Avian adenoviruses

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Summary
Adenovirus infections are ubiquitous in commercially farmed birds, and probably in all avian species. There is a wide range of virulence, in some cases even within the same serotype. While many infections are subclinical and appear to be of little economic or welfare importance, significant outbreaks of disease associated with adenovirus do occur. These diseases are not of public health significance.

Keywords

Introduction

Classification
From a veterinary viewpoint, the avian adenoviruses can be divided into three groups, i.e. groups I, II and III.

Group I, or conventional adenoviruses, share a common group antigen, distinct from the mammalian adenovirus group antigen. These viruses grow readily in avian cell cultures and have been isolated from chickens, turkeys, geese, ducks, quail, pigeons, ostriches and other avian species. Fowl adenoviruses can be divided into at least twelve serotypes. A major problem in classification has been the presence of prime strains and strains of broad antigenicity. Five groups (A-E) have also been distinguished on the basis of restriction endonuclease analysis using two enzymes (58). The fowl adenoviruses not only infect chickens, but also turkeys and many other species. Turkeys, geese and ducks are affected by adenoviruses that do not grow or only grow poorly in chicken cell cultures and require a homologous cell type. At least three serotypes have been isolated from turkeys, and these grow in turkey but not chicken cells. A study of the relationship between isolates found in the United States of America (USA) and Northern Ireland, and between these turkey isolates and other avian strains, remains to be undertaken.

Three serotypes have been isolated from geese (57) and one from Muscovy ducks (Cairina moschata) (4).

Group II adenoviruses include the viruses of turkey haemorrhagic enteritis (THE), marble spleen disease (MSD) and group II splenomegaly of chickens. These viruses share a common antigen which is distinct from the group antigen of mammalian and group I avian adenoviruses.

Group III viruses, the egg drop syndrome (EDS) viruses, are widely distributed in waterfowl but can easily infect chickens, resulting in the production of abnormal eggshells.

Until recently, two genera have been recognised within the family Adenoviridae, namely: Mastadenovirus (mammalian strains including human strains) and Aviadenovirus. A third genus has recently been proposed, the genus Atadenovirus (2). Egg drop syndrome virus would be included in the Aviadenovirus genus, together with bovine adenovirus 5, 6, 7 and 8, and ovine adenovirus isolate 287. The position of the avian group II (THE/MSD) viruses in this classification is unclear (1, 19, 20).

A number of reviews have described group I (19, 21, 23, 36), group II (18, 31) and group III viruses (18, 20, 47). These should be consulted for a full reference listing, as only key and recent references are included here.

Aetiology
The adenovirus virion is a non-enveloped icosahedral particle of 70 nm-90 nm in diameter. The particle has 252 capsomeres arranged in twelve triangular faces with six capsomeres along each edge. The nucleic acid is linear, double-stranded deoxyribonucleic acid. The virions have a density in cesium chloride of between 1.32 g/ml and 1.37 g/ml. Adenoviruses replicate in the nucleus, producing basophilic inclusions.

All adenoviruses are resistant to lipid solvents, sodium deoxycholate, trypsin, 2% phenol and 50% alcohol. They are
resistant to exposure at pH 3 to pH 9, but are inactivated by 1.1,000 formalin. The avian adenoviruses appear to be more resistant to thermal inactivation than mammalian adenoviruses. Some strains survive 60°C and even 70°C for 30 min, and an F1 isolate was reported to survive 18 h at 56°C. At present, information on the effect of divalent cations is conflicting. Most workers accept that divalent cations destabilise adenoviruses, but some studies found no effect. Within the group I adenoviruses, only some strains of F1 agglutinate rat erythrocytes.

**Group I (conventional) adenoviruses**

**Epidemiology and pathogenesis**

Adenoviruses are ubiquitous in chickens, as demonstrated by serological surveys and virological studies, and have been isolated from both sick and healthy birds. Adenoviruses have also been isolated from turkeys, geese, ducks, pigeons, budgerigars and a mallard duck (Anas platyrhynchos). Evidence of adenovirus infection has been recorded in gulls, psittacines, owls and hawks. Infection by adenoviruses is likely to occur in all species of birds.

**Transmission**

Vertical transmission is a very important route. Chicks hatching from infected eggs may excrete virus in faeces from the time of hatching, but more typically chicks do not excrete virus until two to four weeks of age. Presumably reactivation of latent virus does not occur until maternal antibody declines. In a broiler flock where chicks originate from different parent flocks, a massive interchange of strains occurs, and concurrent infections of one bird with two or even three serotypes is not unusual. Spread of virus in this way results in peak virus excretion in a flock between four and six weeks. In one study of a layer replacement flock, virus excretion was at a maximum between five and nine weeks, but 70% of birds were still excreting after fourteen weeks. In another study, virus excretion again remained at a high level until fourteen weeks, and eight different serotypes were isolated from seven farms. Birds can re-excrete virus throughout life. Following a period of excretion, the virus appears to become latent, presumably due to the development of local immunity. When the local immunity is lost, after eight to twelve weeks, the virus is unmasked and excretion occurs. Humoral antibody does not appear to play a role in preventing excretion, as adult birds have been found to excrete virus despite high levels of neutralising antibody to the same serotype. Humoral antibody appears to offer little or no protection against infection with a different serotype. Adenoviruses are frequently isolated from hens during the period of peak egg production. This upsurge in virus activity ensures maximum transmission of virus to the next generation, through the egg.

