immunosorbent assay for measuring antibodies against influenza viruses in chickens. Avian Dis., 31, 560-563.
- Naeem, K., 1998. The Avian Influenza H7N3 Outbreak in South Central Asia. In: Proceedings of the Fourth International Symposium on Avian Influenza, 31-35.
- Olsen, B., V.J. Munster, A. Wallensten, J. Waldenstrom, A.D. Osterhaus & R.A. Fouchier, 2006.
- Obreshkov, K., I. Vasilev, B. Natchev, et al., 1978. Diseases of poultry, Zemizdat, Sofia, pp. 30 – 33 Global patterns of influenza a virus is wild birds. Science, 312, 384-388.
- Perdue, M.L., D.L. Suarez & D.E. Swayne, 1999. Avian influenza in the 1990`s. Poult Avian Biol Reviews, 11, 1-20.
- Perroncito, E., 1878. Epizoozia tifoide nei gallinacei, Annali Accad Agri Torino, 21, 87-126.
- Rauw Vaccine 29 (2011) 2590–2600.
- Rojas, H., R. Moreira, P. Avalos, I. Capua & S. Marangon, 2002. Avian influenza in poulty in Chile. Vet Rec., 1 51, 188.

- Senne, D.A., J.E. Pearson, Y. Kawaoka, E.A. Cardrey & R.G. Webster, 1986. Alternative methods for evaluation of pathogenicity of chicken Pennsylvania H5N2 viruses. In Proceedings of the Second International Symposium on Avian Influenza, B.C. Easterday, ed. U.S. Animal Health Association, Richmond, Virginia, 246-257.
- Skeeles, J.K., R.L. Morressey, A. Nagy, F. Helm, T.O. Bunn, M.J. Langford, R.E. Long & R.O. Apple, 1984. The use of fluorescent antibody (FA) techniques for rapid diagnosis of avian influenza (H5N2) associated with the Pennsylvania outbreak of 1983-84. In Proceedings of the 35th North Central Avian Disease Conference, 32.
- Slemons, R.D. & D.E. Swayne, 1990. Replication of a waterfowl-origin influenza virus in the kidney and intestine of chickens. Avian Dis, 34, 277-284.
- Slepuskin, A.N., T.V. Pysina, F.K. Gonsovsky, A.A. Sazonov, V.A. Isacenco, N.N. Sokolova et al, 1972. Haemagglutination-inhibiting activity to type a influenza viruses in the sera of wild birds from the far east of the USSR. Bull Worid Health Org. 47, 527-530.
- Spackman, E., D.A. Senne, T.J. Myers, L.L. Bulaga, L.P. Garder, M.L. Perdue, K. Lohman, L.T. Daum & D.L. Suarez, 2002. Development of a real-time reverse transcriptase PCR assay for type A influenza virus and the avian H5 and H7 hemagglutinin sub-types. J Clin Microbiol, 40, 3256-3260.
- Stallknecht, D.E., S.M. Shane, P.J. Zwank, D.A. Senne & M.T. Kearney, 1990. Avian influenza virus from migratory and resident ducks of coastal Louisiana. Avian Dis, 34, 398-405.
- Stone, H., B.Mitchell & M.Brugh, 1997. In ovo vaccination of chicken embryos with experimental Newcastle disease and avian influenza oil-emulsion vaccines. Avian Dis, 41, 856-863.
- Suarez, D.L. & C.S.Schultz, 2000. Immunology of avian influenza virus: a review. Dev Comp Immunol, 24, 269-283.
- Suarez, D.L., Lee C.W. & Swyane D.E., 2006. Avian influenza vaccination in North America: strategies and Difficulties. In: Schudel A., Lombard M., editors. OIE/FAO International Conference on Avian Influenza. Dev Biol., 124, 117-124.
- Swayne, D.E. & Suarez, D.L., 2000. Highly pathogenic avian influenza. Revue Scientifigue et Technique, 19, 2, 463-482.
- Swayne, D.E., 2003. Vaccines for list A poultry diseases: emphasis on avian influenza. In Brown F., Roth J., editors. Vaccines for OIE List A and Emerging Animal Diseases, Dev Biol., 114, 201-212.
- Van Riel, D., J.M.A. van den Brand, V.J. Munster, T.M. Besteboer, R.A.M. Fouchier, A.D.M.E. Osterhaus & T. Kuiken, 2009. Pathology and Virus Distribution in Chickens Naturally Infected with Highly Pathogenic Avian Influenza A Virus (H7N7) During the 2003 Outbreak in The Netherlands. Vet Pathol., 46, 971-976.
- Villareal, C.L. & A.O. Flores, 1998. The Mexican Avian Influenza (H5N2) Outbreak. In: Proceedings of the Fourth International Symposium on Avian Influenza, 18-22.
- Winkler, W.G., D.O. Trainer & B.C. Easterday, 1972. Influenza in Canada geese. Bull World Health Org., 47, 507-513.
- Xie, Z., Y. Pang, X. Deng. X. Tang & J. Liu, 2005. Development of multiplex RT-PCR for identification of H5 and H7 subtypes of avian influenza virus. Chinese Journal of Veterinary Science and Technolody, 35, 437-440.
- Zarkov, I., 2003. Veterinary virology, Orthomyxoviridae, RIK "Iskra-M-I", Stara Zagora, 265-274.
- Zarkov, I., R. Manvell, W. Shell, & I.L. Bochev, 2006. Isolation of avian influenza virus in Bulgaria. Veterinary Record 158, 3:106-107.
- Zarkov, I. & I. Dinev Ivanov, 2008. Pathological markers of anas plathyrynchos ducklings experimentally infected with an isolates of low pathogenic avian influenza viruses, Proceedings and abstracts, Second Congress of virology, Military Medical Academy, Sofia, 28-31 May, 248-254.