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Original Research

An Experimental Model of *Actinobacillus suis* Infection in Mice

Shivani Ojha, M Anthony Hayes, Patricia V Turner, and Janet I MacInnes*

Actinobacillus suis **is an opportunistic pathogen of high health status swine and is associated with fatal septicemia, especially in neonatal pigs. A practical model of *A. suis* is unavailable currently. However, some evidence suggests that *A. suis* can infect nonporcine species. We therefore hypothesized that a mouse model of *A. suis* infection might be possible. To test this idea, we challenged CD1 mice with 3 strains of *A. suis* (2 porcine [SO4 and H91-0380] and 1 feline [96-2247]) by intranasal and intraperitoneal routes. We also evaluated the effects of coadministration of hemoglobin and immunosuppression by dexamethasone on the susceptibility of mice to *A. suis* infection. The feline and H91-0380 porcine strains induced clinical signs of acute disease and necrotizing pneumonia in mice similar to those seen in pigs. Although few bacteria were recovered, dissemination of *A. suis* was widespread. Generally, mice infected with the feline *A. suis* isolate had more severe clinical signs and higher bacterial titers than did mice infected with either of the porcine strains. Pretreatment of the mice with dexamethasone or addition of 2% porcine hemoglobin to the challenge inoculum appeared to hasten the onset of clinical signs by the porcine strains but had no significant effect on moribundity. These experiments demonstrate that mice can be infected with *A. suis* and subsequently develop pneumonia and bacteremia comparable to that seen in pigs, suggesting that mice may be used as a model for studying infection in swine.**

Abbreviations: CFU, colony-forming units; PBS, phosphate-buffered saline

Actinobacillus suis is a gram-negative capnophilic coccobacillus belonging to the family *Pasteurellaceae*. *A. suis* often resides asymptomatically in the nasopharynx of pigs and can be isolated as a commensal organism from the alimentary and lower genital tract of pigs.[14] *A. suis* can cause fatal septicemia, especially in neonatal piglets, and is associated with a wide range of clinical conditions, including pneumonia, arthritis, enteritis, and abortion, in pigs of all ages.[27] During the past 2 decades, *A. suis* has emerged in North America and other countries as an important pathogen of high health status swine, but the pathogenesis of this infection is still poorly understood.[15]

A. suis has a number of putative virulence factors common to many gram-negative organisms, including iron-regulated outer-membrane proteins, capsule, and lipopolysaccharide. In addition, *A. suis* has several established virulence factors in common with *Actinobacillus pleuropneumoniae*, including iron-uptake mechanisms, urease, and the ApxI and ApxII toxins.[3,28] A recent signature-tagged mutagenesis study suggested that surface structures (including outer membrane protein A), gene products involved in energy metabolism, and unknown genes may play a role in the virulence of *A. suis*.[20]

Although generally considered a pathogen of swine, *A. suis* or *A. suis*-like bacteria have been isolated sporadically from other species, including Canada geese,[16] ostriches,[18] alpacas,[9] cats,[5] calves,[6] and horses.[2,12] In addition, *A. suis* has been recovered from a human wound infection resulting from a pig bite.[7] Although these isolates were reported to be phenotypically similar to the type strain *A. suis* 15557, no genetic analyses were conducted to confirm identification. Further, Jeannotte and colleagues[10] recently demonstrated that 22 of 24 isolates that were phenotypically similar to *A. suis* were not *A. suis* (*sensu stricto*), but the remaining 2 isolates, including the previously described isolate from a cat's lung, were true *A. suis*.[5]

Several *Pasteurellaceae* species have been studied in mice, and these murine models are very useful for investigating bacterial pathogenesis.[11,13,17,23] In light of evidence suggesting that *A. suis* may not be species-specific, we sought to develop a mouse model of *A. suis* infection. We performed challenge experiments with 1 feline and 2 porcine strains of *A. suis*. In experiments lacking any additional infection-inducing treatments, the feline strain emerged as more virulent than the porcine strains. In subsequent challenge experiments, we added porcine hemoglobin (an exogenous iron source and virulence adjuvant) to the porcine strains and immunosuppressed the mice by pretreating them with dexamethasone.

Materials and Methods

Bacterial strains and culture conditions. We used 3 well-characterized *A. suis* strains—SO4 (O1/K1), H91-0380 (O2/K2), and 96-2247 (O2/K?)—in the challenge experiments. *A. suis* SO4 was recovered from a healthy pig in a herd with active *A. suis* disease at the time of isolation, strain H91-0380 originated from a septicemic pig,[27] and strain 96-2247 was isolated from the lungs of a 9-mo-old cat after ovariohysterectomy.[5] All strains were main-

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Department of Pathobiology, Ontario Veterinary College, University of Guelph, Guelph, Ontario, Canada.
Corresponding author. Email: macinnes@uoguelph.ca

Table 1. *A. suis* infection in mice: strains, route of inoculation, challenge dose, and treatments

