Outbreak of fatal salmonellosis in cats following use of a high-titer modified-live panleukopenia virus vaccine

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- In cats, salmonellosis is commonly subclinical but can result in potentially fatal septicemia in immunocompromised individuals.
- Modified-live virus vaccines, particularly those containing panleukopenia virus, may cause transient immunosuppression and should be used with caution because of the possibility of activating subclinical opportunistic infections.

A 14-week-old Persian kitten from a private breeding cattery was brought to the referring veterinarian with a history of acute onset of recumbency and epistaxis 10 days after receiving a high-titer modified-live virus vaccine containing panleukopenia virus, calicivirus, and herpesvirus components.[a] Important physical examination abnormalities included mild dehydration, splenic and mesenteric lymph node enlargement, weakness, and poor responses to external stimuli. Rectal temperature was 39.7 C (103.5 F). Results of a CBC were available the next morning, and included normocytic, normochromic anemia (2.55 X 10^6 RBC/ml; reference range, 5.0 to 10.0 X 10^6 RBC/ml), neutropenia (1,116 neutrophils/ml; reference range, 2,500 to 12,500/ml) with a left shift (360 band neutrophils/ml; reference range, 0 to 300/ml), and lymphopenia (204 lymphocytes/ml; reference range, 1,500 to 7,000/ml). Reticulocyte fraction was 0.2%; nucleated RBC fraction was 4%. Platelet count was low (< 50,000 platelets/ml). Serum biochemical abnormalities included mild hyperglobulinemia (4.84 g/dl; reference range, 2.8 to 4.8 g/dl) and high BUN (49 mg/dl; reference range, 16 to 33 mg/dl) and total bilirubin (5.43 mg/dl; reference range, 0 to 0.9 mg/dl) concentrations. Alkaline phosphatase (0 U/L; reference range, 10 to 350 U/L) and amylase (452 U/L; reference range, 500 to 1,400 U/L) activities were low; alanine transaminase activity (114 U/L; reference range, 10 to 96 U/L) was high. The cat was treated with procaine penicillin G (150,000 U, SC) and discharged with instructions that lactated Ringer's solution (100 ml, SC) be administered every 2 hours. The kitten was found dead the following morning, and necropsy was performed.

Gross pathologic abnormalities included profound enlargement of the mesenteric lymph nodes and spleen, moderate hepatomegaly, and edema of the lungs. Cytologic evaluation of Gram-stained smears of lymph node and splenic tissue revealed large numbers of gram-negative rods. Lymph node, spleen, and intestinal tissue specimens were placed in selenite enrichment broth, and incubated in air at 37 C for 24 hours. Aliquots of the selenite broth were then plated on MacConkey agar plates, and plates were incubated in air at 37 C for 24 hours. Large numbers of *Salmonella* sp belonging to serogroup B were grown from the lymph node and splenic specimens. The organism was identified as *Salmonella typhimurium* at the National Veterinary Service Laboratory in Ames, Iowa. Susceptibility testing indicated that the isolate was susceptible to enrofloxacin, trimethoprim-sulfonamide, amikacin, and gentamicin, but was resistant to ampicillin, tetracycline, chloramphenicol, and first-generation cephalosporin-class drugs. Results of a test of feces for parvovirus antigen[b] were strongly positive. Samples of feces and bone marrow were inoculated on Crandell feline kidney cells, and cultures were examined for cytopathic effects typical of panleukopenia virus, but such effects were not observed.

