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### Κλινική περίπτωση Λοιμώδους Εγκεφαλομυελίτιδας σε ορνίθια κρεοπαραγωγής στην Ελλάδα

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### Βιβλιογραφική αναφορά:

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## **An outbreak of Avian Encephalomyelitis in broilers in Greece**

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## **Κλινική περίπτωση Λοιμώδους Εγκεφαλομυελίτιδας σε ορνίθια κρεοπαραγωγής στην Ελλάδα**

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**ABSTRACT.** *Avian Encephalomyelitis Virus (AEV) is an infectious viral disease, member in the family of Picornaviridae, with a preference for the central nervous system and various parenchymatous organs of chickens. AEV is an enteric infection and can be transmitted by oral ingestion, but also vertically from infected broiler breeders to the chick, resulting in clinical signs at hatching. A flock of 18,000 broilers, located in the Southern part of Ipirus, exhibited sudden neurological signs. Clinical examination of 16 days old chicks showed rapid head tremors, ataxia and paralysis, often falling on their sides and lie with their legs at unusual angles. No gross lesions were noted during post mortem examinations conducted at 16, 20 and 26 days of age, apart from gross lesions on the brain. Morbidity of the flock exceeded 25% and total mortality reached 20.9% at the end of flock's cycle. In order to diagnose the suspected AEV, histological and serological examinations were performed. The results showed typical "flame-shape" proliferation of glia, neuron necrosis and neuronocytophagia in the gray matter and Purkinje cells*

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as well. Also, foci of infiltrating lymphocytes were seen in the brain, the glandular part of pancreas, muscular layer of heart and muscle layer of proventriculus. All observed lesions were characteristic of AEV infection. Serological results in affected flock, conducted by ELISA, showed a consecutive marked increase in serum encephalomyelitis virus antibody titers through time. At 30 days of age onwards, no clinical signs appeared. This clinical case of AEV in broilers was the first case reported in Greece.

**Keywords:** Avian Encephalomyelitis, broilers, ELISA, Greece, histology

**ΠΕΡΙΛΗΨΗ.** Ο ιός της Λοιμώδους Εγκεφαλομυελίτιδας των Πτηνών (AEV) είναι μια μολυσματική ιογενής νόσος, αποτελεί μέλος της οικογένειας *Picornaviridae*, και προσβάλλει κυρίως το κεντρικό νευρικό σύστημα και διαφορετικά παρεγχυματώδη όργανα των πτηνών. Ο AEV είναι μια εντερική λοίμωξη και μπορεί και μεταδίδεται δια της πεπτικής οδού, αλλά και κάθετα από μολυσμένες όρνιθες αναπαραγωγής στους νεοσσούς, με αποτέλεσμα την κλινική εκδήλωση της νόσου μετά την εκκόλαψη. Σμήνος 18.000 ορνιθίων κρεοπαραγωγής, στη Νότια περιοχή της Ηπείρου, παρουσίασε αιφνίδια νευρικά συμπτώματα. Κατά την κλινική εξέταση πτηνών ηλικίας 16 ημερών, παρατηρήθηκε ταχύς τρόμος της κεφαλής, αταξία και παράλυση, πλευρική κατάκλιση και πτηνά να ξαπλώνουν με τα πόδια τους σε ασυνήθιστες στάσεις. Δεν παρατηρήθηκαν μακροσκοπικές αλλοιώσεις κατά τη διάρκεια της νεκροτομικής εξέτασης στην ηλικία των 16, 20 και 26 ημερών, εκτός από αλλοιώσεις στον εγκέφαλο. Η νοσηρότητα του σμήνους ήταν μεγαλύτερη του 25% και η συνολική θνησιμότητα στο τέλος του κύκλου έφτασε το 20,9%. Για να επιβεβαιωθεί η υποψία της AEV, διεξήχθησαν ιστοπαθολογικές και ορολογικές εξετάσεις. Τα αποτελέσματα έδειξαν χαρακτηριστική εμφάνιση “δίκην φλόγας” διήθησης της νευρογλοίας και εκφυλίσες νευρώνων, καθώς και νευροκυττοφαγία στη φαιά ουσία και στα κύτταρα Purkinje του εγκεφάλου. Επίσης, στον εγκέφαλο, το πάγκρεας, το μυοκάρδιο και τη μυϊκή στοιβάδα του αδενώδους στομάχου παρατηρήθηκαν εστίες διήθησης από λεμφοκύτταρα. Όλες οι παρατηρούμενες αλλοιώσεις είναι χαρακτηριστικές της μόλυνσης με AEV. Με τη πάροδο του χρόνου, τα ορολογικά αποτελέσματα ELISA στο μολυσμένο σμήνος κατέγραψαν αξιοσημείωτες διαδοχικές αυξήσεις στους τίτλους των αντισωμάτων του ιού της Λοιμώδους Εγκεφαλομυελίτιδας. Μετά την ηλικία των 30 ημερών, δεν παρατηρήθηκαν κλινικά συμπτώματα. Η συγκεκριμένη κλινική περίπτωση αποτελεί πρώτη αναφορά της AEV σε ορνίθια κρεοπαραγωγής στην Ελλάδα.

