

***Mycoplasma*-associated Polyarthriti s in a Reticulated Giraffe**

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ABSTRACT: A case of *Mycoplasma*-associated polyarthriti s was diagnosed in a captive reticulated giraffe (*Giraffa camelopardalis reticulata*). Recurrent episodes of lameness with temporary response to antimicrobial therapy characterized the disease. After the fifth episode, the giraffe was immobilized for arthrocentesis of the right front fetlock joint. Although the culture was negative, *Mycoplasma* sp. nucleic acid was detected in synovial fluid using polymerase chain reaction (PCR). Twelve weeks after completion of enrofloxacin therapy evidence of *Mycoplasma* sp. was not detectable in the synovial fluid; no relapses occurred after 22 mo. This is the first report of *Mycoplasma*-associated polyarthriti s in a giraffe.

Key words: Giraffe, *Giraffa camelopardalis*, lameness, *Mycoplasma*, polyarthriti s, polymerase chain reaction.

Mycoplasma is the smallest self-replicating prokaryote, and it lacks a cell wall (Gillespie and Timoney, 1981). It is ubiquitous in nature and may cause disease in a variety of wildlife, including elephants (*Loxodonta africana* and *Elephas maximus*; Clark et al., 1980) and Dall's sheep (*Ovis dalli dalli*; Black et al., 1988). Its pathogenicity is well described in domestic ruminants in which it can cause respiratory disease, mastitis, conjunctivitis, and arthritis (East, 1996; Pfützner and Sachse, 1996). This report describes recurrent polyarthriti s associated with *Mycoplasma* sp. in a captive juvenile male reticulated giraffe (*Giraffa camelopardalis reticulata*).

A 16 mo old hand-reared male reticulated giraffe (estimated body weight 400 kg) became acutely lame with swelling of all hock and fetlock joints and reluctance to move in the spring of 2000. The giraffe had been abandoned by its dam at birth; domestic cow colostrum was given to the calf during the first 24 hr of life and it was raised successfully on cow and goat milk.

At the onset of disease the giraffe was housed at a captive breeding facility in Somervell County (Texas, USA; 32°14'11"N, 97°45'17"W) with four juvenile and six adult giraffes. They were rotated between a 30 m×30 m penned yard and a 130 ha enclosure that contained other non-domestic ungulates, including equids, several species of bovids and cervids, and free-ranging wildlife. In addition, this pasture shared 1,000 m of fenceline with a dairy farm of 120 Jersey cows, separated by a 3 m high wire fence. The giraffes' diet consisted of a pelleted feed (25 kg, Mazuri® ADF #16 Herbivore, PMI Nutrition International, Inc., Brentwood, Missouri, USA) and alfalfa and coastal hay (27–30 kg per day).

At the onset of lameness and lethargy, the giraffe was sedated for physical exam, phlebotomy, catheter placement, and treatment. On physical exam, it was febrile (40.4 C, giraffe normal=38.0–38.8 C; Fowler and Boever, 1986) and had swollen hock and fetlock joints. The giraffe was treated intravenously with 900 ml giraffe plasma once, ampicillin sodium (Amp-Equine®, SmithKline Beecham, Exton, Pennsylvania, USA) 22 mg/kg twice a day (b.i.d.) for 12 days, amikacin (Amiglyde V®, Fort Dodge Animal Health, Fort Dodge, Iowa, USA) 15 mg/kg once a day (s.i.d.) for 12 days, and flunixin meglumine (Banamine®, Schering-Plough Animal Health Corporation, Union, New Jersey, USA) 2.5 mg/kg once followed by 1 mg/kg s.i.d. for 4 days. There was neutropenia (neutrophils=551/μl, normal=6,528±3,319/μl), lymphocytosis (lymphocytes=4,882/μl, normal=2,919±1,331/μl), and monocytosis (monocytes=2,362/μl, normal

=354±312/μl) but the serum chemistry profile was within normal limits (International Species Information System, 2002).

