

## Iatrogenic compressive lumbar myelopathy and radiculopathy in adult cattle following injection of an adjuvanted bacterin into loin muscle: histopathology and ultrastructure

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**Abstract.** Compressive lumbar myelopathy is a recognized iatrogenic complication of injecting water-in-oil vaccines into paravertebral sites of laboratory animals and chickens. Herein, we report the histologic and ultrastructural features of a similar complication in a herd of cattle. Iatrogenic posterior paresis developed over 34 days in 56 of 610 cows (9.2%) following injection of a commercial bacterin 11-34 days earlier into *M. longissimus lumborum*. The bacterin was composed of inactivated *Escherichia coli* and *Campylobacter fetus* ssp. *venerealis* in a proprietary adjuvant. Tissues were collected for histopathology from 9 affected cattle that died or were euthanized after clinical signs lasting 6-38 days. A range of tissues, including the injection site lesion and lumbar spinal nerve roots, was obtained for ultrastructural examination from a cow with paresis of 31 days duration. There was locally extensive pyogranulomatous myositis with fibrosis and necrosis in right *M. longissimus lumborum*. Extension of the lesion into the vertebral canal via spinal nerve foramina resulted in focal pyogranulomatous inflammation in epidural fat and in adjacent dura mater. There was axonal degeneration in dorsal, lateral, and ventral columns and chromatolysis of spinal motor neurons in lumbar spinal cord, secondary to compression. A distinctive histologic and ultrastructural feature of pyogranulomata was the presence of osmiophilic material at the center of inflammatory foci, surrounded by macrophages and giant cells that contained intracytoplasmic lipid droplets. Ultrastructural examination of entrapped spinal nerves revealed axonal degeneration and loss of myelinated and unmyelinated fibers, segmental demyelination with remyelination, axonal spheroid formation, and early axonal regeneration. Two cattle also had bilaterally symmetrical acute myodegeneration with sarcoplasmic mineralization and secondary histiocytic myositis in axial and appendicular muscles, probably due to prolonged recumbency and vascular compromise (downer cow myopathy). Paresis probably developed as the result of the combination of a paravertebral injection site and the florid granulomatous response elicited by a constituent in the vaccine, most likely the water-in-oil adjuvant.

Adverse reactions following vaccination include anaphylaxis, iatrogenic infections,<sup>45,56,58</sup> injection site granulomas,<sup>15</sup> endotoxin-induced tissue damage,<sup>14</sup> acute inflammatory demyelinating polyradiculoneuropathy,<sup>4</sup> and neoplasia.<sup>22,48</sup> Adjuvants act nonspecifically to increase immune responsiveness to antigens and are a key component of many veterinary vaccines that contain inactivated bacteria and viruses.<sup>14,28</sup> Adjuvants such as Freund's incomplete adjuvant (IFA), peanut oil, and aluminum hydroxide may cause persistent lesions at the site of injection.<sup>1,2,15,17,18,23,25,36,41,46,53-53</sup>

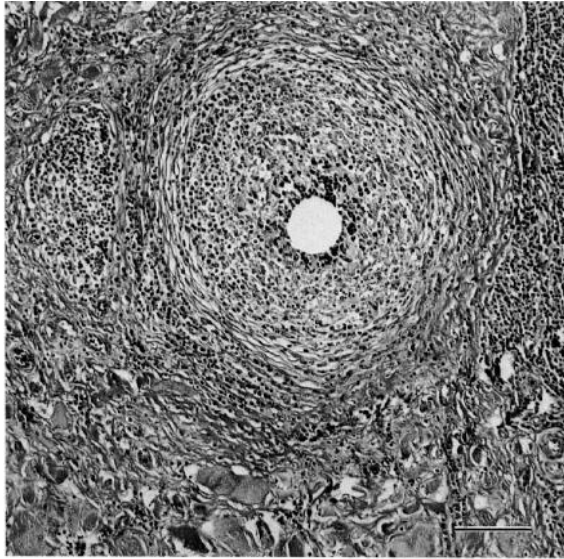
The histologic nature of these lesions has been defined<sup>1,6,17,24,29,41,52,54</sup> and manufacturers of many veterinary vaccines warn of injection site reactions. The risk of injection site reactions following the use of IFA

appears to be higher in vaccines that contain bacterial components<sup>14</sup> and objectionable localized granulomas may occur in cattle following the use of water-in-oil adjuvanted *Campylobacter fetus* ssp. *venerealis* bacterins.<sup>25</sup> There is currently no statutory requirement for manufacturers to undertake prelicensing morphologic studies of injection sites to define the severity and duration of local reactions<sup>16</sup> or to control the endotoxin content in adjuvanted vaccines containing inactivated gram-negative bacteria.<sup>11</sup>

