Fatal, generalized bovine herpesvirus type-1 infection associated with a modified-live infectious bovine rhinotracheitis parainfluenza-3 vaccine administered to neonatal calves

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Abstract

Generalized bovine herpesvirus 1 (BHV-1) infection was diagnosed in six Salers calves from the same herd. The calves had received an intramuscular injection of modified-live infectious bovine rhinotracheitis parainfluenza-3 vaccine between birth and three days of age. The purpose of this study was to determine if the outbreak was associated with the vaccine strain of BHV-1. Analysis of epidemiological data and BHV-1 DNA for restriction fragment length polymorphism was undertaken. Multifocal necrosis in multiple organs was observed on pathological examination, and the presence of BHV-1 in tissues was confirmed by immunohistochemistry. Forty-three calves (aged birth to thirty days) were vaccinated over an 11-day interval. The 10 deaths recorded for vaccinated calves were clustered over a subsequent 14-day interval. Mortality in calves vaccinated between birth and three days of age was significantly higher than in nonvaccinated calves (chi-square test; $p \leq 0.025$), and this mortality was characterized by a greater age at death and duration of illness for vaccinated calves (*t* test; $p \leq 0.001$). The patterns of the restriction fragments, generated by six restriction endonucleases, of BHV-1 isolated from a necropsied calf and from the vaccine were identical, and different from that of a laboratory strain of BHV-1 (P8-2). These findings support the conclusion that newborn calves were susceptible to an intramuscularly injected vaccine strain of BHV-1, and that administration of an intramuscular modified-live infectious bovine rhinotracheitis parainfluenza-3 vaccine to neonatal calves may not be an innocuous procedure.

Résumé

Infection généralisée, fatale, causée par le virus herpès type I suite à l'administration d'un vaccin vivant atténué contre la rhinotrachéite infectieuse bovine, parainfluenza-3 à des veaux néonatals

Une infection généralisée causée par le virus herpès bovin type I (VHG-1) a été diagnostiquée chez six veaux d'un troupeau. Les veaux avaient été immunisés avec un vaccin vivant atténué contre la rhinotrachéite infectieuse bovine, parainfluenza-3 par voie intramusculaire durant les trois premiers jours postpartum. Le but de cette étude était de déterminer si l'apparition de la maladie était associée à la souche du vaccin VHB-1. L'analyse des données épidémiologiques et de l'ADN VHB-1 pour étudier le polymorphisme de la longueur des fragments de restriction ont été effectuées. L'examen pathologique a démontré de multiples foyers de nécrose dans plusieurs organes et la présence du VHB-1 dans les tissus a été confirmée par épreuve d'immunohistochimie. Quarante-trois veaux âgés de 1 à 30 jours ont été vaccinés sur une période de 11 jours. Les 10 mortalités des veaux vaccinés sont survenues en groupe sur une période subséquente de 14 jours. Le taux de mortalité chez les veaux vaccinés durant les trois premiers jours postpartum était plus élevé (chi carré; $p \leq 0{,}025$) que celui du groupe des veaux non vaccinés. De plus, la mortalité survenait à un âge plus avancé et après une plus longue durée de la maladie (test T; $p \leq 0{,}001$). Les caractéristiques des fragments de restriction de l'ADN, obtenues suite à la digestion par six endonucléases de restriction, d'isolats de VHB-1 provenant de tissus d'un veau et du vaccin étaient identiques, mais différaient de ceux provenant d'une souche de laboratoire de VHB-1 (P8-2). Ces résultats sont en accord avec la conclusion que les veaux néonatals étaient susceptibles à la souche du vaccin VHB-1 administré par voie intramusculaire et que l'administration d'un vaccin vivant atténué contre la rhinotrachéite infectieuse bovine, parainfluenza-3 par voie intramusculaire à des veaux néonatals peut ne pas être sans risque.

(Traduit par Dr Thérèse Lanthier)

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Introduction

Bovine herpesvirus 1 (BHV-1) causes a variety of diseases in cattle, including rhinotracheitis, conjunctivitis, infectious pustular vulvovaginitis or balanoposthitis, abortion, meningoencephalitis, enteritis, and a generalized infection in young calves (1). The differences in disease manifestation may be due to viral strain differences, the route of infection, the host's immune status, and management practices (2). A generalized infection in young

calves has been well documented (3–9) but is considered uncommon (1). The lesions in young calves consist of necrotizing foci in multiple organs, including the upper respiratory tract, upper gastrointestinal tract, liver, spleen, lymph nodes, and kidney (1).

