Comparative pathogenicity of different *Actinobacillus suis* O/K serotypes

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Abstract

The pathogenicity of *Actinobacillus suis* serotypes O1/K1 (strain SO4), O1/K2 (strain C84), and O2/K2 (strain H91-0380) was evaluated in specific-pathogen-free (SPF) piglets challenged by intraperitoneal inoculation with approximately 1×10^7 colony-forming units per mL. All 3 strains produced peritonitis, but differences were observed in the composite histopathologic scores ($P = 0.001$) and in their ability to spread ($P = 0.008$) at 7 h post challenge. The O2/K2 strain caused the most severe peritonitis and disseminated most widely to other tissues. Moderate lesions were seen with the O1/K2 strain while the O1/K1 strain caused mild lesions and remained largely localized to the peritoneum. In an attempt to explain the basis of observed differences, the serum sensitivity of 9 *A. suis* strains with different O and K types was assessed. Regardless of the O/K type, all of the isolates tested were serum resistant. Moreover, most *A. suis* isolates grew as well or better in complement-replete sera as they did in complement-depleted sera. These observations indicate that although O2 and K2 strains had a greater propensity to cause a disseminating septic inflammatory response in pigs, they were no more resistant to complement-mediated killing than O1 strains.

Résumé

*La pathogénicité des sérotypes O1/K1 (souche SO4), O1/K2 (souche C84) et O2/K2 (souche H91-0380) d'*Actinobacillus suis *fut évaluée par inoculation intra-péritonéale d'environ* 1×10^7 *bactéries à des porcs exempts de micro-organismes pathogènes spécifiques. Une péritonite fut causée par les trois souches mais des différences furent notées au niveau du pointage des lésions histopathologiques (P = 0,001) ainsi qu'au niveau de la capacité de dissémination 7 h après l'inoculation (P = 0,008). Les lésions les plus sévères de péritonite et la plus grande capacité de dissémination furent obtenues avec la souche O2/K2. Des lésions modérées furent observées avec la souche O1/K2, alors qu'avec la souche O1/K1 les lésions n'étaient que légères et limitées au péritoine. Afin d'essayer d'expliquer l'origine des différences observées, la sensibilité au sérum de neuf souches d'*A. suis *de différents types O et K fut évaluée. Tous les isolats éprouvés se sont avérés résistants au sérum, indépendamment de leur type O/K. De plus, la croissance de la plupart des isolats d'*A. suis *fut aussi bonne sinon meilleure dans du sérum réalimenté en complément que dans du sérum dépourvu de complément. Ces observations indiquent que malgré le fait que les souches O2 et K2 aient une plus grande tendance à causer une réaction inflammatoire septique disséminante chez les porcs, elles ne sont pas plus résistantes que les souches du type O1 à une destruction médiée par le complément.*

(Traduit par le docteur Serge Messier)

Introduction

Actinobacillus suis is an important gram-negative pathogen of high health status swine herds. Various clinical conditions have been associated with *A. suis* infection including septicemia, pneumonia, arthritis, metritis, endocarditis, and erysipelas-like lesions on skin (1–5). In initial reports, *A. suis* was most often described as a causative agent of septicemic disease in very young pigs (6). In recent years, *A. suis* has been reported more often in grower/finisher pigs, animals previously thought to be relatively resistant to this organism (7,8). The susceptibility of these older animals in high health herds is presumably due to differences in specific and cross-reactive immunity and to the lower levels of induction of their innate defense systems (9).

Although early studies suggested that *A. suis* isolates from Canada were homogeneous (10,11), recent work has shown that at least 2 serologically distinct groups of cell surface antigens exist: O1/K1-reactive and O2/K3-reactive. More than 95% of *A. suis* clinical isolates are cross-reactive with O1/K1 or O2/K3 antiserum (12). In a large study that included a limited number of *A. suis* isolates from healthy pigs, all were found to be O1/K1-reactive, whereas more than 80% of the *A. suis* strains submitted for bacterin production (and presumed to be the causative agents of severe disease) were O2/K3-reactive. The O-side chain of O1 antigen is a homopolymer of [→6)-β-D-Glc-(1→6)-β-D-Glc-(1→] while the O2 antigen has a tetrasaccharide backbone of Gal-Glc-Gal-GlcNAc (M. Monteiro, personal communication). Most of the O1 strains analyzed to date also have [→6)-β-D-Glc-(1→6)-β-D-Glc-(1→] in