Horizontal transmission is also important. The virus is excreted in high titres in the faeces. In addition, virus grows in the nasal and tracheal mucosa, conjunctiva and kidneys, and therefore virus could be present in other secretions or excretions. Virus could also be present in semen, which could be important where artificial insemination is used. Excretion of virus in the faeces follows a different pattern in juveniles and adults. In the juvenile, higher titres of virus are excreted for longer periods than in the adult. Lateral spread appears to occur principally by direct contact between birds or indirect contact by people, crates, egg trays and trolleys. Airborne spread probably only occurs over very short distances. True aerosol spread between farms is highly unlikely, but virus in contaminated poultry litter from a depopulated house could present a risk. In broiler houses, infection appears to spread very rapidly, but this is probably due to reactivation of latent virus in many birds throughout the house. When introduction of virus is minimal, as in a specific-pathogen-free (SPF) flock, spread can be very slow.

**Disease**

A wide range of virulence has been reported within the adenoviruses and the viruses are ubiquitous. Many infections are subclinical, in some cases because birds still have some maternal immunity when infected, but in many cases because the viruses have low virulence. The lack of virulence of some strains is illustrated by the fact that many SPF flocks become infected, even during lay, without any signs being observed. However, because latent adenovirus infections often become apparent at approximately two to three weeks of age, and again around peak egg production (i.e. during periods when disease or production problems are rife), adenoviruses have been associated with a range of conditions such as respiratory disease, diarrhoea, reduced egg production, detrimental effects on feed conversion and arthritis. In most of these conditions, the role of the adenovirus, if any, is that of a helper or secondary pathogen, rather than a primary pathogen. Thus, a study in Denmark was unable to detect any effect of adenoviruses on broiler flock performance (16). However, adenovirus is an important pathogen in some outbreaks of disease.

**Inclusion body hepatitis**

Inclusion body hepatitis (IBH) is usually seen in meat-producing birds between three and seven weeks of age, but has also been recorded in birds as young as seven days, and as old as twenty weeks. Classically, IBH is associated with sudden onset mortality which peaks within three to four days and ceases by days five to six, although in some outbreaks, deaths have continued for up to three weeks. Morbidity is low. Affected birds crouch, have ruffled feathers and die or recover within 48 h. Mortality usually ranges between 5% and 10%, but can reach 30%. Within an integrated breeding organisation, disease episodes in broiler flocks have been associated with certain breeder flocks.
The liver is the primary organ affected. Some reports suggest that the target organ is the haemopoietic system, but the aplastic anaemia described was probably due to simultaneous infection with chicken anaemia virus. The liver is pale, swollen and friable, and petechial or ecchymotic haemorrhages may be present. Haemorrhages may also be present in the musculature. Numerous eosinophilic intranuclear inclusions, and infrequently basophilic inclusions, are found in the hepatocytes. For many years, the role of adenoviruses in IBH has been unclear. Many serotypes have been associated with outbreaks of IBH. Adenoviruses are observed in the basophilic inclusions, but the eosinophilic inclusions are composed of fibrillar granular material. Experimental reproduction of IBH using adenoviruses has been inconclusive. Most workers have had no success, but some experimental infections have produced liver lesions and death following parenteral inoculation. However, the hepatocyte nuclei contained basophilic inclusions, rather than the eosinophilic inclusions typical of natural outbreaks.

Recent outbreaks of IBH have been described in Australia in birds under three weeks of age. Mortality was up to 30% and basophilic nuclear inclusions predominated in the hepatocytes. Reproduction of the condition was possible using serotypes 6, 7 and 8 isolated from field cases, administered by natural routes. All isolates were genetically closely-related, possessing a group E genotype. The field isolates were further divided into hypervirulent and mildly pathogenic isolates, using nine endonucleases. Recombination studies indicated that the fibre was responsible for the differences in virulence between isolates.

The serotypes isolated from severe outbreaks of IBH in New Zealand were principally F8 and also F1 and F12. In addition to the liver lesions where eosinophilic inclusions predominated, atrophy of the bursa and thymus was reported, together with aplastic bone marrow. These isolates all belonged to genotype E, but were distinct from the genotype found in Australia.

Necrotising pancreatitis and intranuclear inclusions have been observed in natural cases of IBH, and pancreatitis has occurred in experimentally infected chickens. Gizzard erosions and/or ulceration were present, but no intranuclear inclusion bodies were detected in the gizzard epithelial cells in outbreaks of IBH. Focal necrotising pancreatitis and gizzard erosions with typical adenovirus inclusions containing virus particles in necrotic pancreatic acinar cells and gizzard epithelial cells have also been seen in the absence of IBH. The latter birds were also infected with chicken anaemia virus. Other workers have also noted gizzard erosions, necrotising pancreatitis and mild proventriculitis with wet unformed faeces, in birds orally infected with adenovirus.