Strain	Route	No. euthanized[a]/no. inoculated (no. of CFU/ml)		
		No treatment[b]	Hemoglobin[c]	Dexamethasone[d]
SO4 (porcine)	IN	2/18 ($1.2 \times 10^8 \pm 0.2 \times 10^8$)	4/6 (6×10^8)	2/6 (3.8×10^8)
	IP	5/18 ($1.2 \times 10^8 \pm 0.2 \times 10^8$)	3/6 (6×10^8)	3/6 (3.8×10^8)
H91-0380 (porcine)	IN	9/18 ($1.07 \times 10^8 \pm 0.12 \times 10^8$)	4/6 (3×10^8)	3/6 (1.2×10^8)
	IP	5/18 ($1.07 \times 10^8 \pm 0.12 \times 10^8$)	3/6 (3×10^8)	4/6 (1.2×10^8)
96-2247 (feline)	IN	12/18 ($1.07 \times 10^8 \pm 0.06 \times 10^8$)	1/6[e] (3×10^8)	4/6[e] (1.6×10^8)
	IP	10/18 ($1.07 \times 10^8 \pm 0.06 \times 10^8$)	4/6[e] (3×10^8)	4/6[e] (1.6×10^8)
Control (PBS)	IN	0/6	(PBS + 2% Hb) 0/2	0/2
	IP	0/6	(PBS + 2% Hb) 0/2	0/2

CFU, colony-forming units; HB, hemoglobin; IN, intranasally; IP, intraperitoneally; PBS, phosphate-buffered saline.
[a]Number of animals euthanized by day 3 postinoculation.
[b]Mean challenge dose of 3 individual (no-treatment) trials ± 1 standard deviation. Three trials, totaling 18 mice per group.
[c]2% porcine hemoglobin administered with inoculum.
[d]mice treated with 0.1 ml dexamethasone 72 h prior to challenge.
[e]untreated mice.

tained as glycerol stocks at –80 °C. Growth-curve experiments of the 3 strains confirmed that the rates of replication were comparable, and the number of colony-forming units (CFU) was parallel with OD_{600} readings. For all of the challenges, the 3 strains were streaked liberally on 2 sheep blood agar plates and grown overnight at 37 °C in an atmosphere of 5% CO_2. The next day, bacteria were harvested aseptically into 5 ml of sterile phosphate-buffered saline (PBS), centrifuged at $4500 \times g$ for 15 min, and suspended in 2 ml of PBS. The cell suspensions were adjusted to an OD_{600} of 0.3 to give approximately 1×10^8 CFU/ml. The precise number of CFU was determined by plating 10-fold serial dilutions on blood agar plates (Table 1).

Animals and challenge procedure. Female Crl:CD-1(ICR)BR mice (18 to 20 g) were obtained from Charles River (St Constant, Quebec, Canada) and housed on corncob bedding (Harlan Teklad, Madison, WI) in polycarbonate cages (6 per box) placed in Horsfal units at 23 ± 2 °C, 30% to 70% relative humidity, on a 12:12-h light:dark cycle. Mice were provided with a commercial diet (Global Diet 2019, Harlan Teklad) and water ad libitum. Vendor surveillance reports indicated that the animals were from colonies serologically free from *Bordetella bronchiseptica*, *Citrobacter freundii* 4280, *Corynebacterium kutscheri, Mycoplasma pulmonis*, *Salmonella* spp., *Streptobacillus moniliformis*, *Helicobacter* spp., *Klebsiella* spp., *Pasteurella* spp., *Staphylococcus aureus*, and *Streptococcus* spp. as well as free of ectoparasites, endoparasites, and enteric protozoa. The colonies also were serologically negative for Sendai virus, mouse parvovirus, mouse hepatitis virus, minute virus of mice, reovirus 3, rotavirus, adenovirus, polyoma virus, K virus, mouse cytomegalovirus, lymphocytic choriomeningitis virus, Korean hemorrhagic fever virus (Hantaan), and ectromelia virus. After a 7-d acclimation period during which animals were not handled, mice (n = 6 per group) were weighed immediately before being infected by either the intranasal (IN) or intraperitoneal (IP) route (Table 1). For IN inoculations, animals were anesthetized with methoxyflurane in oxygen (Metofane, Janssen-Ortho, Toronto, Ontario, Canada) and 30 µl of bacterial suspensions containing approximately 10^8 CFU/ml were administered IN with an automatic micropipettor. To prevent swallowing of the droplet, the lower jaw was pressed upward gently. The IP-challenged animals were injected with 0.1 ml of bacterial suspension.

During the first trial, mice were observed for clinical signs for 7 d after inoculation. In all cases, animals that showed no clinical signs gained weight from day 3 postinoculation. Accordingly, in subsequent challenge experiments (2 more trials without treatment and 2 with treatment), mice were euthanized on day 3 postinoculation. In the 2 treatment studies, mice were inoculated with either the porcine *A. suis* strains suspended in PBS containing 2% (wt/vol) porcine hemoglobin (Sigma Chemical, St Louis, MO) or mice were injected subcutaneously with 0.1 ml of dexamethasone (2 mg/ml, Vetoquinol Canada, Cambridge, Ontario, Canada) 72 h prior to bacterial challenge. In every experiment, 2 mock-challenged control animals were included. These animals were treated identically to the test animals except that PBS alone was administered. To minimize animal use, the observations of treated groups (hemoglobin and dexamethasone pretreatment) were compared with those of previous trials done without treatment. All animal procedures and protocols were approved by the University of Guelph Animal Care Committee and were in accordance with the guidelines of the Canadian Council of Animal Care.

