

POST-VACCINAL REOVIRUS INFECTION WITH HIGH MORTALITY IN BREEDER CHICKS

Running title: POST-VACCINAL REOVIRUS INFECTION IN BREEDER CHICKS

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SUMMARY

A broiler breeder flock was subcutaneously vaccinated at the hatchery with a live avian orthoreovirus (ARV) vaccine against viral arthritis. Chicks began to die at three days of age and post-mortem examination revealed massive subcutaneous haemorrhages and edema on the dorsal aspect of the neck at the site of vaccination, a severe necrotic hepatitis and pulmonary edema. Microscopically, the main lesion was a multifocal vacuolar degeneration and necrosis of randomly distributed small groups of hepatocytes, with presence of apoptotic and multinucleated syncytial cells. Necrotic foci were also found in the lungs, as well as a hemorrhagic, granulomatous and heterophilic cellulitis and myositis of the neck, and a generalized depletion and lymphocytolysis of lymphoid organs. At 8 days of age, birds also began to show hock swelling, histologically characterized by a fibrinoleucocytic inflammation of the articulation and tendon sheaths with hyperplasia of the synovial membrane and lymphoplasmocytic infiltration. Polymerase chain reaction and viral culture of livers were positive for ARV. Partial sequencing of the S1 gene from the virus isolate showed 99,2% to 99,8% homology with three vaccinal strain (ARV S1133, 1733 and 2408). Viral particles compatible with reovirus virions were observed at transmission electron microscopy. Investigation at the hatchery revealed that chicks were inadvertently administered a S1133 reovirus vaccine labelled for water administration in 10 to 17 week-old chickens. This human error is most likely the reason for this unusually severe viremic reovirus infection that affected this flock at such an early age.

KEY WORDS:

Avian, chicken, reovirus, hepatitis, vaccine

ABBREVIATIONS:

ARV: *Avian orthoreovirus*

VAT: chicken viral arthritis/tenosynovitis

All reoviruses infecting birds are classified within the species *Avian orthoreovirus* (ARV) of the *Orthoreovirus* genus of the *Reoviridae* family (5). They are commonly found in healthy chickens but some serotypes are known to cause serious diseases such as viral arthritis/tenosynovitis (VAT) and malabsorption syndrome (5). The age of the bird at the time of infection, its immune status, the virus pathotype and the route of exposure are all important to determine the course of the disease and severity of clinical signs, ranging from asymptomatic to severe lack of performance or even mortality (5). The ubiquity of this virus in the chicken normal intestinal flora and in the commercial avian housing makes a solid causal relationship difficult to establish between the presence of this virus and a disease as malabsorption syndrome. This is however not the case in VAT, in which many reovirus serotypes were identified as causal agents, such as the S1133 strain which is commonly included in modified-live and inactivated vaccines used to protect against this disease (5). Chickens are most susceptible to reovirus infection at one day of age. This explains why vaccination is performed in breeders to protect newborns from this infection, which can be transmitted vertically (5). Experimental infection of one day-old chicks with a reovirus VAT strain has been demonstrated to cause liver necrosis in these birds as soon as 24 hours post-infection (9). The lesion was characterized by randomly-distributed foci of vacuolation and coagulative necrosis of individual cells to small clusters of hepatocytes. A prominent feature of the lesion was the presence of scattered multinucleated syncytial cells in necrotic foci (9).

This case report describes clinical signs and multisystemic lesions associated with a subcutaneous injection of a live S1133 reovirus vaccine in broiler breeder chicks.

CASE REPORT AND DIAGNOSTIC PROCEDURES

Flock history

A flock of 11750 broiler breeder chicks (10400 females and 1350 males) were delivered on the afternoon of April 9th2012. On the 24 hrs post-delivery visit, chicks appeared normal and active with a crop filling of 99% of the examined birds. The owner called on the evening of April 11th complaining of an unusual increase in mortality with almost 1% dead chicks picked in late afternoon.

The next morning (April 12th), more than 900 chicks were found dead (7,7%). A mild lack of uniformity was observed in the flock. Many chicks appeared listless, piling up along the cardboard partitions. Those found moribund or dead were in ventral decubitus with their head extended.

