Systemic adverse reactions in young Simmental calves following administration of a combination vaccine

John A. Ellis, Choon Yong

Abstract — Combination vaccines containing viral and bacterial antigens are commonly used in veterinary practice and have been associated with adverse reactions. A group of young Simmental calves developed fever and depression following administration of a mixed vaccine, and 1 died with pulmonary edema, suggesting that endotoxins or other bacterial components may interact synergistically with some adjuvants to cause an enhanced pathologic inflammatory response in some individuals.

Résumé — Réactions systémiques indésirables chez de jeunes veaux Simmenthal à la suite de l'administration d'un vaccin polyvalent. Les vaccins polyvalents contenant des antigènes viraux et bactériens sont utilisés fréquemment en pratique vétérinaire et ont été associés à des réactions indésirables. Un groupe de jeunes veaux a manifesté des symptômes de fièvre et de dépression à la suite de l'administration d'un vaccin polyvalent contenant des antigènes viraux et bactériens. Un animal est décédé à la suite d'œdème pulmonaire, suggérant que les endotoxines ou d'autres composantes bactériennes puissent réagir de façon synergique avec certains adjuvants pour causer une augmentation de la réponse inflammatoire pathologique chez certains individus.

(Traduit par docteur André Blouin)

Can Vet J 1997; 38: 45–47

Each year, millions of doses of mixed vaccines containing viruses and bacteria are used in cattle. During the process of fulfilling federally mandated licensing requirements, these vaccines have been shown to be safe in the target animals and to stimulate immune responses to the constituent pathogens, to at least the level achieved when the individual components are administered in a single component vaccine. Over the years since the development of combination vaccines for use in livestock and other species, there have been reports of adverse effects associated with vaccination. Unfortunately, most reports of adverse reactions following vaccination have been anecdotal, and relatively few have been documented in the scientific or professional literature, making it difficult to assess mechanisms by which certain vaccine components, or combinations thereof, may cause adverse reactions. More information regarding adverse reactions would be useful for practitioners making recommendations on the use of current vaccines, and in the design of improved vaccines. Herein, we report an adverse reaction following vaccination of a group of young calves with a mixed vaccine.

Twenty-one, suckling, apparently healthy, Simmental calves approximately 7 to 30 d of age were vaccinated in the morning, IM, in the rear limbs with a combination vaccine (Virabos 4 + HS, Vetrepharm, London, Ontario) containing inactivated bovine viral diarrhea virus, parainfluenza-3 virus, bovine respiratory syncytial virus, a *Haemophilus somnus* bacterin, and an adjuvant (Immunostim, Vetrepharm, London, Ontario) containing mycobacterial cell wall components. It took approximately 1 h to vaccinate the calves and no adverse reactions were noted at the time of vaccination, or immediately thereafter. Calves were checked by the owner in the early afternoon and appeared normal. Later in the afternoon, approximately 8 to 10 h after vaccination, 1 calf was found dead, and the remaining 20 calves were severely depressed and listless. Calves were reluctant to stand, refused to suck, and invariably had large, hot swellings at the site of injection. Calves appeared stiff and sore when persuaded to move. Rectal temperatures were taken in several calves and all were ≥40°C. Approximately 12 h after vaccination, 15 of the 20 calves were easily caught and treated with long-acting penicillin (Penlong XL, rogar/STB, London, Ontario), 33 000 IU/kg body weight, and dexamethasone (dose not specified in history). Five of the calves were not as severely depressed and escaped treatment. The next morning the calves were considered to be almost normal clinically, with improved dispositions and normal temperatures.