Infection with infectious bursal disease virus (IBDV) has been suggested as a major predisposing factor in the development of IBH. However, in New Zealand, and in the early cases in Northern Ireland, IBDV was absent. Furthermore, spontaneous IBH has been reported in SPF birds free of IBDV.

Adenoviral IBH has been recorded in pigeons, kestrels and a merlin (Falco columbarius), and in day-old turkeys from which turkey adenovirus serotype 2 was recovered. Pancreatitis was also found in some of the pigeons.

Hydropericardium syndrome

In 1987, a new syndrome named hydropericardium syndrome (HPS) or Angara disease was recognised in Pakistan. The disease has subsequently been recognised in India, Kuwait, Iraq, Mexico, Central and South America, Japan and Russia. The disease in Central and South America has been diagnosed as IBH. Hydropericardium syndrome differs from IBH only in that the mortality rate and the incidence of hydropericardium are much higher.

The disease principally affects meat-producing birds between three and six weeks of age, with mortality from 20% to 80%. Hydropericardium syndrome also occurs in breeding and laying flocks, with lower mortality rates. The disease is characterised by the accumulation of clear fluid (up to 10 ml) in the pericardium. Pulmonary oedema, an enlarged liver and pale enlarged kidneys are usually present. In addition, multifocal coagulative necrosis of the liver is observed, with mononuclear cell infiltration and intranuclear basophilic inclusions in the hepatocytes. The serological response to Newcastle disease vaccination is impaired.

The disease is considered to be the result of infection with adenovirus type 4 or 8 although some workers consider that other factors may be involved.

An HPS-like disease has been reported in pigeons, and broilers injected with liver from affected pigeons developed HPS.

Disease in turkeys

Adenoviruses have been isolated from clinical outbreaks of respiratory disease, diarrhoea and depressed egg production and more recently, IBH in day-old turkeys (see above). Attempts to reproduce the diseases have generally been unsuccessful.

Disease in waterfowl

Three serotypes isolated from geese failed to reproduce disease in experimentally infected goslings. In a disease outbreak with high mortality associated with hepatitis, adenovirus-like particles were observed in the liver.

In Canada, an isolated parent flock produced two hatches in which mortality in four- to eleven-day-old goslings reached 12% due to respiratory tract disease. A diptheritic...
stenosing tracheitis with occasional bronchitis and pneumonia, in which tracheal epithelial cells contained numerous adenovirus particles, was reported in 10% of seven- to twenty-one-day-old Muscovy ducks (3).

**Disease in guinea-fowl**

Pancreatitis and focal pancreatic necrosis with large basophilic and smaller eosinophilic inclusions have been associated with adenoviral infection of guinea-fowl. Pancreatitis and respiratory lesions have been induced by intranasal inoculation of adenovirus into day-old guinea-fowl. A haemorrhagic disease of guinea-fowl in which adenoviral inclusions were present in the spleen has been reported and reproduced experimentally (25).

**Disease in ostriches**

Adenoviruses have been associated with illness, diarrhoea, pancreatitis, death and poor hatchability in ostriches. An isolate from an ostrich produced pancreatitis in guinea-fowl (5, 13). In a study where three-day-old ostrich chicks were inoculated with an ostrich-derived adenovirus, all inoculates died (33).

**Quail bronchitis**

Quail bronchitis is an acute, highly contagious disease of young bobwhite quail (*Colinus virginianus*). Disease is most severe in one- to three-week-old birds, with morbidity approaching 100% and mortality up to 50%. Antibody has been detected in older birds and in wild quail. Disease has also been seen in Japanese quail (*Coturnix coturnix japonica*) (36).

Quail bronchitis is caused by a type 1 fowl adenovirus which is indistinguishable from chicken isolates. No information is available regarding whether the F1 strain behaves in quail as it does in chickens, where latency and vertical transmission occur. Chickens and turkeys may be experimentally infected with isolates from quail, but develop only very mild symptoms of disease.

Gross lesions in quail bronchitis include evidence of ocular and nasal discharge, mucoid tracheitis and atresaculitis. Occasionally, haemorrhagic exudate is present in the trachea. Histologically, a necrotising tracheitis, proliferative and necrotising bronchitis and pneumonia are observed. Basophilic intranuclear inclusions are common in tracheal epithelial cells. Multifocal necrotising hepatitis, splenitis and bursal lymphoid necrosis leading to atrophy are also seen (35).

**Other diseases in quail**

Two cases of adenoviral inclusion body ventriculitis have been diagnosed in bobwhite quail (12). In coturnix quail, gastrointestinal disease with inclusions in the digestive tract, particularly in the caeca, has been reported recently (52).

**Diagnosis**

A detailed methodology has been described in the literature for group I adenoviruses (18, 22) and for quail bronchitis (36).