The intramuscular injection of neonatal calves with a modified-live infectious bovine rhinotracheitis (ML-IBR) vaccine is not a routine management procedure, and the vaccine strain of BHV-1 is a rarely considered or confirmed cause of generalized BHV-1 infection (5). Modified-live vaccines are commonly used in veterinary medicine, although risks have been documented (10). Disease is associated with residual virulence of the vaccine strain virus or contamination of the product with other infective agents (10). Intramuscular injection of ML-IBR vaccine has been suspected of causing abortion in cows that were immunologically naive to BHV-1 (11,12). Vaccine strains of BHV-1 have been shown to cause oophoritis in cattle when injected intravenously (13,14). Recent publications have implied that the administration of ML-IBR vaccines to calves as young as one day old is innocuous, protective (15), and may be a treatment choice during an epizootic of IBR in neonatal calves (9).

A breeder of purebred Salers experienced higher than expected calf losses during the spring of 1987. Six calves were submitted for postmortem examination, and all were determined to have generalized BHV-1 infection. The calves had been injected intramuscularly with a modified-live bovine infectious rhinotracheitis-parainfluenza type 3 (IM-ML-IBR/PI_3) vaccine within three days of birth. This report presents the pathological, epidemiological, and virological findings from this outbreak.

Materials and methods

Pathology

Six calves were submitted to the Airdrie Veterinary Laboratory for postmortem examination. The clinical history on the calves was brief; the owner reported that three calves were weak and depressed, two calves had scours, and one calf was in respiratory distress. The calves did not respond to antibiotic therapy. After routine gross examination, selected tissues were fixed in 10% neutral buffered formalin, processed for standard paraffin embedding prior to sectioning at 4 μm, and stained with hematoxylin and eosin (HE). Selected tissues were frozen at −70°C or submitted immediately for bacteriological examination. After the tissue sections had been examined microscopically, frozen tissues were submitted for virological examination. Immunohistochemical staining was performed on serial sections of tissue blocks from which the sections stained with HE had been cut. The BHV-1 antigens were detected with an avidin-biotin complex immunoperoxidase method (16). Tissue sections were stained with a cocktail of three monoclonal antibodies to various proteins of BHV-1 (17).

Three negative controls were included during the staining procedure. Tissue sections were stained with the monoclonal antibody to BHV-1 omitted, the monoclonal antibody to BHV-1 substituted with an irrelevant monoclonal antibody, and the monoclonal antibody to BHV-1 substituted with monoclonal antibodies to parainfluenza-3 (PI_3). As a positive control, tissue sections from a feedlot animal with confirmed BHV-1 tracheitis were included in the staining procedure.

Epidemiology

An interview was conducted with the farmer during the disease outbreak to investigate the management of the herd. The herd consisted of 149 purebred Salers cows with 21 heifers and 128 cows pastured separately. They were fed oat greenfeed, grass hay, and mineral and vitamin supplements, and were bedded on straw. The cows had not been vaccinated for BHV-1 for at least two years, although calves were routinely given an IM-ML-IBR/PI_3 vaccine at weaning. Scours in calves, aged from birth to three weeks, began in calves out of heifers and spread to calves out of cows. Initially, the calves responded to fluid therapy and antibiotic treatment. Intramuscular ML-IBR/PI_3 vaccine had been recommended as an additional treatment to help prevent the spread of the enteritis. Fecal and postcolostral serum samples were collected from two calves in the herd, and examined for *Salmonella* spp. and BHV-1 titer (ELISA), respectively. One calf was vaccinated with IM-ML-IBR/PI_3 vaccine at one day of age and the serum sample was collected six days later. This calf died of generalized BHV-1 infection nine days postvaccination and was submitted for postmortem. The second calf was not vaccinated, and the serum sample was collected at one day of age. When calving was finished, the farmer listed the number of calves that died and the cause of death (unconfirmed by postmortem except for the above-mentioned six calves) and the number of calves that had been vaccinated (IM-ML-IBR/PI_3 vaccine) with the calf survival/death information. The data were analyzed using the chi-square test to determine if mortality in calves vaccinated between birth and three days of age was different from that in calves vaccinated between four and thirty days of age; and if mortality in vaccinated calves of the age groups described was different from that in unvaccinated calves. The length of illness, age of dam, and age at death were compared for vaccinated and nonvaccinated calves using Student's *t* est.

