CEVA HANDBOOK
OF POULTRY DISEASES

10 EGG DROP SYNDROME' 1976

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DEFINITION

Egg Drop Syndrome 1976 (EDS 76) is an infectious disease in layer hens manifested by a sudden drop in egg production, failure to reach peak production, irregularly-shaped eggs, soft-shelled or shell-less eggs and depigmentation.

HISTORY AND SYNONYMS

As the name suggests, the first description of EDS 76 dates back to the mid-1970s in Holland layer hens (Van Eck et al., 1976). The original outbreak in layers was probably provoked by a contaminated vaccine against the Marek's disease cultured in duck embryo fibroblasts. The virus infects breeder flocks and spread to other flocks via infected eggs. Ducks and geese are reservoirs of the virus, also known as duck adenovirus A. Although it has been eradicated from most commercial breeders, duck adenovirus A has become endemic in many chicken flocks worldwide. EDS 76 outbreaks are rarely caused by virus transmission from ducks or geese, neither directly or via contaminated water. Until a decade ago, it was thought that duck adenovirus A was not pathogenic for ducks and geese. In 2001 however, the virus was isolated after a respiratory disease outbreak in goslings and the disease was experimentally reproduced through experimental infection of one-day old ducklings. Synonyms of the virus are duck adenovirus 1 (DAdV-1), egg drop syndrome (EDS) virus, egg-drop-syndrome-76 (EDS-76) virus and adenovirus 127.

CHARACTERISTICS AND CLASSIFICATION OF THE PATHOGEN

The etiological agent is an adenovirus of group I. It belongs to genus Atadenovirus, family Adenoviridae on accounts of its high adenine-thymidine (AT) content. The EDS virus (EDSV) is a typical adenovirus able to agglutinate erythrocytes in chickens, turkeys, ducks, geese, pigeons and peacocks. It does not agglutinate erythrocytes in a large number of mammals. Only one EDSV serotype has been established, but by means of restriction analysis, 3 genotypes have been determined. The first genotype includes isolates from European chickens over a period of more than 11 years. The second includes duck isolated from the US and third – Australian isolates from chickens survived EDS (Todd et al., 1988).

EDSV replicates attaining high titers in duck kidneys, duck embryo liver, duck embryo fibroblast cultures and chick embryo hepatocytes. High titers are also observed in anserine cells. In chick embryo hepatocytes, the peak of the virus and intracellular haemagglutinin titres are attained approx. 48 h after infection, whereas the peak of extracellular haemagglutinin titers occurs after about 72 h. The virus grows very well in duck or goose eggs, making them the best system for production of antigens for vaccines (McFerran & Smyth, 2000).

EDSV is a haemagglutinating non-enveloped DNA virus, 74–80 nm in diameter, replicating in the nuclei of host cells. EDSV isolates could be distinguished via restriction endonuclease analysis of the virus DNAs. As such, European isolates were found to differ from Australian strains. The epidemiological significance of this finding was outlined by Todd et al. (1988), who found that over an 11-year period, no differences were discovered between European viruses. The differences with the Australian isolates (which contained a small deletion at one terminus) provided evidence for the non-European origin of Australian EDS outbreaks.

The EDS virus is inactivated at 60°C for 30 min. The infectivity is lost after treatment with 0.5% formaldehyde or 0.5% glutaraldehyde.

EPIDEMIOLOGY

The behaviour of EDSV in chickens is unique compared to that of other adenoviruses. After penetrating through the gastrointestinal or nasal mucosa, the local virus replication is followed by a transient viraemia. The site of virus replication is the pouch shell gland, and to a lesser extent the reproductive tract (Yamaguchi et al., 1981; Smyth et al., 1988). If the embryo or the chicken is infected at an early age, the virus remains latent until the sexual maturity onset. Thus, the virus transmission to the next generation is guaranteed, with persistence of the virus in laid eggs for over 3 weeks (Smyth & Adair, 1988). The virus is excreted through the cloaca and originates from the oviduct. Unlike other avian adenoviruses, the replication of EDSV does not occur in the gastrointestinal tract. Therefore, the presence of the virus in faeces is caused by contamination with oviduct exudate (Adair & Smith, 2008).

Birds of all ages are susceptible. It has been established that white layers exhibit a higher decline in egg production, where as brown layers suffer from a higher percentage of eggs with defective eggshells (McFerran & Smyth, 2000). Japanese quails (Coturnixcoturnix japonica) are susceptible and develop the classic clinical signs (Das & Pradhan, 1992). The attempts for experimental infection of turkeys and pheasants did not result in any clinical signs of EDS (McFerran & Smyth, 2000). Recently however, a natural outbreak of EDS among turkeys was reported in Croatia. After observing the characteristic egg production drop, poor quality of eggshells, reduced fertility and hatchability rates without clinical disease, the involvement of EDSV in the etiology of the condition was suggested. Subsequent extensive serological monitoring through the haemagglutination inhibition test has proved the presence of the virus in 94.4% and 55.1% of analysed serum samples in 2 turkey flocks. The serological evidence for EDSV infection was confirmed by PCR detection of the viral genome in turkey serum. After vaccination of turkeys flocks at the age of 18 and 25 weeks, the production of eggs

attained and even exceeded the usual levels (Bidin et al., 2007).