**Virus isolation**

The preferred sample is faeces or colon with faeces. If a particular organ has obvious lesions, for example, the liver in IBH, or the trachea in quail bronchitis, this should also be included. Virus is frequently present in bursa of Fabricius, nasal mucosa, pharynx, trachea, lung and kidney. A 10% suspension of the specimen is made in cell culture media or bacteriological broth. In both cases, antimicrobial agents such as 1,000 international units (IU) of penicillin/ml and 1,000 µg streptomycin/ml should be added. The suspensions can be stored at 4°C or -20°C or below until required. Isolation is usually undertaken in cell cultures. For chickens, chick embryo liver or chick kidney cells are best. Chick embryo fibroblasts are insensitive and chick embryo liver cell cultures must show a predominance of epithelial cells. These cells are also suitable for preliminary isolation attempts from other species. However, some adenoviruses that affect turkeys, and probably other avian species, only grow in the cells of homologous species. Therefore, where possible, the homologous cell type should be used, for example, turkey kidney when investigating turkeys. One difficulty is the lack of SPF eggs for most species other than chickens. Because of the widespread distribution of adenoviruses and the presence of virus in eggs, an SPF source is virtually essential. If unavailable, SPF chicken eggs may be the only choice. Following inoculation, the cell cultures should be observed for fourteen days before being discarded. This usually involves one blind passage. Uninoculated cells should be treated in the same manner, to check for the presence of latent virus. Both rolled cultures and flask cultures are equally sensitive. Frequently, more than one adenovirus serotype, or more than one virus is isolated, for example adenovirus and reovirus. To acquire pure cultures, the use of plaque purification or the limiting dilution techniques often associated with the use of neutralising antisera is necessary.

If adenovirus is present, round cells which detach from the glass are observed. As a routine practice, all isolates should be checked for the presence of haemagglutinins, to exclude Orthomyxoviridae and Paramyxoviridae. Adenoviruses of group I and II do not agglutinate fowl erythrocytes. The most rapid method of confirming the presence of adenovirus is indirect immunofluorescence. If available, direct examination of disrupted cell preparations with the electron microscope is also a rapid method of recognition, as the virus morphology is typical. However, if the serotype is to be established, the isolate must be typed against the standard antisera.

Embryonated eggs, inoculated by the allantoic route are not sensitive, except in the case of virus types 1 and 5. Laboratory isolates have been successfully propagated in eggs following inoculation of the yolk sac.
Modern biochemical methods can be employed, but are of limited value. Polymerase chain reaction (PCR) techniques may be inappropriate because latency makes it impossible to determine if a positive result is due to the disease currently being investigated or an earlier infection. However, genotyping may be a valuable tool to distinguish between pathogenic and non-pathogenic strains.

Serological detection
The double immunodiffusion (gel precipitation) test has been widely used. However, the low cost in materials and labour is probably the only advantage of this test. The main disadvantages are lack of sensitivity and detection of group antigen. The test has been used widely to monitor SPF flocks for freedom from adenovirus infection where only group antigen detection is required. However, in many cases, the test has remained negative when birds in SPF flocks have become infected. This has been confirmed by experimental studies which have demonstrated that birds undergoing a primary infection as a result of natural exposure may not respond with precipitin antibodies. The apparent sensitivity of the test in the field is a result of the birds being infected with two or more strains. The sensitivity of the test can be increased by using a pool of antigen prepared from three different serotypes.

The serum neutralisation test is used to detect type-specific antibody. This is time consuming and expensive, even using the microtitre technique, because a minimum of twelve serotypes must be used when testing chicken sera.

The test of choice to monitor SPF flocks is the enzyme-linked immunosorbent assay (ELISA). Little benefit is derived from using a test to detect group antibody in commercial birds, given the widespread extent of infection.

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Public health importance
Group I adenoviruses do not naturally infect mammals and therefore no public health implications exist.

Prevention and control
The widespread distribution of group I adenoviruses throughout the world means that eradication would not be possible. Furthermore, some strains may be able to move between domestic and wild birds. Until recently, development of vaccines has not been a priority because of the absence of important diseases caused by adenoviruses. Since the recent outbreaks of IBH and HPS, development of vaccines has been attempted with varying success. A formalin inactivated liver suspension with liquid paraffin adjuvant is reported to be highly effective against HPS (39). Some other inactivated vaccines have also given good results (44).

No trade implications exist for infections with conventional adenoviruses. Obviously, movement of birds or eggs from flocks infected with the highly virulent viruses associated with HPS or the recent outbreaks of IBH to uninfected areas would not be wise. However, at present, testing for these conditions is not possible. Thus, type 8 viruses belonging to restriction enzyme group E have been associated with new variant IBH, but similar viruses have also been isolated from normal, healthy birds. The best option is certification that the birds, or in the case of eggs, the parents, have not demonstrated signs of HPS or new variant IBH.

Group II adenoviruses
Group II has three known members, namely: turkey haemorrhagic enteritis virus (THEV), marble spleen disease virus (MSDV) and avian adenovirus group II splenomegaly virus (AASV) of chickens. These viruses share a common antigen, which is distinct from that shared by the group I or conventional avian adenoviruses, and from mammalian adenoviruses.

Convalescent THEV serum protects pheasants against MSD. A single serological type of group II viruses appears to exist and isolates are classified only as to the source (e.g. THEV or MSDV). Isolates can be distinguished from one another by restriction endonuclease analysis and monoclonal antibody affinity.

Infectivity resists heating for 1 h at 65°C, but is destroyed after 1 h at 70°C. The viruses demonstrate a wide range of virulence, ranging from highly virulent to non-virulent.

