Autoimmune myasthenia gravis in a ferret

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Case Description—A 7-month-old neutered male ferret was evaluated for episodic pelvic limb weakness of 2 weeks’ duration.

Clinical Findings—Neurologic examination revealed flaccid tetraparesis with decreased spinal reflexes suggestive of a neuromuscular disease. Results of hematologic and CSF analyses, thoracic radiography, and abdominal ultrasonography were unremarkable. Electodiagnostic testing revealed subtle spontaneous activity localized to pelvic limb interosseous muscles, unremarkable motor nerve conduction velocities, and lower than typical compound muscle action potential (CMAP) amplitude for tibial nerve stimulation only. A severe decremental response of the CMAP was detected with repetitive nerve stimulation (45.5% at the third ulnar nerve). An esophagogram revealed mild megaesophagus. Intravenous neostigmine methylsulfate administration resulted in immediate resolution of muscle weakness. Cross-reacting anti-acetylcholine receptor (AChR) antibodies were detected in serum (0.35 nmol/L) by use of a canine- and feline-specific muscle extract. Clinical signs and ancillary test results were diagnostic of acquired myasthenia gravis.

Treatment and Outcome—Pyridostigmine bromide was administered (1 mg/kg [0.45 mg/lb], PO, q 8 h), resulting in complete remission of clinical signs. However, 1 month after the diagnosis, the ferret was euthanized because of recurrence of weakness despite anticholinesterase treatment.

Clinical Relevance—To the authors’ knowledge, this is the first report of acquired myasthenia gravis in a ferret and the first identification of anti-AChR antibodies in this species. Autoimmune myasthenia gravis should be considered in ferrets when weakness and flaccid paresis suggest a neuromuscular disease. Electodiagnostic testing, anticholinesterase challenge, and AChR antibody titer determination were helpful for diagnosis of this condition. (J Am Vet Med Assoc 2009;235:1462–1466)

A 7-month-old castrated male Fitch ferret weighing 1.2 kg (2.6 lb) was admitted to the Frégis Veterinary Hospital Center exotic animal service for evaluation of 3 episodes of pelvic limb weakness during the previous 2 weeks. The ferret’s appetite had not changed, it was vaccinated against distemper and rabies, and it lived in a home environment.

A physical examination performed when the ferret was first admitted revealed nothing remarkable except for a slightly high rectal temperature (39.4°C [102.9°F]). Blood glucose concentration was within reference limits (150 mg/dL; reference limits, 94 to 207 mg/dL). Thoracic and abdominal radiography revealed nothing unusual. At this point, the owners declined further investigation. Tollemalic acid (4 mg/kg [1.8 mg/lb], SC) was administered, and amoxicillin (20 mg/kg [9 mg/lb], PO, q 12 h) was prescribed.

The ferret was reevaluated 1 week later. During the previous week, it had numerous episodes of reluctance to move associated with pelvic limb weakness. The ferret’s appetite was slightly lower than typical, and its rectal temperature remained high (39.5°C [103.1°F]). During neurologic examination, paraparesis was evident. Postural reactions were delayed in the pelvic limbs only. Extensor muscle tone and withdrawal reflexes in the pelvic limbs were mildly decreased, whereas patellar reflexes were adequate. Results of a cranial nerve examination were unremarkable. Clinical signs were consistent with a lower motor neuron disease affecting the pelvic limbs. Differential diagnoses included nervous system disorders of inflammatory (meningomyelitis, radiculoneuropathies), or disseminated idiopathic myositis), degenerative (low lumbar or lumbosacral intervertebral disk disease), congenital (vertebral or spinal cord malformation), or neoplastic origin. Weakness of metabolic origin (hypoglycemia, hypokalemia, hypercalcemia, hypercalciemia, or anemia) and abdominal disease (tumor, lithiasis, peritonitis, or inflammatory bowel disease) were also considered because ferrets can easily lose the characteristic upward arch in their back secondary to non-neurologic disorders.

Venipuncture was performed while the ferret was briefly anesthetized via facial mask with isoflurane (3%...
to 4% in oxygen). Results of a CBC and serum biochemical analysis that included glucose measurement and of serum protein electrophoresis were within reference limits. Results of abdominal ultrasonography and spinal radiography were unremarkable. Despite lacking a definitive diagnosis, the owners declined additional diagnostic tests. Administration of prednisolone (0.5 mg/kg [0.23 mg/lb], PO, q 12 h) and omeprazole (1 mg/kg [0.45 mg/lb], PO, q 24 h) was empirically added to ongoing treatment with amoxicillin.