The calf that died was 1 of the older calves (approximately 20 d of age). At initial examination, approximately 2 h after death, there was moderate swelling at the injection site. Necropsy was performed approximately 24 h after death. The diaphragmatic lobes of the lungs were heavy, wet, and reddened. The kidneys were dark red and there was bilateral perirenal hemorrhage. Examination of the musculature of the back and hindquarters did not reveal any gross evidence of hemorrhage, edema, or needle marks. Gross lesions in other organ systems were not observed. Bacterial cultures of selected organs revealed scant growth of contaminants,

Department of Veterinary Microbiology, Western College of Veterinary Medicine, University of Saskatchewan, 52 Campus Drive, Saskatoon, Saskatchewan S7N 5B4 (Ellis); Saskatchewan Agriculture and Agri-Food Canada, Veterinary Diagnostic Laboratory, 4840 Wascana Parkway, Regina, Saskatchewan S4S 0B1 (Yong).

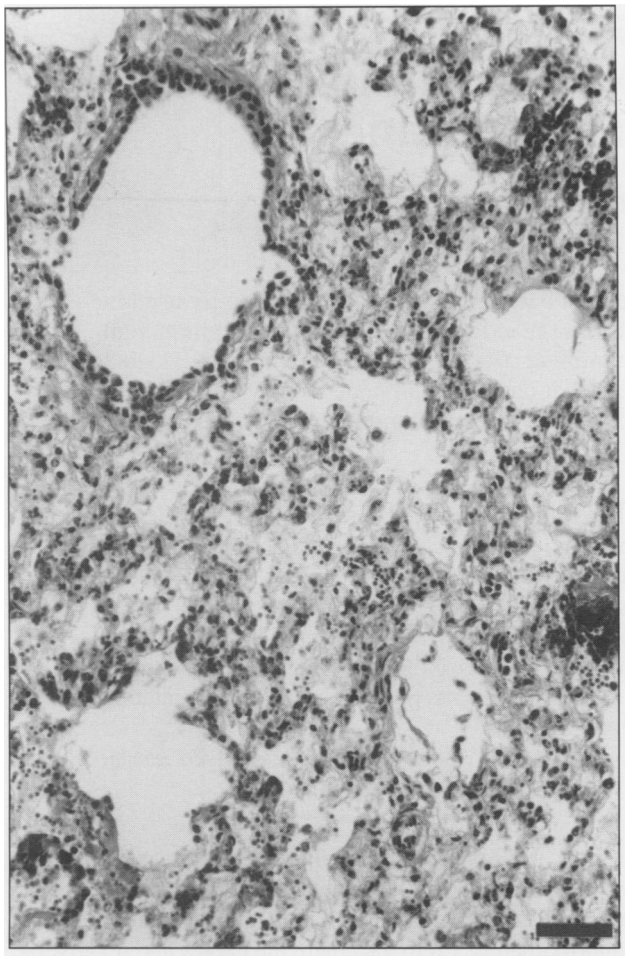

Figure 1. Section of lung from a calf that died approximately 8 h after vaccination. Note multifocal edema, intra-alveolar hemorrhage, and mild infiltration of granulocytes and alveolar macrophages. Hematoxylin and eosin stain. Bar = 88 μm.

nonhemolytic *Escherichia coli* and alpha group streptococci, from liver, lung, and brain. There was moderate growth of *Clostridium sordelli* from renal tissue, also considered a contaminant. Histologically, there were mild autolytic changes in the lungs. In addition, multifocally there was intra-alveolar hemorrhage, edema, and infiltration by granulocytes and pulmonary macrophages, indicative of an acute inflammatory process. The kidneys were congested and moderately autolytic. Only mild autolytic changes were found in sections of heart, small intestine, adrenal gland, brain, and skeletal muscle.

The observed clinical signs and pulmonary lesions in the calf that died could be compatible with a type I hypersensitivity (anaphylactic) reaction (1) or cytokine-mediated injury, as may occur after exposure to bacterial endotoxins (2–4). The apparent 100% morbidity and the prolonged (hours) duration of onset of clinical signs after vaccine administration indicate that an anaphylactic reaction to a vaccine component was probably not responsible for the observed adverse reactions. Type 1 hypersensitivity reactions usually commence within minutes after administration of an allergen and involve only 1 or a few animals in any given population of outbred animals that have not previously been sensitized (1).