Guinea fowl may be naturally infected, exhibiting the specific symptoms, although an attempt at experimental reproduction with a poultry isolate ended in failure (Watanabe & Ohmi, 1983).

Based on the available evidence, EDSV appeared to be a conventional adenovirus in waterfowl. These bird species were frequently infected with EDSV. Antibodies to EDSV were detected in numerous wild as well as domestic ducks, including Muscovy ducks and geese (Gulka et al., 1984; McFerran & Smyth, 2000). The virus has also been isolated from healthy domestic ducks as well as from diseased ducks, but the disease could not be reproduced with the isolate. Similarly, a virus has been isolated in a duck flock with drop in egg production, but the experimental reproduction was not successful, so the virus detection in this case was considered accidental, even if the virus is common among these birds (Adair & Smith, 2008). The infection is also common in geese, but both goslings and geese have been experimentally infected without clinical signs of disease or any disturbances in egg production (Zsak et al., 1982).

In 2010, severe outbreaks with sharp drop in egg production, impaired feed conversion and disease of the ovary and the oviduct were reported in some big duck-producing regions in China. A new flavivirus, called BYD virus, closely related to the Tembusu virus was isolated.

Three models of infection distribution have been associated with EDS. The classical model is observed after infection of primary breeder flocks. Their progeny remains healthy and does not produce antibodies until sexual maturity is reached. In the period between the beginning and the peak of egg production, abnormal eggs and antibodies appear. This dynamic can be observed after use of vaccines contaminated with a latent EDSV, if duck cells are used.

The infection of the original breeder flock can be eradicated. The virus may, however, go on to infect commercial layer flocks and become endemic in some regions. Dissemination usually passes via contaminated egg trays and vehicles, as the virus is also present on the surface of affected eggs. In many instances, the equipment if not cleaned and disinfected before returning to egg packing premises.

Flock-to-flock infection transmission by workers may also occur. Experiments carried out to establish the role of lateral transmission of EDSV have concluded that infected eggs posed a serious risk which should be taken into account before egg tray reuse (Smyth & Adair, 1988). The third model is the sporadic outbreak which occurs when poultry at the farm are in contact with domestic or wild waterfowl. This contact may be either direct or via drinking water. The virus may be shed with faeces, although this excretion route leads to low titers. Such outbreaks are limited until the infection spreads to other flocks to become endemic (McFerran & Smyth, 2000).

As the transmission of EDSV is primarily vertical, association with specific breeder flocks is occasionally made. Horizontal transmission occurs slowly in battery systems and rapidly in floor housing systems.

CLINICAL SIGNS AND PATHOLOGY

The first sign is the loss of egg pigmentation, rapidly followed by the appearance of soft-shelled, shell-less of deformed-shell eggs. If defective eggs are discarded, the remaining ones have no problem with fertilisation and hatching. The drop could be sudden or prolonged. Usually, it lasts for 4-10 weeks and egg production is reduced by about 40%. It mainly occurs in the period between 50% and peak egg production. Apart from inactive ovaries and oviduct atrophy, other lesions are not discovered. The replication of the virus in epithelial cells of oviduct glands results in severe inflammatory and dystrophic changes in the mucous coat. In some outbreaks, numerous undersized eggs are produced. In some cases, the egg albumen is watery, while in others it its not. Transient diarrhoea, probably due to the increased amount of transudate in the oviduct, may be observed. EDSV does not cause an overt illness (Adair & Smith, 2008). In lateral infections, the course of the disease may be somewhat different. Poor-quality eggshells are observed rather than a drop in egg production. A careful analysis of layers reared in battery

cages may reveal that this occurs simultaneously in a few cages only. The rate of spread is influenced by several factors, such as the number of initially affected birds, and the position of affected cages in relation to egg, feed and excreta transportation belts (McFerran & Smyth, 2000).

Gross lesions in natural infections may include inactive ovaries and oviducts, although this is not always the case. After experimental reproduction, oedema and exudate in the oviduct were reported between post-infection days 9 and 14. A moderate splenomegaly, atretic follicles and eggs at various stages of formation in the abdominal cavity have also been reported (Smyth, 1988; Taniguchi et al., 1981).

The primary microscopic lesions are observed in the pouch shell gland – the site of virus replication. It occurs more specifically in the nuclei of superficial epithelial cells of oviduct mucosa. Some researchers have established intranuclear inclusion bodies by the 7th day after inoculation. Typically, heterophilic infiltration of lamina epithelialis and lamina propria, oedema of the mucosa and desquamative catarrh of the surface epithelium are observed. Three days after abnormal eggs appear, inclusions bodies are not found. At a later stage with progression of lesions, mononuclear cells predominate among the heterophilic infiltrate. A regeneration of the desquamated epithelium also occurs.

In many instances, naturally affected birds do not exhibit inclusion bodies or the lesions specific for the acute phase of inflammation, due to the transient character of infection and the fact that not all birds are simultaneously affected (Adair & Smith, 2008).