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their capsular polysaccharide (CPS) (M. Monteiro, personal communication). Although (1→6)-β-D-glucan, also called pustulan, has not been reported in other prokaryotes, it is a major component of fungal and lichen cell walls (13–15). As a result, many animals have antibodies to this polysaccharide in the absence of exposure to *A. suis* (12). In contrast, 2 different CPS, both containing significant amounts of sialic acid, have been found in association with *A. suis* O2 strains (M. Monteiro, personal communication). Sialic acid is present in the capsules of various septicemic bacterial pathogens such as *Escherichia coli*, *Klebsiella pneumoniae*, and *Streptococcus suis* and is known to be a poor immunogen (16–18). Given the epidemiological data and differences in cell surface antigenicity, we hypothesized that *A. suis* O2-containing strains are more virulent than O1-containing isolates.

Materials and methods

Bacterial strains and growth conditions

Three strains were used for the challenge studies. *Actinobacillus suis* SO4 was isolated from a healthy pig in a herd with active *A. suis* disease at the time of isolation. Strain SO4 is an O1/K1 strain with both CPS and LPS composed of [→6)-β-D-Glc-(1→6)-β-D-Glc-(1→] (M. Monteiro, personal communication). Strain H91-0380 originated from a septicemic pig. It is an O2 (Gal-Glc-Gal-GlcNAc), K2 (sialic acid-containing) strain. *Actinobacillus suis* C84 was recovered from a healthy pig at slaughter and is an O1/K2 strain. The growth rate, hemolysin production (19), and protein profiles (20) of these strains were evaluated and, by these criteria, the 3 strains are identical.

For the serum resistance studies, 6 additional *A. suis* isolates were used: B49 (O1/K1), ATCC 15557 (O1/K1), H89-1173 (O2/K3), H91-0406 (O2/K3-reactive), VSB 3714 (rough/K?), and Q95-8196 (untypable). The sources of these strains and serotype characteristics have been described previously (11,12). The 2 bovine fecal *Escherichia coli* isolates, 2204F and 2463F, used as controls in these experiments were the generous gift of C. L. Gyles, University of Guelph. *Escherichia coli* 2204F is serum-sensitive while *E. coli* 2463F is serum-resistant, although the basis of these different serum-sensitive phenotypes is not known (21). Bacterial cultures were routinely grown on blood agar plates and incubated for 18 h at 37°C in an atmosphere of 5% CO_2. For preparation of the challenge inocula, cells were washed off heavily streaked plates in 5 mL of phosphate-buffered saline (PBS), harvested by centrifugation (27 000 × *g*, 5 min, 4°C), and resuspended in PBS to an optical density at 650 nm (OD_{625}) of 0.3. This suspension was diluted 10-fold to a final concentration of approximately 10^7 colony-forming units per mL (cfu/mL). To reduce bacterial mortality, the inoculum was kept on ice until used. The viable cell concentration of the inoculum was determined by plate counting (22).

Animals

Twenty-four, 3-week-old castrated male Yorkshire-cross piglets were used in this study. The animals were obtained from a commercial specific-pathogen-free (SPF) herd that had no history of *Actinobacillus suis* and/or *A. pleuropneumoniae* infection. Upon arrival, the pigs were weighed, ear-tagged, and throat swabs were collected. After an acclimatization period of 3 d, blood samples were collected from the retro-orbital sinus into a sterile Vacutainer tube (Becton-Dickinson Canada, Mississauga, Ontario) using a 16-gauge needle. The blood was allowed to clot at room temperature prior to centrifugation at 800 × *g* for 15 min (GLC-1 centrifuge, Sorvall, Du Pont Canada Inc., Mississauga, Ontario). Aliquots (1 mL) of serum were stored frozen at −20°C. All sera were tested for the presence of hemolysin antibodies using an ELISA to ApxI and ApxII from *A. pleuropneumoniae* (23). Based on sequencing and immunological studies, these toxins are virtually identical to $ApxI_{var.suis}$ and $ApxII_{var.suis}$ (11). In addition, sera were tested for O1/K1 and O2/K3 antibodies by immunoblotting. While all pigs showed a positive reaction to hemolysin, there were no antibodies to either O1/K1- or O2/K3-reactive antigens or to serotypes 1, 5, or 7 CPS of *A. pleuropneumoniae*. In addition, no *A. suis* or *A. pleuropneumoniae*-like colonies were cultured from the throat swabs of piglets prior to the challenge, so the source of these cross-reactive antibodies is not known at this time. Regardless, the presence of these antibodies did not seem to effect the outcome of these challenges as similar results were obtained in 4 pilot studies with antibody-negative animals.

