

Myositis, lameness, and recumbency after use of water-in-oil adjuvanted vaccines in near-term beef cattle

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Abstract. A producer administered 2 US Department of Agriculture–licensed adjuvanted veterinary vaccines (inactivated bovine rotavirus–coronavirus vaccine; *Clostridium perfringens* type C–*Escherichia coli* bacterin-toxoid) into muscles of the left and right hips of 469 pregnant beef cows. Within 24 hours, 5 cattle were recumbent, and another 2 had non–weight bearing pelvic limb lameness (1.5% affected; 7/469). During the next 10 days, 50% of the herd developed firm swellings up to 24 cm in vaccination sites in muscles of the hip. Histological samples revealed granulomatous myositis with intralesional oil. Lesions resolved slowly during the next 6 months. Six cattle were injected experimentally with the vaccines. None became lame, but all developed foreign body granulomatous myositis similar to those in the affected herd. The maximum diameter of experimentally induced lesions in muscle at necropsy 60 days after injection with the recommended dose of the bacterin-toxoid vaccines was 12 cm. Histological examination revealed pyogranulomatous myositis, fibrosis, and myonecrosis. The inactivated viral vaccine induced milder granulomatous myositis with intralesional lipid and scant fibrosis. Acute transient lameness on the ranch was attributed to use of 2 irritating biological vaccines in the hip muscles of cows that were close to parturition.

Introduction

Injection site reactions are an important source of loss for the North American beef industry. The annual cost of trim to the industry is estimated to be \$55 million in the United States and \$19 million in Canada.^{10,28} To reduce the cost of trim, producer organizations in North America recommend that biological, antibiotic, vitamin, and mineral products be formulated for subcutaneous use whenever possible and administered in the neck.³⁰ The recommendations has been moderately effective. Injection site lesions at slaughter in choice cuts decreased over 8 years (1991–1998) from 22% to 10%.²⁹

Reducing the incidence of injection site lesions requires that manufacturers of biological and antibiotic compounds commit to less irritating formulations.³⁰ Intramuscular vaccines are popular with some manufacturers. Beef producers in our area are generally tolerant of postvaccination swellings and lameness in cattle. Requests for veterinary assistance occur only when large numbers of cattle are affected or when swellings resolve slowly. Published accounts of postvaccination episodes in cattle are sparse.^{18,19,24,25} By contrast, extensive documentation, discussion, and concern sur-

round postvaccinal complications due to the use of aluminum-adjuvanted vaccines in companion animals.¹⁷

Adjuvants (Latin: *adjuvare*, to help) are used to boost active host immune responses to antigens in vaccines.³² Clinical effectiveness of adjuvanted vaccines depends on an appropriate balance between toxicity and adjuvanticity.¹³ Unlike veterinary vaccines and their recognized efficacy notwithstanding, water-in-oil adjuvants are no longer used for human vaccines because of their irritancy in tissue, particularly with regard to more slowly absorbed mineral oil mixtures.^{2,6–9,13–16,26,31,32}

This report describes an adverse reaction in late-term beef cattle after injection of 2 licensed veterinary vaccines into muscles of the hip.

Case report

On January 9, 2002, a producer in northwestern Nebraska vaccinated 469 adult Angus and Angus-cross cows 10 days before calving was due to start. Cattle were injected with an inactivated combination bovine rotavirus–coronavirus vaccine (2 ml/animal; vaccine A)^a and an inactivated combination *Clostridium perfringens* type C–*Escherichia coli* bacterin-toxoid (1 ml/animal; vaccine B)^b in muscles of the left and right hips, respectively. Both vaccines were white oily liquids formulated for use in healthy pregnant cattle. According to label directions, vaccines A and B should be given 8–10 weeks and 1–3 months before calving, respectively. Stated contents are inactivated infectious agents, a proprietary adjuvant,^c a mercuric preservative (thimerosal), and (vaccine A only) amphotericin B,

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Table 1. Lesions in cattle after vaccination with adjuvanted vaccines.*

Animal no.	Days post-vaccination	Size of lesion		Oil present (grossly)	Recumbency after vaccination	Histopathology	Creatine Kinase (U/liter) ref: <110 U/liter	Lactic dehydrogenase (U/liter) ref: 694–1445 U/liter
		Left hip (cm)	Right hip (cm)					
1	12	12.0 × 8.4	None	No	No	PGM + F	26	7245
2	12	24.0 × 12.0	10.0 × 7.8	No	No	GM + F	43	7040
3	12	10.1 × 10.3	5.0 × 7.0	Yes	Yes	LHM + F	110	6231
4	12	10.0 × 10.7	None	No	Yes	LHM	63	5600
5	12	10.2 × 8.7	5.0 × 7.0	No	Yes	LHM + F	273	5690
6	12	6.8 × 8.1	11.1 × 11.5	Yes	No	GM + F	38	5322
7	25	17.0 × 14.0	10.0 × 9.0	Yes	†	GM + F	NE	NE
8	34	NE	NE	NE	Yes	NE	NE	NE
9	60	9.0 × 4.0	None	NE	No	PGM + F	NE	NE
10	140	2–4 cm	None	No	No	GM + F	113	780
11	140	2–4 cm	None	No	No	GM + F	100	1010
12	140	2–4 cm	None	No	No	F	104	840
13	140	2–4 cm	None	No	No	LHM + F	98	920

* PGM = pyogranulomatous myositis; F = fibrosis; GM = granulomatous myositis; LHM = lymphohistiocytic myositis; NE = not examined or tested.