These birds had been vaccinated *in ovo* at the hatchery against Marek disease and laryngotracheitis (plus gentamicin). They had also received, at one day of age, a arthritis/tenosynovitis vaccine (plus ceftiofur) by the subcutaneous route as well as a coccidial vaccine by spray.

Following a necropsy performed on site, feed was removed and replaced, water lines were flushed and amoxicillin and vitamin K were added to water to mitigate the clinical signs measures.

Fourteen (14) chickens, aged 3 day-old, were then submitted to the Laboratoire d'épidémiosurveillance animale du Québec (LÉAQ), Saint-Hyacinthe, Canada, on April 12th (submission 1).

As mortality continued to increase, reaching 37,63 % on one floor (males), and as bacteriological results were inconclusive, twenty (20) more birds were sent to the lab the next day on April 13th (submission 2). A third submission was done on April 17th following the histopathological results, with the 8 day-old chicks clinically developing lameness. Ten chicks were submitted (submission 3).

Necropsy and histopathology

A gross necropsy was performed on each submitted bird. Post-mortem examination of chicks from submissions 1 and 2 revealed a mild to moderate subcutaneous hemorrhage on the dorsal aspect of their neck (figure 1) but also a severe hepatitis in all of them. Their liver was moderately enlarged, pale, mottled, had a yellowish color and contained myriads of white pinpoint foci and/or petechiae (figure 2). Other lesions observed in these birds were a severe pulmonary edema with presence of fluid in the trachea, a large and mottled spleen, hydropericardium and an unabsorbed yolk sac with an abnormal greenish and liquid content.

Tissues were collected on three selected animals from each submission, fixed in 10% neutral buffered formalin, trimmed, embedded in paraffin, sectioned at 3–5 μm and stained with hematoxylin, phloxin, eosin and saffron (HPES). Microscopically, the main lesion was a multifocal vacuolar degeneration and necrosis of randomly distributed small groups of hepatocytes (figure 3). Some multinucleated syncytial cells were present in necrotic foci and occasionally appeared apoptotic. Rare hyaline thrombi were found in sinusoids and a mild to moderate accumulation of mononuclear leucocytes occurred in

periportal areas. Apart from severe congestion and edema of the lungs, discrete and randomly distributed necrotic foci were also discovered in the lung parenchyma, associated with a mild exudation of erythrocytes and/or fibrin in the air capillaries and small airways. In the cervical area, moderate to severe infiltration of the subcutaneous tissue by macrophages and heterophils was observed, as well as diffuse hemorrhage (figure 4). Some small vessels were thrombosed and/or necrotic and there was a mild fibrin exudation in the subcutaneous tissue. The underlying muscle showed degenerated and atrophic fibers and was infiltrated by a mixed and moderate population of leucocytes. A moderate lymphocytolysis and lymphoid depletion were apparent in all lymphoid tissues (thymus, cloacal bursa and spleen) (figures 4 and 5). A periarteriolar fibrin exudation was present in the spleen of some birds. Other lesions included a mild mononuclear inflammation in the pericardium and/or perivascular areas of the myocardium of many birds (figure 6). Rare accumulations of necrotic cells (leucocytes or astrocytes) were also observed around capillaries in the cerebral cortex and cerebellum of one individual, as well as rare foci of gliosis.

In submission 3, mononuclear periportal infiltration of liver was more prominent and macrophages had started to accumulate around necrotic foci. Many birds showed typical lesions of septicemia as fibrinous, heterophilic and histiocytic pericarditis, exudation of fibrin in spleen ellipsoids and hyaline thrombi in liver sinusoids. A new lesion was also present, as these birds showed a mild hock swelling grossly. Microscopic examination of the tibiotarso-metatarsal joint and gastrocnemius tendon sheath revealed a mild fibrinoleucocytic exudate in the joint, as well as a mild proliferation of the synovial

membrane. The underlying connective tissue was moderately infiltrated by lymphocytes and plasma cells (figure 7).

Bacteriology

Two to three fresh tissues and or swabs of three selected birds from each submission were sent to the LÉAQ Bacteriology Laboratory for culture and identification. Columbia blood agar and MacConkey agar plates were inoculated and incubated for 48 hours at 35 ± 2 °C.