Challenge procedures

Various doses and routes of infection were evaluated in preliminary studies. From these experiments it was found that systemic *A. suis* disease could be reproduced by intraperitoneal (IP) injection of relatively high numbers of the bacteria. As this challenge system produced acute disease, the severity of infection and early pathological responses were evaluated by examining the spread of the organism within the animal and the gross and histopathologic changes 7 h post infection. For the challenge, piglets were randomly assigned to 3 test groups of 7 piglets each and a control group of 3 piglets. One mL of bacterial suspension (1×10^7 cfu/mL) was added to 2 mL of PBS and injected IP using a 21-gauge needle. Control animals received 3 mL of PBS. All animal work was done in accordance with recommendations of the Canadian Council on Animal Care.

Histopathologic and bacteriologic examination

Approximately 7 h post infection, animals were euthanized by intravenous (IV) injection of pentobarbital sodium (Euthansol; Schering Canada Inc., Pointe-Claire, Québec). At necropsy, the amount of peritoneal fluid was measured and all animals were examined for gross pathological lesions. Swabs of the peritoneum, pleura, pericardium, heart, stifle joint, and hock joint were taken with sterile rayon-tipped swabs (Culturette, Becton-Dickinson) and streaked directly onto blood agar plates. Liver, spleen, lung, heart, kidney, peribronchial lymph node, enteric lymph node, and inguinal lymph node were collected and further processed for bacteriologic culture. The surface of each tissue was seared and opened with a sterile blade then a swab was taken and plated on blood agar. Bacterial growth was from 0 to 4, where 0 was no growth, 1 was < 50 colonies, 2 was approximately 50–100 colonies, 3 was > 100 colonies, and 4 was confluent growth.

Tissue samples were taken from stomach, duodenum, spiral colon, omentum, and mesentery for histopathologic examination.

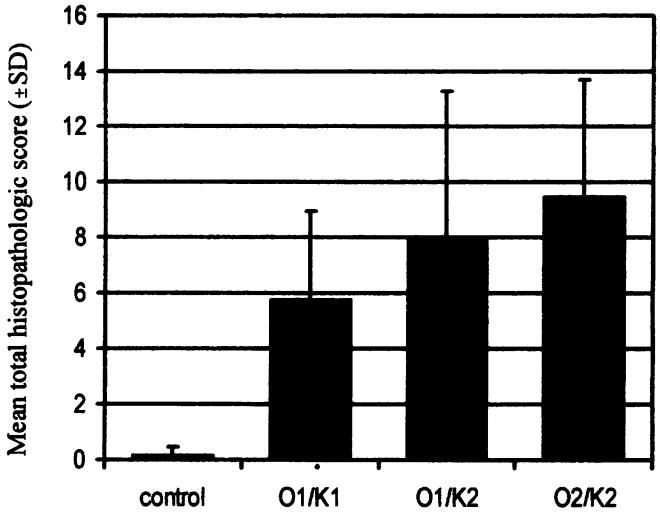

Figure 1. Mean total histopathologic score of all tissues from pigs challenged with different *Actinobacillus suis* strains. Error bars indicate standard deviation. Scores were derived by adding individual scores (0 to 3) for omentum, gastric serosa, splenic mesentery, colonic serosa, mesentery, and the presence of bacterial clumps. Maximum score = 18. Number of animals (*n*) in each group was 7, except for the control group, where *n* = 3. The observed linear trend was significant (*P* = 0.001).