Virology

Analysis of BHV-1 DNA for restriction fragment length polymorphism (RFLP) was performed on BHV-1 from three sources: a) virus isolated from a homogenized lymph node of a calf that died from generalized BHV-1 infection eight days after injection with the IM-ML-IBR/PI_3 vaccine (isolate #V1702-87); b) IM-ML-IBR/PI_3 vaccine (Serial number 291132, Tech America Biologics Corp., A Tech America Company, Omaha, Nebraska, USA); and c) the P8-2 strain of BHV-1, which was initially isolated from a field case of infectious bovine rhinotracheitis and has been maintained in the laboratory at the Western College of Veterinary Medicine for several years. Madin-Darby bovine kidney cells (MDBK), in Corning 150 cm^2 tissue culture flasks, were infected with BHV-1 at an approximate multiplicity of infection of 0.1 infectious units per cell. The vaccine virus was treated with an antiserum (Wellcome Research Laboratories, Beckenham, Kent, UK) specific for the SF-4 strain of PI_3 before innoculation of MDBK cells. Four days later, virus was recovered by centrifuging cul-

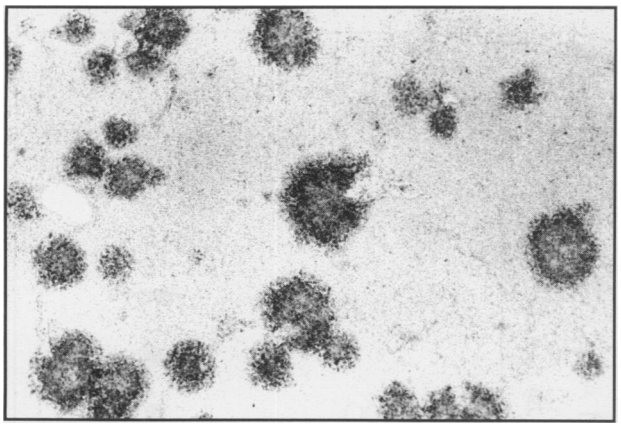

Figure 1. Photomicrograph of the liver of an affected calf (×25). The BHV-1 viral antigen is stained (black, granular appearing material) at the sites of necrosis. Avidin biotin peroxidase complex method, formalin-fixed, paraffin-embedded tissue.

ture supernatants and cell lysates from three flasks at 85,000 × *g*. Virus was resuspended in 2 mL of tris-ethylene diamine tetraacetic acid (TE) buffer (Tris 0.01M, pH 7.5; EDTA 0.001M), and N-lauroylsarcosine (sodium salt) (Sarkosyl, Sigma Chemical Co., St. Louis, Missouri, USA) was added to the 0.5% level and proteinase K to 200 µg/mL level. These lysates were extracted once with phenolchloroform (1:1) and viral DNA was purified by banding in cesium chloride gradients, as previously described (17). About 250 ng of DNA from each sample were digested with one of several restriction endonucleases, including *Eco*RI, *Hind*III, *Hpa*I, *Nco*I, *Pst*I, and *Bgl*II (Bethesda Research Laboratories-Gibco, Ontario). The enzymes were used as suggested by the manufacturer. The digests were electrophoresed through 0.5% agarose gels, which were then stained with ethidium bromide and photographed.