Culture in conventional cell cultures such as turkey kidney or chick embryo liver is not possible. Growth occurs in a turkey lymphoblastoid B cell line derived from a Marek's disease induced tumour, the MDTC-RP19 cell line (28, 54). Virus has also been grown in turkey peripheral blood leukocytes.

Disease
Turkey haemorrhagic enteritis
Turkey haemorrhagic enteritis virus is distributed widely throughout the world. Antibody studies demonstrate that a high proportion of adult domestic turkeys have been infected, although a study of wild turkeys reported no evidence of infection. Guinea-fowl and psittacines may be naturally infected. Other gallinaceous birds such as peafowl, bobwhite quail and chukars can be infected. Lesions develop in the latter, but deaths have not been reported. A serological survey of forty-two species of wild birds indicated no evidence of a reservoir outside the Galliformes (9).

Turkey haemorrhagic enteritis usually occurs in turkeys between six and eleven weeks, although a case has been described in 2.5-week-old poults. Turkeys under thirteen days old appear to be resistant to infection in the absence of maternal immunity, presumably because target cells have not adequately matured. No upper age limit exists for infection.
Transmission is faecal-oral. Virus is present in faeces for several weeks and further bursts of excretion may occur when local antibody wanes. The virus is very resistant and can easily be carried from farm to farm by humans. Infection is also liable to recur in successive flocks in the same house, unless cleansing and disinfection is meticulous. No evidence of egg transmission has been found.

The virus replicates initially in the lymphoid cells of the intestinal tract and bursa of Fabricius. Virus can be detected one day post infection (dpi), peaks at 4 dpi-7 dpi and remains detectable up to 15 dpi in the intestinal tract. Virus is recoverable from the bursa between 2 dpi and 7 dpi. Virus is present in plasma from 2 dpi, and virus replicates are detected in the blood leukocytes from 3 dpi to 18 dpi. The spleen is the major site of viral replication. Antigen is detectable in the spleen from 2 dpi, reaches a peak at 6 dpi and is no longer detectable at 18 dpi (27). Reports as to the amount of antigen in the intestine are conflicting, and the intestinal pathology may be immune-mediated (48). Apoptosis occurs in approximately half of the immunoglobulin M+ cells at 3 dpi but not in cluster of differentiation 4+ (CD4+) and CD8+ T lymphocytes, and occurrence of apoptosis is not restricted to infected cells. The role of apoptosis in the pathogenesis of THEV is not clear, but this may be the cause of the immunosuppression (34, 48).

Experimentally, the incubation period is five to six days following oral infection. In natural outbreaks, virtually all birds become infected, as demonstrated by the development of antibodies. Mortality ranges from zero to over 60% with an average of 10%-15%.

Classically, the onset of disease is sudden. Birds are depressed, have bloody droppings and may die suddenly. Death usually occurs within 24 h of the appearance of the first signs of disease, or the bird recovers. Signs of disease within a flock last approximately six to ten days. Outbreaks due to less virulent strains are less spectacular. All strains, including those previously thought to be apathogenic, are immunosuppressive. Therefore, infection with THEV may allow paramyxovirus type II, Chlamydia, Staphylococcus and E. coli to cause disease (11).

In a breeding organisation in Northern Ireland which has a very high standard of hygiene, breeding turkeys remained uninfected until commencement of lay. The turkeys developed a clinical condition similar to EDS, with loss of eggshell colour, thin shelled and shell-less eggs, and this was associated with seroconversion to THEV. The birds remained apparently healthy.

Birds which have died from THE are often pale due to blood loss. Sudden death is often indicated by feed in the crop and good body condition of the carcase. The small intestine is usually distended, the mucosa is congested and the lumen filled with feed and blood (Fig. 1). In some cases, a yellow fibrinonecrotic membrane may be present. The lesions are more prominent in the proximal small intestine. If sick birds are killed, the spleens are found to be enlarged, friable and marbled or mottled. Where birds have died as a result of infection, the spleens tend to be smaller and the mottling is less apparent.

Fig. 1
Duodenum from a turkey with haemorrhagic enteritis
The upper specimen shows a less acute form

Following experimental infection, proliferation of the white pulp surrounding the splenic ellipsoids occurs from day three onwards. This progresses to large irregular islands of white pulp, grossly visible around days five to six. At around three to four days, lymphoblasts, probably B cells, with intranuclear inclusions are prominent (Fig. 2). Inclusions are also present in the splenic mononuclear phagocytes. By day four or five, the white pulp begins to become necrotic, and by day six to
seven the white pulp is completely involuted with only occasional plasma cells appearing in the red pulp. Lymphoid depletion also occurs in the thymus and bursa of Fabricius between days three and nine (27).

Severe congestion of the intestinal mucosa, degeneration and sloughing of the villous epithelium and haemorrhages in the villous tips are also observed. One group reported that the blood vessels in the lamina propria were intact and the erythrocytes appeared to escape from the vessels by diapedesis (41). Increased numbers of lymphoreticular cells with intranuclear inclusions are present in the lamina propria, in addition to mast cells, plasma cells and heterophils.