Endotoxins are constituents of the cell wall of many gram-negative bacteria (5). They are macromolecular complexes comprising lipopolysaccharide (LPS), proteins, and phospholipids (5). Lipopolysaccharide is composed of a polysaccharide of variable chain length and a covalently bound lipid component, termed lipid A (5). It is recognized that the lipid A portion of the molecule is responsible for the biological activities of LPS (5). Endotoxins have been shown to have pleiotropic physiological effects when administered to cattle, including vasoactive effects that can be manifested clinically in pulmonary lesions, similar to those found in the calf that died following vaccination (2–4). These bacterial toxins stimulate the production of "proinflammatory" cytokines, such as interleukin-1 (IL-1), IL-6, and tumor necrosis factor (TNF), primarily by macrophages (4,6). However, it is becoming apparent that endotoxin is just one of numerous so-called "modulins" of gram-negative and gram-positive bacteria that have these cytokine-inducing inflammatory effects.

It is currently thought that inflammatory mediators (cytokines) produced by the host are primarily responsible for the pathological effects seen in septicemia due to gram-negative infections, and in experimental models of endotoxin administration (4,6). In addition, the lipid A component of endotoxins has documented, potent adjuvant effects (7). Some of the same cytokines, such as IL-1 and TNF, are thought to mediate both pathologic and adjuvant effects (4,6,7). Whether or not a particular animal experiences potentially beneficial, adjuvant, or life-threatening shock-like effects following endotoxin administration is probably related to the dose of endotoxin (4,6,7). *Haemophilus somnus*, a specific component of the vaccine that was administered to the calves, is a gram-negative bacterium that contains lipooligosaccharides (8). It has been proposed that many of the lesions associated with naturally acquired *H. somnus* infections in cattle are mediated by the endotoxin activity of the lipooligosaccarides (8).

There are numerous factors, in addition to dose, that can affect the response to endotoxins, including the age, genetics, and immune status of the recipient (2,6). The affected calves in this case were all ≤30 d of age. An older calf on this farm that was given the same vaccine apparently experienced no untoward effects. This particular mixed vaccine is not recommended for use in calves less than 3 mo of age. Therefore, the young age of these calves or low concentrations of endotoxin-specific colostral antibodies may have been a determining factor in the adverse response to vaccination. There is substantial data from experiments in laboratory rodents that breed or strain can significantly affect the response to parenterally administered endotoxins (6). Unfortunately, similar comparative data are not available for cattle or other domestic species.

Virtually all of the available data concerning the effects of parenterally administered endotoxin in cattle are based on experiments in which endotoxin, usually from *E. coli*, was given, IV, in saline (2). There is scant information on how the kinetics of endotoxin release or the effects of endotoxin may be altered by administering endotoxin in commercially available vaccines, the chemical composition of which is markedly different

from saline. Neither is there much information concerning the comparative potency of various endotoxins or other proinflammatory components (5) from gram-negative bovine pathogens, such as *P. haemolytica* and *H. somnus*, that are included in mixed vaccines. Although it is known that breakdown of bacterial cells releases endotoxins (4,5), how prolonged storage of vaccines may affect the levels of available endotoxin in various formulations is poorly documented. Furthermore, there is scant information concerning how components of *Clostridium* spp, such as bioactive exotoxins, may interact with endotoxins in vaccines. Currently federal regulatory agencies in Canada and the United States do not have mandated limits for endotoxin in vaccines, neither do they routinely monitor vaccines for endotoxin concentrations, except in the case of reported complaints or adverse reactions.