Clinical signs. After IN or IP inoculation with *A. suis*, mice were weighed daily and evaluated clinically at least twice daily (Figure 1 B). Clinical conditions were scored based on 1 point each for ruffled hair coat, hunched back, marked lethargy, isolation seeking behavior, or weight loss of 15% from pre-inoculation weights, for a maximal possible score of 5. Mice were euthanized by IP injection of barbiturate solution (Euthansol, 340 mg/ml, Schering Canada, Pointe-Claire, Quebec, Canada) when weight loss exceeded 15% weight loss, the clinical score was 3 or greater, or at the end of the experiment.

Sampling of tissues and microbiologic scores. At necropsy, the entire heart, 90% of the spleen, left lobe of lung, left lobe of liver, and left kidney were collected and macerated aseptically, and the tissue paste was spread evenly on blood agar plates. Peritoneal swabs collected during postmortem examination were plated directly on blood agar plates. *A. suis* bacteria recovered from these cultures were identified on the basis of their colonial morphology and hemolytic phenotype. The number of bacteria recovered was scored as: 0, no bacteria recovered; 1, 1 to 5 colonies; 2, 6 to 10 colonies; 3, 11 to 100 colonies; 4, more than 100 colonies.

Histopathology. Tissues (lung, liver, spleen, and right kidney)

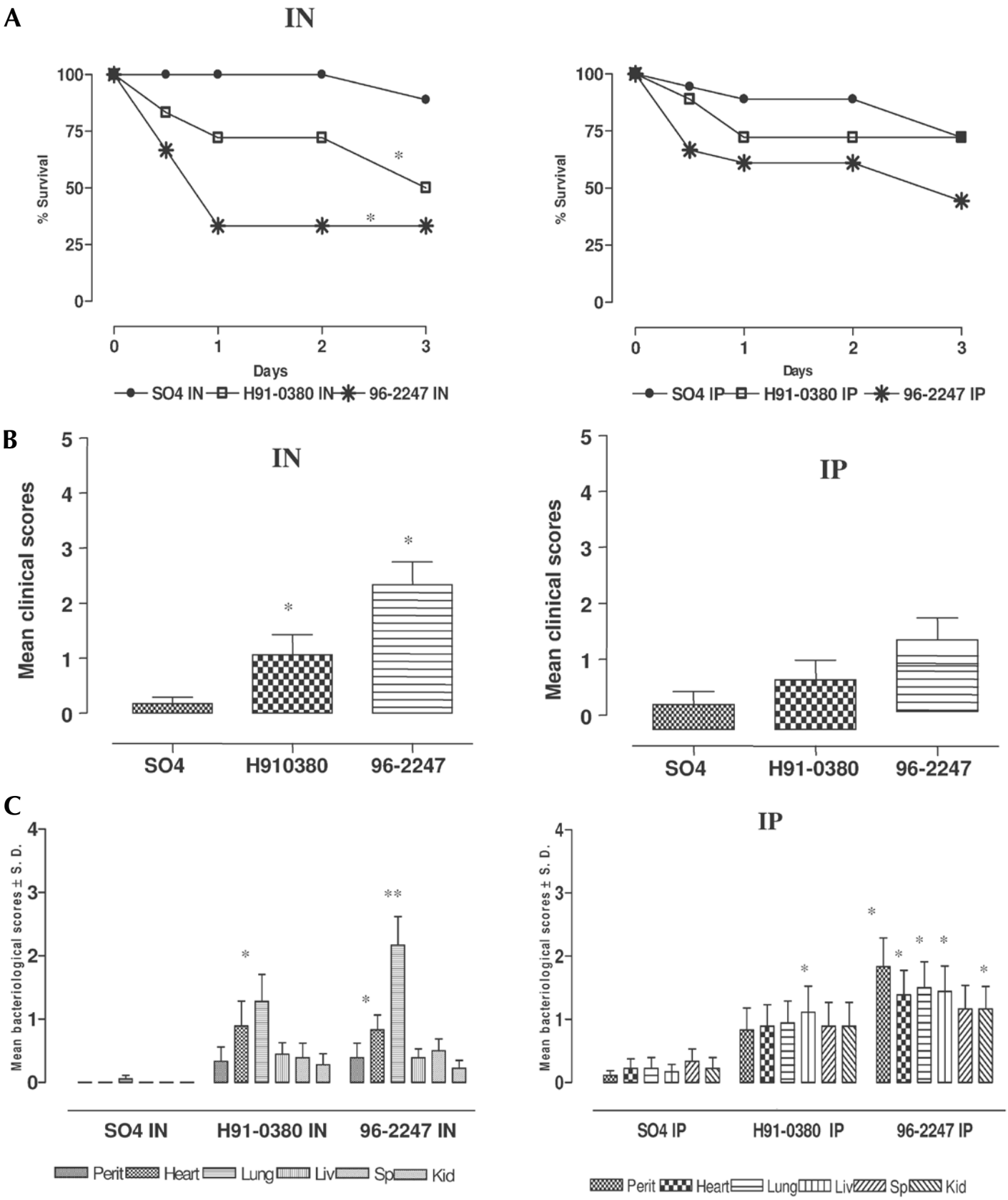

Figure 1. (A) Survival curves, (B) clinical scores, and (C) bacteriologic scores of CD1 mice (n = 6 per group) infected with *A. suis* strains SO4, H91-0380, and 96-2247 by the intranasal route (IN; left) or intraperitoneal route (IP; right) in 3 no-treatment challenges. Data are presented as mean ± 1 standard deviation. Bacteriologic scores were evaluated for the following tissues: peritoneum (perit), heart, lung, liver (liv), spleen (sp), kidney (kid).

for histopathology were collected and fixed in 10% neutral buffered formalin, paraffin-embedded, sectioned, and stained with hematoxylin and eosin. Information regarding treatment group was removed from slides prior to microscopic evaluation, and a pathologist scored lung histopathology from 0 to 4 (0, no pathology; 1, minimal [focal] changes; 2, mild [scattered, multifocal]; 3, moderate [no more than 25% of section affected, or locally extensive]; 4, marked (>25% section affected, multifocal or locally extensive]) according to the presence and severity of edema, hemorrhage, leukocyte infiltration, and necrosis, with a maximum tissue score of 16.