**Diagnosis**

The spleen is the preferred organ for virus isolation, but faeces also contain large amounts of virus. The lymphoblastoid B-cell line of turkeys (MSTC-RP19) is inoculated. If cell culture is not available, then five- to ten-week-old antibody-free turkeys can be given material orally or by the intravenous route. Birds usually die approximately three days after the intravenous injection and five or six days after oral infection. Birds which are infected but still alive at six days usually have enlarged spleens.

Traditionally, diagnosis has been made using affected spleen as antigen in a double immunodiffusion test. More sensitive tests such as the immunofluorescent test, ELISA, restriction endonuclease and PCR are now being used increasingly.

Antibody can be first detected three to four days after infection using the ELISA. This antibody is long lasting; in one flock, 83% of the birds were still positive forty months after initial testing. Due to lower sensitivity, the double immunodiffusion test becomes positive only after two weeks. Further details are provided by Pierson et al. (32).

Enlargement of the spleen in turkeys can be caused by THEV, but can also be due to reticuloendotheliosis or lymphoproliferative diseases. Blood in the intestine gives a strong indication of THE, and demonstration of antigen in the spleen provides the proof.

Vaccines are used in many areas. A tissue culture attenuated vaccine has been used extensively, but such vaccines have been reported to be immunosuppressive. Vaccines derived from the spleen of birds with THE or MSD have been used as vaccines, but both types are also immunosuppressive. A recombinant fowl pox vaccine which afforded good protection under laboratory conditions and which did not cause immunosuppression has recently been reported (6).

**Marble spleen disease**

Marble spleen disease is observed in pheasant production operations throughout the world. Marble spleen disease occurs naturally in three- to eight-month-old birds, but has been experimentally reproduced in adult pheasants. Infection, as indicated by antibody development, approaches 100%. Birds are often found dead, but depression, weakness, nasal discharge and dyspnoea may also be observed. Mortality ranges from 2% to 20%, usually occurring over a period of ten to fourteen days, but can continue for several weeks.

Antigen is present in spleen, liver, lung, bone marrow and kidney, but in contrast to THE infection, no antigen is detectable in the intestine (11). Bursectomy protects against the disease and an age-related resistance occurs below six weeks of age, which is unconnected with the presence of maternal antibody. This indicates the importance of B lymphocytes in the disease process. T lymphocytes are important in controlling MSD infection (11). Infection with MSD impairs both the humoral and cell mediated responses. The effect on the humoral response is more pronounced and lasts several weeks.

The spleens of pheasants which have died as a result of MSD are usually enlarged and mottled or marbled. In naturally occurring cases of the disease, the lungs are congested and oedematous (which is thought to be the cause of death through asphyxiation). In contrast to HEV, no evidence exists of intestinal bleeding or lesions. The splenic weight is significantly elevated between 6 dpi and 10 dpi. Histological changes in the spleen are similar to those in THE. Splenic necrosis and numerous large intranuclear inclusions are usually observed in birds which have died from the disease (Fig. 3). Necrosis may also be observed in the lungs.

![Fig. 3](image)

**Avian adenovirus group II splenomegaly**

Antibody to avian adenovirus group II splenomegaly is widespread, but associated disease is not a major problem. Mortality is unusual, although 8%-9% mortality has been recorded in mature chickens.
Infection is recognised as splenomegaly in broilers at slaughter and as splenomegaly with pulmonary oedema/congestion in adults. The disease can be important as a cause of condemnation at slaughter because of enlarged spleens. Histologically, the splenic lesions are similar to those reported in TJE.

Public health importance
The group II adenoviruses pose no threat to public health, as no record exists of infection of mammals by these viruses.

Control
The viruses are widely distributed throughout the world and therefore import restrictions would not be justified.

Group III adenoviruses (egg drop syndrome viruses)

Aetiology
The EDS virus (EDSV) is a typical adenovirus, except that it agglutinates erythrocytes of chickens, turkeys, ducks, geese, pigeons and peafowl. The virus does not agglutinate erythrocytes from a wide range of mammals. It partially shares an antigen with F1 adenoviruses (24).

Only one serotype of EDSV has been recognised, but three genotypes have been recognised using restriction analysis. One genotype encompasses isolates obtained from chickens in Europe over a period of eleven years. The second encompasses isolates from ducks in the United Kingdom and the third from chickens in Australia suffering from EDS (50).

Egg drop syndrome virus grows to high titres in duck kidney, duck embryo liver or duck embryo fibroblast cultures and chick embryo liver cells. The virus grows less well in chick kidney cells and grows poorly in chick embryo fibroblast cultures. Growth in turkey cells is poor and no growth could be detected in a range of mammalian cells. The virus grows to high titres in a range of goose cells.

In chick liver cells, peak virus and intracellular haemagglutinin titres are reached approximately 48 h after infection, and peak extracellular haemagglutinin titres at approximately 72 h.

The virus grows very well in SPF embryonated duck or goose eggs and this is the best system for producing antigen for vaccine or haemagglutinins, as titres of 1/16,000-1/32,000 are produced.

