INVESTIGATION OF EXPERIMENTAL ENTEROCOCCUS 
FAECALIS AMYLOID ARTHROPATHY IN CHICKENS

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Received for publication May 23, 2007

Abstract

In this study, 25 five-week-old layer ISA brown chickens were used. Twenty animals were inoculated intravenously with 0.1 ml of Enterococcus faecalis EFAA-01 strain at 10⁹ CFU doses. Five chickens (control group) received 0.1 ml of 0.1% NaCl. After the treatment, the chickens were kept under observation for 10 weeks. Necropsy was performed on animals that died during the observation period and the rest of birds were sacrificed at the end of the experiment by cervical dislocation. Dullness, closing of the eyes, loss of wing feathers, decrease in feed and water consumption, cachexia, and decreased growth rate were all noticed. The most outstanding clinical finding was bilateral or unilateral swelling of the femoro-tibial and tibio-metatarsal joints, which resulted in lameness. Macroscopically, thickenings associated with unilateral or bilateral amyloid accumulation were observed in femoro-tibial, tibio-metatarsal, and hip joints. Microscopically, amyloid accumulation was noticed in the vessel walls and the surrounding matrix, and the matrix surrounding the chondrocytes in the femoro-tibial, tibio-metatarsal, and hip joints. At scanning electron microscope examination, amyloid substance in fibrillary form was observed.

Key words: layer chickens, amyloid arthropathy, E. faecalis, pathology.

As a result of extracellular accumulation in fibrillar form in various tissues and organs of precursor or autologous soluble proteins, due to the loss of parenchymal cell and micro-anatomic structure, the impairment occurring in the organs is called amyloidosis (10). The amyloid arthropathy, a special form of amyloidosis, is characterised by the accumulation of amyloid in joints. The arthropathy was widely described in chickens (12, 13, 16, 17), dogs (6), and mice (23). Amyloid accumulation was detected in humans with multiple myeloma (9) and chronic haemodialysis (1). The amyloid types were defined as AL type (9), ß2-microglobulin (Aß2M) (19), and transthyretin (ATTR) (2). No such amyloid types were observed in domestic and wild animals (14).

Enterococci in poultry have been isolated from the cases of endocarditis, meningitis, ovarian inflammation, fibrinous arthritis, and tendosynovitis (11) as well as of amyloid accumulation in the joints and amyloid arthropathy in chickens (12, 13). Bacteria (i.e., E. faecalis, S. aureus, E. coli, S. enteritidis, and M. synoviae) and viruses (i.e., reovirus and chicken anaemia virus) may cause amyloid arthropathy in chickens, especially in brown layer chickens (12, 13). In most cases, the arthropathy is caused by E. faecalis (12). This strain was also shown to cause systemic and usually articular accumulation in chickens (24). The most comprehensive studies related to amyloid arthropathy in chickens were carried out by Landman et al. (12, 13, 15, 16). Landman et al. (13) reported for the first time in 1994 the fact that E. faecalis caused spontaneous amyloid arthropathy in brown layer chickens. They observed that the arthropathy was characterised by growth retardation, lameness, and unilateral or bilateral swelling of tibio-metatarsal, femoro-tibial and hip joints at considerable level. On necropsy, orange-coloured periarticular deposits were observed in the affected joints. Weigert van Gieson and Congo-red staining revealed that these deposits were amyloid ones. In addition, amyloid accumulation was observed in the internal organs.

The molecular structure of amyloid fibrils has been first described by Cohen and Calkins (5) in 1959 by the use of a transmission electron microscope (TEM). The TEM was used also by Peperkamp et al. (20) who demonstrated extracellular accumulation of amyloid fibrils in chickens.

Joint lesions cause lameness and adverse affect on animal performance and productivity (13). Economic losses due to joint lesions in poultry reached about 80-100 million dollars (18). Similarly, amyloid arthropathy, especially in brown layer chickens, affected about 20-30 % of European chicken flocks (12). The relationship between arthritis and amyloid arthropathy of
enterococcal etiology, has gained importance in the recent years (4, 12, 13, 16, 20) around the world and in our country (8, 22, 26). We aimed to investigate, for the first time in Turkey, the ability of amyloidogenic and arthropathic strain of E. faecalis to cause amyloid arthropathy and arthritis, and to describe pathogenic findings observed under SEM.

