An inherited lower motor neuron disease of pigs: clinical signs in two litters and pathology of an affected pig

Donal O’Toole, James Ingram, Val Welch, Katie Bardsley, Tom Haven, Carol Nunamaker, Gerald Wells

Abstract. A chronic progressive neurodegeneration, called hereditary porcine neuronal system degeneration (HPNSD), was recognized in a swine herd in Devon, England. Adult pigs that were presumed carriers of the dominantly inherited trait for HPNSD were transferred from England, where a breeding colony was maintained for 9 years, to the Wyoming State Veterinary Laboratory (WSVL) for study. Two litters of affected piglets were born to 2 carrier sows at the WSVL. Clinical signs of muscular tremors, paresis, or ataxia developed at 12-59 days of age in 4 of 6 liveborn pigs. Three other pigs were stillborn. In the 4 affected liveborn pigs, clinical signs progressed and included symmetrical (3 pigs) or asymmetrical (1 pig) posterior paresis, bilateral knuckling of metatarsal-phalangeal or carpal joints, poor exercise tolerance, and in 1 pig, marked hind limb hypermetria. A 34-kg gilt exhibiting clinical signs of muscular tremors and posterior paresis and clinical signs for 22 days was euthanized and examined postmortem at 83 days of age. Apart from decubitus ulcers, gross lesions were absent. Microscopically, perikaryal vacuolation and osmiophilic lipid droplets were observed in atrophic alpha motor neurons in the spinal cord. There was axonal (Wallerian) degeneration in sulcomarginal and dorsal spinocerebellar tracts. Axonal degeneration also involved ventral but not dorsal spinal nerve roots, and was present in eight peripheral nerves sampled for histopathology. Changes in skeletal muscles were consistent with denervation atrophy and were most pronounced in M. tibialis cranialis of the 6 muscles sampled. Immunohistochemical staining of spinal cord for phosphorylated and nonphosphorylated neurofilaments did not reveal abnormal patterns, unlike some well-characterized inherited motor neuron diseases in other species.

Motor neuron diseases in pigs are rare. Reports of porcine lower motor neuron disease have described neurofilamentous accumulations in perikarya and proximal axonal internodes similar to those reported in similar disorders of other species including humans. In 1983, a distinctive paretic disease of young pigs (2-20 weeks postnatal life) was recognized on a farm in Devon, England. Pigs were transferred to the Central Veterinary Laboratory (CVL) of the Ministry of Agriculture, Fisheries and Foods, where a breeding colony was established. Over the subsequent 9 years, data were accumulated on the clinical signs, pathology, and inheritance of the disorder (Wells G, O’Toole D, Wijeratne WVS, unpublished data). Ultrastructural studies of perfusion-fixed affected pigs revealed abnormal mitochondria and lipid droplets in the perikarya of some alpha motor neurons in the ventral horn of spinal cords. With the exception of 2 brief reports, there is no published account of this disease, which we have called hereditary porcine neuronal system degeneration (HPNSD).

The purpose of this report is to describe the clinical features in 2 HPNSD-affected litters and to document the presence of significant lesions in alpha motor neurons, peripheral nerves, and skeletal muscles. We also report failure to isolate viruses or to visualize scrapie-associated fibrils (SAFs), as seen in some chronic neurodegenerations, in tissues from 1 pig with typical clinical signs.

Materials and methods

Herd history and clinical assessment. A 4.5-yr-old boar (no. PB122), a 2.5-yr-old sow (no. PD104) and an 8-yr-old sow (no. PX561) were shipped from the HPNSD colony at the CVL in Addlestone, England, to the Wyoming State Veterinary Laboratory (WSVL) in Laramie, Wyoming, in January 1992. While in England, the 3 crossbred pigs (Large White x Landrace) consistently produced litters with clinical signs of HPNSD and were therefore presumed carriers of the HPNSD trait. Both sows had mild clinical signs suggestive of neuromuscular disease. The young sow had a poor response to the sway test in both pelvic limbs. The older sow had difficulty rising from a sitting or recumbent position and
negotiated low obstacles, such as steps in her path, with difficulty. After quarantine for 30 days at an animal import center, the pigs were kept in isolation from other animals, including other pigs, at the WSVL. The sows were mated to the boar. Following mating, routine husbandry and vaccination practices for commercial swine were followed. Two weeks prior to farrowing, the diet of both sows was changed to a proprietary ration for pregnant and lactating pigs. After 1 day after farrowing, without having shown any clinical signs of serious disease. Her 3 piglets were raised by hand using a proprietary milk replacer ration for pigs. No gross or histologic lesions were found in the 3 stillborn pigs, and examination included the brain, spinal cord, and skeletal muscles. The dead sow had markedly enlarged thyroid glands (54 g) that had histologic changes of thyroidal hyperplasia. Microscopic examination of the brain and spinal cord did not reveal abnormalities, but autolysis comprised evaluation. Liveborn pig nos. 1-6 were examined regularly for evidence of neuromyopathy, and their movements were recorded on videotape. Still images were obtained from videotape using a frame grabber with an interactive computer program.\(^5\) Approximately 3 days after birth, blood and cerebrospinal fluid were collected for HE-stained sections. Tissues from pig no. 3 were collected for HE-stained sections.

