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Mycoplasmosis Associated Perosis Type Skeletal Deformity in a Saker Falcon Nestling in Hungary

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ABSTRACT: A wild, 3-wk-old saker falcon (*Falco cherrug*) nestling showing uncoordinated movements and a perosis type tarsometatarsus deformity was found abandoned; it was euthanized a week later on 29 May 1997 after an unsuccessful attempt to rehabilitate it. Gross pathological findings included congestion of parenchymal organs and a lateral bowing of the left tarsometatarsal bone. Histopathology revealed initial interstitial hepatitis, focal catarrhal pneumonia, and dyschondroplasia in the epiphysis of the left tarsometatarsus. Mycoplasmas were isolated from the lungs, trachea, bone marrow and brain. A polymerase chain reaction (PCR) assay was performed for the detection of the mycoplasmal 16S rRNA gene. The resulting 262 base pair PCR product was sequenced and compared to the available mycoplasmal sequences but no identical corresponding sequences were found. However, 98% similarity was found to the *Mycoplasma buteonis* 16S rRNA and the isolate also was positive by immunoblotting against reference sera to the same species.

Key words: Case report, *Falco cherrug*, *Mycoplasma buteonis*, perosis, saker falcon.

Mycoplasmas have been isolated from birds of prey on several occasions (Furr et al., 1977; Bölske and Mörner, 1982; Poveda et al., 1990a, 1990b, 1994; Panangala et al., 1993) and some of these isolates were established as new species (Panangala et al., 1993; Poveda et al., 1994). A serological study conducted on a range of wild bird species detected seropositivity to *M. gallisepticum* in two species of birds of prey (Ziedler et al., 1995). The birds in these reports were all adults and pathological changes, if present, ranged from sinusitis to pneumonia and airsacculitis.

On 29 May 1997, a live 3-wk-old saker falcon (*Falco cherrug*) nestling was presented to the Wildlife Health department of the Central Veterinary Institute (Budapest, Hungary). Ornithologists of the Nature Conservation Authority (Budapest, Hungary) had found the bird a week earlier, under a nest located in an agricultural area 60 km east of Budapest in Heves County, Hungary (47°40′N, 19°40′E). Their efforts to hand raise the obviously ill nestling had failed due to its worsening neurological symptoms and dyskinesia. Therefore, a detailed diagnostic procedure was requested.

After the clinical examination and a short observation period the bird was humanely killed by cervical dislocation. Blood was collected, and necropsy was performed immediately after death. Tissue samples were taken from the lungs, liver, heart, brain and the affected proximal epiphysis of the left tarsometatarsal bone and were fixed in 10% formaldehyde solution. The bone sample was decalcified in 15% EDTA solution. Routine histologic sections were prepared and stained with hematoxylin-eosine. Samples were taken aseptically from the liver, lungs, bone marrow, brain and trachea for bacterial and mycoplasma culture. All samples were homogenized in approximately 10 times volume of physiological buffered saline solution. The above organ samples were cultivated on 5% sheep blood agar (Merck, Darmstadt, Germany) at 37 C under both aerobic and anaerobic conditions, as well as on a Tryptic Soy Agar (DIFCO Laboratories, Detroit, Michigan, USA) based Haemophilus medium at 37C in 10% v/v CO_2. For mycoplasma isolation, 0.2 ml aliquots were transferred from each suspension into 2 ml of modified Hayflick's broth (Whitford et al., 1994) and were also plated directly onto modified Hayflick's agar medium (Whitford et al., 1994) and then

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incubated at 37C, in 5% v/v CO_2. The isolated mycoplasma strain was clone purified three times using disposable 0.2 μm Minisart membrane filters (Sartorius Corp., Edgewood, New York, USA).