Material and Methods

The study was conducted on 25 five-week-old ISA brown layer chickens. Out of these, five animals were separated as the control group at the end of a week acclimatisation period. The experimental group was inoculated intravenously (v. ulnaris) with EFAA-01 strain of E. faecalis isolated from an amyloid arthropathy case, at 0.1 ml 10^9 CFU dose. The control group received 0.1% NaCl applied by the route mentioned above. After the treatment, the chickens were kept under observation for 10 weeks.

The necropsy was performed on animals that died during the observation period and the rest of birds were sacrificed at the end of the experiment using the method of cervical dislocation. Tissue samples were taken from the joints. The samples were fixed in 10% formalin for 24 h. Then, the samples were decalcified in 36.8% formic acid and 6.8% sodium formate. Finally, the samples were post-fixed and embedded in paraffin.

For the SEM, tissue samples taken from joints were fixed in Karnovsky's fixative (2.5% gluteraldehyde and 2% paraformaldehyde, 0.1 M cacodylate buffer, pH 7.3) and post-fixed with 2% osmium tetroxide. The samples were washed with distilled water and dehydrated by passing through the graded acetone. Finally, tissue samples were dried in the critical point and coated with gold palladium and investigated by the SEM (3).

Results

The chickens showed clinical signs of dullness and weakness two days after inoculation of the agent. They had tendency to closing eyes, losing feather, staying close to each other, and their feed and water consumption dropped. A week later, difficulties in walking and standing up were observed (Fig. 1a). As a result of the drop in food and water consumption, the animals became very weak and cachexic. Later, the animals were unable to stand and lay down all the time, having feather contaminated with faeces. The most important clinical sign one week after the inoculation was bilateral swelling of femoro-tibial and tibio-metatarsal joints in 12 animals and unilateral in 2 animals (Fig. 1b). Two of the animals in this group were excluded from the study due to cannibalism. The birds that showed clinical signs during the 10-week observation were sacrificed by cervical dislocation and necropsied.

At necropsy examination, bilateral thickening and swellings in femoro-tibial and tibio-metatarsal joints were observed in 12 cases and the same unilateral changes were observed in 2 cases. Unilateral swelling and thickening in the hip joints were observed in 9 cases and bilateral in 2 cases. In 5 cases, the articulationes digitorum was affected. On the cut surface of these swollen joints, it was observed that joint cavities and synovial recesses were filled completely with orange-coloured substance (Fig. 2a), and inner ligaments of the joints became thickened due to this accumulation. It was noticed that the surface of condylus lateralis and condylus medialis of the femur and tibia were covered with an orange-coloured proteinaceous material, and that the capsule surrounding the joint was destroyed (Fig. 2b).

The outer ligaments of the joint, the tendon of musculus quadriceps femoris and distal ends were filled with the same substance. In addition, bilaterally in 9 of 11 cases and unilaterally in 2 cases, material accumulation was also seen in articularia sacroiliaca, cavum articulare and capsula articularis.

It was observed that cartilage surface of these affected joints was coated with amyloid substance, and the cartilage surfaces were eroded and these erosions became deeper in some birds. Synovial fluid was increased in volume and contained floccular orange-colour proteinaceous material (Fig. 2a).

Hypertrophia in synovial membrane villi of femoro-tibial, tibio-metatarsal, and hip joints and destruction in articular cartilage were observed rarely. Homogeneous, amorphous amyloid accumulations of eosinophilic colour were detected in the hypertrophic synovial villi, articular recesses, articular capsule, and adjacent tendon sheaths. The adjacent connective tissue to these joints was thickened due to the amyloid accumulations. Amyloid was present in the vessel walls and the surrounding matrix tissue and also in the matrix tissue that contained chondrocytes in the vicinity (Fig. 3). It was also detected that amyloid accumulated between ligaments and muscle cells in this region.

In synovial cavity and articular recesses, heterophil leukocytes, plasma cells, and free erythrocytes were observed. In addition to oedema, lymphocyte, histiocyte, plasma cell, and heterophil leukocyte infiltrations in both the synovial villi and synovial membranes were detected. Infiltration of inflammatory cells, and degenerative changes in muscle tissue and tendons were present.