It may be that endotoxins contained in the vaccine alone did not cause the adverse effects seen in these calves. Administration of a *P. haemolytica* bacterin (Oneshot, Pfizer Animal Health, Exton, Pennsylvania, USA) and a mixed viral vaccine (CattleMaster-4, Pfizer Animal Health) simultaneously in the hind limbs of 9-day-old calves was recently reported (9). Aside from local swellings noted at the site of bacterin injection in a minority of the calves 24 h after administration, no adverse systemic effects were observed. It is known that the *P. haemolytica* bacterin contains high levels of endotoxin activity (10). These different clinical outcomes in young calves subsequent to administration of vaccines with endotoxin activity suggest that other factors may be responsible for the adverse reactions observed in this case, such as an interaction between endotoxin and other vaccine components. One of the components of the adjuvant in the vaccine used in this case is unspecified constituents of mycobacterial cell walls. Mycobacterial components have been shown to have potent adjuvant effects in other species (7). Furthermore, in murine models, it has been shown that a synthetic mycobacterial adjuvant, mauramyl dipeptide (MDP), acts synergistically with endotoxins to stimulate enhanced secretion of TNF, IL-1, and IL-6, which can result in enhanced shock-like disease (7). Whether or not similar phenomena occur in cattle and other species of interest to the clinical veterinarian is not known. Alternatively, proinflammatory components of *H. somnus*, other than endotoxin (5), may have acted alone or in concert with adjuvant to stimulate cytokine secretion and be primarily responsible for the adverse reactions.

In this case, corticosteroids were administered to clinically affected calves, all of which recovered. Although these pharmacologic agents have been advocated for the treatment of septic shock (11), recent evidence from humans suggests that in order to be effective, steroids must be administered prior to endotoxic insults (4) because, for example, dexamethasone acts by inhibiting transcription of messenger RNA in macrophages and other cells, thereby preventing TNF production. Therefore once macrophages have been exposed to cytokine-stimulating components of bacteria, their TNF response is not prevented and the treatment is ineffective. Current treatment of endotoxin-induced lung injury and sepsis is supportive, and aimed primarily at maintaining compromised hemodynamics (4,11). Current research in therapy for cytokine-mediated acute lung injury induced by bacterial components is aimed at inhibiting the host's response to proinflammatory mediators (4). CVJ

References

1. Landiges WC, Dickinson EO, Gorham JR. Clinical and pathological comparison of the pulmonary response during experimentally induced anaphylaxis in sheep and cattle. Am J Vet Res 1974; 35: 389–392.
2. Cullor JS. Shock attributable to bacteremia and endotoxemia in cattle: Clinical and experimental findings. J Am Vet Med Assoc 1992; 200: 1894–1902.
3. Slocombe RF, Mulks M, Killingsworth CR, Derksen FJ, Robinson E. Effect of *Pasteurella haemolytica*-derived endotoxin on pulmonary structure and function in calves. Am J Vet Res 1990; 51: 433–438.
4. Welbourn CRB, Young Y. Endotoxin, septic shock and acute lung injury: Neutrophils, macrophages and inflammatory mediators. Br J Surg 1992; 79: 998–1003.
5. Henderson B, Wilson M. Modulins: A new class of cytokine-inducing, pro-inflammatory bacterial virulence factor. Inflamm Res 1995; 44: 187–197.
6. Alving C. Lipopolysaccharide, lipid A and liposomes containing lipid A as immunologic adjuvants. Immunobiol 1993; 187: 430–446.
7. Galanos C, Feudenberg MA. Mechanisms of endotoxin shock and endotoxin hypersensitivity. Immunobiol 1993; 187: 346–356.
8. Inzana TJ, Iritani B, Gogolewski RP, Kania SA, Corbeil LB. Purification and characterization of lipooligosaccharides from four strains of "*Haemophilus somnus*." Infect Immun 1988; 56: 2830–2837.
9. Ellis JA, Hassard LE, Cortese VS, Morley PS. Effects of perinatal vaccination on humoral and cellular immune responses in cows and young calves. J Am Vet Med Assoc 1996; 208: 393–400.
10. Cullor JS. Safety and efficacy of gram-negative bacterial vaccines. Proc Annu Meet Am Assoc Bovine Prac 1993; 26: 13–18.
11. Haskins SC. Management of septic shock. J Am Vet Med Assoc 1992; 200: 1915–1924.