Posterior paresis due to compressive myelopathy and radiculopathy is a recognized side effect of using adjuvants in paravertebral injection sites in guinea pigs.<sup>29</sup> Compressive cervical myelopathy and severe granulomatous myositis is occasionally recognized following subcutaneous injections of vaccines in the necks of 1-day-old chickens (R. P. Chin, personal communication). The purpose of this report is to document the histologic and ultrastructural features of similar iatrogenic lesions that developed in adult cattle following injection of an adjuvanted bacterin into *Musculus longissimus lumborum*.

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**Figure 1.** Injection site lesion in right *M. longissimus lumborum*; cow with moderate bilateral posterior paresis for 38 days. Note central clear space suggestive of extracted lipid, surrounded by neutrophils and epithelioid macrophages. There is fibrosis in adjacent endomysium. HE. Bar = 100  $\mu$ m.

### Materials and methods

**Case history.** The clinical signs, clinical pathology, and findings at necropsy in this group of paretic cattle have been reported elsewhere.<sup>34</sup> Posterior paresis developed in 56 of 610 pregnant cows (9.2%) on a ranch in Wyoming. Some 11-34 days before clinical signs developed, cattle were injected with an inactivated bacterin composed of *Escherichia coli* and *C. fetus* ssp. *venerealis* in a proprietary adjuvant<sup>a</sup> The injection site used was the right loin. Affected animals initially dragged the right hind foot, with progression in some cattle to bilateral posterior paresis of variable severity over the next 7-10 days. Various treatments were attempted. Tail-head epidural injections of corticosteroids were given to many affected cattle, including 5 of the 7 cattle submitted for necropsy; 1 of the 7 cows was untreated with corticosteroids, and the status of the remaining cow was unknown. By 5 mo after vaccination, 19 cattle had died or were euthanized, 5 cattle had persistent neurologic deficits, and the remaining 32 cattle recovered sufficiently to be returned to the herd.

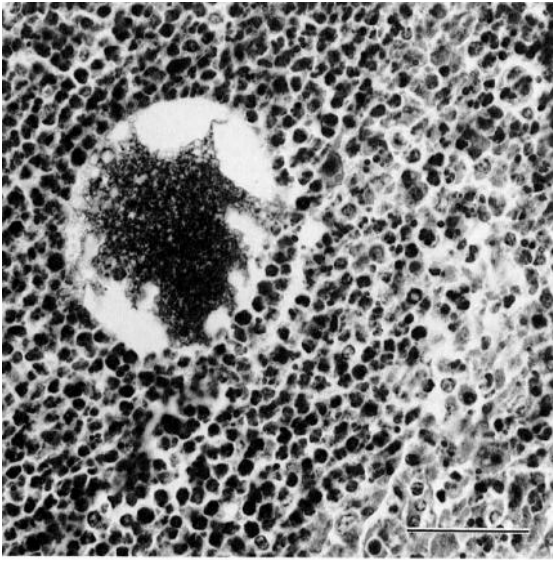
**Histopathology and ultrastructure.** Seven affected cattle with moderate posterior paresis (2 cows), severe posterior paresis (4 cows), or quadriplegia (1 cow) were submitted alive to the Wyoming State Veterinary Laboratory (WSVL). Cattle were euthanized after clinical signs lasting 10-38 days and examined postmortem. Particular attention was paid to the injection site lesion in right *M. longissimus lumborum* and its association with spinal nerve roots, spinal nerve foramina, and the lumbar epidural lesion. In addition, various separately identified muscles were collected on the ranch from 2 cattle that died or were euthanized after clinical signs lasting 24 and 36 days, respectively, fixed in phosphate-buffered neutral 10% formalin (NBF), and processed routinely for light microscopy. The 9 cattle whose tissues were examined were 3-8 years of age, and each had been vaccinated

