Mycoplasma iowae Associated with Vertebral Osteochondrosis In Commercial Turkeys

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ABSTRACT

Turkey eggs of the same primary breed from two different multiplier breeder sources were incubated and hatched at the same time by a commercial meat turkey company. Female turkeys from each breeder source were raised together in two separate locations under different management systems. The general purpose of this trial was to compare the production performance of poults from two breeder sources in different facilities and management systems. Swollen hock joints (arthritis) and vertebral lesions occurred at very low incidences in turkeys from both multiplier breeder sources. Herein, we describe the association of *Mycoplasma iowae* (MI) with vertebral osteochondrosis and osteomyelitis. MI was identified by culture/immunofluorescence and MI-specific PCR from some of the vertebral lesions but not from swollen joints. This is the first report of MI associated with vertebral osteochondrosis in turkeys, which should now be considered in the differential diagnosis of this lesion.

INTRODUCTION

Husbandry practices, metabolic and infectious diseases, and rapid growth may all play a role in the development of skeletal diseases in poultry (1). Bone abnormalities occur in all ages of growing poultry with the incidence ranging from 0.5 to 25% (1). The estimated annual cost in 1994 due to skeletal problems in the US turkey industry was $32-40 million (2). Skeletal problems in poultry include: tibial dyschondroplasia, spondylolisthesis, chondrodystrophy, osteomyelitis and synovitis, MS infection, and others (2). MI infection in turkey breeders causes reduced hatchability (2-5%), embryo mortality (in later stages of incubation), and leg deformities (3, 4). Trampel (4) described 17-day-old turkey poults with leg weakness, dehydration, chondrodystrophy of the hock joints, clear fluid in hock joint spaces, valgus deformities and shortening of the tarsometatarsal bones, and curled toes associated with MI. Experimentally in chickens and turkeys, MI has also induced airsacculitis, tenosinovitis, and arthritis, rupture of digital flexor tendons, rotated tibia and cartilage erosion (3, 4).

CASE REPORT

Turkey flocks. A commercial turkey company obtained eggs of the same primary breed from two different multiplier breeder companies (A and B). Poults from each breeder company were separated according to sex at the hatchery and females of each were placed at production facilities in two locations. One production facility was a typical commercial farm (COM) that received 8,000 turkeys from each breeder source. The other production facility was the Teaching Animal Unit (TAU) poultry house at NCSU CVM which received 1,000 turkeys from each breeder source. Each TAU turkey that was found dead or culled was identified with a wing band number and necropsied. COM dead and culled turkeys were necropsied at intervals.

Bacteriology. Samples of hock joint exudate were taken at necropsy with sterile cotton tipped swabs, cultured on blood and MacConkey's agar and incubated aerobically at 37C for bacterial isolation and identification.

Mycoplasma culture and MI identification. At necropsy, swollen hock joints and vertebral lesions were incised with sterile blades. Joint fluids/exudates were sampled using sterile cotton tipped swabs and inoculated to Frey's mycoplasma broth with 15% swine serum (FMS) (5). Each inoculated broth contained 2 ml of FMS. One ml was used for mycoplasma culture and 1 ml was used for molecular identification of MI by PCR (6). Inoculated broths were incubated aerobically at 37°C with periodic transfer of aliquots to FMS agar. Mycoplasma colonies on agar were identified as MI by direct immunofluorescence (5) using fluorescein-conjugated rabbit antiserum provided by SH Kleven (University of Georgia, Athens, GA), and isolates in broth were identified as MI by PCR.

Histopathology. Samples of spinal column were taken at necropsy from affected turkeys and placed immediately into 10% neutral buffered formalin. Fixed tissues were trimmed, decalcified in 10% formic acid, processed by standard paraffin embedding procedures, sectioned, and stained with hematoxylin and eosin (H&E) for histopathologic examination.

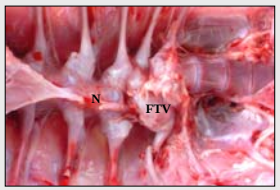

Fig. 1. Female turkey, 39-days-old, culled due to stunting and lameness. Caudal thoracic vertebrae are severely deformed because of lesions in the caudal notarium (**N**) and free thoracic vertebra (**FTV**). A swab sample of the intervertebral space was cultured for *Mycoplasma* spp. but not tested initially for *Mycoplasma iowae* (MI) by PCR. *Mycoplasma* sp. was isolated in culture from the intervertebral space within the lesion, and identified as MI by direct immunofluorescence and PCR.