Histologic abnormalities were detected in the small intestine, mesenteric lymph nodes, spleen, liver, and lungs. The heart, tongue, bone marrow, and pancreas did not have any detectable histologic abnormalities; the kidneys contained incidental cortical and medullary cysts. Small intestinal abnormalities included mild to moderate, subacute to chronic, patchy fibrosis and histiocytic infiltration secondary to crypt necrosis. In mesenteric lymph nodes, there was moderate, acute multifocal follicular central necrosis, with numerous short rod-shaped bacteria within lesions. Additionally, there was severe, acute diffuse sinusoid draining of cell debris, fibrin, degenerate neutrophils, and bacteria, essentially effacing the nodular architecture. Splenic abnormalities included severe, acute multifocal to coalescing fibrinoid necrosis, with cell debris scattered throughout the necrotic areas and bacteria either free or contained within phagocytes. Arteriolar and venular thrombosis was detected; thrombi contained fibrin, blood cells, cellular debris, and bacteria. Within vessels, there were marginated neutrophils and monocytes. Hepatic lesions consisted of acute, severe multifocal hepatocellular necrosis with some foci coalescing but with a random distribution of lesions. Short

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Funding provided by the University of California, School of Veterinary Medicine, Center for Companion Animal Health, and the Veterinary Medical Teaching Hospital.

rod-shaped bacteria were observed within necrotic lesions, as well as within Kupffer's cells in sinusoids not associated with necrotic lesions and extracellularly within sinusoids. There was mild to moderate, acute to subacute, necrotizing, diffuse, lymphoplasmacytic and histiocytic pericholangitis. In the lungs, lesions consisted of diffuse, moderate, acute to subacute, histiocytic and neutrophilic interstitial pneumonia and bacteria. Moderate, diffuse interstitial and alveolar edema was also evident. Pathologic and microbiologic findings suggested that death was a result of pulmonary edema and disseminated intravascular coagulopathy secondary to *Salmonella* endotoxemia. Gastrointestinal tract lesions were suggestive of recovery from panleukopenia virus infection.

After the breeder was informed of the findings, she revealed that 4 other kittens had died in the previous month, each within 1 to 2 weeks after being vaccinated with a modified-live virus vaccine. Three partial carcasses were preserved in her freezer. All 3 of the frozen carcasses had mesenteric lymphadenopathy, and *Salmonella* sp was isolated from mesenteric lymph nodes. The kittens also had villus crypt necrosis and secondary fibrosis.

Samples of rectal contents were obtained from the remaining 40 cats and kittens in the cattery by inserting sterile cotton swabs deep into the rectum. Samples were placed in selenite broth and incubated. Twelve of the 40 animals in the cattery were kittens that were < 5 months old and had received the modified-live virus vaccine at 5 and 8 weeks of age; *S typhimurium* was isolated from rectal contents of 3 of the 12. The remaining 28 animals were older cats that had been vaccinated 11 months earlier with a modified-live feline viral rhinotracheitis and calicivirus and killed panleukopenia virus vaccine[c] and had never received the modified-live panleukopenia virus vaccine; *S typhimurium* was not isolated from any of the older cats. A CBC was performed on 2 of the 3 kittens from which *S typhimurium* was isolated. All values were within reference limits; WBC counts were 10,000/ml and 6,300/ml.

Pulsed field gel electrophoresis was used to determine relatedness between the *Salmonella* isolates.[1,d] Conditions for DNA isolation and digestion with endonuclease enzymes were as recommended by the manufacturer. Plugs were incubated with 20 U of restriction enzyme in 150 ml of restriction enzyme buffer overnight at 37 C. Gels were 1.2% agarose in 0.5X tris-borate-EDTA buffer, and were run at 6 V/cm for 22 hours at 14 C, with a linearly ramped pulse time varying from 5 to 50 seconds at an angle of 120°. All isolates had identical banding patterns after digestion with *Not*I or *Apa*I. The 12 surviving kittens were treated with enrofloxacin (5 mg, PO, q 12 h) for 10 days. Samples of rectal contents collected 3 and 20 days after discontinuation of the drug did not yield *Salmonella* sp.