annually with the same product 1-6 times. Tissues were fixed in NBF, Zenker's acetic acid (eyes), or Bouin's solution (eyes). Following fixation, tissues were dehydrated, embedded in paraffin, sectioned at 5  $\mu$ m, and stained with hematoxylin and eosin (HE). Selected tissues were stained with Wilder's technique for reticulin, Gram's stain for bacteria, Steiner's silver stain for microorganisms, an acid-fast stain for mycobacteria, Alcian blue for acid mucins, von Kossa's stain for calcium, and Bielschowski's stain for axons and neurofibrils, and selected levels of spinal cord were stained immunocytochemically for ubiquitin using a published procedure<sup>43</sup> and commercially available antibody (Mab 221M).<sup>b</sup> Formalin-fixed pieces of the epidural mass and the mass in *M. longissimus lumborum* from 4 cattle were sliced 1-2 mm thick, postfixed at room temperature in osmium tetroxide until tissues became dark gray (1-2 hr), and processed routinely in ascending concentrations of alcohol for embedment in paraffin. Samples of the following tissues from a paretic cow with clinical signs for 31 days were fixed in NBF for electron microscopy: dorsal and ventral spinal nerves at lumbar cord segments L2 and L3, spinal ventral horn at L3, lateral column of lumbar cord at L2, epidural mass at L2, and the injection site lesion in right *M. longissimus lumborum*. Following fixation in NBF for 5 days, samples were diced, transferred to a solution of 2% glutaraldehyde-2% paraformaldehyde in 0.1 M phosphate buffer for 8 hr, washed in buffer, postfixed in 1% osmium tetroxide in 0.1 M phosphate buffer containing 1.5% potassium ferricyanide<sup>32</sup> in 0.1 M phosphate buffer, washed in buffer, dehydrated, and embedded for oriented sections in epoxy resin. Semithin (1  $\mu$ m thick) sections were cut and stained with toluidine blue (TB). Ultrathin sections (70-90 nm thick) were cut with a diamond knife on an ultramicrotome, contrasted with uranyl acetate-lead citrate, and examined using an electron microscope.

**Endotoxin assay.** Two unopened bottles of vaccine that were stored at 4 C following vaccination of cattle (lot serial numbers 27008 and 27009) were tested in duplicate for endotoxin concentration using a kinetic turbidimetric *Limulus* amoebocyte lysate assay.<sup>c</sup>

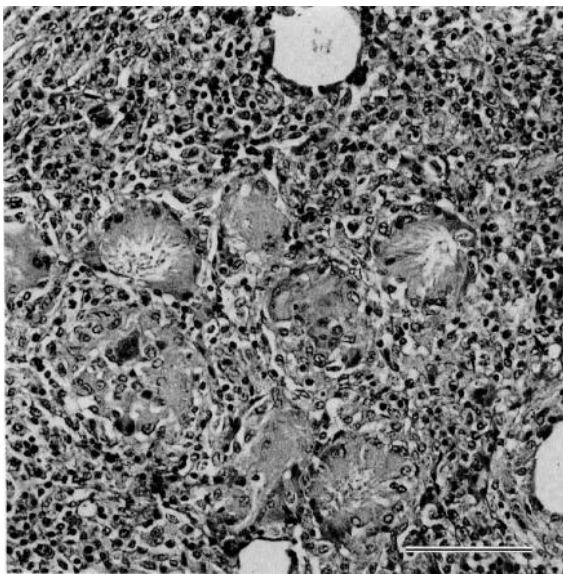
### Results

The histologic appearance of lesions was identical in *M. longissimus lumborum*, a lumbar aortic lymph node, and lumbar epidural fat. In conventional HE-stained preparations, there were multiple granulomatous or pyogranulomatous inflammatory foci that contained central optically empty circular spaces 35-540  $\mu$ m in diameter (Fig. 1). Fixation of thinly sliced lesions in osmium tetroxide before tissues were dehydrated in alcohol demonstrated that these spaces contained granular osmiophilic material (Fig. 2). Epithelioid macrophages surrounding this material contained fine round osmiophilic lipid-like intracytoplasmic droplets. Associated with some granulomas, there were multinucleated giant cells that contained intracytoplasmic lipid-like droplets and fan shaped arrays of acicular spaces, suggestive of extracted material (Fig. 3). Plasma cells and small lymphocytes, often in aggregates, were com-