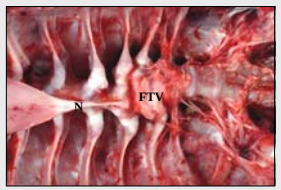

Fig. 2. Female turkey, 57-days-old, culled due to stunting. There is severe deformity of the thoracic vertebrae involving the caudal notarium (**N**) and free thoracic vertebra (**FTV**). A swab sample of the intervertebral space was initially negative for *Mycoplasma iowae* (MI) by PCR. However, *Mycoplasma* sp. was isolated in culture, and identified as MI by direct immunofluorescence and PCR.

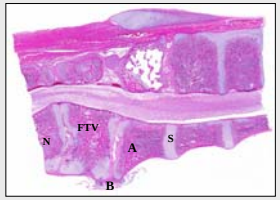

Fig. 3. Saggital section illustrates compression of the spinal cord caused by distortion of the ventral bodies of several thoracic vertebrae. Necrosis of the physeal cartilage (**A**) and dyschondroplasia (**B**) in the ventral aspect are illustrated. Higher magnifications of regions **A** and **B** are provided in **Figs. 4** and **5**.
FTV = Free thoracic vertebra, **N** = Notarium, **S** = Synsacrum

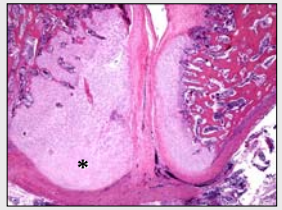

Fig. 4. Dyschondroplasia (region **B** in **Fig. 3**) is characterized by a plug of cartilage in the ventral aspect of a thoracic vertebra (*). Articular cartilage at the junction of T6 and T7 is necrotic and the ventral intervertebral ligament is expanded.

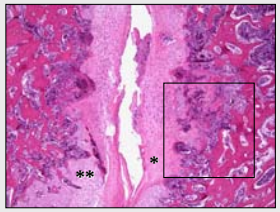

Fig. 5. Necrosis and degeneration of articular cartilage (region **A** in **Fig. 3**) is accompanied by osteomyelitis in the region enclosed by the box. The area adjacent to the * is illustrated at higher magnification in **Fig. 6**. The area adjacent to ** is illustrated at higher magnification in **Fig. 7**.

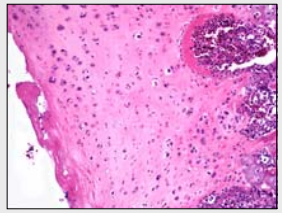

Fig. 6. Osteochondrosis is apparent as lesions including necrosis and degeneration of the articular cartilage and increased eosinophilia of the cartilage matrix.

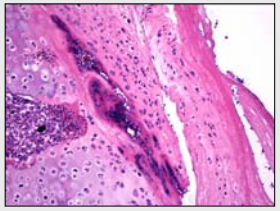

Fig. 7. Necrosis, an eosinophilic streak containing necrotic debris, and separation or tearing of the physeal cartilage are all features of osteochondrosis.

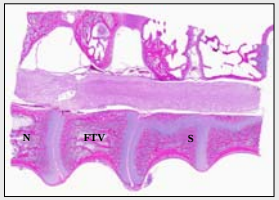

Fig. 8. Sagittal section illustrates persistence of cartilage as irregular bands traversing the length of several vertebrae. There is distortion of some vertebral bodies and increased thickness of the ventral intervertebral ligament due to osteochondrosis.
FTV = Free thoracic vertebra, **N** = Notarium, **S** = Synsacrum

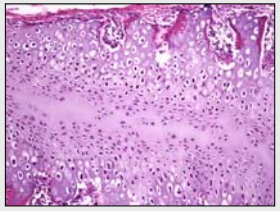

Fig. 9. Features of osteochondrosis include a persistent transverse band of cartilage having a wide zone of disorganized chondrocytes and a prominent area of acellular cartilage matrix.