Two weeks later, the ferret was readmitted to the hospital because of nonambulatory tetraparesis of recent onset. When examined, the ferret was in lateral recumbency and could not support any weight on its limbs. Rectal temperature was within reference limits (37.7°C [99.8°F]). Results of neurologic examination suggested tetraparesis with limited voluntary movements in the thoracic limbs, absence of movement in the pelvic limbs, and ventral flexion of the head and neck. Postural reactions were absent in all 4 limbs. Extensor muscle tone and withdrawal reflexes were mildly decreased in all 4 limbs, whereas patellar reflexes were unremarkable. The palpebral reflex was adequate but weakened after repetitive stimulation. The lesion was neuroanatomically localized to the neuromuscular system. At this point, differential diagnoses were refined to include a disorder of neuromuscular transmission (ie, myasthenia gravis) and a neuropathy or myopathy of inflammatory, degenerative, metabolic, toxic, or paraneoplastic origin.

Additional serum biochemical and hematologic analyses were performed, including measurement in serum of ionic balance status, CK activity, and calcium and phosphorus concentrations. Subtle hyperkalemia (6 mEq/L; reference limits, 4.3 to 5.3 mEq/L) was detected but was believed to be secondary to hemolysis of the blood sample. All other values were within reference limits. The ferret was hospitalized and received nursing care, IV administration of fluids (0.9% NaCl and 5% glucose solutions; 3 mL/kg/h [1.4 mL/lb/h]), and prednisolone (0.5 mg/kg, PO, q 12 h). The following day, the ferret was anesthetized with isoflurane and cisternal CSF was obtained with a 22-gauge spinal needle. Results of the CSF cell count were unremarkable. Results of PCR assays of the CSF sample to detect Aleutian disease and distemper viruses were negative.

On day 3 of hospitalization, electrodiagnostic testing was performed while the ferret was anesthetized with isoflurane. Rectal temperature was maintained at > 37.0°C (100.5°F). Electromyographic evaluation of the head, paravertebral, and limb muscles was performed with a concentric needle electrode. For electromyography, stimulation of the right bicipital and left ulnar nerves was performed by use of monopolar needle electrodes, and recording was performed with surface cutaneous electrodes (alligator clips). Electromyography revealed sparse spontaneous activity (librillation potentials) only in the interosseous muscles of the pelvic limbs. Compound muscle action potential amplitudes resulting from distal and proximal stimulations of the tibial nerve were slightly lower than typical for this nerve (3.4 and 4.5 mV for the distal and proximal CMAP amplitudes, respectively; typical mean values, 7.1 ± 3.1 mV and 7.6 ± 3.3 mV, respectively) without temporal dispersion. Motor nerve conduction velocities were also unremarkable for the tibial and ulnar nerves (46.9 and 48.9 m/s, respectively; typical mean values, 53.0 ± 8.8 m/s and 46.7 ± 11.5 m/s, respectively). Supramaximal repetitive nerve stimulation at a frequency of 3 Hz induced a severe decremental response (41.2% for right and 44.9% for left tibial nerves and 45.5% for the ulnar nerve at third stimulation; typical value, < 1%). These findings were suggestive of a disorder of neuromuscular transmission. Despite the mild decrease of tibial nerve CMAP amplitude, existence of a neuropathy (axonopathy) or myopathy was judged unlikely because of absence of generalized electromyographic abnormalities and unremarkable electroneuromyographic findings for the thoracic limbs.

After the ferret recovered from anesthesia, iohexol was administered PO and thoracic radiographs were obtained. Radiography revealed that the esophagus was mildly enlarged, but the mediastinal appearance radiographically normal. Neostigmine methylsulfate (0.04 mg/kg [0.02 mg/lb], IV) was then slowly injected. The clinical status of the ferret improved dramatically during the injection, and the ferret was clinically normal during the subsequent 5 hours. Serologic testing for anti-AChR antibodies at the Comparative Neuromuscular Laboratory of the University of California-San Diego yielded a positive result (0.35 nmol/L, compared with < 0.06 nmol/L in 4 clinically normal ferrets and 1 with an acquired megasophagus) when a canine- andeline-specific muscle extract was used as antigen. These abnormalities were diagnostic of acquired myasthenia gravis.