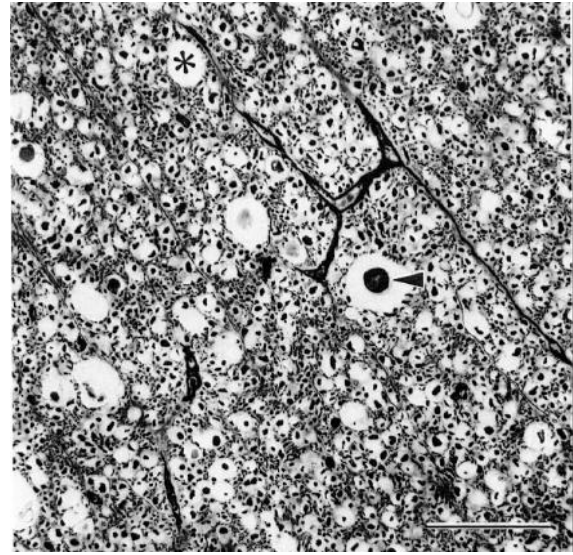


**Figure 2.** Injection site lesion; same cow as in Fig. 1. Granular osmiophilic material is partially retained at the center of the pyogranuloma, following secondary fixation of tissue in osmium tetroxide-potassium ferricyanide prior to tissue processing. HE. Bar = 50  $\mu$ m.

mon adjacent to granulomas, accompanied by various degrees of fibrosis. Extensive myonecrosis, hemorrhage, and edema were present in right *M. longissimus lumborum*. No bacteria or fungi were detected in lesions that were examined using special stains. In areas of lumbar spinal cord at L3-L5 that were associated with epidural fibrosis and granulomatous inflamma-

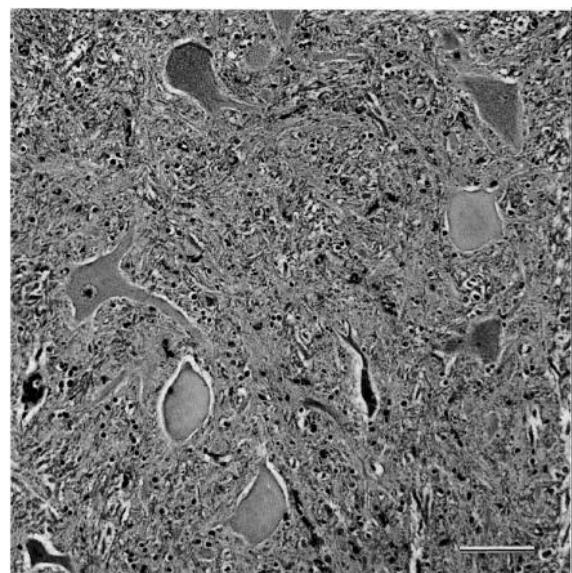


**Figure 3.** Granulomatous inflammation in epidural space of lumbar vertebra; cow with moderate bilateral posterior paresis for 22 days. Note unstained acicular spaces in cytoplasm of giant cells, suggestive of material that was extracted during tissue processing. HE. Bar = 100  $\mu$ m.

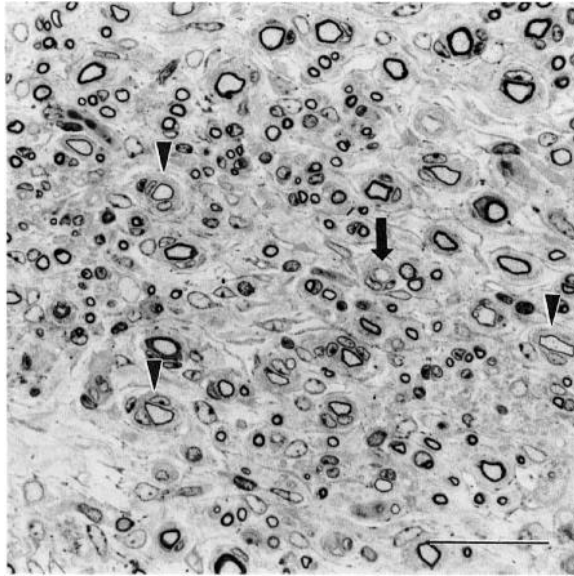


**Figure 4.** Lateral column of lumbar spinal cord; cow with severe bilateral posterior paresis for 31 days. Note marked axonal swelling (arrowhead) and vacuolar degeneration (asterisk). Bielschowsky. Bar = 50  $\mu$ m.

tion, there was mild to severe Wallerian degeneration in all funiculi, accompanied by macrophage infiltration, ubiquitin-positive argyrophilic axonal swelling, and gliosis (Fig. 4). Neuronal chromatolysis was present in many lumbar alpha motor neurons (Fig. 5). In some right dorsal and ventral lumbar spinal nerve roots, there were axonal spheroids, nerve fiber loss, inappropriately thin myelin sheaths relative to axonal caliber, Biingner band formation, ingress of macrophages, and