Statistics. The differences of least-square means of bacteriologic scores for 3-way interactions (route × strain × organ) were estimated and tested for statistical significance using SAS software;[24] *P* values less than 0.05 were considered as statistically significant. Fisher Exact tests were performed to determine the effects of hemoglobin and dexamethasone on the survival of mice. Cochran–Mantel–Haenzel statistics were applied to test the effect of *A. suis* strains and route of administration on the survival time and clinical scores of animals. The bacteriological scores of the different organs obtained from first trial (no-treatment) and trials with treatment were analyzed by using the Kruskal–Wallis test.

Results

Intranasal challenge. In no-treatment challenge experiments (3 trials), mice infected IN with the feline *A. suis* isolate, 96-2247, had the highest clinical scores, and 6 moribund animals had to be euthanized within 6 h after inoculation. By 24 h postinoculation, all but 6 mice were euthanized (Figure 1 A, B). Animals infected with *A. suis* H91-0380 (O2/K2) IN also had high clinical scores, with 5 mice euthanized within 24 h after inoculation (Figure 1 A, B). The most commonly observed clinical signs of distress were hunched back, ruffled hair coat, and lethargy. Strain SO4 was the least virulent by the IN route, with no animals showing signs of infection before 48 h postinoculation. Compared with SO4, significantly higher numbers of CFU of the feline strain were recovered from lung ($P \leq 0.0001$) and heart ($P = 0.037$); recovery of bacteria from lung also differed significantly ($P = 0.002$) between H91-0380 and SO4, whereas no marked difference was found among strains in other organs (Figure 1 C).

The survival period of mice infected IN with O2 strains (H91-0380 and 96-2247) was significantly ($P = 0.001$) shorter than that for those infected with the O1 strain (SO4), and clinical scores were significantly ($P = 0.004$) higher for O2- than O1-challenged mice (Figure 1 A, B). The mock-challenged control animals remained healthy and always had a clinical score of 0.

Intraperitoneal challenge. Mice infected IP with *A. suis* 96-2247 again had the highest clinical scores, greatest systemic spread of bacteria, and lowest survival rate of the 3 strains tested, although compared with infection by IN route, clinical scores were lower (Figure 1 A-C). Animals infected with H91-0380 had lower clinical scores than those infected IN, and only 5 mice were euthanized by day 3 postinoculation (Figure 1 A, B). Only 5 of 18 mice infected with SO4 showed clinical signs. In the 3 trials conducted without treatment, 1 animal (clinical score, 4) was euthanized at 6 h postinoculation in 1 experiment, whereas 4 others with 15% weight loss and ruffled coat were euthanized by day 3 postinoculation (Figure 1 A, B). Strains 96-2247 and H91-0380 could be recovered from all tissues after challenge by the IP route, but SO4 was recovered rarely (Figure 1 C).

Recovery of bacteria differed significantly ($P \leq 0.05$) between 96-2247 and SO4 in all organs except spleen in mice challenged IP, whereas recovery was higher for H91-0380 than SO4 only for liver ($P = 0.018$). No significant difference was noted between feline and porcine O2 strains, except in peritoneal recovery ($P = 0.012$) from IP-challenged mice. Significant differences were not detected between survival time of O2-infected mice and O1-inoculated animals or among the clinical scores of IP-challenged mice.

Histopathology. Because *A. suis* is a respiratory pathogen of swine and because higher clinical scores, more gross lesions, and greater bacterial recovery occurred for IN-infected mice, histopathology scoring was restricted to lungs. Some mice euthanized within 72 h after inoculation showed areas of hemorrhagic and consolidated lung (IN group) and exudate at the IP inoculation sites. Microscopically, acute focal to locally extensive necrotizing pneumonia was present in lungs of mice infected with O2 *A. suis* strains by the IN and IP routes, a characteristic lesion observed during natural disease in pigs. Locally extensive necrosis with congestion, moderate to marked neutrophilic infiltrates, and colonies of bacteria were seen in affected areas. In places, alveoli were flooded with proteinaceous fluid (edema), fibrin, and multifocally, septal blood vessels were thrombosed (Figure 2 A, B). Multifocal necrotic foci were evident grossly in the livers of 3 mice inoculated with 96-2247 IP. Several mice given O2 *A. suis* strains by the IP route had foci of acute hepatocellular necrosis with marked neutrophilic infiltrates. Many infected mice had marked splenomegaly due to congestion. No gross or histologic lesions were present in mice inoculated with the SO4 strain. The total histopathology score in lungs did not differ significantly between mice infected with feline O2 strain compared with the porcine O2 ($P = 0.86$; Figure 3).