The ferret was discharged on day 5 of hospitalization. Pyridostigmine bromide was prescribed (1 mg/kg, PO, q 12 h), and corticosteroid treatment was tapered gradually over the following week. The ferret had signs of diarrhea during the first days after discharge, which resolved by addition of diosmectite powder to its food. When the ferret was reevaluated 1 week later, results of neurologic examination were unremarkable, but the beneficial effect of treatment with pyridostigmine bromide was of short duration as reported by the owners. Frequency of drug administration at the same dosage was increased to 3 times daily and resulted in a complete clinical remission. However, 1 month after diagnosis, the ferret was euthanized by the referring veterinarian because of recurrence of clinical signs while still receiving the drug. Postmortem examination at the Frigis Veterinary Hospital Center was not permitted by the owners.

**Discussion**

Myasthenia gravis is a disorder of neuromuscular transmission resulting from an autoimmune attack against postsynaptic nicotinic AChRs (acquired myasthenia gravis) or from genetic structural or functional abnormalities of AChRs (congenital myasthenia gravis). Congenital myasthenia gravis is rare and has been reported only for a few domestic breeds of dogs, cats, and cattle. Congenital myasthenia gravis has an auto-
Clinical signs in the ferret of this report included intermittent pelvic limb weakness that progressed to a nonambulatory tetraparesis. As is evident in other species, intermittent or episodic clinical signs can appear early in the course of myasthenia gravis but can progress to continuous generalized weakness. Regurgitation and dysphagia were not evident in the ferret. Two forms of acquired myasthenia gravis are recognized in dogs and cats. In the local form, regurgitation (esophageal weakness), dysphagia (pharyngeal weakness), or cranial nerve abnormalities (facial muscle weakness) are the main clinical signs, and limb muscle weakness is not apparent. In the generalized form, limb weakness with or without involvement of esophageal, pharyngeal, or facial muscles is typical. An acute fulminating form of myasthenia gravis in dogs and humans has also been reported and is characterized by acute, rapidly progressive respiratory failure, megaesophagus, and tetraparesis to tetraplegia.

A concurrent disease affecting the neuromuscular system, particularly a neuropathy, also remains a possibility in the ferret of this report. The use of corticosteroids prior to worsening of clinical signs may also have contributed to progression of muscle weakness. One study revealed only 13% of dogs with generalized myasthenia gravis did not have clinical signs of pharyngeal weakness, or cranial nerve abnormalities (facial muscle weakness) and limb muscle weakness is not apparent. In the generalized form, limb weakness with or without involvement of esophageal, pharyngeal, or facial muscles is typical. An acute fulminating form of myasthenia gravis in dogs and humans has also been reported and is characterized by acute, rapidly progressive respiratory failure, megaesophagus, and tetraparesis to tetraplegia.

The clinical signs in the ferret were compatible with a generalized form of myasthenia gravis with subclinical megaesophagus and mild facial weakness. Whereas the rapid progression into severe paralysis with recumbency was consistent with an acute fulminating form, respiratory failure and regurgitation were not obvious. Mildly depressed flexor reflexes and muscle tone were also unusual findings that are rarely associated with myasthenia gravis.

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The reference criterion (gold standard) for diagnosis of immune-mediated myasthenia gravis in animals is detection of serum antibodies against AChRs in muscle by immunoprecipitation radiomunoassay. This test is highly specific and sensitive for autoimmune myasthenia gravis. In generalized forms of the disease, false-negative results occur in approximately 2% of dogs. The assay is fairly species specific, but in the ferret, cross-reacting anti-AChR antibodies (0.35 nmol/L) were detected by use of a canine and feline muscle extract. Although reference limits for this assay have not yet been established for ferrets, in the past in our laboratory, serum anti-AChR antibody titers from 5 ferrets were all < 0.06 nmol/L. The serum antibody titer in the ferret reported here could have been reduced by the prednisolone administered before testing was performed.

Detection of a high titer of cross-reacting antibodies against AChR was considered consistent with acquired myasthenia gravis. Additional research is needed to establish reference limits for the anti-AChR antibody assay in ferrets. Use of a ferret-specific antigen to perform the immunoprecipitation should increase assay sensitivity and provide a more accurate titer to establish the diagnosis.