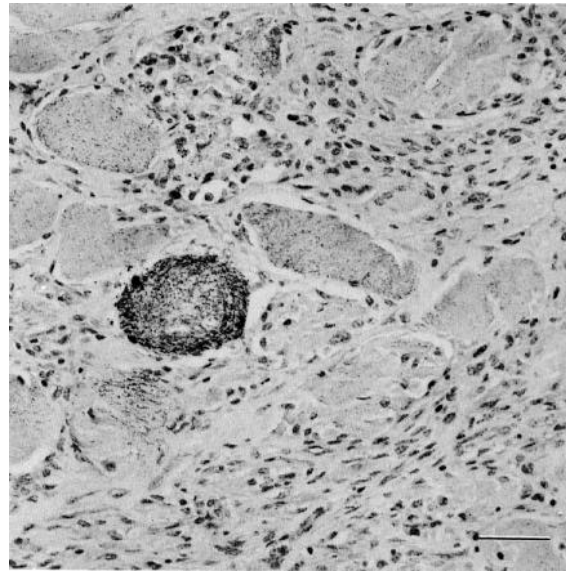
**Figure 5.** Ventral horn of lumbar spinal cord; cow with severe bilateral posterior paresis for 10 days. Note marked neuronal chromatolysis in 3 alpha motor neurons. HE. Bar = 100  $\mu$ m.



**Figure 6.** Lumbar spinal nerve root at L2-L3; cow with severe bilateral posterior paresis for 31 days. Note loss of myelinated nerve fibers resulting in relative expansion of the endoneurial compartment, axons with disproportionately thin myelin sheaths (arrowheads), and naked axon (arrow). TB. Bar = 50  $\mu$ m.

limited nerve fiber regeneration (Fig. 6). Lesions in selected major nerves of the hind limb (*Nn. ischiadicus, tibialis, peroneus, plantaris lateralis*) were either minimal or absent. No lesions were found bilaterally in *N. radialis*, which was the only forelimb nerve that was examined. In 2 cattle with clinical signs for 6 and 24 days, respectively, there was moderate or severe locally extensive bilateral myodegeneration with punctate sarcoplasmic mineralization and secondary histiocytic myositis (Fig. 7). Affected muscles, which corresponded to pale muscles noted at necropsy, included *Mm. triceps brachii, semitendinosus, semimembranosus, tibialis cranialis*, and *psaos major*.

Ultrastructural examination of the centers of granulomas revealed pools of homogenous lightly osmophilic matrix and trilamellar material, invested by epithelioid macrophages (Fig. 8). Trilamellar material was 30 nm thick and consisted of a central 5-nm-thick electron-lucent line flanked by irregularly beaded electron-dense material (Fig. 8, inset). The periphery of granulomas consisted of lipid-laden macrophages and multinucleated cells and a mixture of plasma cells, neutrophils, and small lymphocytes (Fig. 9). In spinal nerve roots, most nerve fibers with inappropriately thin myelin sheaths contained a single axon that was surrounded by 1-5 Schwann cell profiles, suggesting segmental demyelination with early remyelination<sup>50</sup> (Fig. 10). Axonal spheroids occurred in myelinated and unmyelinated axons and contained disordered arrays of neurofilaments mixed with mitochondria, membra-