**Pathology.** At 83 days of age, pig no. 3. Immediately after death, blood and cerebrospinal fluid were also collected from the control pig for thyroxine and lactate-pyruvate levels, respectively.

For resin-embedded sections, samples of skeletal muscles (as above), 8 peripheral nerves (lateral thoracic, phrenic, vagus, radial, median, ulnar, sciatic, and fibular) and ventral horn of the spinal cord at spinal segments C7 and L2-L4 were fixed in formalin and processed identically to those of pig no. 3. Immediately after death, blood and cerebrospinal fluid were also collected from the control pig for thyroxine and lactate-pyruvate levels, respectively.

**Immunocytochemistry.** Levels of cervical, thoracic, and lumbar spinal cord were fixed for 1 day in neutral phosphate-buffered 10% formalin, dehydrated, embedded in paraffin wax, cut at 5 µm, and stained with dilutions of 4 commercially available murine monoclonal antibodies (MAbs). MAb SMI 31\(^{14}\) reacts with a phosphorylated epitope in extensively phosphorylated neurofilament H and, to a lesser extent, neurofilament M in most mammalian species. MAb SMI 32\(^{14}\),\(^{19}\) reacts with a nonphosphorylated epitope of neurofilament H in most mammalian species. MAb 1273 detects a 65-kD protein in mitochondria and is an immunocytochemical marker for mitochondria in all human cell types. MAb 031\(^{14}\) reacts with double-stranded DNA and is an immunocytochemical marker for both mitochondrial and nuclear DNA. Antibodies were diluted in phosphate-buffered saline (PBS) as follows: 1:400 (SMI 31), 1:200 (SMI 32) 1:100 (1273) and 1:100 (03 1). For antigen retrieval, sections were either pretreated with 0.05% protease XIV\(^{14}\) for 15 min (for MAbs 031 and 1273) or with heat for 10 min using a microwave oven (for MAbs SMI 31 and SMI 32). Staining was detected using an avidin-biotin complex kit (ABC)\(^{14}\) according to the man-
manufacturer’s directions. For negative controls, PBS or an irrelevant MAb was substituted for specific MAbs.

**Scrapie-associated fibrils.** For electron microscopy, 0.5 g of frozen (-70 °C) spinal cord and caudal medulla oblongata from pig no. 3 and the clinically normal pig and caudal thalamus and medulla oblongata from an elk with chronic wasting disease were processed for detection of SAFs and negatively stained. Grids were examined for 20-30 min each, using a transmission electron microscope at 60 kV.

**Virus isolation.** Small (1 g) pieces of spinal cord from pig no. 3 and the clinically normal pig were minced with scissors, mixed with 9 ml sucrose phosphate glutamate buffer, ground using a Tenbröck homogenizer, and centrifuged (2,400 x g for 15 min at 5 °C [x 3]), and aliquots were frozen at -70 °C until attempted virus isolation. Two cell lines, murine neuroblastoma Neuro-2A (CCL-131) and swine testicle (CRL-1746), were cultured as recommended by the supplier and inoculated with 1 ml of tissue suspension. Each flask was incubated for 1 hr at 37 °C and rinsed once, and maintenance medium was added. Uninfected control flasks were processed similarly using 1 ml of medium as inoculum. Flasks were observed daily for 7 days. After 7 days of incubation, each flask was frozen at -70 °C then thawed at 37 °C for 3 cycles to release any cell-bound virus. The supernatant was then used as inoculum for a second blind passage. A third passage was performed in 75-cm² tissue culture flasks, and the supernatant was processed for electron microscopy as previously described.