A decrement of the CMAP amplitude in response to repetitive nerve stimulation was also highly suggestive of myasthenia gravis in the ferret of this report. A decrement > 40% was noticed at the third stimulation when a 3-Hz frequency was used. In our experience, such a decremental response does not occur in healthy ferrets when the same protocol is used. In a study involving 10 healthy ferrets, a decremental response, when present, was always ≥ 8% with a 2-Hz frequency. In dogs, a 3-Hz repetitive stimulation should not produce a decrement > 10%, and a higher value is suggestive of myasthenia gravis. However, the repetitive stimulation test is neither sensitive nor specific. For example, in other animal species, polymyositis can yield a decremental response and botulism can result in a decremental response at slow repetitive stimulations and an incremental response at rapid ones. Nevertheless, a decrease in the amplitude of the CMAPs is evident during electromyography recording in animals with severe polymyositis and botulism.

In the ferret reported here, only a slight decrease in the amplitude of CMAPs was evident with tiibial nerve stimulation. This finding, as well as fibrillation potentials recorded with electromyography, is not a classic feature of myasthenia gravis. In this ferret, fibrillation potentials were sparse and limited to the interosseous muscles of the pelvic limb. Fibrillation potentials, focal decreases in amplitude of CMAP, and neurogenic muscle atrophy have been reported for humans with myasthenia gravis.

Such changes are believed to be secondary to functional interruption of the neuromuscular junction, which may have happened in this ferret. A concurrent neuropathy (axonopathy) was considered less likely because an axonopathy would not have resulted in an extreme decremental response and should have been associated with severe and generalized spontaneous activity on electromyography secondary to denervation. Our laboratory’s reference limits for motor nerve conduction velocities and CMAP amplitudes in ferrets were slightly less than those reported elsewhere. However, such values, most notably the CMAP amplitudes, should be compared cautiously because of different recording methods (monopolar needle electrodes inserted IM vs cutaneous electrodes in our clinic) and numbers of healthy ferrets in the control groups.
The positive result for the neostigmine methyl sulfate challenge was also consistent with a diagnosis of myasthenia gravis. The dosage of long-lasting anticholinesterase drug was used in accordance with recommendations for dogs and cats (0.02 mg/kg [0.01 mg/lb], IV or 0.04 mg/kg [0.02 mg/lb], IM). However, the dose administered IV in our ferret was that recommended for IM administration. Injection of neostigmine methylsulfate resulted in an immediate and complete remission of clinical signs that lasted 5 hours. Although this result is not specific for myasthenia gravis and improvement in muscle strength can be encountered with many myopathic and neuropathic disorders, a dramaticic response with complete resolution of clinical signs appeared consistent with a diagnosis of myasthenia gravis. It is unknown whether a lower dose may have been effective, but the dose used did not yield observable adverse cholinergic effects.

Given the adult age of onset, unequivocal response to neostigmine challenge, decremental response of the CMAP amplitude to repetitive nerve stimulation, and positive results for anti-AChR antibody testing, a diagnosis of acquired myasthenia gravis was made. Other neuromuscular diseases were considered unlikely because of the discrepancy between subtle focal electromyographic changes, the severity of clinical signs, and the initial complete clinical remission. The unremarkable serum CK activity made a myopathy a less likely diagnosis, but serum CK activity is not always high in muscle disease.

Disseminated idiopathic myositis (or myofasciitis) is an emerging disease in ferrets characterized by pyrexia and marked leucocytosis with neutrophilia. Another particular feature of this type of myositis is that serum CK activity is not high, even though muscle damage is believed to be severe. Muscle atrophy in affected ferrets is prominent and was not evident in this ferret. Pyrexia was detected, but results of a CBC were unremarkable. Extensor rigidity-hyperreflexia syndrome was also not consistent with the flaccid paresis we detected. Spontaneous neuropathies are, to the authors’ knowledge, poorly described for ferrets. In this ferret, the absence of muscle atrophy was not consistent with a neuropathy. Histologic examination of muscle and nerve biopsy specimens would have been useful to rule out a concurrent neuropathy or a myopathy. Botulism was not included among the differential diagnoses because of the slow progression and waning of clinical signs, lack of some of the classic clinical signs of botulism (dysphagia, salivation, and respiratory distress before death), and the fact that exposure to the toxin was unlikely considering the ferret’s environment and commercial diet given. Therefore, specific tests for botulism toxin identification were not performed.