Results

Pathology

On gross examination, the lesions varied among the calves. In one calf, there were no specific changes, and one calf had a necrotizing bronchopneumonia. In variable combinations of organ involvement, the remaining calves had multiple, well-demarcated, areas of pallor (necrosis) in the liver, adrenal glands, kidneys, and rumen. This was often accompanied by a mild fibrinous serositis (epicardial, pleural, and peritoneal). Histologically, in all cases, the lesions were similar in character, although they varied in distribution and severity. In affected organs, there were multiple, randomly located, variably-sized, foci of coagulation necrosis. There were few recognizable inflammatory cells in or around the areas of necrosis, except in the brain where there was a mild diffuse nonsuppurative encephalitis (primarily perivascular lymphocytic infiltration) with focal spongiosis and acute neuronal necrosis. Eosinophilic intranuclear inclusion bodies were occasionally seen within adrenal cortical epithelial cells. Five of the six calves had lesions in at least three systems, with the cortex of the adrenal gland the most consistently and severely affected. The liver and kidney were also frequently affected, although the lesions were not as extensive. Less commonly affected were the lung, brain, rumen, small intestine, lymph nodes, and spleen. In the sixth calf, the lung was the only affected organ with necrotizing lesions in the bronchi and alveoli. The first calf examined had additional lesions, including severe fibrinous peritonitis, pleuritis, omphalophlebitis, and hepatic phlebitis, and a *Salmonella* sp. was isolated from the liver, lung, spleen, and intestine. In all cases, there was specific immunohistochemical staining for BHV-1 antigen at the sites of necrosis (Figure 1). There was no specific staining when the monoclonal antibody to BHV-1 was omitted or substituted with antibody specific for PI_3 or irrelevant antibodies. In two of the three cases in which viral isolation was attempted, BHV-1 virus was recovered from the liver.

Table 1. Number of calves that survived and died in the different age groups vaccinated and among the nonvaccinated calves

Group	n	Survival	Death
Vaccinated[a] at day 0[b] to day 3	29	19	10[c,d]
Vaccinated[a] at day 4 to day 30	14	14	0[e]
Nonvaccinated	106	90	16
Total	149	123	26

[a]Intramuscular injection of modified-live infectious bovine rhinotracheitis parainfluenza-3 vaccine
[b]Day 0 = day of birth
[c]Significantly different than day 4 to day 30 and nonvaccinated (chi-square test; p≤0.025)
[d]Generalized BHV-1 confirmed by postmorten examination of six calves
[e]Not significantly different than nonvaccinated (chi-square test; p≤0.900)

Epidemiology

Herd morbidity was 51%, and the mortality was 17% in calves of both cows and heifers. Of the calves that died, eighteen (69%) were from cows (3–10 yr old), three (12%) were from heifers, and five (19%) were from dams of unspecified age. During an 11-day interval (March 04–14), 43 calves received IM-ML-IBR/PI_3 vaccine between birth (day 0) and day 30. Death losses occurred over a 51-day period (February 11- April 02). All 10 vaccinated calves that died did so during a 14-day interval (March 12–25); whereas, in this period, three nonvaccinated calves died. The numbers of calves that survived and died in the different age groups that were vaccinated with IM-ML-IBR/PI_3 vaccine and among the nonvaccinated calves are given in Table 1. Among calves that died (Table 2), vaccinated calves were older and the length of their illness was longer when they were compared with the nonvaccinated calves (*t* test; p≤0.001). The ages of the dams of the vaccinated calves that died were clustered between three and four years, which contrasted sharply with the distribution of the ages of dams of nonvaccinated calves that died (Figure 2). Furthermore, the mean ages of the dams in the two groups were significantly different (Table 2) (*t* test;

Table 2. Disease pattern associated with death in vaccinated and nonvaccinated calves

Group	Age of dam Year[a]	Age at death Days[a]	Length of illness Days[a]
Vaccinated[b]	3.3±0.7[c]	11.8±2.9[d]	4.2±1.8[d]
Nonvaccinated	5.9±3.0	2.3±3.2	1.1±2.1

[a]Values expressed as mean ± SD
[b]Intramuscular injection of modified-live infectious bovine rhinotracheitis parainfluenza-3 vaccine
[c]Significantly different than nonvaccinated (*t* test, $p \leq 0.05$)
[d]Significantly different than nonvaccinated (*t* test, $p \leq 0.001$)

p≤0.05). The calves from which serum was collected had negative titers for antibodies to BHV-1, and their dams were three years old.

Virology

The patterns of the restriction fragments, generated by the six restriction endonucleases, of BHV-1 isolated from the field case (#V1702-87) and from the vaccine were identical. Furthermore, these patterns were different from those of the P8-2 laboratory strain of BHV-1 (Figure 3). Seen with only *Pst*I and *Nco*I restriction endonucleases, the differences were minor and involved one or two bands in each restriction endonuclease digest.