A polymerase chain reaction (PCR) was performed on the isolated mycoplasma strain. A general mycoplasma primer pair designed by Van Kuppeveld et al. (1992) was used for the detection of the 16S ribosomal (r)RNA gene. One μl of broth culture was used as a DNA template. The PCR conditions were as described earlier by Kiss et al. (1997). The amplified DNA product was cloned and sequenced. It was recovered from low melting temperature agarose (Sigma, St Louis, Missouri, USA) with a phenol-based method described by Sambrook et al. (1989). The purified fragment was blunt ligated into the EcoRV site of the pBluescript SK plasmid (Stratagene, La Jolla, California, USA). The double stranded plasmid DNA was sequenced by the dideoxynucleotide chain termination method from both directions with the ALFexpress AutoRead Sequencing Kit (Amersham Pharmacia Biotech, Uppsala, Sweden) using Universal and Reverse primers. The sequence was analysed by programs of the Lasergene software package (DNASTAR Inc., Madison, Wisconsin, USA). A homology search of GenBank and EMBL databases with the BLAST search program (Altschul et al., 1990) was performed. Partial regions corresponding to our sequence were excised from the available mycoplasmal 16S rRNA sequences and were used to generate a multiple alignment.

On clinical examination the bird was in relatively good body condition, weighed 920 g and showed appropriate feathering for its age. Normal behavior patterns such as sleeping, curiosity, fear and begging for food were observed with one exception: when excited, the bird flexed its neck to the sternum with sudden, uncoordinated jerky movements. The left leg was rotated laterally and lacked muscle tone; its digits were limp and ataxic, but deep pain sensation was not affected (Fig. 1). The lower left eyelid was covered with flakes of dry ocular discharge. At necropsy the liver was slightly enlarged and congested, and there were several petechial hemorrhages on its surface. Lungs were congested and contained several distinctive greyish foci. The proximal epiphysis of the left tarsometatarsal bone was bowed laterally at an angle of approximately 30° (Fig. 2). There were no discernible macroscopic lesions in the air sacs or joints. Histopathological changes comprised a marked cerebellar edema with karyopycnosis and lysis of some neurons in the molecular layer; initial interstitial infiltration of the liver by heterophils; and an acute, in some areas subacute, focal catarrhal pneumonia characterized by accumulation of heterophils in the parabronchi and of mononuclear cells in interstitial areas around the blood vessels. There were subtle changes in the epiphysis cartilage of the tarsometatarsus with dystrophy and lysis of chondrocytes confined to areas surrounding nutritional blood vessels (Fig. 3). Dyschondroplasia could be detected in distal areas.

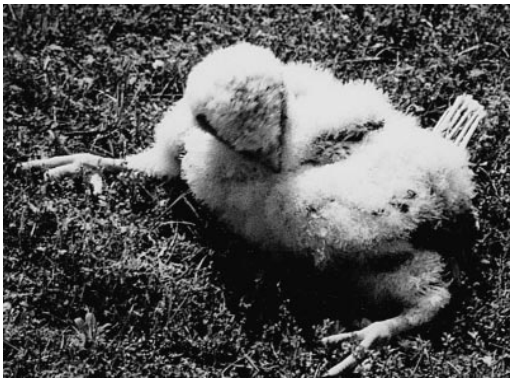


FIGURE 1. Paresis of the left leg and a perosis type tarsometatarsus deformity in a 3-wk-old saker falcon nestling.

There was no significant growth of bacteria on the media used. However, after 24 hr of incubation several typical large, "fried-egg" shape colonies could be observed on all Hayflick's medium agar plates. Biochemical studies (Whitford et al., 1994) showed that the strain ferment-

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FIGURE 2. Lateral bowing, valgus deformity (arrow) in the proximal epiphysis of the left tarsometatarsal bone of a saker falcon nestling.