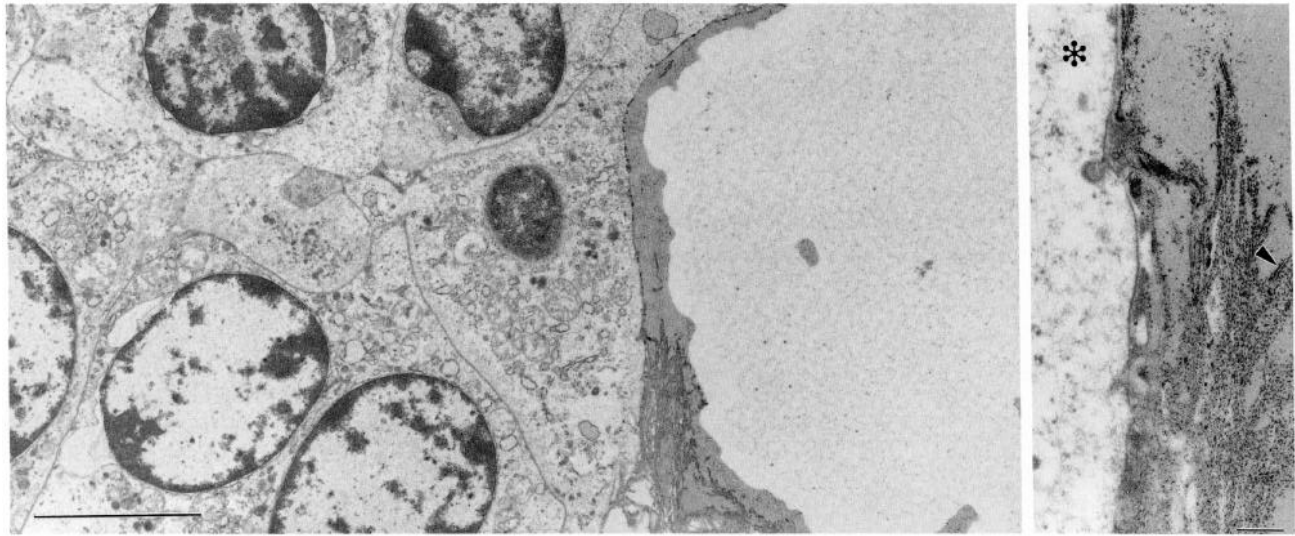
**Figure 7.** Right *M. psaos major*; cow with severe quadriplegia for 6 days. Note diffuse myonecrosis with various degrees of sarcoplasmic mineralization and infiltration of endomysium by histiocytes. Fibroplasia of endomysium is also present. von Kossa, Bar = 50  $\mu$ m.

nous vesicles, and lamellar debris (Fig. 11). Less common features were Büngner bands with or without intracytoplasmic myelin debris in large ovoids in Schwann cells (Fig. 12), clustered myelinated and unmyelinated axons within a single basal lamina indicative of axonal regeneration, small axons with disproportionately thick myelin sheaths signifying axonal atrophy, and lattice-like arrays of 70-80 nm vesicles in myelin indicative of vesicular disruption of myelin.<sup>50</sup> No examples were found of large concentric arrays of Schwann cells ("onion bulbs")<sup>13,21</sup> or of active myelin stripping by macrophages.<sup>13,31,49</sup>

The 2 bottles of vaccine analyzed for endotoxin contained 3,180,000 endotoxin units (EU)/ml (serial no. 27008) and 3,790,000 EU/ml (serial no. 27009).

### Discussion

Posterior paresis in these cattle developed as a result of 2 factors: an unfortunate choice of injection site and induction of severe inflammation in response to 1 or more components in the vaccine. The vaccine used on the ranch was designed for generic intramuscular use, and its data sheet carried no warning about its use in paravertebral sites. In the light of this study such a warning may be appropriate. The company was not forthcoming about constituents in the vaccine, such as adjuvant type, or about the frequency of reported injection site reactions. In the last 10 years, 2 reports of adverse reaction associated with this product were filed with the Veterinary Biologics office of the USDA-APHIS (S. Karli, personal communication); both re-

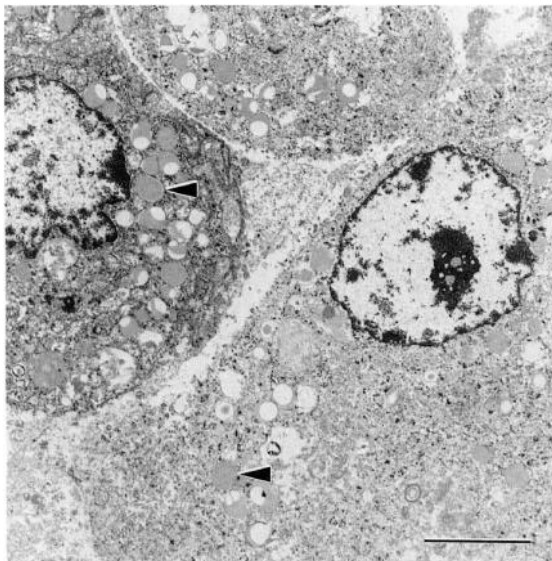


**Figure 8.** Electron micrograph. Right *M. longissimus lumborum*; cow with severe paraparesis for 31 days. At the center of a granuloma similar to that illustrated in Figs. 1 and 2, epithelioid macrophages about an acellular space containing homogenous lightly osmiophilic matrix in which trilamellar material is suspended. Most of the contents in the acellular space were probably extracted during processing. Bar = 5  $\mu\text{m}$ . *Inset:* Higher magnification of trilamellar material (arrowhead), showing cytoplasm of adjacent epithelioid macrophage (asterisk). Bar = 250 nm.