Epidemiology and pathogenesis
The behaviour of the EDSV in chickens appears unique compared to other adenoviruses. After initial entry through the nasal or gastrointestinal mucosa, local viral replication is followed by a transient viremia. The principal site of virus replication is the pouch shell gland (Fig. 4), and replication occurs to a lesser degree elsewhere in the reproductive tract (46, 56). If the embryo is infected, or the chick is infected before sexual maturity, then the virus remains latent until the bird is sexually mature. This strategy ensures transmission of the virus to the next generation, as virus will be present in and on eggs for up to three weeks (45). Virus is excreted through the cloaca and originates in the oviduct. Unlike other adenoviruses, EDSV does not originate from the gastrointestinal tract, as the virus has minimal replication in this organ.

Fig. 4
Shell gland from a hen infected with egg drop syndrome virus
Viral deoxyribonucleic acid (brown) is abundant in the surface epithelium. In situ hybridisation, haematoxylin counterstain

All ages and breeds of chickens are susceptible, although differences in the response may occur. When two brown egg laying strains and one white egg laying strain were experimentally infected, the white egg strain showed a more marked depression in egg production than the brown egg strains. However, the brown egg strains produced more eggs with shell defects.

Information on the pathogenesis of EDSV in waterfowl is scarce, but the available evidence suggests that EDSV behaves as a conventional adenovirus. Waterfowl are frequently infected with EDSV. Thus, studies on birds in the Atlantic flyways in the USA demonstrated antibody in ruddy (Oxyura jamaicensis), ring-necked (Aythya collaris), bufflehead (Bucephala albeola), wood (Aix sponsa), lesser scaup (Aythya affinis), mallard (Anas platyrhynchos), northern shovel (Anas clypeata), gadwall (Anas strepera) and common merganser (Mergus merganser) ducks. Antibody has also been detected in coot (Fulica spp.), grebes, cattle egrets (Bubulcus ibis), Canada geese (Branta canadensis), herring gulls (Larus argentatus), owls, a stork and a swan (14, 42). Antibody is also widespread in domestic ducks, including Muscovy ducks and geese.
Quail are susceptible and develop classical clinical signs (8). Although turkeys and pheasants can be experimentally infected, no signs of disease are observed. Guinea-fowl may be naturally infected and develop typical signs. However, in one study, guinea-fowl failed to show signs of disease after being infected with a fowl isolate (55).

Three syndromes are associated with EDS. The classical form was seen when primary breeding stock became infected. Chicks derived from these flocks remained healthy and did not produce antibody until reaching sexual maturity. At some time between the onset of egg laying and peak production, abnormal eggs were produced and the birds produced antibody. This infection probably initially arose from the use of a vaccine grown in duck cells which contained latent EDSV (18). Infection has since been eradicated from primary chicken breeding stock (18). However, the virus subsequently infected commercial egg-producing flocks and has become endemic in some areas. This is primarily due to the presence of virus on the exterior of eggs, leading to contamination of trays and trolleys. In many cases, this equipment is not adequately cleaned or disinfected before being returned from the egg packing plants to other farms at random. Infection can also be transmitted from flock to flock by humans, such as group advisory staff and workers servicing equipment.

The third category is the sporadic outbreak. This occurs when chickens come into contact with domestic or wild waterfowl. Contact may be direct or through contaminated drinking water. These outbreaks are self-limiting unless infection is spread to other flocks, when the outbreaks become the focus of an endemic cluster.

**Clinical signs**

The first sign is loss of shell colour in pigmented eggs. This is quickly followed by the appearance of thin shelled, soft shelled or shell-less eggs (Fig. 5). The thin shelled eggs often have a rough sandpaper-like appearance or a granular roughness at one end. If the obviously affected eggs are removed, fertility and hatchability are not affected. Small eggs have been described in some outbreaks, but were not reported in experimental infections. Some workers have described watery albumen, but others found normal albumen. Egg drop syndrome is probably a misnomer as much of the apparent drop is due to the production of shell-less eggs, as the numbers of shell membranes in the litter will testify, and consumption of the thin-shelled and shell-less eggs by birds.

If the disease develops as a result of reactivation of latent virus then the production of usable eggs is reduced by approximately 40%; this usually occurs when the flock is coming into lay and production is between 30% and peak production. Production does not return to predicted levels until four to ten weeks later and compensation often occurs later in lay resulting in an overall loss of eggs of ten to sixteen eggs per bird.

If the birds become infected by lateral spread when in lay, the clinical picture may appear different because transmission can be very slow, especially if the birds are in cages. Poor egg production may be reported rather than a marked decline. A careful inspection will reveal that only birds in a few cages are producing abnormal eggs at any one time. The speed of spread is influenced by a number of factors, such as the number of birds initially infected and the position of the affected cages with respect to the flow of the belts transporting the eggs, feed and faeces.

Affected birds appear healthy; some workers have described inappetence and dullness but most have not reported this. Transient diarrhoea has been described by some, but this is probably exudate and fluid excretion from the oviduct.

Although one study reported oral infection of day-old chicks causing increased mortality in the first week of life (7), most workers have found no signs of disease either in chicks or growing birds infected with virus (47), or commercially in the very large numbers of chicks hatching from infected eggs (18, 21).