### Results

**Clinical findings.** The progression of clinical signs in pig no. 3 was typical of the disease in affected pigs. From birth to 58 days, she developed normally and had a normal gait. At 59 days, tremors developed bilaterally in the muscles of the thighs. At 66 days, exercise tolerance diminished markedly, and brief exertion resulted in hyperventilation, inability to move, and a dog-sitting posture. Muscular weakness of the pelvic limbs was pronounced when the pig turned tightly and was accompanied by awkward crossing of the pelvic limbs. Intermittent bilateral knuckling of the metatarsal-phalangeal joints developed at 77 days when the pig trotted or ran. At 80 days, this knuckling was constant (Fig. 2b), and after 20-30 paces the pig collapsed on her left pelvic limb. At 82 days, she was nearly paraplegic and dragged herself forward using only her forelegs. Cutaneous pressure sores developed over the pastern joints, and she was euthanized at 83 days of age.

In the 3 remaining liveborn pigs, clinical signs developed at 59 (no. 1), 41 (no. 2), and 12 (no. 5) days of age. Early signs were bilateral or unilateral muscular fasciculations after exercise (nos. 1, 5) or bilateral hind limb ataxia (no. 2). In 1 pig (no. 5), progression of clinical signs was rapid, and she was euthanized at 21 days. Pig no. 2 developed posterior paresis, bilateral carpal knuckling, and marked posterior hypermetria at 81 days (Fig. 2a). There was no clinical deterioration until the pig was found dead at 132 days; because of autolysis, the brain and spinal cord were unsuitable for detailed histological study, but lesions typical of neurogenic atrophy were found in skeletal muscles, including M. tibialis cranialis. Pig no. 1 was euthanized by anesthesia-whole body perfusion at 130 days for ultrastructural studies that will be reported elsewhere. The 2 surviving pigs (nos. 4, 6) remained healthy at 7 months of age.

Throughout the period of clinical illness, pigs remained alert, ate normally, and continued to gain weight. Phonation, vision, swallowing reflexes, and lingual strength were unaffected.

**Pathology.** Pig no. 3 weighed 34 kg at necropsy at 83 days, and the control pig weighed 38 kg at 84 days. Apart from decubitus ulcers, gross lesions, including evidence of muscular atrophy, were absent. Histologically, approximately 10-20% of alpha motor neurons in all spinal cord segments were atrophic (Fig. 3a-3d). Many contained fine (1-3 µm) perikaryal vacuoles, which were evident in both HE- and TB-stained prep-
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Figure 3. Photomicrographs of changes in motor neurons in ventral horn of lumbar spinal cord of HPNSD-affected pig. HE. Bar = 10 µm. a. Atrophic neuron with loss of Nissl substance and reduced perikaryal cytoplasm. Note adjacent mononuclear cells, suggestive of early neuronophagia. b. Atrophic neuron with faint eosinophilic intracytoplasmic inclusions, some with a halo (arrowheads). c. Markedly atrophic neuron with cytoplasmic vacuolation and scalloped plasmalemma. d. Atrophic vacuolated neuron. Note eccentric nucleus that is poorly stained due to loss of detectable heterochromatin (arrowhead).

Figure 4. Photomicrograph of 3 alpha motor neurons in lumbar spinal cord of HPNSD-affected pig. There are distinct unstained cytoplasmic vacuoles (arrow) and osmiophilic droplets in the perikaryon of 1 neuron. TB. Bar = 10 µm.

arations (Figs. 3c, 3d, 4), and small (1-2 µm) perikaryal osmiophilic lipid droplets (Figs. 4, 5). There was reduced Nissl substance and reduced perikaryal cytoplasmic volume in the atrophic motor neurons (Fig. 3a-3d). Some atrophic neurons contained lightly eosinophilic inclusions with a distinct halo (Fig. 3b). Mild multifocal gliosis was present in ventral horns and was associated with neuronal atrophy. A single swollen chromatolytic neuron was found. Bielschowsky staining patterns of spinal cord for neurofibrils and immunocytochemical staining patterns for neurofilaments (Fig. 6a, 6b) and for mitochondrial markers were identical to those of the clinically normal pig. Marchi preparations revealed degenerating myelin in dorsal
spinocerebellar and the sulcomarginal tracts. There was corresponding vacuolar degeneration in lateral and ventral columns of the spinal cord in HE preparations (Fig. 7). Macrophages, some with pyknotic nuclei, occurred in these vacuoles. A few axonal spheroids were present in lateral and ventral columns. Mild vacuolar degeneration was also present in cerebellar foliar white matter and in spinocerebellar tracts of the medulla oblongata.