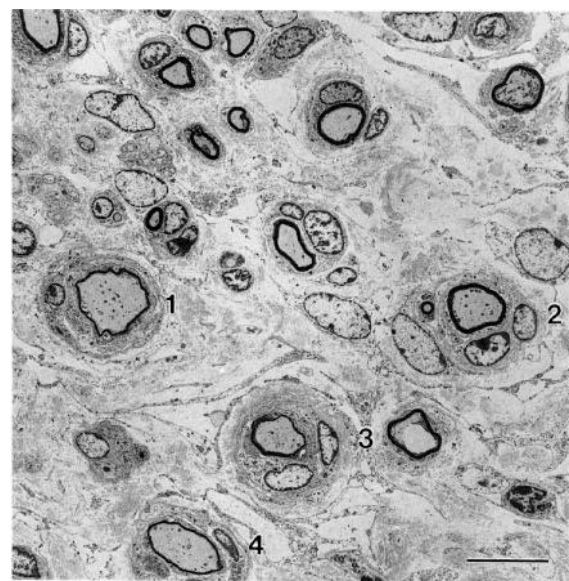
ports were of local injection site swellings. In 1989, one of us (KG, unpublished observations) reported to the company that postinjection abscesses developed in 70 of 74 cattle following vaccination with the product. The company attributed other reports in 1989 and 1990 of injection site reactions to bacterial contamination and “overresponse of the immune system” (information provided by the Veterinary Biologics Field

Office via USDA/APHIS Freedom of Information Office, Hyattsville, MD). Veterinary diagnosticians contacted in 7 states in January 1994 (MT, CO, NE, CA, MN, OR, MO) were not aware of adverse effects associated with this product, including posterior paresis.

In spite of its locally irritant properties,<sup>1,54</sup> some manufacturers prefer adjuvants such as IFA in their vaccines because of the better and more prolonged



**Figure 9.** Electron micrograph. Right *M. longissimus lumborum*; cow with severe paraparesis for 31 days. Note partly extracted intracytoplasmic lipid-like droplets (arrowheads) in 3 macrophages. Bar = 5  $\mu\text{m}$ .



**Figure 10.** Electron micrograph. Right lumbar spinal nerve root at L3; cow with severe paraparesis for 31 days. Note 4 (1-4) thinly myelinated axons encircled by multiple profiles of Schwann cell processes that suggests remyelination following segmental demyelination. The endoneurial compartment is expanded. Bar = 10  $\mu\text{m}$ .



**Figure 11.** Electron micrograph. Right lumbar spinal nerve root at L3; cow with severe paraparesis for 31 days. Note the markedly swollen naked axon that contains numerous neurofilaments. Bar = 5  $\mu$ m. Inset: Higher magnification of neurofilaments (arrow) in axoplasm. Bar = 50 nm.

immunity they induce, as compared with other adjuvants such as aluminum hydroxide.<sup>7,9,35</sup> IFA is an approximately 50:50 mixture of water and low-viscosity mineral oil that is emulsified using hydrophobic surfactants such as mannide monooleate.<sup>14,38</sup> Depending on the refinement of the oil and the emulsifier, the complex mixture may consist of straight chain, cyclic, and branched hydrocarbons, peracylated carbohydrates, mannide monooleate, carbohydrate polymer, conjugated dienes, and cyclic fatty acids.<sup>38</sup> Components of IFA, such as straight-chain hydrocarbons, may persist in injection sites for up to 10 months.<sup>38</sup> Because of its irritant effects in tissue and concerns about carcinogenicity,<sup>14,38,48</sup> IFA is not used routinely in vaccines licensed for use in people in the United States,<sup>14</sup> and its use in animals has been questioned.<sup>6</sup> It is difficult to identify which vaccine constituent caused tissue damage in the absence of information from the company about the concentration of potentially irritating constituents in the vaccine, such as free fatty acids and esters<sup>14,20,35</sup> and short (<C<sub>12</sub>) straight-chain hydrocarbons.<sup>57</sup>

The demonstration of osmiophilic lipid-like material<sup>27</sup> at the center of pyogranulomas and the absence of stainable infectious organisms in tissue sections indicated that the reaction was most likely directed toward a component in the vaccine. Attempts to stain tissues immunocytochemically for the two major antigenic constituents in the vaccine (*C. fetus* and



**Figure 12.** Electron micrograph. Right lumbar spinal nerve root at L3; cow with severe paraparesis for 31 days. Büngner band with redundant basal lamina (arrowhead) and large whorls of myelin-derived debris in cytoplasm of Schwann cells. Bar = 2.5  $\mu$ m.