To determine whether hematologic changes and intestinal lesions observed at necropsy could be associated with the modified-live panleukopenia virus vaccine, 4 six-week-old specific-pathogen free cats that had not previously been vaccinated were experimentally inoculated with the vaccine, according to the manufacturer's instructions. Two other cats that were not vaccinated were observed as contact controls. Transient diarrhea and mild increases in rectal temperatures were observed in all 4 vaccinated cats 4 and 5 days after inoculation. For vaccinated cats, mean WBC count on day 6 (mean ± SD, 10,600 ± 1,600 cells/ml) was significantly ($P = 0.01$) less than mean WBC count prior to inoculation (17,500 ± 2,450 cells/ml). Two vaccinated cats and the 2 control cats were euthanatized on day 6. At necropsy, 1 of the vaccinated cats had numerous colonic crypt abscesses; the other vaccinated cat and the 2 control cats did not have any important lesions. The 2 remaining vaccinated cats had neutrophilia on day 10 (WBC count, 22,100 and 25,400 cells/ml). They were euthanatized 14 days after inoculation, and at necropsy, both were found to have patchy subacute fibrosis and lymphohistiocytic infiltration in regions of crypt necrosis.

Kittens in this cattery appear to have been subclinically to fatally infected with *S typhimurium* from an unknown source. Salmonellosis in cats can range from a subclinical infection to a chronic, nonspecific illness,[2] to severe peracute disease,[3] as in the kitten described in this report. Clinical signs are inconsistent but may include hematochezia, diarrhea, vomiting, fever, and, in the terminal stages, endotoxic shock.[2,3] One report[4] documented *S choleraesuis* pneumonia and probable septicemia in a cat without gastrointestinal tract abnormalities. It is likely that *Salmonella* bacteremia most often develops after gastrointestinal tract infection and dissemination of the organism, and organisms are often recovered from mesenteric lymph nodes but not from feces.[5] The variability in clinical signs among infected cats in the cattery described in this report may have been, in part, age-related or a result of environmental factors. However, it was interesting that only the kittens that were inoculated with the modified-live panleukopenia virus vaccine developed fatal salmonellosis.

Hematologic abnormalities typically associated with systemic salmonellosis include leukopenia, a left shift, and abnormalities in hepatic enzyme activities or function tests.[2,3] Hemograms of the kittens in this report fit this pattern, but it is not clear what contribution the panleukopenia virus vaccine may have had in reducing leukocyte counts as well. Histologically, paratyphoid nodules have been found in the livers of animals with severe generalized *S typhimurium* infection. These nodules are characterized by histiocytic and mononuclear infiltration with or without necrosis, and *Salmonella* organisms are often visible within hepatic Kupffer's cells, alveolar macrophages, and small venules.[6] Widespread thrombosis, probably associated with disseminated intravascular coagulopathy secondary to gram-negative endotoxemia, has also been described in association with *Salmonella* bacteremia in cats.[2,7]

Many *Salmonella* strains have been isolated from cats, including *S newport*, *S infantis*, *S manhattan*, and *S havana*,[8] but the most common isolate is *S typhimurium*.[9,10] Strains differ in regard to pathogenesis and virulence, with invasive disease often associated with certain plasmids carried by *S typhi*, *S dublin*, and *S typhimurium*.[11] The clinical and pathologic findings

in the kitten described in this report, particularly the pulmonary edema, were reminiscent of findings with peracute *S dublin* infection in cattle.[12]

Published treatment protocols for human patients with salmonellosis include administration of a trimethoprim-sulfonamide combination, ampicillin, or chloramphenicol. First-generation cephalosporin drugs reportedly have poor efficacy in vivo,[2,12] and resistance to ampicillin, sulfonamides, and tetracycline is common.[9] Recently, *S typhimurium* DT104, an emerging phenotype with resistance to multiple drugs including ampicillin, chloramphenicol, streptomycin, sulfamethoxazole, and tetracycline, has been isolated from humans and domestic animals, including cats.[13,14] Patients with acute enteritis secondary to salmonellosis are often not treated, in part, because of the risk of inducing a chronic carrier state[15] and, in part, because some human medicine studies suggest that treatment may not reduce the severity or duration of clinical illness.[16] Possible induction of chronic carriage of *S typhimurium* in cats treated with enrofloxacin (7.5 mg, q 24 h) orally has been reported,[17] but infection completely resolved after 14 days of parenteral treatment with enrofloxacin. In contrast, continued shedding was not observed in cats in the present report after oral enrofloxacin treatment.