† Cow became recumbent immediately before parturition, 22 days after vaccination.

penicillin, and streptomycin. Vaccines had been stored appropriately and were used before the printed expiry dates. One year earlier, the producer used the same vaccines immediately before parturition with no untoward effects. Labels on vaccine A and B contained the warning: “This product may cause persistent swelling at the site of injection.” The owner decided to inject muscles of the hip for reasons of operator safety and ease of processing, and because there was no statement on the vaccines advising against use of hip muscles. Cattle were injected within 10 days of parturition because this was done 1 year earlier without adverse consequences. Vaccines were administered using 2.54-cm-long needles that were changed when blunt (after ~10–20 animals). Cattle were also injected intramuscularly in the left hip with 5 ml of vitamin A and D preparation^d and subcutaneously in the neck with 5 ml of a *C. perfringens* type C and D vaccine.^e

At 8:00 AM the next day, the owner found 2 cows recumbent. Three more cows became recumbent later that day. That evening, 2 cows developed non-weight bearing lameness of the right pelvic limb. Discreet areas of heat and swelling up to 20 cm diameter developed at vaccination sites in muscles of the right and left hips. The 5 recumbent cattle were treated with flunixin meglumine^f and oxytetracycline,^g injected into muscles of the neck. These 5 animals gradually became ambulatory over the next 4 days after intensive use of hip slings. Cattle remained weak and lame, and fell periodically. The number of animals in the herd with visible swellings in the hips increased from 25–33% at 6 days to 50% at 10 days postvaccination. A cow was found recumbent with both pelvic limbs in extension 22 days after vaccination. She could drag

herself forward using the thoracic limbs. Gross swellings were present in both hips at the vaccination sites, and there was no response to intravenous calcium borogluconate. She delivered healthy twin calves, developed ruminal bloat, and died (cow No. 7, Table 1). Lesions in the hips of injected cows in the herd resolved slowly over the next 6 months. By 127 days postvaccination, approximately 25% of the herd had visible swellings. All surviving cattle recovered completely by 1 year after the episode.

The suspect adverse reaction was reported to the US Department of Agriculture (USDA) Center for Veterinary Biologics (CVB).

Materials and methods

Samples from Nebraska herd. Six affected cows were examined clinically 12 days after vaccines A and B were administered (animal Nos. 1–6, Table 1). They included 3 of the 5 animals (Nos. 3–5) that were recumbent after vaccination. Lesions were measured, and biopsy samples were collected for histology from swollen muscles of the left and right hips. Attempts to aspirate fluid for cytology and bacterial culture were successful in 1 of the 6 animals (No. 2). Samples were collected for chemical analysis of selenium (whole blood) and trace elements (serum). Additional samples were collected from 3 cows that died 1–2 mo after vaccination. One animal died 3 days after parturition (No. 7; 25 days postvaccination), a second cow broke a leg when she fell on ice (No. 8; 34 days postvaccination), and a third died while being handled (No. 9; 60 days postvaccination). Samples of liver were taken for trace element analysis from animal Nos. 7 and 8. Samples of hip muscle including injection sites were collected from animal Nos. 7 and 9. Samples of muscle from the hip were collected by surgical biopsy from 4 cows, 140 days after vaccination (Nos. 10–13). Unopened

Table 2. Lesions in cattle after experimental injection with adjuvanted vaccines.

Animal no.	Necropsy (days post-vaccination)	Injected with		Visible swelling	Residual vaccine in lesion	Lesion size at necropsy (cm)	Histopathology	
		Left hip (volume)	Right hip (volume)				Left hip	Right hip
13	10	vaccine A (10 ml)*	none	No	Yes	4.5 × 4.0	PGM†	normal
14	10	none	vaccine B (5 ml)‡	No	Yes	6.0 × 6.0	normal	PGM + F§
15	21	vaccine A (10 ml)	none	Yes	Yes	7.0 × 4.0 × 4.0	PGM	normal
16	21	none	vaccine B (5 ml)	No	Yes	15.0 × 6.0 × 5.0	normal	PGM + F
17	65	vaccine A (2 ml)	vaccine B (1 ml)	No	No	7.0 × 3.0 × 2.0 (left hip) 11.5 × 7.5 × 2.4 (right hip)	PGM	PGM + F
18	65	vaccine A (2 ml)	vaccine B (1 ml)	No	Yes	9.0 × 4.0 × 1.5 (left hip) 12.0 × 6.0 × 6.0 (right hip)	PGM	PGM + F
19	NE	saline (2 ml)	saline (2 ml)	No	NE	NE	normal	normal

* Vaccine A: inactivated bovine rota virus–coronavirus vaccine.