**Λέξεις ευρετηρίασης:** Ελλάδα, ELISA, Ιστολογία, Λοιμώδης Εγκεφαλομυελίτιδα, ορνίθια κρεοπαραγωγής

## INTRODUCTION

Avian encephalomyelitis virus (AEV) is an infectious disease with a preference for the central nervous system and other parenchymatous organs of chickens. Recent characterization of AEV genome indicates that it is more closely related to *Hepatitis A virus* (Marvil et al., 1999; Todd et al., 1999), than to enteroviruses (Butterfield et al., 1969), and it had been provisionally classified as a tentative species in the genus *Hepatovirus* (Van Regenmortel et al., 2000) in the family *Picornaviridae* (Marvil et al., 1999; Todd et al., 1999). Recently AEV has now been reclassified as a unique virus and placed as the sole member of the *Tremorvirus* genus (King et al., 2012).

AEV is essentially an enteric infection and is transmitted between birds by oral ingestion, but it can also be transmitted vertically from infected breeding females through the egg to the chick, result-

ing in clinical signs at hatching (Calnek, 2008). Due to the great stability of the virus, contaminated areas may remain infectious for long periods. It affects mainly young chicks and is characterized by neurological signs like ataxia and rapid tremors of the head giving rise to the former name of “epidemic tremor” (Tannock & Shafren, 1994; Suarez, 2013). The disease is controlled in poultry by vaccination of breeding flocks during the growing period to prevent this vertical transmission.

Although AEV has a virtually worldwide distribution in domestic fowl, to the best of our knowledge, no clinical case of AEV has previously been reported in Greece in scientific literature. The present study describes the clinicohistopathological investigation of an AEV outbreak in broilers affecting a commercial flock in Arta, Greece.

## CASE DESCRIPTION

### Flock Details

The reported clinical case occurred in a commercial broiler farm located in Arta, in the Southern part of Ipirus region, Greece. Two broiler flocks, 18,000 and 15,000 birds, were housed in different houses under intensive and controlled environment system. The farm was surrounded by other farms in a relatively close distance, but kept high standards of biosecurity and held complete records of production data. The placement of one day-old chicks was done in two stages: (1) 18,000 day-old chicks, purchased from a local hatchery and (2) 15,000 day-old chicks, purchased from another hatchery 4 days later from the latter placement, and hatched: the first flock (Flock 1) from a non-vaccinated and the second flock (Flock 2) from a vaccinated against AEV broiler breeders flock, respectively.

### Clinical History

Both flocks were vaccinated against Infectious Bronchitis and Newcastle Disease at day 1 in the hatchery and no health or production problems were observed, except an increase in mortality due to omphalitis derived from poor hatchery management. Therefore, the production data (Body Weight and Feed Conversion Ratio) were accordingly to the breed standards, but a total mortality of 5.2% was recorded up to two weeks of age. At 16 days of age of Flock 1, daily mortality raised up to 1%, and the birds showed neurological signs which gradually

spread to the rest of the flock and total morbidity exceeded 25%. Supplementation of Vitamin E and selenium was continuously given for 7 days with no signs of improvement. However, no symptoms of the condition were manifested in Flock 2. In the next two weeks period, the daily mortality fluctuated from 1 to 2% daily and reached 19.7% totally in Flock 1.