Wallerian degeneration was detected in ventral spinal nerve roots (Fig. 8b). Dorsal spinal nerve roots were unaffected (Fig. 8a). All 8 peripheral nerves and intramuscular nerves in M. tibialis cranialis had evidence of Wallerian degeneration (Fig. 9). In the sciatic nerve, the severity of axonal degeneration ranged from marked in some fascicles to minimal in others. Lesions indicative of denervation atrophy\(^1^6\) were present in all 6

**Figure 5.** Photomicrograph of alpha motor neuron in lumbar spinal cord of an HPNSD-affected pig, demonstrating small osmiophilic droplets (arrowhead). TB. Bar = 10 µm.

**Figure 6.** a, upper; b, lower. Immunocytochemical staining for neurofilaments in lumbar spinal cord of HPNSD-affected pig. Bar = 25 µm. a. There are no abnormal phosphorylated neurofilaments in perikarya of 2 neurons (1, 2). Large and small axons are stained (arrowheads). b. Normal pattern of nonphosphorylated neurofilaments in perikarya.
Figure 7. Photomicrograph of sulcomarginal area of ventral columns of cervical cord of HPNSD-affected pig, separated by ventral median fissure. Note vacuolated myelin, empty sheaths, and rows of macrophages (arrows), indicating axonal degeneration. HE. Bar = 100 µm.

Figure 8. a, upper; b, lower. Photomicrophages of spinal nerve rootlets in lumbar spinal cord of HPNSD-affected pig. HE. Bar = 50 µm. a. Dorsal rootlet shows no evidence of axonal degeneration. b. Ventral rootlet shows axonal degeneration with intravacuolar macrophages (arrows). Note swollen axon (asterisk).

muscles and were most severe in M. tibialis cranialis (Fig. 10a-10c). Ultrastructurally, many Schwann cells in peripheral nerves were devoid of an associated axon and contained myelin remnants at different stages of degradation (Fig. 11). Cords of proliferated Schwann cells within a single basement membrane (Büngner bands) were common. Some axons were markedly swollen, with flocculent axoplasmic contents and few or no neurofilaments and microtubules (Fig. 12). Macrophages that contained lamellar debris and osmiophilic lipid droplets occurred in endoneurium, often adjacent to blood vessels. Skeletal muscle fibers had ultrastructural features typical of advanced atrophy. The thyroid gland contained some abnormally large (800 µm) follicles. Other tissues were normal.
Figure 9. Photomicrograph of axonal degeneration in a fascicle of sciatic nerve of HPNSD-affected pig. Note widespread degeneration with Büngner band formation (arrow). The number of remaining myelinated fibers is reduced markedly. TB. Bar = 50 µm.

SAF/virus isolation/clinical chemistry. SAP-like structures were absent in spinal cord samples from the affected and the control pig and were present in homogenates of brain from the elk with chronic wasting disease. No viruses were detected. Circulating thyroxine levels were 5.76 and 6.12 µg/dl in the affected and control pig, respectively. Lactic acid levels in cerebrospinal fluid were 10.8 and 11.6 meq/l in the affected and control pig, respectively. Pyruvate levels were 1.2 and 0.8 mg/100 ml in the affected and control pig, respectively.

Discussion
Clinical signs in affected pigs and the histopathologic lesions found in 1 pig were consistent with progressive neuronal degeneration of varying severity in the progeny of this boar. The term hereditary porcine neuronal system degeneration was originally selected for the disease to reflect its genetic basis and because neuronal degeneration occurs in at least 2 cell populations: in the somata and axons of ventral horn alpha motor neurons and in the axons of sensory (proprioceptive) neurons of the dorsal spinocerebellar tract, among oth-
Clinical signs were however largely attributable to progressive degeneration of lower motor neurons. Failure to isolate viruses is consistent with the probability of a genetic basis. The vacuolar changes in atrophic neurons were not typical of a spongiform encephalopathy, and the absence of SAF fibrils further tends to rule out a prion-type disease.