† PGM = pyogranulomatous myositis.

‡ Vaccine B: bacterin-toxoid *Clostridium perfringens* type C–*Escherichia coli*.

§ F = fibrosis.

|| NE = not examined; histopathology evaluation based on serial biopsy samples taken at 7–28 days postinjection.

bottles of vaccines A and B remaining on the Nebraska ranch were tested for aerobic bacteria and for endotoxin concentration using the gel-clot method.^h

Experimental vaccination. In an effort to reproduce the syndrome in the source herd, and to monitor and compare the temporal development of lesions, vaccines A and B were tested in 7 cattle as shown (Table 2).

Four 6-mo-old calves were injected intramuscularly with 5 times the dose of the vaccines used on the Nebraska ranch (vaccine A into left hip, animal Nos. 13 and 15; vaccine B into right hip, animal Nos. 14 and 16). Injections were given using new 18-gauge, 3.81-cm-long needles with the entire volume (10 ml of vaccine A, or 5 ml of vaccine B) deposited in 1 site per animal. The rationale of this study was to establish whether adverse reactions could be detected clinically. Ten times the commercial dose is the recommended calf safety test for vaccine licensing (Code of Federal Regulations [CFR]—Animals and Animal Products; §113.41 Calf Safety Test). Five instead of 10 times the commercial dose was used because the latter required injection of 20 ml of vaccine A, which exceeds current recommendations for injectable product delivered per site into muscle,³⁰ and due to concern about inducing unacceptably severe reactions. Vaccine was injected using 3.81-cm-long needles rather than the shorter length needles used on the ranch to ensure that all of the vaccine was delivered intramuscularly. Rectal temperatures were recorded daily for the duration of the study. Injection sites were palpated daily. Calves were euthanized and examined postmortem at 10 ($n = 2$) and 21 days post-injection ($n = 2$).

In a second study, 3 healthy yearling animals that were not previously vaccinated with vaccines made by the company were used (animal Nos. 17–19, Table 2). Two were injected with the recommended dose of vaccine (2 ml of vaccine A, and 1 ml of vaccine B; animal Nos. 17 and 18) into muscles of left and right hips, respectively. The remaining animal No. 19 was used as a control and injected with 2 ml and 1 ml of sterile phosphate-buffered saline into right

and left hip muscles, respectively. The vaccine was injected at right angles to the skin using new 3.81-cm-long needles. The production serial of vaccines were the same as those used on the ranch. Biopsy samples of muscle at injection sites of animal Nos. 17–19 were collected for histopathology using a biopsy instrument immediately before vaccination and 7, 14, 21, and 28 days after vaccination. Before biopsy, hair was clipped and shaved, subcutaneous tissues were infiltrated with 2% lidocaine solution, skin was disinfected with surgical scrub solution followed by ethyl alcohol, and the biopsy sample was collected by an 18-gauge automatic cutting biopsy needle.ⁱ At 65 days, animal Nos. 17 and 18 were euthanized and examined postmortem; control animal No. 19 was not euthanized. The rationale of this study was to attempt to reproduce the clinical syndrome in the Nebraska ranch, to monitor progression of histological lesions over time, to establish the size of lesions after 60 days (after which time, the vaccinated animals may go for slaughter), and to establish whether vaccine was grossly detectable at that time. The vitamin A and D product used on the herd was not given because the goal of the study was to see the effects of the vaccines alone.

Pathology and toxicology. Samples of muscle that were collected by biopsy or at necropsy on the ranch from animal Nos. 1–12 were fixed in phosphate-buffered, neutral 10% formalin, dehydrated, embedded in paraffin wax, sectioned on a microtome at a thickness of 5–7 μm , and stained with hematoxylin and eosin (HE). Selected samples of fixed tissue were postfixed in osmium tetroxide solution to retain oil-based vaccine during processing.¹⁹ Sections were stained to demonstrate bacteria (Gram's), mycobacteria (acid-fast stain), and fungi (periodic acid–Schiff stain). Samples from major organ systems were collected from 6 animals injected experimentally with vaccine (Nos. 13–18) and processed for histology. Samples of blood and liver were processed and analyzed for trace elements including selenium and copper by inductively coupled plasma mass spectrometry (Elan 6100, Perkin Elmer, Norwalk, CT) after digestion in 3 ml



Figure 1. Injection site lesion at Nebraska ranch. Swelling in left hip of cow 12 days after intramuscular injection of vaccine A. The measuring scale is in centimeters.