The giraffe's lameness improved initially with treatment. However, a relapse of swollen fetlock joints occurred 2 wk after the first episode. This cycle of response to antibiotic therapy with concurrent relapse was repeated three more times. Radiographs of the front fetlock joints of the standing giraffe taken 4 wk after the onset of disease did not reveal any obvious abnormalities. Throughout the course of disease various serologic and hematologic tests for common domestic animal diseases were negative. Tests included bovine viral diarrhea serology using the modified complement fixation technique (Texas Veterinary Medical Diagnostic Laboratory [TVMDL], College Station, Texas; Angulo and Eugster, 1974), *Chlamydia* serology using the direct complement fixation technique (TVMDL; Palmer et al., 1969), and *Ehrlichia* polymerase chain reaction (PCR) on whole blood according to Breitschwerdt et al. (2002) at North Carolina State University (College of Veterinary Medicine, Raleigh, North Carolina, USA).

Various antibiotic regimens were tried, including intravenous ceftiofur sodium (Naxcel®, Pharmacia and Upjohn, Kalamazoo, Michigan, USA), oral trimethoprim sulfamethoxazole (Mutual Pharmaceutical Company, Inc, Philadelphia, Pennsylvania, USA), oral rifampicin (Hawkins Chemical, Inc, Minneapolis, Minnesota, USA), and oral tetracycline (Tetracycline Soluble Powder 324, Agripharm, Memphis, Tennessee, USA). These antibiotic therapies resulted in only temporary remission of clinical signs.

Five months after the initial episode of lameness, the giraffe relapsed with swollen fetlock joints for the fifth time. Using medetomidine (Domitor®, Pfizer Animal Health, Exton, Pennsylvania) and ketamine (Ketaset®, Fort Dodge Animal Health), the giraffe was immobilized (Bush et al., 2001) for arthrocentesis of the

right front fetlock joint. The synovial fluid had elevated white blood cell count (5,470/μl, normal=103.5±14.23/μl) which was predominately neutrophils (87%, normal=6.0±1.2 %) and high red blood cell count (8,000/μl, normal=0–few) compared to normal synovial fluid analysis in cattle (Van Pelt and Conner, 1963). Total protein (1.0 g/dl) was slightly below normal as compared to large animal synovial fluid (Blood and Radostits, 1989). Despite the inflammatory appearance of the synovial fluid, no bacteria were cultured using standard bacterial and *Mycoplasma* techniques (including *Mycoplasma* agar and broth techniques; Goll, 1994; Ruhnke and Rosendal, 1994).

Mycoplasma sp. was detected in the synovial fluid using genus-specific PCR (Harasawa et al., 1986). The DNA was extracted from joint fluid using proteinase K digestion, phenol/chloroform extraction, and ETOH precipitation. Conditions for PCR were standard, including a MgCl₂ final concentration of 1.5 mM and thermocycling temperatures of 94 C (30 sec), 57 C (30 sec), and 72 C (45 sec), for 35 cycles. A strong 600 bp amplicon was detected on a 2% agarose gel stained with ethidium bromide. Sequencing of the DNA was performed on purified PCR product (Core Sequencing Facility, College of Veterinary Medicine, Texas A&M University, College Station, Texas) but did not result in sequence data that could be matched to any previously identified *Mycoplasma* spp.

The giraffe was treated with oral enrofloxacin (Baytril® Taste Tabs™, Bayer Corporation, Agricultural Division, Animal Health, Shawnee Mission, Kansas, USA) 10 mg/kg s.i.d. for 14 days and the clinical signs resolved. Evidence of *Mycoplasma* sp. infection was not detected in synovial fluid by PCR 12 wk after enrofloxacin treatment. Twenty-two mo after completion of the enrofloxacin treatment, the giraffe remained normal.