In tissues from submission 1, a mild growth of non-hemolytic *E.coli* was observed in one or two samples per bird, admixed with other bacterias (*Enterococcus spp.* and/or *Klebsiella oxytoca* and/or *Streptococcus spp.* and/or *Proteus spp.*).

Tissues from birds of submission 2 were all negative or yielded the growth of rare alpha-hemolytic *Streptococcus*. In submission 3, a mild growth of *E.coli* and rare other bacteria occurred from the pericardial swab of some birds but all other organs were sterile.

Transmission electron microscopy

Selected thin sections of liver from birds from submissions 2 and 3 were fixed in a 2,5% glutaraldehyde solution with 0,1M cacodylate buffer pH 7.4. After 2 hours at room temperature, tissues were washed 3 times in 0.1 M cacodylate buffer with 4% sucrose, pH 7.4, and stayed in this solution at 4°C until analysis. Tissues were then rinsed in 0.1M cacodylate buffer and postfixed in 1% osmium tetroxide (EMS, PA, USA) in cacodylate

buffer, for 1 hour at 4°C. After being washed in buffer, tissues were dehydrated in graded ethanol, infiltrated and embedded in Epon 812 (MECALAB, Québec, Canada), according to standard technique (8). Ultrathin sections were obtained using a Reichert Ultracut S ultramicrotome and mounted on naked copper grids (MECALAB, Québec, Canada). Sections were then stained with uranyl acetate and lead citrate and examination was performed with a Philips CM 100 transmission electron microscope. Numerous viral particles were visualized in the cytoplasm of necrotic hepatocytes, measuring around 85 nm in diameter, with an icosahedral symmetry and a double-shelled capsid (figure 8). These viral particles were compatible with reovirus virions as previously described. (1)

Viral culture and PCR

Liver tissues homogenates were obtained from birds with lesions from submissions 2 and 3. They were inoculated in the chorioallantoic sac of specific pathogen free 10 days-old embryonated chicken eggs, as well as in primary chicken kidney cell line (REP) and incubated at 37°C with 5% CO₂ atmosphere (3). An ARV real-time RT-PCR assay (qRT-PCR) was performed on the tendons of these birds and on cell culture lysates. Briefly, the primers and probe used within this qRT-PCR assay are REO-F: 5'-GTGCAGCCMTGGACAACAC -3'; REO-R: 5'-AGCTGCGCCGGATGTGTT -3'; and REO-P: 5'-(6-FAM) GTGCGTGTGGAGTTTCYCG (BHQ3) -3' respectively. These primers and probe are targeting a highly conserved region of the S4 gene of the ARV genome.

An ARV strain was isolated from embryonated eggs as well as from the REP cells in culture. A cytopathic effect was observed in REP inoculated cells after a few days of incubation. The presence of ARV particles within allantoic fluids and cell culture lysate was confirmed by TEM of negatively stained samples. The qRT-PCR tested tissues were ARV positives. Furthermore, the cell culture lysate was also ARV qRT-PCR positive. Thereafter, a partial sequence of the S1 gene (823 nucleotides in length) of the ARV isolate (named FMV12-1382537) was obtained. Sequence analyses of the FMV12-1382537 partial S1 gene revealed strong homology (99,2%, 99,5% and 99,8%) with three known attenuated ARV vaccinal strains (S1133, 2408 and 1733, respectively).

Final information from the hatchery

Further information was received from the hatchery in the week following the onset of mortality. A S1133 reovirus vaccine labelled for drinking water administration in 10 to 17 week-old chickens was inadvertently subcutaneously administered to the newly hatched chicks. Because mortality was over 50% and continued to worsen with remaining chicks extremely unthrifty, the entire flock was humanely culled. This was a single event and no other flock were reportedly affected.