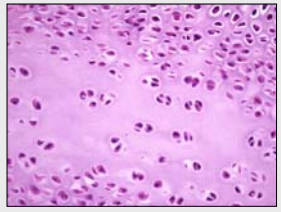

Fig. 10. Increased amounts of cartilage matrix, variations in size and shape of chondrocytes, and disorganization of chondrocytes are features of osteochondrosis.

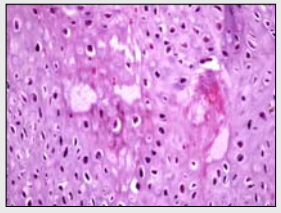

Fig. 11. Focal necrosis of cartilage characterized by shrunken chondrocytes, changes in staining patterns, and loss of cartilage matrix are lesions of osteochondrosis.

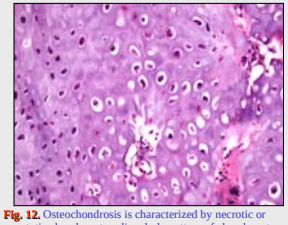

Fig. 12. Osteochondrosis is characterized by necrotic or apoptotic chondrocytes, disorderly pattern of chondrocytes, and eosinophilic streaks containing cellular debris. This pattern is thought to represent a progression in severity from the pattern illustrated in **Fig. 11**.

Results and Discussion. Eight turkeys (4 TAU and 4 COM) were identified at necropsy with swollen hocks or vertebral lesions (**Figs. 1** and **2**). Swollen hocks were noted at 12 and 15 days of age in three turkeys: 2 TAU-breeder source A, and 1 COM-breeder source B. MI was not identified by culture and/or PCR of joint exudates from any of these three turkeys. However, *E. coli* + *Enterobacter* sp., and *Staph*. sp. were isolated from 2 turkeys respectively. Therefore, bacterial infections were the likely causes of lameness, swollen joints, and arthritis that were observed in these young turkeys.

Vertebral lesions (**Figs. 1** and **2**) were noted in 5 turkeys necropsied between 39 and 65 days of age: 2 TAU-breeder source A, 2 COM-breeder source A, and 1 COM-breeder source B. MI was identified by culture and/or PCR from spinal lesion swabs of 3 turkeys (2 TAU-breeder source A, and 1 COM-breeder source B). ***This is a low number of positive results, but it suggests that a possible source of MI infections may be the primary breeder.*** In two of three MI positive cases, the initial PCR tests from spinal lesion swabs were negative, yet the organism was isolated in culture and identified by immunofluorescence and repeat PCR. In another case, initial PCR from the lesion was not done, but the organism was isolated in culture and follow-up PCR was positive. In one case the initial MI PCR was negative and the mycoplasma culture was contaminated, thus not allowing for isolation or follow-up PCR. Therefore the MI status of this case may be considered uncertain. And in the final vertebral lesion case, MI PCR and mycoplasma culture were both negative – so the organism was not identified in this case. Again this is a small data set, but the ***results show that mycoplasma culture of spinal lesion samples was more sensitive than MI PCR, and in these cases identification of MI associated with these lesions would have been missed if the only test used on lesion samples was PCR.***

Typical examples of vertebral lesions noted at necropsy are shown in **Figs. 1** and **2**. Histopathology and interpretations are shown in **Figs. 3** to **12**. ***The term osteodystrophy commonly is used to refer to this gross lesion because of the distortion in shape of the affected vertebrae. The histologic lesions illustrated are more consistent with osteochondrosis. Lesions of dyschondroplasia and osteomyelitis were also present. We believe that the term osteochondrosis should be used for this combination of lesions.***

CONCLUSIONS

- Low incidences of stunting, lameness, swollen hocks/arthritis, vertebral lesions occurred in turkeys of the same primary breed from two multiplier breeder companies.
- Swollen hocks/arthritis occurred in turkeys between 12 and 15 days of age, with no evidence of MI involvement.
- Vertebral lesions were noted in turkeys between 39 and 65 days-of-age, and MI was identified in turkeys from both multiplier breeder companies, suggesting that the source of MI infection may be the primary breeder.
- Mycoplasma culture of spinal lesion samples was a more sensitive diagnostic method than MI PCR, however PCR did confirm the identity of mycoplasmas isolated.
- The histologic lesions are consistent with osteochondrosis, with dyschondroplasia and osteomyelitis also present.
- MI should be considered in the differential diagnosis of turkeys with vertebral osteochondrosis.

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