Figure 2. Injection site lesion at Nebraska ranch. Incision of swelling similar to that in Fig. 1, 25 days after intramuscular injection of vaccine A. Note leaking white oil consistent with vaccine (arrow).

Figure 3. Injection site lesion at Nebraska ranch. Portion of muscle and skin with injection site reaction of cow 25 days after vaccination. White oil consistent with vaccine is present in muscle and subcutaneous adipose tissue (arrowhead). There is extensive hemorrhage and necrosis. Bar: 1 cm.

Figure 4. Injection site lesion at Nebraska ranch. Muscle and skin with portion of injection site reaction in hip muscle of cow 60 days after vaccination. Note extensive ramifying areas of fibrosis (asterisk) in superficial muscle. Bar: 1 cm.

each of hydrogen peroxide and nitric acid at 180 C (MDS 2000, CEM Corp, Mathews, NC) using a standard Wyoming State Veterinary Laboratory protocol.

Results

The 6 cows examined on the ranch at 12 days post-injection were bright and alert. There were 6.8–24.0-cm firm intramuscular swellings in right or left hips (or both) of the 6 cows that were examined clinically. The largest lesions were in the left hip (Fig. 1; Table 1). Lesions in the left hips of 2 cows (Nos. 3 and 6) leaked white oil onto skin as the biopsy instrument was withdrawn (Fig. 2). Samples of muscle collected from left and right hips from 2 cows that died at 25 and 60 days postvaccination had extensive fibrosis, panniculitis, hemorrhage, and necrosis, with intrale-

sional oil (animals No. 7 and 9; Figs. 3, 4). The maximum size of lesions in the 2 animals was 9.0 cm and 17.0 cm, respectively. The only other gross lesion in animal No. 7, which died 25 days postvaccination, was bloat. The cause of death of animal No. 9, which died 60 days after vaccination, was not established. No gross lesions were present in thoracic or abdominal cavities. Four cows had clinically palpable, mild (2–4 cm) lesions in muscles of the left hip 140 days after vaccination.

Histologically, samples of muscle collected 12 days postvaccination had varying degrees of fibrosis and myositis with intralesional clear vacuoles consistent with oil (Table 1). In most animals there was severe granulomatous or pyogranulomatous myositis and replacement fibrosis. Many lipid droplets were partly or