DISCUSSION

The liver lesions described here are similar to those previously observed in chicks experimentally infected at one-day-old by a VAT and an enteric strain of reoviruses (9,

13). Hepatic necrosis is a feature of natural reovirus infection in young chicks, poults, and psittacines (2, 12, 14). Moreover, the lesions in the myocardium are similar, although less severe, to those reported in turkey poults and broiler breeder chick infected by a reovirus (12). Reoviruses are also known to induce atrophy of lymphoid tissues, especially of the cloacal bursa (4, 7, 10). Lymphoid depletion and lymphocytolysis were obvious in our case, not only in the cloacal bursa but also in the thymus and spleen. A novel strain of avian reovirus has also been reported to cause foci of gliosis in the cerebrum of young chicks and this lesions was also observed in one of our birds (13). The presence of multinucleated syncytial cells in liver foci of degeneration and necrosis is interesting as ARV are well known to induce fusion of cells in culture. This phenomenon contributes to apoptosis of the infected cells (6, 11). These syncytial cells have also been observed in chicken hepatocytes and turkeys cardiomyocytes infected experimentally or naturally by ARV (9, 12). The similarity of these lesions to previously described reovirus infections in young birds, the visualization of reovirus particles in degenerated hepatocytes using TEM, the virus isolation and its detection by PCR in livers and tendons all support the hypothesis that the lesions in our case were caused by a reovirus.

Mortality and lesions started two days after vaccination with a live reovirus vaccine , and a subcutis and muscular reaction at the site of vaccination was observed. Because the reovirus isolated from these birds showed a very high homology with vaccinal strains, and because of the case history, the vaccine in this case is most likely the source of the mortality and lesions observed. Further investigation revealed that a S1133 reovirus vaccine labelled for drinking water administration in 10 to 17 week-old chickens was

inadvertently subcutaneously administered to the day-old chicks. Since chickens are most sensitive to the pathogenic action of the ARV at a day of age, we postulate that a live vaccinal strain, even attenuated, could have the potential to induce such dramatic lesions in susceptible birds (5). It is interesting to note that, in one study, chicken orally inoculated with a reovirus strain rarely died (3% mortality) compared to those who were inoculated intramuscularly (54% mortality) (13). Furthermore, even if visceral lesions were similar in both groups of this study, lesions developed later in orally-infected chicken than in the intramuscularly-infected group (13). In another study, birds orally infected with some reoviruses strains failed to develop lymphoid lesions compared with subcutaneously-infected birds. Moreover, in the latter group, reovirus infection resulted in substantial mortality in birds but no mortality was recorded in groups infected by the oral route (10). This could indicate that parenterally administered reovirus vaccines could be more challenging for the young bird immune system than a vaccine given by water.

This is a most interesting example of a human error that generated major losses in broiler breeder chicks. It also demonstrates that a live-attenuated reovirus vaccine, given to birds of an inappropriate age and by an inadequate route, has the potential to cause a viremia with subsequent severe lesions and mortality.

Figures

Figure 1. Numerous 3-day-old chicks had a subcutaneous hemorrhage on the dorsal aspect of the neck.

Figure 2. Liver, 3-day-old chick. Multifocal to confluent white pinpoint foci of necrosis are scattered throughout the liver parenchyma, as well as small dark spots compatible with hemorrhage.

Figure 3. Liver, 3-day-old chick. A well-delineated focus of necrosis and vacuolar degeneration of hepatocytes. Note the multinucleated syncytial hepatocytes in the lesion (arrows). Bar: 50 μm .

Figure 4. Subcutaneous tissue, neck, 3-day-old chick. The interstitial tissue and the underlying muscle are distended by edema and a mixed inflammatory infiltrate. The thymus (in the left upper corner) contains numerous apoptotic lymphocytes. Bar: 50 μm .

Figure 5. Cloacal bursa, 3-day-old chick. There is a diffuse and moderate lymphocytolysis in the follicles. Bar: 50 μm .

Figure 6. Myocardium, 3-day-old chick. A diffuse, mild to moderate infiltration of mononuclear leucocytes occurs in the interstitium. Bar: 50 μm .

Figure 7. Gastrocnemius tendon sheath, 8-day-old chick. There is a moderate to marked mixed leucocyte infiltration all along the synovial membrane (arrows.) Bar: 200 μ m.

Figure 8. Liver, 3-day-old chick. Transmission electron microscopy. Viral particles with a morphology compatible with reovirus are found in the cytoplasm of infected hepatocytes. Bar: 100 nm.

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