Effect of the addition of hemoglobin. *A. suis* SO4 given IN in the presence of 2% porcine hemoglobin demonstrated increased virulence, although this effect might have been the result of the higher challenge dose (6×10^8 versus 1×10^8). The survival rate of mice differed significantly ($P = 0.18$) between those in the untreated trial and those given hemoglobin IN. Of the 6 mice, 4 were euthanized by day 2 postinoculation, and bacteria could be recovered readily from the lungs of affected animals (Figure 4 A–C). Compared with that after inoculation of H91-0380 IN alone, the onset of clinical signs was hastened when this strain was administered in the presence of hemoglobin, but there was no significant effect on overall moribundity. In the group infected IN with strain 96-2247, only 1 animal demonstrated clinical signs, and bacterial recovery was much lower than for other strains (Figure 4 A–C).

The virulence of SO4 also was enhanced when hemoglobin was administered IP, and clinical signs appeared sooner than when SO4 was inoculated alone. However no bacteria could be recovered (Figure 4 A through C), but the difference in the survival was not significant. The IP challenge using the porcine O2 strain with hemoglobin led to increased recovery of bacteria, mortality, and clinical scores ($P < 0.05$) , as compared with the challenge without hemoglobin (Figure 4 A–C), but the difference in survival was not significant. When 96-2247 was given IP without hemoglobin, 4 mice were euthanized by 24 h after inoculation, and the organism was recovered from all tissues (Figure 4 A–C).

Effect of dexamethasone pretreatment of mice. Administration of *A. suis* SO4 by either IN or IP to dexamethasone-pretreated mice did not cause a statistically different change in mortality or in the time to appearance of clinical signs (Figure 5 A, B). The

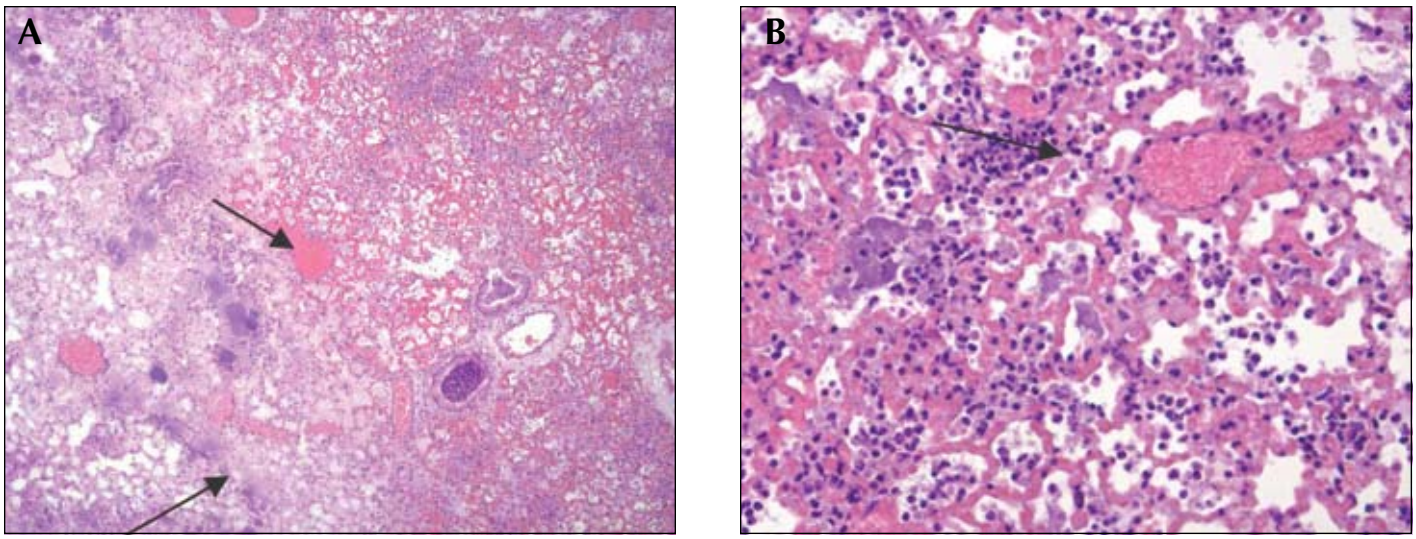

Figure 2. Tissue sections from CD1 mice infected with *A. suis* (n = 6 per group). (A) Section of lung from mouse infected with *A. suis* 96-2247 (O2) 18 h previously. A focal area of necrosis with bacteria, a moderate neutrophilic infiltrate, and congestion (arrow) are present. Magnification, ×50. (B) Section of lung from CD1 mouse infected with *A. suis* H91–0380 (O2/K2). Focal thrombus formation with marked neutrophil and macrophage infiltrate are present (arrow). Magnification, ×200. Hematoxylin and eosin stain.