Several autoimmune disorders can occur concomitantly with acquired myasthenia gravis. In dogs, thymocytopenia, hemolytic anemia, inflammatory bowel disease, inflammatory myopathies (polymyositis and masticatory muscle myositis), dysautonomia, hypothyroidism, and hypoadrenocorticism have been recognized. In this ferret, hematologic abnormalities were ruled out. The ferret did not have clinical signs of gastrointestinal disease. To the authors’ knowledge, dysautonomia and hypothyroidism in ferrets have not been reported. Although hypoadrenocorticism in ferrets has not yet been described, it could have been considered given the weakness and hyperkalemia in the ferret. However, serum sodium concentration was within the reference range, and hyperkalemia could have resulted from hemolysis. Few other immune-mediated disorders have been reported for ferrets. A pemphigus-like syndrome has been described, possibly related to vaccination or drug treatment. In the ferret reported here, a history of drug exposure prior to the initial onset of clinical signs was not identified.

Acquired myasthenia gravis can also manifest as a paraneoplastic syndrome. The most common associated type of neoplasia is thymoma because of the proximity of AChR antigen to myoid cells within the thymus. Approxi matively 3% of dogs with myasthenia gravis have a cranial mediastinal mass, compared with 26% of cats with myasthenia gravis. In dogs and cats, other forms of neoplasia have rarely been associated with myasthenia gravis (eg, anal sac adenocarcinoma, cholangiocellular carcinoma, osteogenic sarcoma, and cutaneous lymphoma). Thymoma has been reported, but clinical signs in the affected ferrets were those of a respiratory distress. No tumors were detected in this ferret.

Infectious diseases that cause neurologic signs were also considered because of the initial pyrexia in this ferret. For this reason, CSF was obtained before specific tests for neuromuscular dysfunction were performed. Meningitis was ruled out by the unremarkable results of a CSF cell count. However, prednisolone administration could have influenced the result of this test. Protein concentration was not measured in CSF because of the small amount of fluid available. Evidence of Aleutian disease or distemper was considered; however, results of PCR assays for the agents that cause these diseases were negative. Toxoplasmosis has been detected in black-footed ferrets and could have been a cause of pelvic limb weakness; however, this disease is rare in ferrets and is commonly associated with other clinical signs (eg, anorexia, depression, and signs of CNS involvement) and biological abnormalities (eg, anemia and hyperglobulinemia) that were not detected in this ferret. Cryptococcosis can also cause meningitis in ferrets but was deemed unlikely given results of the CSF analysis and initial resolution of some clinical signs without treatment for this condition. Serologic testing for toxoplasmosis and cryptococcosis was not performed. Because a concurrent disease condition was not identified, the trigger for initiation of the disorder remained unknown in this ferret.

The dosage of anticholinesterase drug used in the ferret of this report was extrapolated from data in other species. Pyridostigmine bromide, a long-acting anticholinesterase drug, was used and dosage was based on the lower dose recommended for dogs (1 mg/kg, PO, q 12 h). Although corticosteroid administration in dogs with myasthenia gravis is controversial, it may be used when a satisfactory response is not achieved with an anticholinesterase drug and supportive care. Myasthenia gravis may spontaneously resolve in dogs, but whether the same is true for other species is unknown. In this ferret, response to pyridostigmine bromide alone...
was judged satisfactory when the ferret was reevaluated 1 week after the initial evaluation. Moreover, a worsening of weakness was observed during the initial immunosuppressive treatment prior to diagnosis. It is known that in myasthenic dogs and humans, prednisone administered at immunosuppressive dosages may result in an increase in muscle weakness during the first few weeks of treatment, which may have happened with the ferret. Therefore, we chose to treat the ferret long-term with pyridostigmine bromide only. In carnivores, adverse effects of this type of treatment include gastrointestinal signs. In this ferret, diarrhea developed only during the first week after diagnosis and resolved with treatment for clinical signs, without interruption of the anticholinesterase drug. Because the owner elected to euthanize the ferret without further adjustment of the anticholinesterase drug, it is known that in myasthenic dogs and humans, prednisone administration prior to diagnosis. It is known that in myasthenic dogs and humans, prednisone administration prior to diagnosis.

References