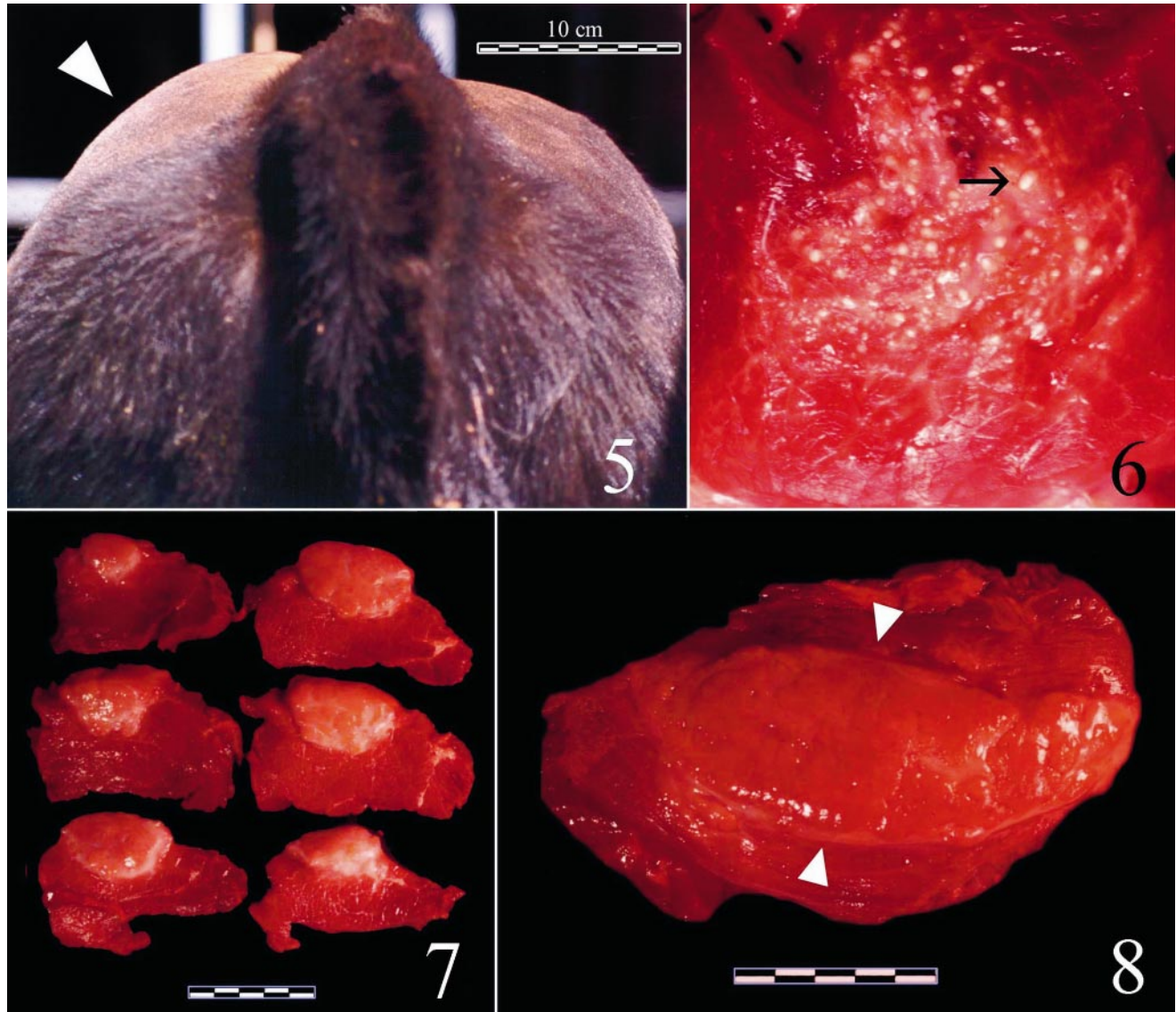


Figure 5. Experimental vaccination. Posterior view of hip muscles 21 days after injection of a calf with 5× dose of vaccine A. Hip muscles are asymmetrical due to swollen left *M. biceps femoris* (white arrowhead).

Figure 6. Experimental vaccination. Incised *M. biceps femoris* 21 days after injection with vaccine B. Beads of white liquid (arrow) exude from muscle.

Figure 7. Experimental vaccination. Sliced incised lesion of *M. biceps femoris* 65 days after injection with vaccine B. No white oil is visible. Bar: 1 cm.

Figure 8. Experimental vaccination. Higher magnification view of cross-sectioned *M. biceps femoris* 65 days after vaccination with vaccine B. Lesion is well demarcated and partly encapsulated (white arrowheads). Bar: 1 cm.

completely phagocytosed by foreign body giant cells. Cytology samples collected from animal No. 2 consisted of erythrocytes and scant nucleated cells, of which >95% were neutrophils. No bacteria or other infectious agents were present, and no pathogenic bacteria were cultured. Granulomatous or pyogranulomatous lesions with fibrosis were present at 25 and 60 days postvaccination. By 140 days postvaccination, granulomatous myositis persisted in 2 of 4 cows ex-

amined, with varying degrees of myositis or fibrosis (or both) in the other 2 (Table 1).

The results of the experimental study are shown (Figs. 5–12; Table 2). None of 6 animals injected with the vaccines became ill, febrile, or lame. Palpable swellings developed in 4 animals, 1–4 days postinjection. A visible lesion developed in 1 animal (animal No. 15), which attained maximum size (8.0 × 9.0 cm) 18 days postinjection, after which it decreased slightly