Prolonged fecal shedding of *Salmonella* sp by cats, particularly *S typhimurium*, has been associated with increased risk of human infection.[8] Infection with *S typhimurium* is common in humans, cattle, and poultry[18] and a high proportion of healthy cats and cats with diarrhea (particularly those from random sources) reportedly excrete *Salmonella* sp in their feces.[8,10] Results of pulsed field gel electrophoresis of isolates from the kittens in this report indicated that the isolates were indistinguishable, suggesting that they were derived from a common source. However, we could not identify that source. The breeder reported that she fed raw chicken to 1 adult house cat when she prepared meals for the family, but did not feed raw chicken to the kittens. In addition, the house cat was not found to be infected with *Salmonella* sp, and it did not have contact with kittens in the cattery.

Panleukopenia virus infection causes gastroenteritis with vomiting and diarrhea and is accompanied by profound leukopenia. Immunosuppression is a result of disruption of the gastrointestinal barrier, loss of all leukocytes classes, and reduced responsiveness of T-lymphocytes to mitogens.[19] Vaccination may produce some of the effects of clinical panleukopenia virus infection, including a moderate reduction in leukocyte count and immunosuppression.[20] Survival time of dogs with parvovirus infection given a modified-live parvovirus vaccine was shorter than that for dogs given a killed-virus vaccine and even for unvaccinated dogs, presumably as a result of immunosuppression.[21] Shedding of the virus has been reported for dogs given modified-live parvovirus vaccines,[22] and the positive panleukopenia virus antigen test results for the kitten in this report suggest that the kitten was shedding panleukopenia virus. A possibility exists that panleukopenia virus antigen in the feces of this kitten was a result of natural infection, but this was unlikely because the kitten was maintained in a closed, well-managed cattery, in which all cats > 6 weeks of age were adequately vaccinated, and clinical panleukopenia virus infection had never been previously observed.

Immunosuppressive disorders, including lymphoma,[6] FeLV infection,[7] and diabetes mellitus,[7] are risk factors for clinical salmonellosis,[23] and salmonellosis has often been associated with panleukopenia virus infection.[10] Disruption of the mucosal barrier secondary to panleukopenia virus infection may facilitate systemic dissemination of *Salmonella* organisms.

Pathologic lesions attributable to panleukopenia virus infection in the kittens in this report were mild to moderate, and typical of the crypt effacement and subsequent fibrosis associated with this infection. Kittens had been vaccinated when quite young (typically 6 weeks of age), and although the manufacturer's product literature states that safety studies were performed on kittens as young as 4 weeks of age, it is possible that the immunosuppressive effects of the vaccine (probably related to the panleukopenia virus component, but possibly also related to the calicivirus and herpesvirus components) were partially age-related. It would be interesting to determine whether recently vaccinated cats had increased mucosal permeability and altered responses to antibodies and mitogens, compared with unvaccinated cats. Importantly, any immunosuppressive effects associated with vaccination would likely not be associated only with use of this manufacturer's product, but would be expected after use of any similar modified live virus vaccine.

The temporal association between vaccination and development of pathologic lesions of panleukopenia and systemic salmonellosis in kittens in this cattery suggests, but does not prove, that the vaccine may have facilitated development of fatal salmonellosis in subclinical carrier cats. Even though we cannot prove that vaccination played any role, we wanted to present these data to alert readers to the possibility of similar incidents.

[a]Trivalent IN IO vaccine, Heska, Fort Collins, Colo.
[b]Parvovirus Probe Test, IDEXX Laboratories, Westbrook, Me.
[c]FVRCP, Solvay, Mendota Heights, Minn.
[d]CHEF-DR III System, Bio-Rad Laboratories, Hercules, Calif.

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