### Clinical Examination

During the clinical inspection of the Flock 1 at 16 days of age, the main symptoms observed were depression and a variable tremor, in some cases very marked and evident, especially when the chicks were upright. Exciting or disturbing the chicks brought on the tremor of the head and neck, the frequency and magnitude of which varied. The chicks also showed ataxia and paralysis, falling on their backs and unable to right themselves easily and they also had an inclination to sit on their hocks (Figure 1), and to sit or lie with their legs at unusual angles. When disturbed, the chicks tended to move about with splayed legs and wings, often falling on their sides (Figure 2) and many of the chicks showed curling of the toes, but also some refused to move. Postmortem examination of the chicks confirmed the total absence of feed in the digestive tract and revealed lack of gross abnormalities in the proventriculus, gizzard, pancreas/duodenum, myocardium or peripheral nerve tissues. Birds with neurological signs showed gross lesions on the brain with cerebellar swelling, edema, hemor-



Figure 1. Clinical symptoms.

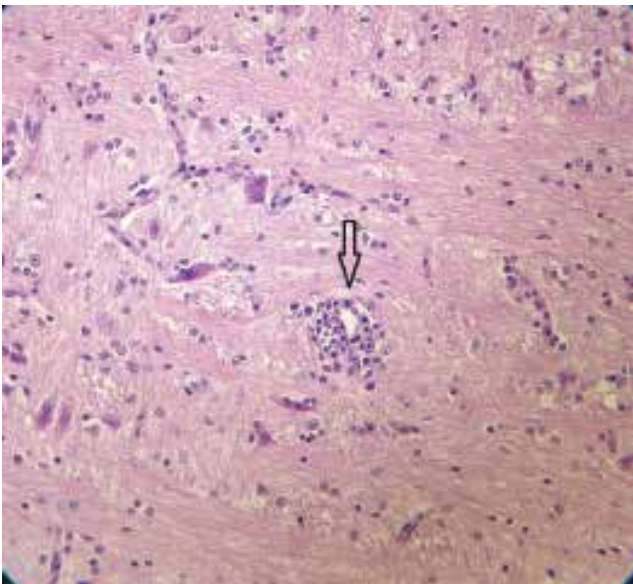


Figure 2. Chick falling on the side.

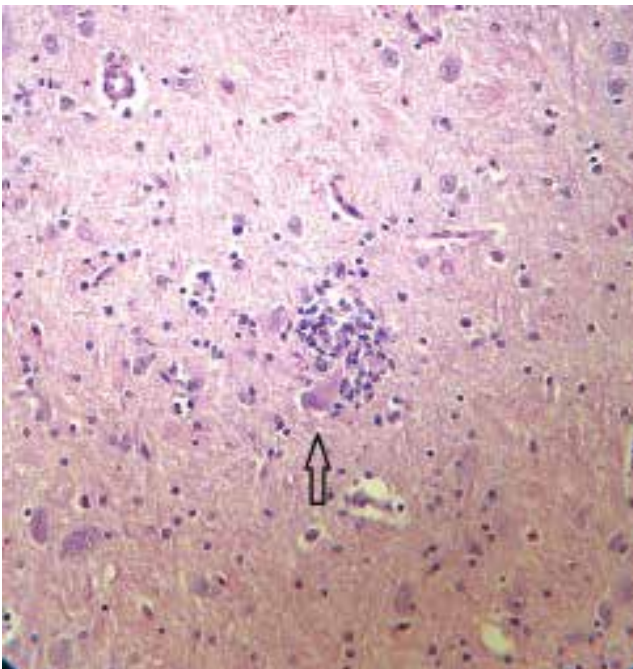




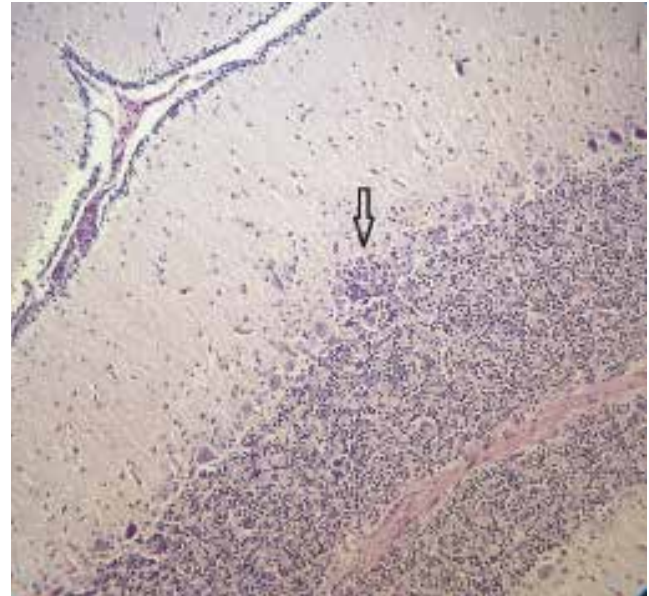
**Figure 3.** Gross lesions on the brain.