Determination of the species of the *Mycoplasma* in this case was not possible us-

ing PCR; nonetheless, the clinical signs in this giraffe resembled those of mycoplasmosis in domestic animals. *Mycoplasma mycoides* ssp. *mycoides* in goats is often characterized by fever, multiple swollen joints, reluctance to move, and relapses after antimicrobial treatment (East, 1996). *Mycoplasma bovis* causes similar signs in young cattle (Pfützner and Sachse, 1996). Many cases of *Mycoplasma*-associated polyarthritis in domestic animals are also associated with respiratory disease (East, 1996; Henderson and Ball, 1999). Clinical signs of respiratory problems were not observed in this case.

This giraffe's joint fluid did not resemble the fibrinopurulent polyarthritis often observed in domestic goats with *Mycoplasma putrefaciens* infection (Rodríguez, 1994). However, it was not examined until almost 5 mo after onset of clinical signs and after numerous antibiotic treatments. Thus, the synovial fluid analyses may reflect chronic *Mycoplasma* arthritis.

Culture of joint fluid is considered the gold standard for identifying pathogens because it allows identification of species and antibiotic sensitivity. However, due to their fastidious nature *Mycoplasma* spp. can be difficult to culture (Clark, 1994) resulting in many false negative cultures (Simecka et al., 1992). Possible reasons for the inability to culture the *Mycoplasma* sp. in this case include insufficient numbers of organisms present in the joint fluid and previous antibiotic therapy.

Polymerase chain reaction is considered to be a more sensitive method of detecting low levels of *Mycoplasma* than culture (Simecka et al., 1992). It also is a more rapid test; it can usually be completed within 36 hr, whereas several weeks are often needed for *Mycoplasma* culture (Simecka et al., 1992; Ghaderoni et al., 1997).

Remission of clinical signs was only temporary until enrofloxacin therapy was initiated. Enrofloxacin is bactericidal and has good penetration of synovial fluid; fluoroquinolones have been shown to have ex-

cellent efficacy against various *Mycoplasma* spp. (Cooper, 1993; Plumb, 1999).

The route of infection was not identified in this case. Possible sources of infection include transmission via *Mycoplasma*-infected colostrum or milk, contact with a subclinical carrier (giraffe, non-domestic ungulate, or domestic cow), or environmental exposure through contaminated litter (soil, bedding, wild bird transmission) (Pfützner and Sachse, 1996). However, if the giraffe had been fed *Mycoplasma*-infected colostrum or milk as a neonate, clinical signs would have been expected earlier than seen in this case. Stress may influence manifestation of infection and was indicated as a predisposing factor to *Mycoplasma* infection in a herd of goats (Rosendal et al., 1979; East et al., 1983) and Dall's sheep (Black et al., 1988).

Transmission from conspecifics is another possible source of infection. However, all other giraffes housed with this animal have remained healthy. Although *Mycoplasma* spp. tend to be highly species-specific (Clark, 1994), non-domestic ungulate or domestic cow-giraffe transmission cannot be ruled out. One case report of *Mycoplasma ovipneumoniae* implicated asymptomatic domestic sheep as the source of an outbreak of *Mycoplasma pneumoniae* in captive Dall's sheep (Black et al., 1988). There have been no documented cases of mycoplasmosis in other ungulates in the enclosure shared with the affected giraffe. While transmission from domestic cows on the adjacent farm is possible, polyarthritis or *Mycoplasma* mastitis had not been observed on the farm in the past year.

Diseases of wildlife can be challenging to diagnose because of the dearth of information about disease processes and the appropriateness of adapting assays from domestic species. Polymerase chain reaction can be a useful diagnostic tool because of its rapidity and high sensitivity and specificity (Pfützner and Sachse, 1996). It will become a more useful tool as new molecular techniques such as ran-

dom amplified polymorphic DNA and arbitrarily primed-PCR, which allow typing of *Mycoplasma* spp. and improve isolation results, become more widely used (Rawadi, 1998). Documentation of *Mycoplasma*-associated polyarthritis in this reticulated giraffe raises interesting epidemiologic issues regarding disease transmission among animals in mixed-species exhibits at zoological facilities or where wildlife and domestic animals interact.

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