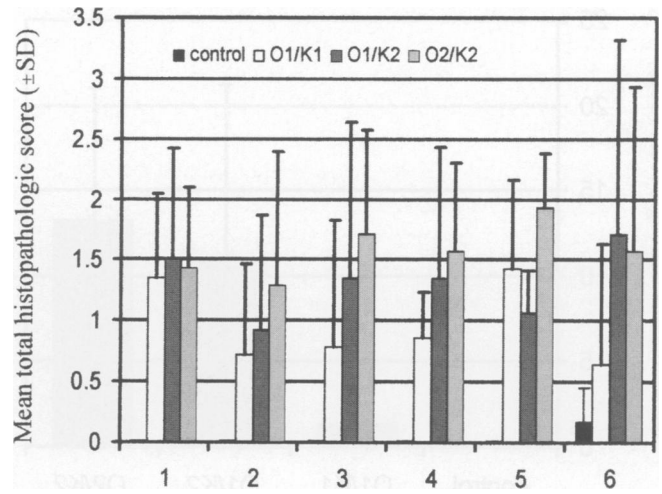

Figure 2. Mean total histopathologic score of individual tissues. 1 — omentum (*P* = 0.01); 2 — gastric serosa (*P* = 0.04); 3 — splenic mesentery (*P* = 0.01); 4 — colonic serosa (*P* = 0.003); 5 — mesentery (*P* = 0.0002); and 6 — presence of bacterial clumps (*P* = 0.06). Error bars indicate standard deviation. *P* values are shown for the observed linear trends. Number of animals (*n*) in each group was 7, except for the control group, where *n* = 3.

These specimens were fixed in 10% buffered formalin, embedded in paraffin, sectioned (5 μm), and stained with hematoxylin and eosin. Histological samples of the different tissues were scored blindly on a scale of 0 to 3 with 0 for normal, 1 for mild, 2 for moderate, and 3 for severe histopathologic changes. The presence and size of bacterial clumps in these specimens were also evaluated.

Serum killing assay

A modification of the method of Taylor was used to measure serum resistance (24). Five mL of Mueller-Hinton (MH) broth was inoculated with 3 colonies from blood agar plates and grown to OD_{578} of 1.0. Five hundred μL of this bacterial suspension was subcultured in 10 mL of pre-warmed MH broth and grown to mid-log phase (OD_{578} = 0.5). Cells were harvested from 1 mL of this suspension in a microcentrifuge (27 000 × *g*, 2 min, 20°C). The cell pellets were washed once in buffered saline-gelatin (BSG), pH 7.35, spun again, and suspended in BSG to a concentration of 3×10^6 cells/mL, as determined from previous growth curve experiments. Buffered saline-gelatin contains 8.5 g NaCl, 0.3 g KH_2PO_4, 0.6 g $NaHPO_4$, 10 mL 1% gelatin and 990 mL H_2O (25). Blood was obtained from 3 clinically healthy 4-week-old SPF pigs from the University of Guelph Research Station at Arkell. Samples were immediately placed on ice and allowed to clot for 1.5 h. Sera were then collected by centrifugation at 800 × *g* for 15 min at 4°C (GLC-1 centrifuge), pooled, aliquoted, and stored at −70°C. As demonstrated by immunoblotting, these antisera contained antibodies to pure *A. suis* O1 and O2 lipopolysaccharides. The pooled sera were used within 3 wk. Immediately before use, individual aliquots were thawed on ice. For each experiment, one aliquot of serum was heat-treated (56°C, 30 min) in order to inactivate both the alternative and the classical complement pathways (heat-inactivated; HI), one was chemically treated (EGTA and $MgCl_2$; final concentrations of 10 mM) to inactivate the classical complement pathway only (classical complement pathway inactivated; CPI) and the third aliquot was left untreated (normal; N).

Table I. Number of *Actinobacillus suis* used in challenge experiments and the volume of peritoneal fluid at euthanasia

Group	Challenge dose (cfu/mL)	Peritoneal fluid mean ± SD (mL)
PBS	—	1.2 ± 1.0
O1/K1 (*A. suis* S04)	1.5×10^7	15.6 ± 12.8
O1/K2 (*A. suis* C84)	9.0×10^6	33.4 ± 29.1
O2/K2 (*A. suis* H91-0380)	6.9×10^6	34.0 ± 26.5

cfu — colony-forming units; PBS — phosphate-buffered saline; SD — standard deviation

To measure serum resistance, 125 μL of bacterial suspension (3×10^6 cells/mL) was added to 250 μL of N, HI, or CPI sera and incubated with agitation (50 rpm) at 37°C for 3 h. Twenty-μL samples were taken from each tube at 0, 1, 2, and 3 h and serially diluted in 180 μL of BSG. Two 10-μL aliquots of the 10^{-2} to 10^{-7} dilutions were then spotted on BHI plates, incubated overnight, and the colonies counted. Percentage viability at each time point was calculated as number of surviving organisms divided by the initial count × 100%. To determine the error associated with these percentages, the standard error of the mean cfu/mL was transformed to reflect the error associated with the % viability (% of the standard error of the mean = standard error of the mean at a certain time point × 100/initial cfu per mL). All strains of *A. suis* were assayed independently at least 3 times in duplicate. *Escherichia coli* 2204F and 2463F were always assayed in parallel.