Discussion

This report demonstrates the hazard of administering an IM-ML-IBR vaccine to calves less than three days of age without sufficient knowledge of the BHV-1 immune status of the herd. The use of BHV-1 vaccines are highly recommended in dairy and beef herd vaccination programs, and the IM-ML-IBR vaccines are considered to be relatively safe, inexpensive, and efficacious, and to rapidly elicit an immune response that provides lifelong immunity after a single dose (15). The risks of abortion and/or vaccine-induced respiratory disease (from recrudescence and shedding of vaccinal virus) are considered minimal, providing the in-contact cattle populations have been properly immunized (15). On the basis of experimental evidence that IM-ML-IBR vaccine elicits an active immune response in one-day-old calves, it has been recommended that an initial BHV-1 vaccination may be administered at any age (9,15). However, practitioners and farmers should be aware of the risks of administering an IM-ML-IBR vaccine to newborn calves, which, in this investigation, were shown to be considerable.

Results of the RFLP analysis are consistent with the conclusion that the mortality was associated with the vaccine strain BHV-1 virus. Extensive use of DNA fragmentation patterns has been made to "fingerprint" herpesvirus isolates. Such information has been used to study the spread of virus during nosocomial outbreaks (18,19), as well as possible vaccine-induced epizootics (12). However, isolates of BHV-1 are relatively homogeneous in their fragmentation patterns (20), and this invariance often makes it easier to support claims of difference rather than identity. Thus, in this investigation, while the RFLP data confirms the possibility that the vaccine and field case (#V170287) isolates shared identity, it does not prove that they were the same virus. Therefore, conclusions drawn from the RFLP results must be corroborated by epidemiological and pathological findings.

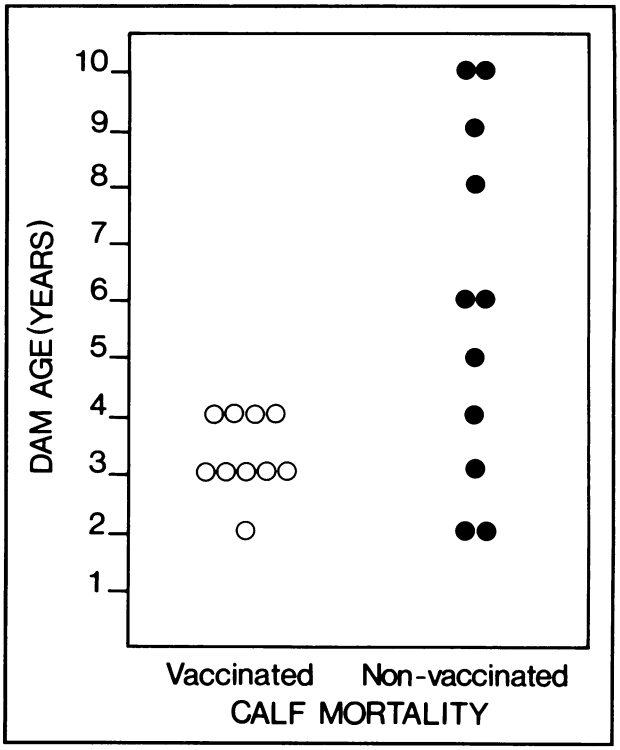

Figure 2. Scatter plot graph comparing the age of the dams of the vaccinated (IM-ML-IBR/PI_3) and nonvaccinated calves that died. Each dot represents one calf.

The epidemiological data support a vaccine-induced BHV-1 epizootic. Mortality in vaccinated calves was significantly higher than in nonvaccinated calves. The disease patterns (Table 2) were very different between the vaccinated and nonvaccinated calves, suggesting that calves in the two groups died of different diseases. Vaccinated calves had a longer duration of illness, were older at death, and their deaths were clustered in a 14-day period. The pattern of death loss in nonvaccinated calves is compatible with that of the common perinatal diseases resulting in mortality during the first week of life (21). Although we cannot absolutely exclude the possibility that deaths in vaccinated calves were the result of field strain BHV-1 exposure, it is unlikely. The occurrence of generalized BHV-1 infection in calves reflects waning maternal antibody (8); therefore, abortions and respiratory disease in cows often accompany the disease in calves (1,4,9). This was not seen in this investigation.