Amyloid fibrils were observed on the superficial layer surrounding the articular cartilage (Fig. 4a, 5a). The damage of the articular cartilage (Fig. 4b), depending on amyloid accumulation and very dense clusters of E. faecalis (Fig. 5b) were seen. Moreover, polymorphonuclear cell infiltration was also detected in the synovial cavity.
Fig. 1  
a. The appearance of affected chickens, which are unable to stand with a tendency for closing eyes, and losing feathers.  
b. Unilateral swelling of tibio-metatarsal joints (arrows).

Fig. 2  
a. Deposites of orange coloured amyloid materials in the cavum articulare and capsula articularis within tibio-metatarsal joints.  
b. Amyloid deposits in the periaricular capsule (arrows).

Fig. 3.  
Amyloid deposits in the matrix surrounding chondrocytes (arrows). 100x.

Fig. 4  
a. Amyloid fibrils on the superficial layer surrounding the articular cartilage (arrow). 3000x  
b. The damage of the articular cartilage. 1200x.
Discussion

Amyloid arthropathy has been described with bilateral or unilateral swelling of tibio-metatarsal, femoro-tibial, and hip joints and arthritis and tendosynovitis in chickens (13, 15, 16). In another study, it was observed that the lameness occurred in ISA brown layer chickens one week after intravenous injection of 10^9 CFU/ml dose of amyloidogenic and arthropathic E. faecalis strain (16). Following intravenous injection of the agent, it was reported that lesions developed bilaterally in femoro-tibial and tibio-metatarsal joints (12, 16).

In various experimental studies on the role of E. faecalis strains in amyloid arthropathy, it was demonstrated that strain causes lameness (12, 13, 15, 16). Accordingly, in the current investigation, in 14 chickens inoculated with an amyloidogenic and arthropathic strain of E. faecalis, bilateral or unilateral swelling of the joints and a severe lameness were observed. The development of lameness and hence the difficulty of feed and water consumption, and growth retardation was previously observed in same animals (12, 16).

Landman et al. (16) reported that intravenous inoculation of an amyloidogenic, and arthropathic strain of E. faecalis at 10^9 CFU to brown layer chickens caused amyloid arthropathy and therefore severe growth retardation in these animals was observed. We also observed a drop in body weight in chickens inoculated with amyloidogenic and arthropathic strain of E. faecalis compared to the control group (from the day of agent inoculation until the end of the experiment). Bilateral or unilateral thickening in femoro-tibial, tibio-metatarsal, and hip joints were previously reported in those chickens inoculated with amyloidogenic and arthropathic strain of E. faecalis (12, 13, 15, 20).

In presented study, femoro-tibial and tibio-metatarsal lesions were observed bilaterally in 12 cases and unilaterally in 2 cases and in hip joints the same lesions were observed bilaterally in 2 cases and unilaterally in 9 cases. Articulationes digitorum, articulatio humeri, articulatio cubiti, and articulatio carpometacarpica et intercarpica were also described to be affected (12, 16). However, smaller joints are less affected (16, 20). In this study, only in 5 cases, articulationes digitorum were seen to have been affected.

According to other authors bone epiphysis was bigger than normal with increased synovial liquid that contained clear, floccular orange-colour, proteinaceous material, and the joint surface was eroded (13, 14, 20). Amyloid material was observed on the superficial layers of the articular cartilage, in periarticular capsule, and especially in the synovial recesses (12-14, 20).

It was also reported that hardening of joints, orange-coloured rough swellings in meniscus and decaying at the meniscus border were previously observed (13, 16). In addition, it was reported that amyloid accumulation was also observed in the tendon sheaths of tibio-metatarsal joints (16, 20). The macroscopical findings of this study were similar to those of the previously published articles.

Microscopically, amyloid deposition was detected in hypertrophic synovial villi of the femoro-tibial and tibio-metatarsal joints, arthropitic recesses, and the adjacent tendon sheaths, on the superficial layer surrounding articular cartilage, in the walls of blood vessels. Besides, in some areas, amyloid deposits in matrix belonging to cluster of chondrocytes were found. Amyloid accumulation was also described in the undetermined periarticular fibrous tissues, between the collagenous fibrils of cruciate ligaments, and in the meniscus (13, 15, 16, 20).

It was reported that inflammatory changes characterised by heterophil leukocyte containing exudate in the synovial cavity, infiltration of plasmocytes, mononuclear cells, and heterophils in the synovial villi were also observed at amyloid arthropathy (12, 13, 15, 16, 20). Similar findings were demonstrated in the present study as well.

References