**Figure 4.** Cerebellum with perivascular cuffing of lymphocytes in the molecular layer.



**Figure 5.** Cerebellum with "Flame-shape" proliferation of glia.

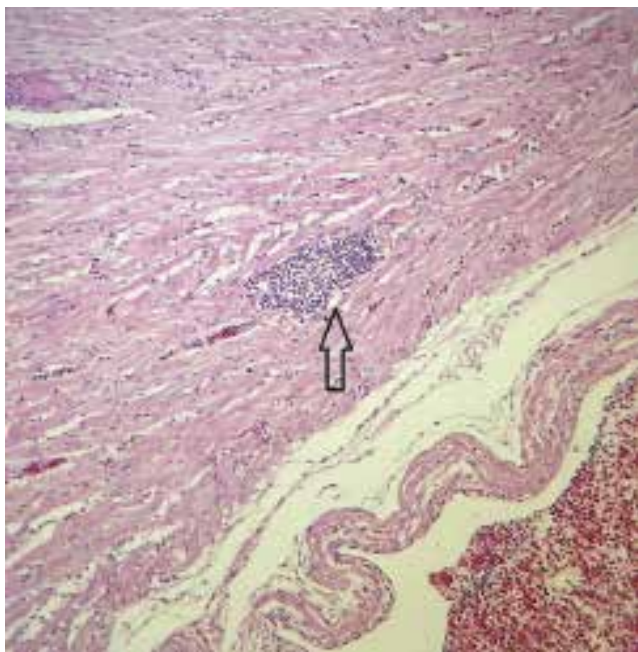


**Figure 6.** Nucleus cerebellaris with infiltration and gliosis.

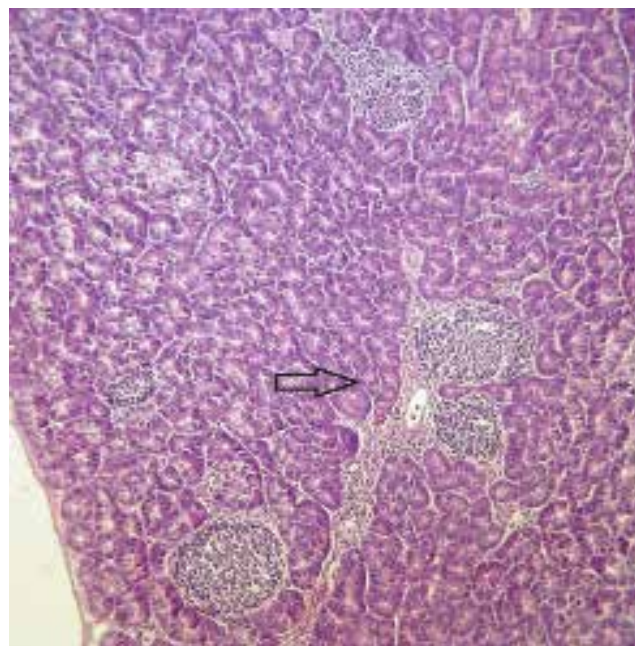
rhage and coning of the swollen cerebellum into the foramen magnum (Figure 3). No gross abnormalities were noted in post mortem examinations at days 20 and 26 as well. No symptoms neither gross lesions were noted in Flock 2 during the whole flock cycle.

### Sample Collection and Laboratory Examination Histology

Due to the initial AEV suspicion and for further differential diagnosis, samples for histology were taken at 20 days of age. Following the clinical examination of Flock 1, live birds showing neurological signs were humanely euthanized and specimens of brain, small intestine and pancreas, heart, proventriculus and gizzard were taken from 10 dead birds and were fixed in 10% buffered neutral formalin for histological examination as described by Ono et al. (2003). Histological results revealed various microscopic lesions in all specimens which are characteristic for AEV and some of them are considered to be pathognomonic. In cerebellum, mainly in the grey matter and in the Purkinje cells, neuron-necrosis, neuronocytophagia, lymphocytic inflammation (Figure 4) and appearance of typical "flame-shape" proliferation of glia as well (Figure 5) were seen. Perivascular infiltration and gliosis was also noted in the nucleus cerebellaris (Figure 6). In the myocardi-



**Figure 7.** Aggregates of lymphocytes in the myocardium.



**Figure 8.** Several follicles of lymphocytes are present in pancreas.

um (Figure 7), particularly the atrium, and pancreas (Figure 8), foci of infiltrating lymphocytes were considered to be the result of AEV. Finally, a significant increase, which is a pathognomonic lesion, of dense lymphocytic foci in the muscular wall of proventriculus (Figure 9) was found.