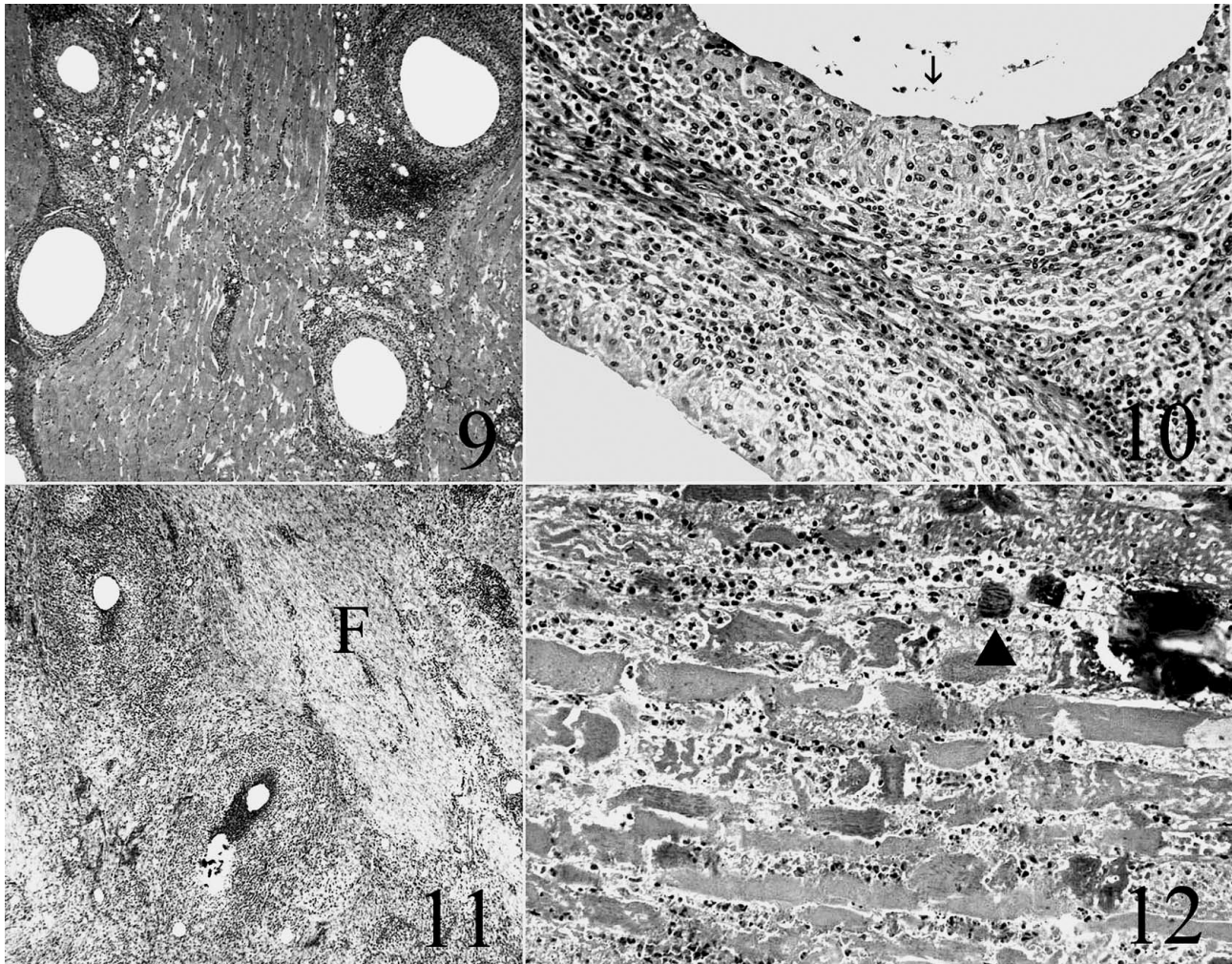


Figure 9. Experimental vaccination. Low-power micrograph of *M. biceps femoris* 21 days after injection with vaccine A. There are ramifying granulomas in endomysium. Large clear spaces correspond to product extracted during tissue processing. Fibrosis is minimal. HE.

Figure 10. Experimental vaccination. Higher magnification of type of lesion illustrated in Fig. 9 due to vaccine A. The reaction consists predominantly of epithelioid macrophages. Whisps of membranous material (arrow) are present in oil droplet spaces. HE.

Figure 11. Experimental vaccination. Low-power micrographs of injection site lesion in *M. biceps femoris* 21 days after injection with vaccine B. There are widely spaced small clear spaces corresponding to extracted vaccine oil; compare with large pools of retained oil due to vaccine A in Fig. 9, taken at same magnification. There is severe diffuse fibrosis (F). HE.

Figure 12. Experimental vaccination. Higher magnification of injection site lesion in *M. biceps femoris* 21 days after injection with vaccine B. Note mineralization (arrowhead), necrosis, and histiocytic myophagocytosis. HE.

(8.5 × 8.0 cm) until euthanasia and necropsy at 21 days (Fig. 5). At necropsy, the 4 animals given 5 times the recommended dose of vaccine had bilateral, grossly visible lesions containing intralesional oil (Fig. 6; Table 2). The largest lesions were due to vaccine B and were 6.0 cm at 7 days postvaccination and 15 cm at 21 days postvaccination. There was mild subcapsular and cortical granulomatous lymphadenitis in pre-femoral and sublumbar lymph nodes of the 4 calves. No lesions were found elsewhere.

Both yearling cattle given the recommended dose of vaccine and examined 65 days later had bilateral lesions in *M. biceps femoris* (Fig. 7). Lesions were pale,

discreet, firm, and cigar shaped, with the long axis parallel to muscle fibers. The lesions in 1 animal contained oil. Maximum dimensions were 12 cm (vaccine B) and 9 cm (vaccine A)(Fig. 8). Microscopically, lesions due to the 2 vaccines were distinct from each other, based on examination of surgical biopsy samples at 7–28 days postvaccination, as well as at necropsy 65 days after vaccination. Vaccine A induced ramifying pyogranulomatous myositis that dissected between muscle bundles and was associated with minimal fibrosis. Pyogranulomas contained large, clear central spaces consistent with the presence of lipid (Fig. 9). The lipid stained positively with osmium tetroxide.