Statistical analysis

The challenge experiment data were analyzed using the random permutation tests, which are analogous to the analysis of

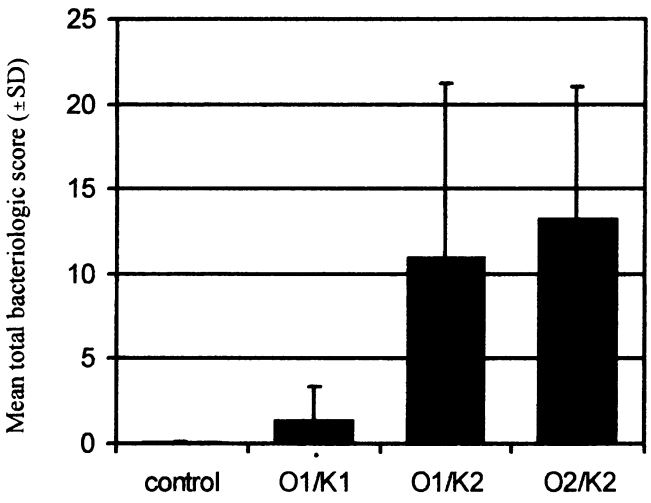

Figure 3. Mean total bacteriologic score of tissues from pigs challenged with different *Actinobacillus suis* strains. Error bars indicated standard deviation. Scores were derived by adding individual scores (0 to 4) for the 14 different sites (see Materials and methods). Maximum score = 56. The observed linear trend was significant ($P = 0.008$). Number of animals (n) in each group was 7, except for the control group, where $n = 3$.

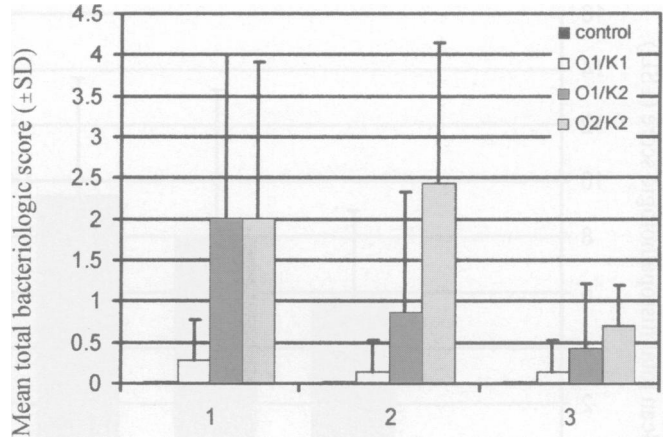

Figure 4. Mean total bacteriologic score of tissues from pigs challenged with different *Actinobacillus suis* strains. 1 — peritoneum ($P = 0.03$); 2 — spleen ($P = 0.01$); and 3 — peribronchial lymph node ($P = 0.06$). Error bars indicate standard deviation. P values are shown for the observed linear trends. Number of animals (n) in each group was 7, except for the control group where $n = 3$.

variance (ANOVA) test. For this analysis, the strains were first ordered in the pre-planned sequences: control, O1/K1, O1/K2, and O2/K2 and then a general contrast analysis for each outcome of interest was done (26). Data from serum killing assays were analyzed using PROC GLM (SAS v6.12, SAS Institute, Cary, North Carolina, USA). These data were first transformed to $\log_{10}$ and then fitted to the $\log_{10}(CNH) = \beta_0 + \beta_1 T + \beta_2 T^2$.

Results

Clinical and pathologic observations

Pigs were challenged with approximately 1×10^7 cfu of the different *A. suis* strains by the IP route (Table I). Depression, vomiting, and weakness were observed 2 to 3 h after infection. At necropsy, a large amount of opaque, serous to serosanguineous fluid was present in the peritoneal cavities of pigs infected with serotypes O2/K2 (34 ± 26.5 mL) and O1/K2 (33.4 ± 29.1 mL). Less fluid (15.6 ± 12.8 mL) was found in the animals inoculated with *A. suis* O1/K1. The linear trend observed was significant ($P = 0.03$) (Table I). The most marked gross pathologic changes were observed on serosal surface of the spiral colon and included pronounced edema with clumps of fibrin loosely adherent to the tissue surface. In addition, petechial to ecchymotic hemorrhages were present on the serosal surface of the entire colon. The control animals were grossly normal.