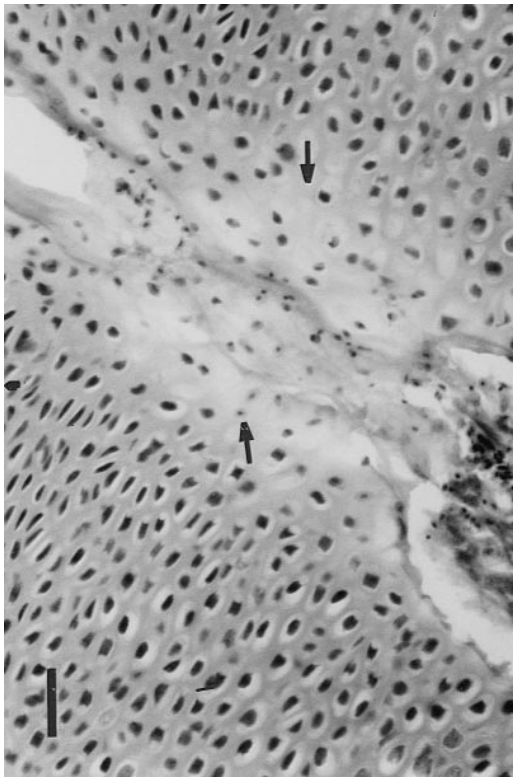


FIGURE 3. Dystrophy and lysis of chondrocytes (arrows) around blood vessels in the growth plate cartilage of the proximal epiphysis of the left tarsometatarsus of a saker falcon nestling. H&E stain. Bar = 40 μm.

ed glucose and that it was sensitive to digitonin (7–8 mm zone of inhibition). The strain did not show phosphatase activity, did not hydrolyse arginine or urea, neither did it reduce tetrazolium chloride. There was no film and spot production. The isolate proved positive by immunoblotting to reference sera against *M. buteonis* (performed by J. M. Bradbury, Department of Veterinary Pathology, Faculty of Veterinary Science, University of Liverpool, UK).

The PCR resulted in a 262 base pair (bp) DNA product (not shown). After sequencing, the homology search of the GenBank and EMBL databases identified no 100% similarity of the 262 bp sequence with any mycoplasmal 16S rRNA so far sequenced. Comparison with the corresponding partial regions showed the following similarities among the best matching mycoplasma species: *M. crocodyli* 97.0%, "*M. lacerti*" 96.3%, *M. edwardii* 96.3%, *M. leonicaptivi* 96.6%, *M. felis* 94.8%, *M. bovirhinis* 94.8%, *M. gallinaceum* 95.9%, *M. synoviae* 94.8%, and *M. sturni* 95.9%. Comparison with the unpublished sequence of *M. buteonis* 16S rRNA (K.-E. Johansson, pers. comm.) revealed a 98.0% similarity. It is important to note that these results are based on partial rRNA analysis and therefore of informative value only.

Perosis type skeletal deformities later associated with generalized *M. meleagridis* infection were first reported in turkey poults and named Turkey Syndrome-65 (TS-65) (Working Party, 1965). The role of

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M. meleagridis in TS-65 as a causative agent was proved experimentally (Wise et al., 1974). The syndrome was also reproduced by *M. gallisepticum* (Wannop et al., 1971; Wise and Fuller, 1975) and *M. iowae* (Bradbury and Ideris, 1982; Bradbury et al., 1988) infections in turkey poults and with an unidentified mycoplasma strain in pheasants (Wise and Fuller, 1976). Several attempts have been made to clarify the pathogenesis of the disease but none gave definite answers. According to most theories, transovarially transmitted mycoplasmas or very early generalized mycoplasma infections may deprive the young birds' metabolism of substances essential in bone development (Wise and Fuller, 1975; Bigland and Warenyicia, 1978; Yamamoto, 1997). A clinical perosis case attributed to nutritional deficiencies also was reported in a captive lanner falcon (*Falco biarmicus*) by Kummerfeld (1982).

This case highlights the possibility of vertical spread and potential pathogenicity of mycoplasmas in free living falcon populations. It is difficult to estimate the real pathogenicity of the isolated mycoplasma strain and to predict the chances for recovery of diseased nestlings. If breeding pairs remain infected for more than a year, the disease could significantly affect breeding success. Pathogenicity testing of the isolated mycoplasma strain and further observations of affected falcons are necessary to obtain at least partial answers to the above questions.

Although the DNA sequence analysis involved only a part of the 16S rRNA gene, its results along with the result of the immunoblot assay suggest that the isolated mycoplasma strain is *M. buteonis*. The final answer can only be given after obtaining the complete sequence of the 16S rRNA gene.

We are grateful to J. M. Bradbury for performing the immunoblot assay and to K.-E. Johansson for comparing our sequence data with the *M. buteonis* 16S rRNA sequence. This work was partly supported by the OTKA A312 grant of the National Research Fund of Hungary. We thank J. Bagyura for presenting us the case, V. Sztojkov, F. Rátz and R. Glávits for their helpful advice during the diagnostic work and reviewing the manuscript.

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