### Serology

Fourteen (14) blood samples for serology were also taken from affected Flock 1 at 20, 26 and 42 days of age and were tested using a commercial AEV enzyme-linked immunosorbent assay (ELISA) (BioCheck Baselines, 2012). Each serum sample was prepared and tested by ELISA according to Smart et al. (1986). Blood samples for serology were also taken from non-affected Flock 2 at 42 days of age at the slaughterhouse. Serological results for both flocks are presented in Table 1.

### Treatment and outcome

Although hylelectrolytes, multivitamins, vitamin E and selenium were supplemented in the water in an attempt to boost the health of birds, no major improvement in health and production data was noted. Flock 1 was slowly recovered after two weeks from the initiation of symptoms, reached lower body

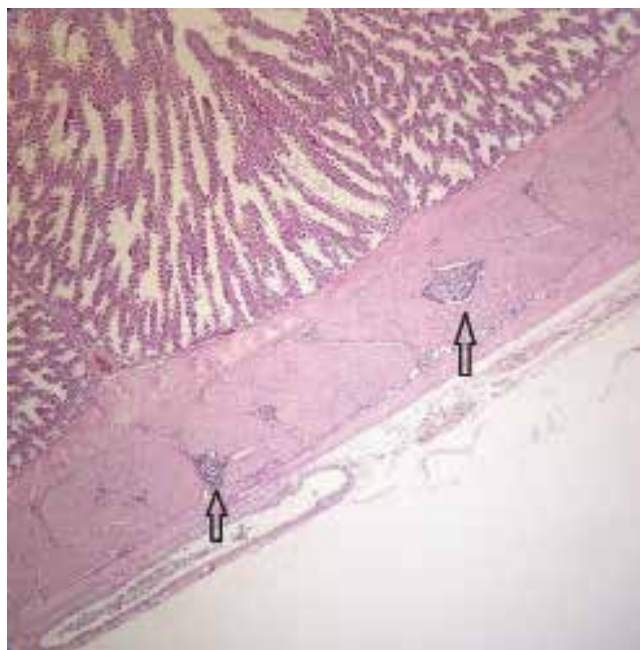
weights and production data compared to non-affected Flock 2 and with an accumulated mortality 20.9% at 42 days of age at the slaughterhouse.

### DISCUSSION

In the current study, an outbreak of AEV in broilers was described. AEV occurs virtually worldwide (Tannock and Shafren, 1994; Van der Heide, 1970). Nearly all chicken flocks eventually become infected with the virus, but the incidence of clinical disease is very low unless a breeder flock is not vaccinated and becomes infected after the commencement of egg production. Avian encephalomyelitis virus has a limited host range. Chickens, pheasants, coturnix quail, pigeons and turkeys have all succumbed to naturally occurring infection (Toplu and Alcigir, 2004; Butterfield, 1975; Van der Heide, 1970). The naturally occurring disease in turkeys is essentially the same as that in chickens (Hohlstein et al., 1970). In Greece, no data on the presence of AEV in broilers has been previously recorded in the scientific literature.

The clinical signs of depression, fine tremor of the head in a variable degree, ataxia varied from slight incoordination to sitting on the hocks, and lateral recumbency (Jana et al., 2005), paralysis, as well as





**Figure 9.** Dense lymphocytic foci in muscular wall of proventriculus. This lesion is pathognomonic for AEV.

absence of gross lesions in various organs examined or peripheral nerve tissues were typical of epidemic tremor and resembled those reported in cases of AEV in domestic fowl chicks (Calnek, 2008). Studies conducted by Calnek et al. (1960) demonstrated that the incubation period in chicks infected by embryo transmission was 1–7 days, whereas chicks infected by contact transmission or oral administration had a minimum incubation period of 11 days. Thus the disease pattern may involve a reduction in hatchability, clinical signs in vertically infected chicks, which appear during the first 10 days of life, and signs in those infected after hatching, which are seen

at 2–5 weeks (Calnek et al., 1960; Calnek, 2008). In this study, the first appearance of clinical signs was noted at 16 days of age, which strongly indicates the horizontal transmission of AEV either at hatchery or through other route. Also, gross lesions on the brain, resembling those which occur during encephalomalacia were recorded, but unsuccessful supplementation of Vitamin E and selenium eliminated this disease from differential diagnosis list from the beginning.