Histopathologic examination

The most prominent histopathologic changes were observed on the omentum, mesentery, and serosal surfaces of abdominal organs. In contrast to tissues from control animals, large numbers of neutrophils and macrophages, extensive fibrin aggregates, patchy hemorrhages, and cellular debris were present in tissues of the *A. suis*-challenged animals. Clumps of rod-shaped bacteria embedded in the fibrin aggregates were also seen. Depending on the *A. suis* strain, these histopathologic findings varied in severity. The mean histopathologic score for all tissues in each challenge group is shown in Figure 1. The mean scores for individual tissues are shown in Figure 2. While there was no change observed in tissues of the pigs in the control group, animals inoculated with the O2/K2 strain had marked, diffuse peritonitis (mean total score of 9.5) (Figure 1). The intensity of peritonitis was less severe in the pigs infected with the O1/K2 strain (mean total score of 7.9) and in the pigs inoculated with the O1/K1 isolate (mean total score of 5.8). The observed linear trend was significant ($P = 0.001$). Histopathologic changes in animals challenged with the O2/K2 strain were most prominent in the gastric serosa, splenic mesentery, colonic serosa, and mesentery (Figure 2). Severe lesions on omentum and the presence of bacterial clumps were observed following challenge with the *A. suis* O1/K2 isolate, whereas histopathologic lesions in the O1/K1-infected group were otherwise mild, except in the mesentery where they were moderate. There were significant linear trends observed for the gastric serosa ($P = 0.04$), splenic mesentery ($P = 0.01$), colonic serosa ($P = 0.003$), mesentery ($P = 0.0002$), and omentum ($P = 0.01$), but not for the presence of bacterial clumps ($P = 0.06$).

Bacteriologic examination

Tissues were either sterile or yielded pure cultures of *A. suis*. The mean total bacteriological scores of all tissues are shown in Figure 3. A strong linear trend was seen ($P = 0.008$). *Actinobacillus suis* O2/K2 was recovered from tissues with the highest frequency followed by the O1/K2 and O1/K1 strains. The ability of the different strains to spread through the tissues was assessed by examining 3 distinct sites: peritoneum, spleen, and peribronchial lymph node (Figure 4). While a significant linear trend in the isolation rate of different serotypes was seen in peritoneum ($P = 0.03$) and spleen ($P = 0.01$), differences observed for peribronchial lymph nodes were not statistically significant ($P = 0.06$). The *A. suis* O2/K2 strain, however, was recovered with higher frequency from peribronchial lymph