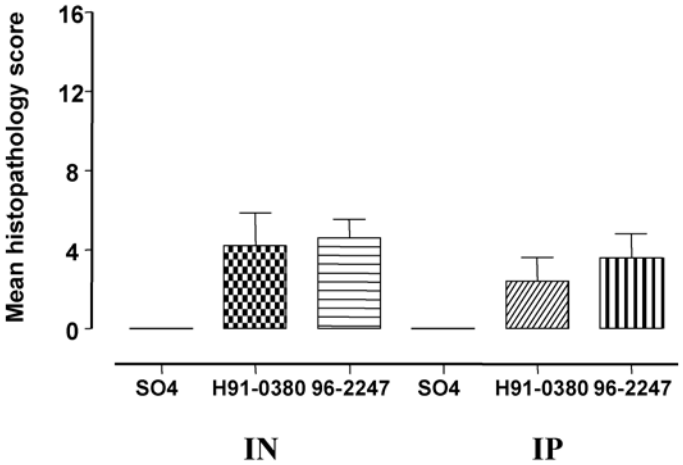

Figure 3. Histopathologic scores for lung from mice infected with various *A. suis* strains by intranasal or intraperitoneal routes (n = 6 per group). Data are presented as mean ± 1 standard deviation.

organism was recovered in greater numbers from few of the tissues sampled from dexamethasone-treated animals (Figure 5 C). A marked and deleterious effect was noted when H91-0380 was administered to dexamethasone-treated mice IP or, to a lesser degree, IN (Figure 5 A, B). The recovery of H91-0380 from peritoneum was increased in dexamethasone-treated animals challenged IP. Untreated mice inoculated with strain 96-2247 either IN or IP had collectively the highest mortality and clinical and bacteriologic scores (Figure 5 A–C).

Discussion

All *A. suis* strains tested were recovered more readily from systemic sites when bacteria were administered IP rather than IN, but bacteria could also be recovered from the peritoneal cavity, heart, and lung after IN challenge, demonstrating the dissemination of *A. suis* from the upper respiratory tract. Similarly, spread from the peritoneal cavity was shown by the presence of large numbers of bacteria in almost all the tissues sampled after challenge by the IP route. Although clinical signs generally were seen earlier in IP-challenged mice, by 24 h after inoculation, the route of infection did not markedly influence the severity of disease. Mice exposed IN had pneumonia analogous to natural infection in pigs, whereas more mice exposed IP had focal hepatic necrosis. In a study of 12 different serotypes of *A. pleuropneumoniae* in mice, the organism was recovered from systemic tissues of lung, liver, heart, and spleen after IP infection, but in contrast to *A. suis*, *A. pleuropneumoniae* was localized in the lungs after IN inoculation.[13] In natural disease, *A. pleuropneumoniae* remains localized within the respiratory tract, whereas *A. suis* spreads systemically, although the factors that determine invasion by *A. suis* are not known.[14]

Pathogenic members of the family *Pasteurellaceae* often acquire iron from host protein iron complexes by using siderophore-independent, receptor-mediated mechanisms.[25] This process is often species-specific: *A. suis* can acquire iron from porcine, but not human or bovine, transferrin.[1] Accordingly, we hypothesized that the virulence of the porcine strains in mice might be limited due to the inability of the bacteria to acquire sufficient iron; we therefore added porcine hemoglobin to the bacterial suspensions for inoculation. Given the presence of 2 hemolysins, ApxI and ApxII, in *A. suis*,[28] we anticipated that porcine hemoglobin would be a readily used source of iron. The addition of hemoglobin appeared to enhance the onset of clinical signs and markedly affected clinical scores, although this effect could be due in part to the higher challenge dose. In particular, systemic spread occurred when hemoglobin was given in conjunction with the porcine O2 strain IP. This result is in agreement with the findings of Rodriguez and colleagues,[23] who demonstrated that in challenge experiments with *Haemophilus influenzae* type B in BALB/c mice, coadministration of hemoglobin had a marked effect on the lethality induced by the bacteria. Similarly, a fatal infection developed in C57BL mice after IP challenge with *Neisseria meningitidis* B2b or *H. influenzae* type B plus mucin–hemoglobin.[4] The increased susceptibility to infection in these cases was attributed to interference