The inflammatory infiltrate consisted of epithelioid macrophages with peripheral nodular aggregates of small lymphocytes (Fig. 10). Lesions due to vaccine B were associated with myonecrosis, mineralization, and fibrosis (Figs. 11, 12). Clear spaces in pyogranulomas were smaller and scantier than those associated with vaccine A (compare Fig. 9 with Fig. 11). No lesions were present in lymph nodes draining affected muscle (prefemoral, iliac, and sublumbar lymph nodes) or in other tissues.

Vaccine A contained 0.3 endotoxin units (EU)/ml. Vaccine B contained 2 million EU/ml. No bacteria were grown in culture from either vaccine.

Selenium concentrations in whole blood from animal Nos. 1–6 were 0.36–0.47 $\mu\text{g/ml}$ (reference range: 0.12–0.5 $\mu\text{g/ml}$). Serum copper was 0.57–0.84 $\mu\text{g/gl}$ (reference range: 0.6–1.2 $\mu\text{g/ml}$). Serum iron, manganese, molybdenum, and zinc were within reference ranges (data not shown). Hepatic copper and selenium were 25.2 $\mu\text{g/gm}$ and 0.69 $\mu\text{g/gm}$ in animal No. 7 and 16.59 $\mu\text{g/gm}$ and 0.53 $\mu\text{g/gm}$ in animal No. 8 (reference range: 25–100 $\mu\text{g/gm}$ copper; 0.25–0.75 $\mu\text{g/gm}$ selenium). Hepatic concentrations of iron, manganese, molybdenum, and zinc were within reference ranges in both animals (data not shown).

Discussion

Lameness and recumbency in the Nebraska herd was attributed to 2 factors: the use of irritating water-in-oil adjuvanted vaccines and bilateral injection into hip muscles of near-term cattle. The lesions induced by vaccination were histologically similar to those in an earlier report when a related vaccine injected into paravertebral muscles induced severe foreign body myositis and pachymeningitis.¹⁹ Water-in-oil adjuvanted vaccines are rarely used in humans because of their irritancy. They remain widely used for food animals in the United States.¹³ The size of lesions in ranch cattle in this study was comparable with those produced experimentally when 10 adult cattle were vaccinated with a related vaccine^j and examined 21 days later (G. Mason, personal communication). In that study, which was sponsored by a commercial competitor, the geometric mean size and weight of lesions at necropsy in muscles of the neck at 21 days were 25.5 cm and 783 gm, respectively (Pfizer Animal Health Technical Bulletin Feb 2001). Locally irritating products should not be injected into muscles of the hip in late-term cows. Industry advocates recommend that muscles of the neck be used as an injection site for intramuscular products.²³ The episode reported here underscores the recommendation.

Lameness and large intramuscular lesions were not induced in calves and yearling cattle when an attempt was made to reproduce the syndrome seen in the ranch

herd. Several factors may explain this. None of the experimental animals were in late pregnancy. Near-term cows are more likely to become lame or recumbent (or both) because of myositis affecting muscles of the hip, compared with nongravid animals. Experimental cattle, unlike those in the Nebraska herd, were not vaccinated previously with either product. Hypersensitivity reactions are reported or suspected to occur when water-in-oil-adjuvanted vaccines are used in people and animals.^{13,24} Failure to induce grossly visible swelling in all experimental animals may be attributable to more consistent experimental placement of vaccine into muscle. There was probably more variability, including subcutaneous placement of vaccine, in animals on the ranch (Fig. 3), with the result that gross swelling was more readily detected than in the experimental animals.

Representatives of the company reported they were unaware of problems after use of either vaccine in herds elsewhere, and offered several explanations for the episode. These included poor injection technique (delivery of vaccines into subcutaneous tissue, rather than muscle), concurrent administration of the vitamin A and D product in muscles of the hip, low nutritional copper and high nutritional selenium status in the herd, hypocalcemia (in 1 recumbent cow), and use of vaccines too close to parturition. Biopsy samples taken from injection sites 12 days after vaccination demonstrated that vaccine was consistently present in muscle. The small size of biopsy samples precluded any conclusion about concurrent subcutaneous placement. The administration of a vitamin A and D product into muscles of the left hip may explain why larger lesions tended to occur on that side at the ranch. Laboratory analysis did not confirm the company's suspicion of low dietary copper and high dietary selenium. The ranch is located in a seleniferous area of northwestern Nebraska, but we are not aware of an association between high dietary selenium, low dietary copper, and postvaccination granulomatous myositis. Blood and hepatic concentrations of selenium were within reference ranges, and none of the cows in the Nebraska herd had clinical selenosis.^{20,21} Both vaccines were given to cows within 3 weeks of parturition. There was a recommendation on the product labels that vaccines A and B be given to pregnant cows 8–10 weeks and 1–3 months before calving, respectively. There was no warning about vaccinating near-term cattle.