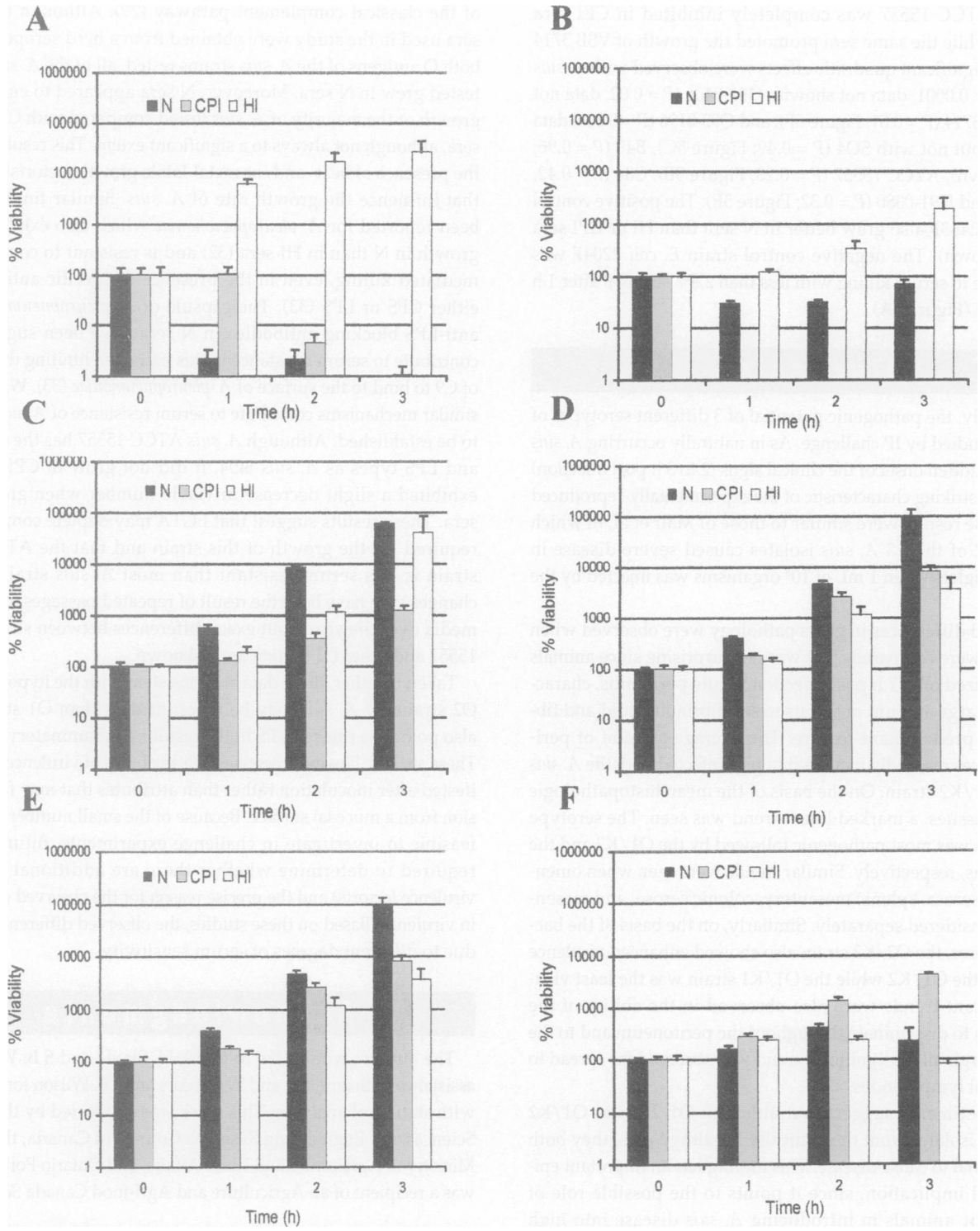

Figure 5. Resistance of (A) *Escherichia coli* 2204F (B) *Actinobacillus suis* ATCC 15557 (O1/K1), (C) S04 (O1/K1), (D) C84 (O1/K2), (E) H91-0380 (O2/K2), and (F) VSB 3714 (rough/K?) to killing with 50% normal (N) sera, 50% heat-inactivated (HI) sera, and with 50% classical pathway-inactivated (CPI) sera. Error bars indicate % of the standard error of the mean.

nodes compared with O1/K2 isolate. Only low numbers of *A. suis* SO4 (O1/K1) were recovered from these 3 sites.

Serum killing assays

Serum killing assays were performed on 9 strains with N (normal), HI (heat-inactivated), and CPI (classical pathway inactivated) sera. The results are shown as % viability (Figure 5). All *A. suis* strains grew well in N sera over a period of 3 h with the exception of ATCC 15557 (O1/K1), which showed an initial decrease (75%) in viability (Figure 5B). The viability of ATCC 15557 in N sera did, however, increase at the end of the incubation period. In general, *A. suis* strains grew more slowly in HI and CPI sera than in N sera. The

growth of ATCC 15557 was completely inhibited in CPI sera (Figure 5B) while the same sera promoted the growth of VSB 3714 (Figure 5F). Significant quadratic effects were observed with *A. suis* H89-1173 ($P < 0.0001$; data not shown), H91-0406 ($P = 0.02$; data not shown), VSB 3714 ($P = 0.01$; Figure 5F), and Q95-8196 ($P = 0.008$; data not shown), but not with SO4 ($P = 0.49$; Figure 5C), B49 ($P = 0.96$; data not shown), ATCC 15557 ($P = 0.23$; Figure 5B), C84 ($P = 0.42$; Figure 5D), and H91-0380 ($P = 0.32$; Figure 5E). The positive control strain *E. coli* 2463F also grew better in N sera than HI or CPI sera (data not shown). The negative control strain *E. coli* 2204F was fully sensitive to serum killing with less than 2.5% survival after 1 h of incubation (Figure 5A).