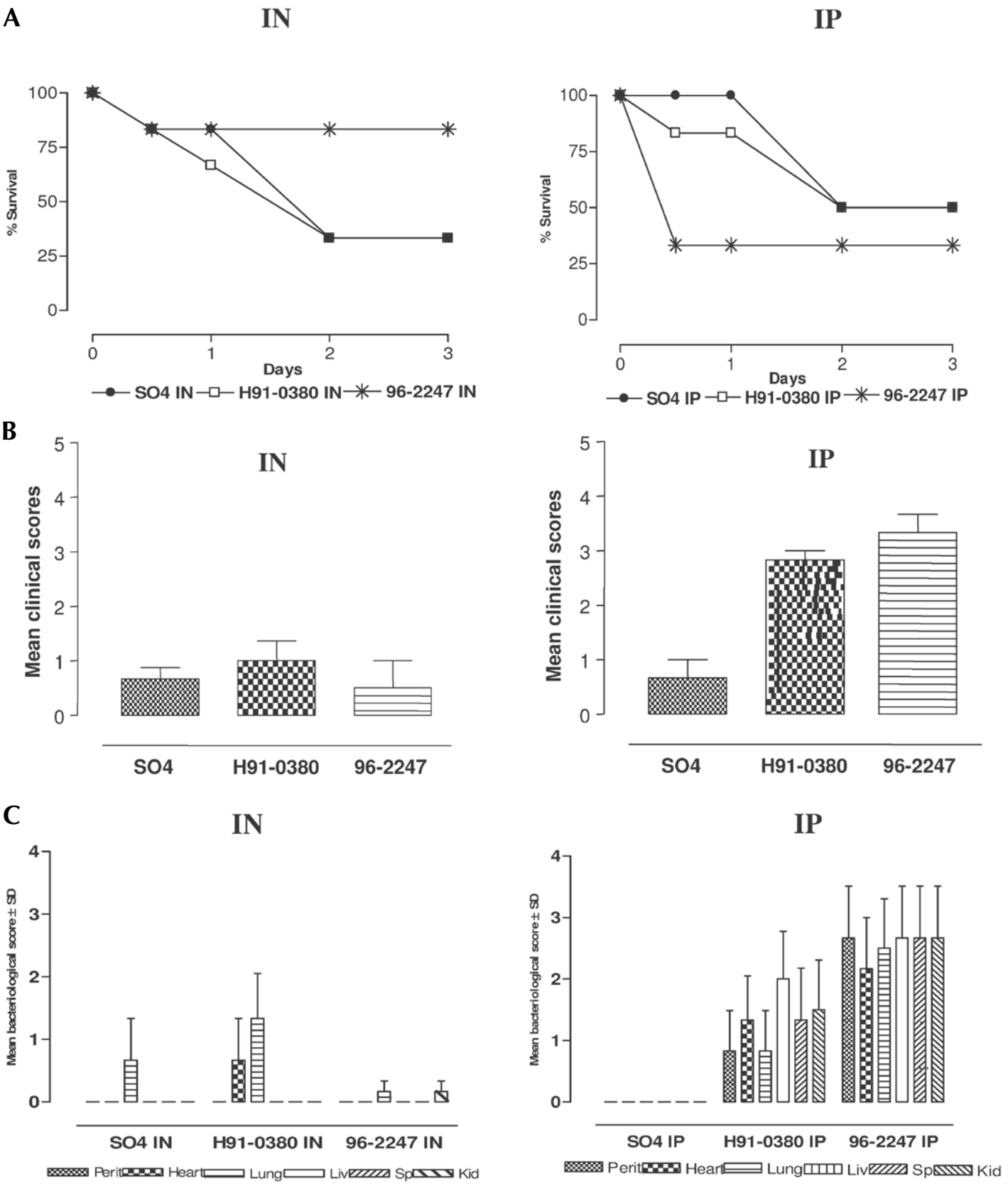

Figure 4. Survival curves (A), clinical scores (B), and bacteriologic scores (C) of CD1 mice (n = 6 per group) infected with *A. suis* strains SO4 with 2% hemoglobin, H91-0380 with 2% hemoglobin, and 96-2247 in PBS alone by the intranasal route (IN; left) or intraperitoneal route (IP; right). Bars represent mean ± 1 standard deviation.