The presence of intracytoplasmic vacuolation and atrophy in alpha motor neurons was a distinctive feature in the spinal cord of 1 affected pig with typical clinical signs. Earlier electron microscopic studies of well-fixed spinal cords from 4 affected pigs following whole-body perfusion revealed large vacuolar mitochondria that corresponded to vacuoles seen with the light microscope (O’Toole D, Wells G, unpublished data). This was the rationale for using 2 immunocytochemical markers for mitochondria in this study and for testing lactate and pyruvate levels in cerebrospinal fluid. Immunocytochemical examination did not reveal evidence of abnormal mitochondria, and marked elevations of lactate and pyruvate levels in the affected pig were absent. Preliminary attempts to stain for other mitochondrial components such as cytochrome c in formalin-fixed spinal cord from normal pigs were unsuccessful. Mitochondrial encephalopathies are well recognized in humans, but none are associated with motor neuron disease in any species. Additional morphologic and biochemical studies are required to establish the subcellular basis and significance of vacuolation in lower motor neurons in HPNSD-affected pigs.

Lesions in the affected pig distinguish this disease from reported porcine spinal neuronopathies. The time of onset and clinical signs resembled those seen in outbreaks of bilateral posterior ataxia and weakness in pigs. There was no evidence in conventional, Bielschowski, or immunocytochemical preparations of accumulated phosphorylated neurofilaments in perikarya and proximal axonal internodes, unlike previously described lower motor neuron diseases of Yorkshire and Hampshire pigs. A posterior paresis.

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**Figure 11.** Electron micrograph. Intramuscular nerve branch in M. tibialis cranialis from HPNSD-affected pig. Five nerve fibers (1-5) are degenerate and myelin debris has accumulated in cytoplasm of Schwann cells (Büngner bands). Mild axonal swelling in unmyelinated fibers and focal disarray of myelin lamellae in other nerve fibers are artifacts of immersion fixation. Bar = 10 µm.

**Figure 12.** Electron micrograph. Sciatic nerve of HPNSD-affected pig. Normal myelinated nerve fiber (1) has intra-axonal neurofilaments. Nearby, the swollen axon of a nerve fiber (2) is devoid of neurofilaments and contains amorphous flocculent material (large arrowhead). Presumptive axolemma forms discontinuous blebs (small arrowheads). Note intracytoplasmic lamellar material (asterisk) in Schwann cell. Bar = 2.5 µm.
associated with widespread neuronal degeneration in the brain and spinal cord was reported in Large White pigs in the USSR, but unlike HPNSD, clinical signs were present at birth.\textsuperscript{13} Toxic myelopathies in pigs resulting in posterior paresis can be induced with selenium and 6-aminonicotinamide, but degenerative lesions are restricted to spinal cord intumescences and comprise poliomyelomalacia.

Muscular changes were typical of denervation atrophy and were consistent with the widespread axonopathy in spinal nerve roots and peripheral nerves. Distinctive axonopathic abnormalities, as reported in other species,\textsuperscript{18} were absent. Mild follicular abnormalities in the thyroid gland of the paretic pig were probably incidental, and thyroid hormone levels were normal. The significance of thyroidal hyperplasia in the sow that died unexpectedly is unknown.

This disease is likely to be rare and of limited economic importance. Its main significance resides in it being a readily reproduced progressive disease of lower motor neurons that results in paresis in a domesticated species. Progressive upper and lower motor neuron diseases in humans have been studied for over 100 years, and their pathogenesis remains almost entirely unknown because in general only the endstage lesion in human cadavers can be examined morphologically.\textsuperscript{15,20,24} Genetically determined motor neuron diseases in\textsuperscript{1} animals have been useful in understanding the progression of neuronal loss\textsuperscript{12,18} and for testing treatment strategies, such as slowing the progression of neuronal degeneration using ciliary neurotrophic factor in the pmn mouse.\textsuperscript{30} The availability of genetic disorders affecting motor neurons in animals also makes it possible to test hypothetical pathogenetic mechanisms, such as the role of secondary antegrade degeneration,\textsuperscript{2} autoimmune to calcium channels,\textsuperscript{13} and deranged glutamate metabolism.\textsuperscript{9,27}

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Sources and manufacturers
a. True Vision, Indianapolis, IN.

References