**Diagnosis**

**Selection of specimens**

Selection of the correct specimen is very important. If birds are vertically infected, then no antibody will develop until sexual maturity. Therefore, to certify that a breeding flock is free of vertically transmitted virus, testing should be undertaken no earlier than thirty-two weeks of age. In the absence of clinical signs, selection of the correct bird for sampling poses a problem. In a cage unit, this problem can be overcome by selecting a cage in which affected eggs are present. If all the birds in the cage are examined, then at least some birds will have antibody and may have virus. The pouch shell gland is the organ of choice for histology,
immunochemistry or virus isolation, but pathognomonic lesions and viral antigen are present only for a short time. If blood is to be collected for serology, then the birds bled should be those from the cages in which defective eggs have been produced for the longest time. On litter, the problem is more difficult. To isolate virus, or to detect antigen or lesions, the simplest method is to feed affected eggs to antibody-free hens held individually in cages. The eggs produced by these birds should be examined daily and testing should be performed when a bird produces abnormal eggs. Examination of randomly selected cloacal swabs has been successful in some cases.

Serological tests
The haemagglutination inhibition (HI) test is the method of choice. A 1/10 serum dilution is mixed with an equal volume of a solution containing four haemagglutinating units of antigen. The mixture is allowed to react for 15 min at room temperature and then one volume of an 0.8% fowl erythrocyte suspension is added. Other tests, such as the ELISA and serum neutralisation, are available, but the HI test is rapid, inexpensive and accurate.

Virus isolation
A 10% suspension is made from the pouch shell gland and the supernatant inoculated onto cell cultures or embryonated duck eggs. Suitable cells, in order of preference, are duck cells, chick embryo liver or chick kidney cells. At least fourteen days incubation (one blind passage) are required after inoculation. If the cells degenerate, the supernatant should be checked for the presence of haemagglutinins using a 0.8% fowl erythrocyte suspension. If agglutination occurs, the isolate can be confirmed by an HI test using specific antiserum.

Antigen detection
Antigen can be detected in the pouch shell gland, during the time that defective eggs are produced, using immunofluorescent techniques on frozen sections or the avidin-biotin-peroxidase technique on formalin fixed tissue sections. In situ hybridisation may also be used.

Public health importance
Infection with EDSV has no public health significance.

Control
Basic breeding stock should be free of infection, and many breeding organisations are free at all levels. Given that EDSV is transmitted vertically and that birds do not develop antibody until sexual maturity, certification of freedom from vertically transmitted virus is not possible until the flock has been in lay for a number of weeks. The HI test is satisfactory, but the appropriate time for blood testing varies according to the type of parent. For broiler breeders, sampling at approximately thirty weeks of age would be acceptable, although thirty-five weeks was chosen in eradication programmes to allow a generous safety margin. Given the severe economic effects of the disease and the difficulty of excluding the virus if using an egg packing station serving infected flocks, many commercial egg producers routinely vaccinate flocks using a commercial inactivated vaccine which is very effective in controlling disease when administered correctly. Apparent failure of vaccines to protect appears to be due to poor vaccination techniques (53).

Risks from imported eggs and processed chickens
Since avian adenoviruses may be vertically transmitted, imported hatching eggs could give rise to infected chicks. Although adenoviruses occur world-wide, diseases such as hydropericardium syndrome do not. Therefore prudent measures would include checking the history of the supply flock, and rejection of eggs from a region or organisation where serious adenoviral diseases are present. Similarly, non-fertile eggs and hatchery waste eggs may also be infected, and these should not be recycled into poultry food.

Viraemia usually occurs in the early stages of adenovirus infections, and since adenovirus may be found in many visceral organs, adenoviruses could theoretically be present in processed chicken. However, flocks infected with significant adenoviruses will show evidence of disease and accordingly should not be slaughtered for human consumption. Therefore, the risk of importing very pathogenic adenoviruses with processed chicken should be low. Adenoviruses have been recovered from the faeces of normal chickens and the potential exists for contamination of carcasses in the processing plant. However, while adenoviruses may remain viable for some time, in contrast to some significant bacterial contaminants, the virus will not multiply on the carcass. Thus, while the risk of acquiring significant infection from uncooked poultry meat or offal appears small, care should nonetheless be taken to ensure that such poultry meat or offal are not recycled to avian species.
Adénovirus aviaires

J.B. McFerran & J.A. Smyth

Résumé
Les adénovirus sont des agents pathogènes ubiquistes affectant les oiseaux des élevages industriels, et probablement toutes les espèces aviaennes. La virulence varie considérablement d'une souche à l'autre, parfois au sein du même sérotype. Nombre d'infections sont inapparentes et leur incidence économique ou sanitaire semble faible ; toutefois, les adénovirus peuvent être à l'origine de graves épizooties. Ces dernières sont sans conséquence pour la santé publique.

Mots-clés

Adenovirus aviares

J.B. McFerran & J.A. Smyth

Resumen
Las infecciones por adenovirus son ubicuas entre las aves de explotación comercial, e incluso posiblemente entre todas las especies de aves. Existen niveles de virulencia muy variables, a veces incluso para un mismo serotipo. Aunque muchas de las infecciones son de carácter subclínico y parecen irrelevantes en términos económicos o de bienestar de las aves, a veces se declaren brotes infecciosos de cierta consideración asociados a la presencia de adenovirus. Estas enfermedades son de importancia menor en lo que a salud pública se refiere.

Palabras clave

References

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