*E. coli*) were unsuccessful (D. Haines, personal communication), probably because of extraction of vaccine components during routine tissue processing for light microscopy. Amorphous or crystalline material suggestive of aluminum oxide particles<sup>15,36,42</sup> was absent in giant cells, and histologic lesions in loin muscle and epidural fat were more consistent with published descriptions of tissue reactions to water-in-oil adjuvants<sup>1,6,17,24,29,41,52,55</sup> which have been variously described as "oil granulomas"<sup>18</sup> and "paraffinomaes."<sup>42</sup> The vaccine contained a high concentration of endotoxin, which could have contributed to the intensity of local inflammation and tissue necrosis.<sup>1,14</sup> One of the 2 lots of vaccine tested was assayed independently by USDA-APHIS, and nearly identical levels of endotoxin were found (J. H. Payne, personal communication). Systemic signs of endotoxemia<sup>10</sup> were not however recognized in vaccinated cows.

Paralysis in these cattle was due to a combination of compressive lumbar myelopathy and unilateral or bilateral lumbar radiculopathy. Migration along tissue planes following vaccination is a recognized property of water-in-oil adjuvants,<sup>6</sup> and the presence of osmiophilic material at the center of extradural granulomas suggests that product migrated into the vertebral canal, probably via intervertebral foramina. Expanding space-occupying epidural masses due to chronic inflammation accounts for progression of clinical signs in some cattle. Unlike acute spinal compression injury, which primarily involves gray matter, chronic compression injury affects chiefly white matter of the spinal cord and results in axonal degeneration and demyelina-

tion.<sup>12,33</sup> Neuronal chromatolysis in ventral horn neurons probably resulted from axonal degeneration in spinal nerve roots and spinal nerves. Spinal nerve roots are particularly susceptible to compression injury because of their thin root sheath and paucity of endoneurial connective tissue.<sup>19</sup> The nature of changes in nerve fibers following compression is determined by the severity and chronicity of the insult.<sup>44</sup> Mild injury results in segmental demyelination with characteristic polarized changes in paranodal areas,<sup>40</sup> whereas more severe and/or prolonged compression induces Wallerian degeneration, particularly of large-caliber myelinated fibers.<sup>3,37,51</sup> The combination of Wallerian degeneration and segmental demyelination with remyelination in spinal nerve roots in this study reflects the severity of spinal nerve injury following epidural compression and entrapment in vertebral foramina by granulomatous tissue. The presence of swollen axons packed with neurofilaments may reflect either axonal injury or attempted regeneration.<sup>30</sup> At least 5 cattle in this study were injected epidurally with corticosteroids. Corticosteroids such as methylprednisolone acetate caused iatrogenic meningitis and adhesions when injected intrathecally in people;<sup>39,47</sup> thus, these steroid injections may have aggravated lesions present in some cattle. However, the clinical history, the use of a sacrococcygeal injection site, the character of lesions, and the presence of identical lesions in one cow that received no epidural injections make it unlikely that corticosteroids had any significance in inducing lesions.

Two parietic cattle also had bilaterally symmetrical acute myodegeneration involving selected axial and appendicular muscles. One of the 2 cows was quadriparetic, and widespread acute myodegeneration probably contributed to this animal's illness. The cause of myodegeneration was not established. We suspect that these lesions were the result of ischemia (downer syndrome myopathy)<sup>26</sup> in cattle that were recumbent for prolonged periods in sub-zero (-25 C) temperatures. The histologic character of lesions, including the presence of mineralization suggestive of intramitochondrial calcification, resembled myopathy due to selenium-vitamin E deficiency.<sup>5</sup> The presence of normal selenium and vitamin E levels in liver from 1 of the 2 cows affected by severe myopathy makes the deficiency etiology unlikely.

Paravertebral intramuscular injection sites should be avoided by veterinarians and animal owners who use biological products that are known to be locally irritating.

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#### Sources and manufacturers

- a. Pilivib Shield@ (adjuvant: Xtend III™), Grand Laboratories, Larchwood, IA.
- b. Biomeda Corp., Foster City, CA.
- c. Associates of Cape Cod, Woods Hole, MA.

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