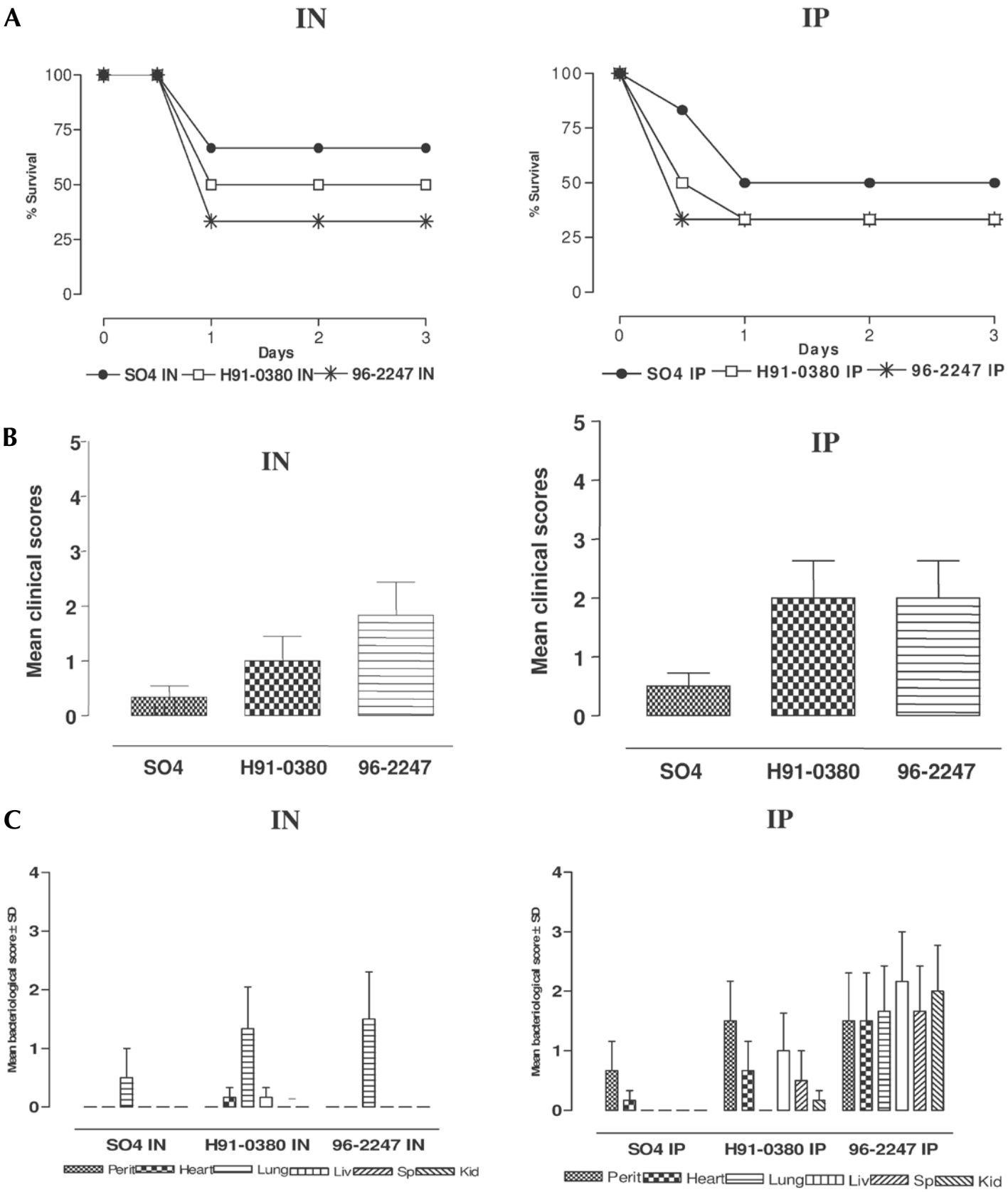

Figure 5. (A) Survival curves, (B) clinical scores, and (C) bacteriologic scores of dexamethasone-treated CD1 mice infected with *A. suis* strain SO4 or H91-0380 and of untreated mice given strain 96-2247 by the intranasal route (IN; left) or intraperitoneal route (IP; right). Mice were pretreated with 0.02 mg/kg dexamethasone IP.

by enhancement factors with the phagocytic processes of macrophages, thereby reducing clearance by the host.[4]

Susceptibility to some bacterial infections may increase when animals are stressed, for example, after weaning, parturition, or transport.[8] Corticosteroids, particularly dexamethasone, have often been used experimentally to study the effects of stress on bacterial infection.[22] Bacterial clearance from the biologic systems usually is slowed after administration of corticosteroids, and the antimicrobial action of macrophages is impaired.[22] As with the coadministration of iron, dexamethasone pretreatment of mice increased the pace of infection, but the systemic spread of bacteria did not differ significantly, except for greater peritoneal recovery of strain H91-0380 when administered IP. This finding is in contrast to some other recent infection models. For example, *Listeria monocytogenes* is reported to persist longer in the liver and spleen of dexamethasone-treated Swiss CD1 mice,[22] and dexamethasone is routinely used for the maintenance of *Cryptosporidium parvum* oocysts in mice.[21] In our experiments, bacteria were recovered in large numbers only from treated mice euthanized on the day of infection. There was no significant recovery of the organism from systemic sites later during the experimental period, suggesting that dexamethasone-pretreatment does not impair the mechanism(s) by which mice clear *A. suis*.

Consistent with an earlier study,[17] we found that *A. suis* could cause disease in mice. Unfortunately, the previous report presents few details, and their results or methods cannot be compared with those of the current study. With 1 exception, the feline strain 96-2247 (O2/K?) appeared to be the most virulent of the strains tested. Of the 2 porcine strains, H91-0380 (O2/K2) generally caused more severe disease and did so more rapidly than SO4 (O1/K1). These results are consistent with a previous report that O2 strains of *A. suis* are more likely associated with severe disease in swine than are O1 strains and that in acute IP challenge of pigs, *A. suis* H91-0380 (O2/K2) is somewhat more virulent than C84 (O2/K1) and markedly more virulent than SO4 (O1/K1).[26] Slavic and colleagues[26] suggest, however, that the association of virulence with the O2 antigen may be related more to the presence of a sialic acid-rich capsule than to the O antigen itself. Although the chemical structure of capsular polysaccharide of strain 96-2247 has not been determined, it is likely to have sialic acid as well.[19] That said, morbidity in mice and severe pulmonary lesions also may result from the synergistic action of endotoxin and hemolysin, but adhesins, outer membrane proteins, and other factors could also be associated with the greater virulence of O2 strains.

The mouse model described here has many of the features of natural infection with *A. suis* in pigs, but researchers using this model should be aware of the potential for species-related differences in cell and tissue tropism and clinical signs during infection. For example, characteristic clinical signs in infected pigs—such as cyanosis, petechial hemorrhages, paddling of limbs, and swollen joints—did not manifest in this murine model. In addition, gross lesions of endocarditis and miliary abscesses in systemic organs were not seen. With further refinements of infectious dose and treatments, modeling some of the other characteristic clinical signs of *A. suis* disease in mice may be possible. Despite these differences, we believe that this model is a highly suitable alternative for examining variations in virulence of different strains of *A. suis*, including isogenic mutants.

In summary, we have demonstrated that *A. suis* given either IN or IP in the absence of enhancement factors causes disease in mice. Although mice are relatively resistant to *A. suis* challenge and require a high challenge dose to induce infection, they rapidly develop clinical, gross, and histologic evidence of bacterial lesions, and these lesions are morphologically similar to those that occur during natural *A. suis* infections in swine. Given the difficulty and expense associated with the reproduction of *A. suis* disease in swine, mice may provide a useful alternative for future virulence and vaccine studies. The results of this study also support the conclusion that at least some strains of *A. suis* are not species-specific, reinforcing the need for effective biosecurity in swine operations.

Acknowledgments

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