Grossly, lesions resembled the injection site category of "scar with nodules" used to classify injection site lesions in muscle.²⁸ The histological character of lesions was typical of severe inflammatory reactions that occurs in response to water-in-oil vaccines.¹ Oil droplets were smaller and less numerous with vaccine B, indicating quicker absorption or dispersal in mus-

cle. Both lesions progressed over time to form locally extensive intramuscular fibrosis ("clear scars," as defined by the Colorado system).²⁸ Vaccine was grossly visible in muscle at 60 and 65 days postvaccination, by which time vaccinated animals may go for slaughter because the withholding time is 60 days. This suggests that the time to slaughter should be extended or that the vaccines be reformulated for more rapid dispersal and absorption.

It was not possible to account for differences in the character of histological lesions associated with the 2 vaccines in the absence of information from the company about their composition. Vaccine B had a higher concentration of endotoxin. There are no published studies associating severity of fibrosis due to oil-in-water vaccines with endotoxin concentration, although an association has been proposed.¹ According to the USDA, the presence of endotoxin in vaccine B at a concentration of 2 million EU/ml is not linked to adverse reactions (D. E. Starling, personal communication). Other cellular components of *E. coli* or *C. perfringens* (or both) may be fibrogenic. An organomercurial preservative, thimerosal, was present in both vaccines. Thimerosal is occasionally associated with delayed-type hypersensitivity reactions in people.^{3-5,12}

The USDA CVB is responsible for ensuring the safety, purity, and efficacy of veterinary biologics. The CVB received no other reports of adverse reactions to either vaccine. Of an estimated 10,000 adverse reaction reports toward veterinary biological products each year in the United States, <1% are reported directly to the CVB.²² Producers in the United States can purchase most veterinary vaccines directly from manufacturers and administer them without the intervention of a veterinarian. In such situations, producers lack detailed information about the vaccines they are using, other than what is provided on the label or vaccine insert or in promotional advertising, or know how to report suspected adverse reactions and vaccine failures to the CVB. Personnel at the CVB did not consider lesions in the Nebraska herd unduly severe, given that there was a warning on the vaccine of persistent swellings at sites of injection, and because the producer elected to vaccinate cattle close to term.

Producers and veterinarians rely on the USDA and vaccine manufacturers for accurate, timely data about safety and efficacy of vaccines and alerts about postmarketing problems. Current prelicensing safety tests for inactivated bacterial vaccines are unlikely to detect all but the most overt, consistently induced adverse reaction. Prelicensing studies of vaccines are not specifically designed to detect adverse vaccine reactions.¹¹ The evaluation is performed in rodents (CFR 9—Animals and Animal Products; §113.33 (b) Mouse Safety Tests and §113.38 Guinea Pig Safety Test). Although

there is provision for a calf safety test under the Code of Federal Regulations, it is not mandatory for inactivated bacterial vaccines. That test involves injecting 2 calves with 10 times the standard dose of vaccine, followed by daily observation for 21 days (CFR—Animals and Animal Products; §113.41 Calf Safety Test). A vaccine is considered satisfactory if neither calf develops unfavorable reactions during 3 weeks of observation. Necropsy and histological examination are not required as part of the protocol, although companies may elect to do this. The severity of reactions induced by vaccines, particularly intramuscular products, may be difficult to assess without postmortem and histological examination.

This situation may be redressed in part when federal regulations governing the collection and dissemination of adverse reaction reports in the United States are changed. The USDA recently proposed to veterinary biological manufacturers that the latter record and transmit all reports of suspect adverse reactions received from the field to the CVB. This would be an efficient way to document and share adverse vaccine reaction reports, particularly those occurring at low to moderate frequency, provided that annual summary data are made publicly available by the CVB, as happens in the UK, Sweden, and Canada.^{11,27} Sharing summary adverse reaction data with diagnosticians, veterinarians, and producers would go some way toward developing a more transparent postmarketing surveillance system for US veterinary biological products.

Acknowledgements

We thank the Wohlers family for cooperation in conducting the study. It was supported by the Wyoming Agricultural Experiment Station.

Sources and manufacturers

- a. Scour Bos[®] 4, Grand Laboratories, Inc., Larchwood, IA.
- b. Bovine Pilishield[®] + C, Grand Laboratories, Inc., Larchwood, IA.
- c. Xtend III[®], Grand Laboratories, Inc., Larchwood, IA.
- d. Vita-Jec A & D 500, Agripharm, Walco International Inc., Grapevine, TX.
- e. Toxoid BarVac CD, Boehringer Ingelheim, St. Joseph, MO.
- f. Banamine, Schering-Plough Animal Health, Omaha, NE.
- g. Bio-Mycin C, Schering-Plough Animal Health, Omaha, NE.
- h. Cape Cod Associates, East Falmouth, MD.
- i. Allegiance Tru-Cut[®] biopsy needle, MWI Veterinary Supply Company, Denver, CO.
- j. Scour Bos[®] 6, Grand Laboratories, Inc., Larchwood, IA.

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