DIAGNOSIS

The appearance of eggs with impaired quality, and an overall drop in egg production are suggestive for EDS 76. The diagnosis is supported by some serological studies and is confirmed after isolation and identification of the virus. In many instances, no antibodies are detected in infected flocks until egg production approaches levels between 50% and peak production. If the infection is vertically transmitted, antibodies are not detected until the age of sexual maturity. This is why, to certify that a given breeder flock is free of infection which could be transmitted vertically, tests should be performed after 32 weeks of age. The selection of appropriate specimens for analysis is essential. When clinical signs are not present, the choice of birds for testing could be challenging. For birds reared in batteries, those in cages with defective eggs must be tested. In such birds, antibodies and viruses may well be found. Pouch shell glands are a suitable site for sample collection for histological and immune-histochemical examination, but viral antigen and pathognomonic lesions are present only for a short time. If blood samples are obtained, they should be collected from those layers producing abnormal eggs for the longest period of time. In free range birds on litter, this is more complicated. In order to isolate a virus, determine an antigen or lesions, the simplest method is to separate and feed antibody-free hens in individual cages. The eggs produced by these layers are then examined on a daily basis and, after the appearance of defective eggs, the respective tests should be conducted. Sometimes, the tests of random cloacal swabs could be successful (McFerran & Smyth, 2000).

For EDSV isolation and identification, a supernatant prepared from 10% pouch shell gland suspension could be inoculated in cell cultures

of embryonated duck eggs laid by EDSV-free birds. Most appropriate for this purpose, in order of preference, are duck cells, chick embryo liver or chick kidney cells. Embryonated chick eggs are not suitable. Embryonated duck or goose eggs are preferred for propagation as only a few fowl viruses can grow in these environments. After inoculation, incubation of at least 14 days is necessary. In case of cell degeneration, the supernatant should be checked for presence of haemagglutinins with 0.8% suspension of chick erythrocytes. If agglutination occurs, the isolate may be confirmed by haemagglutination inhibition (HI) test using a specific antiserum (McFerran & Smyth, 2000).

Applicable serological tests include HI, ELISA, immunofluorescent assay (IFA), serum-neutralisation (SN) etc. The HI test is preferred, as in cases where birds are infected with different adenovirus serotypes it gives the most accurate results. Furthermore, the HI test is rapid, accurate and inexpensive. For detection of EDS viral genome, polymerase chain reaction (PCR) is used. After experimental infection of oestrogen-treated chickens with EDSV, swab samples were collected from the oviducts, spleens and cloacae for PCR testing either directly or following a single passage in embryonated duck eggs. The tests were 98% sensitive in antigen detection. Therefore, the authors recommend the use of PCR for diagnosis of EDSV infections due to the easy assay protocol and the lack of requirements for special reagents. It is even recommended to use PCR directly on tissue homogenates and to repeat PCR-negative samples after passage through embryonated duck eggs (Dhinakar Raj et al., 2003).

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DIFFERENTIAL DIAGNOSIS

The differential diagnosis of EDS in poultry includes other conditions which may lead to a drop in egg production and poor quality of egg-shells. In terms of infectious diseases, EDS should be distinguished from infectious bronchitis (IB) and egg apical abnormalities (EAA), also known as "glass top eggs" provoked by *Mycoplasma synoviae*. IB affects the second and final thirds of oviduct mucosa and results in watery albumen of eggs. In EAA, abnormal eggs are produced, mainly with defects of the sharper pole, but there is no sharp reduction in egg production.

PREVENTION AND CONTROL

Biosecurity

The main route of transmission of infection is vertical. Therefore, attempts should be focused to EDSV-free breeder flocks. The eradication of EDS infection includes production of chickens from infection-free breeder flocks. With regard to lateral transmission, measures should be taken to improve hygiene and management practices aimed at restricting the use of contaminated egg trays, personnel compliance to high hygiene standards and vehicle disinfection. It should be remembered that infected eggs are a potential source of infection. Farms with mixed-age flocks, despite the regular vaccinations, are always at risk of infection due to the possibility for lateral transmission before vaccinated flocks become immune to EDSV.

Vaccines

A routine method for protection of egg-type flocks from EDS infection is the use of commercial oil-adjuvant inactivated vaccines. They are usually applied at 14-16 weeks of age, intra-muscularly or subcutaneously. During the egg laying period, vaccines are not applied. The vaccinal immunity lasts about one year (Adair & Smith, (2008).

In general, embryonated duck eggs are used for EDSV vaccine production. This procedure carries a risk of spreading other pathogens, such as avian influenza virus. This is why a recombinant vaccine has been developed, in which an EDS viral protein is expressed in *E. coli*. A single immunisation with the recombinant protein induces formation of EDS viral antibodies over at least 20 weeks.

For a single dose, 64 mg protein is required. Two shots of the recombinant protein before beginning of lay induces higher antibody titers compared to titers produced after administration of inactivated vaccines, which persist for 50 weeks. The vaccine has no side effect on egg production, egg quality and weight (Gutter et al., 2008).

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