Discussion

In this study, the pathogenic potential of 3 different serotypes of *A. suis* was studied by IP challenge. As in naturally occurring *A. suis* disease, the sudden onset of the clinical signs (2 to 3 h post infection) was the most striking characteristic of the experimentally-reproduced disease. These results were similar to those of Mair et al, in which they found 2 of their 3 *A. suis* isolates caused severe disease in 13-day-old piglets when 1 mL of 10^8 organisms was injected by the IP route (27).

No marked differences in gross pathology were observed when the 3 strains were compared. This was not surprising since animals were euthanized only 7 h post infection. Acute peritonitis, characterized by a large amount of serous to serofibrinous fluid and fibrin, was the predominant feature. The average amount of peritoneal fluid was markedly increased in pigs infected with the *A. suis* O2/K2 or O1/K2 strain. On the basis of the mean histopathologic score of all tissues, a marked linear trend was seen. The serotype O2/K2 strain was most pathogenic followed by the O1/K2 and the O1/K1 strains, respectively. Similar trends were seen when omentum, gastric serosa, splenic mesentery, colonic serosa, and mesentery were considered separately. Similarly, on the basis of the bacteriologic scores, the O2/K2 strain also showed enhanced virulence followed by the O1/K2 while the O1/K1 strain was the least virulent. Significant trends were also observed in the ability of the O2/K2 strain to disseminate throughout the peritoneum and to the spleen. A marginally insignificant trend was observed for spread to peribronchial lymph nodes.

These experiments suggest that although O1/K1 and O1/K2 strains were isolates from the clinically "healthy" pigs, they both have a potential to cause disease. This finding has an important epidemiological implication, since it points to the possible role of asymptomatic animals in introducing *A. suis* disease into high health status herds. It also emphasizes the need to develop a simple serodiagnostic test to *A. suis*-infected animals.

The role of O and K types in the virulence potential of *A. suis* was further assessed in serum-mediated killing assays. While in most gram-negative bacteria the length, distribution, and structure of the O-polysaccharide chain of smooth LPS type determines the degree of serum resistance (28,29) in some, capsule can also contribute (30,31). However, the protective role of cell surface antigens in serum resistance may, in some instances, be overcome by the presence of specific antibodies to the same components due to activation of the classical complement pathway (29). Although the swine sera used in the study were obtained from a herd seropositive for both O antigens of the *A. suis* strains tested, all of the *A. suis* strains tested grew in N sera. Moreover, N sera appeared to enhance the growth of the majority of *A. suis* tested compared with CPI and HI sera, although not always to a significant extent. This result suggests the presence of heat- and/or metal-labile growth factor(s) in serum that influence the growth rate of *A. suis*. Similar findings have been reported for *A. pleuropneumoniae* which also exhibits better growth in N than in HI sera (32) and is resistant to complement-mediated killing, even in the presence of specific antibodies to either CPS or LPS (33). The capsule of *A. pleuropneumoniae* and anti-LPS blocking antibodies in N sera have been suggested to contribute to serum resistance in this pathogen limiting the amount of C9 to bind to the surface of *A. pleuropneumoniae* (33). Whether the similar mechanisms contribute to serum resistance of *A. suis* remains to be established. Although *A. suis* ATCC 15557 has the same CPS and LPS types as *A. suis* SO4, it did not grow in CPI sera and exhibited a slight decrease in viable number when grown in N sera. These results suggest that EGTA may deplete component(s) required for the growth of this strain and that the ATCC 15557 strain is less serum-resistant than most *A. suis* strains. These changes may have been the result of repeated passages on artificial media over the years, but exact differences between strain ATCC 15557 and other O1 strains are unknown.

Taken together, these data are consistent with the hypothesis that O2 strains of *A. suis* may be more virulent than O1 strains and also point to a role for CPS in the resulting inflammatory response. These studies, however, assessed the attributes of virulence that manifested after inoculation rather than attributes that may favor invasion from a mucosal surface. Because of the small number of isolates feasible to investigate in challenge experiments, future work is required to determine whether there are additional serotype-virulence factor(s) and the precise reason for the observed differences in virulence. Based on these studies, the observed differences are not due to different degrees of serum sensitivity.

Acknowledgments

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