All samples from chicks with marked clinical signs at 20 days of age showed characteristic histopathological findings. Focal gliosis of the Purkinje and molecular layers was noted, as well as evidence of the Purkinje cell degeneration, as previously described by Hishida et al. (1986) as the most characteristic change in domestic fowl chicks horizontally infected with AEV. Also, foci of infiltrating lymphocytes in heart, pancreas and proventriculus were found in significant numbers, confirming the initial diagnosis of AEV. These findings were greatly correlated with those in the studies of Peckham (1957), Jana et al. (2005) and Butterfield et al. (1969), who reported lymphoid proliferations with follicle formation detected in the muscle layers of the proventriculus, gizzard or duodenum, or in the pancreas or myocardium which occur in naturally infected chickens.

Antibodies to AEV were detected in blood serum by a commercial ELISA test. These results showed an increase in serum avian encephalomyelitis virus antibody titers in sequential serum samples that they were collected at 20, 26 and 42 days of age. A strong positive increase of titers, a decrease of CV and increase of positive samples in relation with time strongly indicated an active infection with AEV.

**Table 1:** ELISA titers, Coefficient Variation (CV) and positive samples of AE in two flocks at 20, 26 and 42 days of age.

No of Flock	20 Days			26 Days			42 Days		
	Titers	CV %	N/S/P*	Titers	CV %	N/S/P	Titers	CV %	N/S/P
<b>Flock 1**</b>	7437	132	0/1/13	9070	91	0/0/14	12296	46	0/0/14
<b>Flock 2***</b>	-	-	-	-	-	-	5657	59	0/0/14

\*N/S/P: Negative/Suspected/Positive.

\*\* Flock 1: Hatching eggs from Non-Vaccinated against AEV Broiler Breeders flock.

\*\*\* Flock 2: Hatching eggs from Vaccinated against AEV Broiler Breeders flock.

These findings are in accordance with those of Smart et al. (1986) who used ELISA to diagnose active infections with AEV by an increase in titer with sequential serum samples.

Although Flock 2 was located very close to Flock 1, neither clinical signs nor significant increase in ELISA titers were noted. AEV is shed in the feces for a period of several days, and because it is quite resistant to environmental conditions, it remains infectious for long periods of time and can be easily transmitted by ingestion (Calnek et al., 1960; Hoekstra, 1964). Shafren and Tannock (1988) found virus in feces from 4–10 days after exposure to a field strain of AEV. Infected litter is a source of virus easily transmitted horizontally by tracking or fomites. Infection spreads rapidly from bird to bird within a pen or house once introduced and from pen to pen on farms where no special precautions are taken to prevent spread (Calnek, 2008). Flock 1 and Flock 2 were hatched from eggs laid by non-vaccinated and vaccinated against AEV broiler breeders flocks respectively. With no maternal protection, Flock 1 was probably horizontally infected and exhibited clinical signs of infection, whereas Flock 2 was not influenced by AEV presence in the farm. Also, ELISA titers of Flock 2 was not increased compared to those of Flock 1. These findings were strongly correlated with those of Calnek et al. (1960) and Westbury and Sinkovic (1978) who reported that passively acquired antibodies can prevent development of disease and prevent or reduce the period of virus excretion in feces.

No satisfactory treatment is known for acute outbreaks in young chicks. Removal and segregation of affected chicks may be indicated under certain conditions, but they generally will not develop into profitable stock (Suarez, 2013). In domestic fowl, AEV is self-limiting and a simple humoral response provides lifelong protection. Therefore, vaccination is widely used to control AE in breeding flocks in order to prevent vertical transmission (Calnek, 1998). In this study, flock suffered a significant economic loss due to the outbreak of AEV, encouraging the owner to demand compensation and to consider new ways of avoiding the disease in the future.

## CONCLUDING REMARKS

In conclusion, AEV may cause severe health and production problems in broiler flocks. This study demonstrated AEV as the cause of an outbreak of neurological disease in Arta, Greece. It is most probable that the entry of AEV via a horizontally transmitted route caused the disease and deteriorated the production indicators. The findings of histopathology and serology for AEV could be considered as a simple laboratory analysis for a positive diagnosis of the disease.

## CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.



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