All IM-ML-IBR/PI_3 vaccinated calves that died were vaccinated between birth and three days of age. This suggests an age-related susceptibility to generalized BHV-1 infection. An increased susceptibility of neonates to systemic herpesvirus infection is recognized in other species, including dogs, humans, guinea pigs, and mice (22,23). Infection may be localized or disseminated, and although all the factors contributing to either limiting the infection or to dissemination are not known (23), neu-

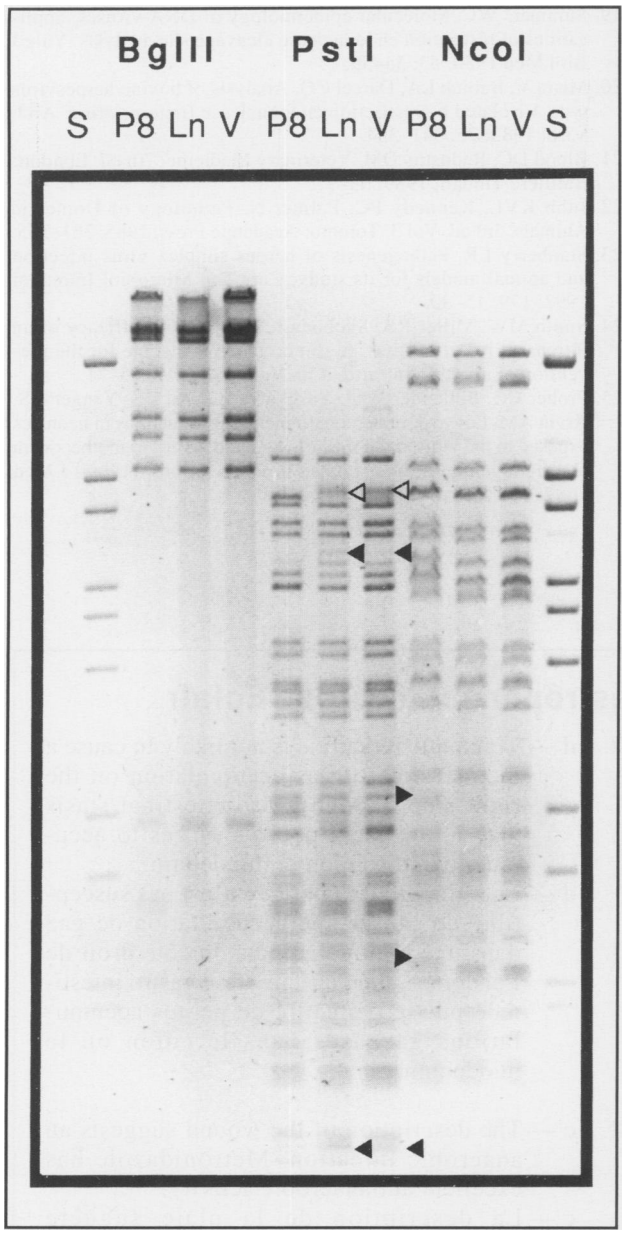

Figure 3. Restriction endonuclease fragment pattern of DNA from the P8-2 strain of BHV-1 (P8), virus isolated from lymph node (Ln) and vaccine (V). DNA from purified virus was digested with either *Bgl*II, *Pst*I, or *Nco*I. DNA fragments of known size (14.1kb, 7.2kb, 6.3kb, 4.8kb, 4.3kb, 3.6kb, 2.3kb, 1.9kb, 1.4kb and 1.3kb), produced by digesting bacteriophag e λ DNA with *Bst*EII, were electrophoresed on the same gel as size markers (S). Extra fragments in a digest (▶) or bands of heterogeneous size (▷, "fuzzy") are marked.

tralizing antibody appears to be important in protecting the neonate from infection (8,24,25). In a pertinent study, five BHV-1 seronegative calves that were exposed by aerosol to BHV-1 at 48 h of age died (or were killed for humane reasons), whereas five calves fed colostrum containing BHV-1 serum neutralizing antibodies prior to the aerosol exposure remained healthy (8). Calves in this investigation were from three- and four-year-old dams (Figure 2), suggesting poor colostral transfer of BHV-1 antibodies in this group. Both the young age at which the calves were vaccinated and inadequate BHV-1 antibody intake were probably contributing factors in the death of the vaccinated calves.

The portal of entry of the virus appears to have some effect on the distribution of the lesions. Neonatal calves infected orally, intranasally, or via contact had more extensive lesions in the upper respiratory and gastrointestinal tracts (3,7,8) than was seen in this investigation. In utero infection (24) or intravenous injection (3) of BHV-1 in neonatal calves resulted in prominent lesions in the adrenal glands, liver, and kidneys, results that were similar to the findings in this study. One calf in our investigation had only pulmonary involvement, which suggests there are other factors that modify the distribution of the virus. The clinical and gross pathological findings for each case of generalized BHV-1 infection are not always consistent or pathognomonic because of the described variability of organ involvement. Peritonitis has been described previously (6) and can be confused with a bacterial septicemia. A thorough histological examination is required with the etiological diagnosis being quickly confirmed using immunohistochemistry.

In conclusion, this investigation suggests that newborn calves were susceptible to an intramuscularly injected vaccine strain of BHV-1. Therefore, exposure of neonates (birth to three days of age) to infectious BHV-1 may pose a significant threat, and the risk of vaccine-induced disease is accentuated if the dam's colostrum does not have adequate levels of neutralizing BHV-1 antibody. During an epizootic of IBR within a susceptible herd, supplying colostrum with adequate levels of virus neutralizing BHV-1 antibody to all newborn calves would be a sound recommendation, while the administration of an IM-ML-IBR vaccine to neonates that had received an unknown level of colostral antibody could result in unnecessary calf losses. CVJ

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Answers to Quiz Corner/Les réponses du Test Éclair

1. e — Calibration should be performed every 1–3 months to account for changes in the centrifuge that occur as a result of "wear and tear."
   e — Le calibrage doit être effectué à tous les 1–3 mois pour tenir compte des altérations à la centrifugeuse qui se produisent à la suite de l'usage de l'appareil.

2. d — Antineoplastic therapy is most important to control the cause of hypercalcemia.
   d — Une thérapie antinéoplasique est très importante pour contrôler la cause de l'hypercalcémie.

3. c

4. c — Overheating of cookware coated with polytetrafluoroethylene (Teflon) can cause immediate death in pet birds inhaling the fumes.
   c — Le chauffage excessif des plats de cuisine enduits de polytétrafluoroéthylène (Teflon) peut causer la mort subite des oiseaux qui inhalent les vapeurs.

5. c — Though the cause of laminar ischemia remains elusive, these treatment aims are justified, based on the available information about the animal's status during development of the condition.
   c — Bien que la cause de l'ischémie laminaire demeure intangible, les buts de ce traitement sont justifiés si l'on tient compte des informations disponibles quant à l'état de l'animal au cours de l'évolution de l'affection.

6. b

7. d — Traumatic reticulitis is not likely to cause a distinct area of gas accumulation on the right side, though gastrointestinal stasis may allow small pockets of gas to accumulate in the colon or duodenum.
   d — La réticulite traumatique n'est pas susceptible de causer une accumulation de gaz dans une région distincte du côté droit de l'abdomen, bien qu'une stase gastro intestinale puisse permettre de petites accumulations gazeuses dans le côlon ou le duodénum.

8. c — The description of the wound suggests an anaerobic infection. Metronidazole has excellent antianaerobic activity.
   c — La description de la plaie suggère une infection anaérobique. Le métronidazole possède une excellente activité antianaérobique.

9. d — H2 blockers are not totally effective in protecting against ulceration induced by nonsteroidal antiinflammatory drugs. Sucralfate is effective in allowing ulcers to heal. Affected animals are not helped by kaolin-pectin or milk diets.
   d — Les antagonistes H2 ne sont pas totalement efficaces pour protéger contre l'ulcération induite par des anti-inflammatoires non stéroïdiens. Le sucralfate est efficace pour permettre la guérison des ulcères. Le kaolin-pectine ou les diètes à base de lait n'aident pas les animaux qui souffrent d'ulcères.

10. e — These signs are characteristic of mastitis caused by leptospirae.
    e — Ces signes sont caractéristiques d'une mammite causée par des leptospires.