Enzyme-Linked Immunosorbent Assay for Detection of Antibodies to *Staphylococcus aureus* Cell Walls in Experimental Osteomyelitis

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An enzyme-linked immunosorbent assay was used to evaluate and compare the immunoglobulin G antibody response to *Staphylococcus aureus* cell walls of rabbits with either chronic staphylococcal osteomyelitis or subcutaneous abscesses. Osteomyelitis of the femur was produced by the intramedullary application of a sclerosing agent (3% sodium tetradecyl sulfate) and *S. aureus*. Radiographic evidence of osteomyelitis was observed in 10 of the 13 animals that survived the 10-week experimental period, and the diagnosis was confirmed by histopathology in 8 of the 10 instances. Abscess formation was initiated in a separate group of rabbits by the subcutaneous injection of *S. aureus* cells. All 10 of these rabbits subsequently developed abscesses, which usually resolved spontaneously within 3 to 5 weeks. Elevated levels of immunoglobulin G antibodies to the cell wall antigen were detected in 7 of 10 rabbits with osteomyelitis at 21 days postinfection, and these animals continued to display high antibody levels even at 59 days postinfection. In contrast, elevated levels of anti-cell-wall antibodies were only detected in 1 of 10 rabbits with subcutaneous abscesses. The enzyme-linked immunosorbent assay was found to be a rapid and sensitive serological technique for the detection of cell wall antibodies in this experimental osteomyelitis model and may be useful for the diagnosis of staphylococcal bone infections in humans.

Osteomyelitis, secondary to trauma, surgery, or a contiguous focus of infection, is still an important diagnostic and therapeutic problem for clinicians. Although a variety of bacteria have been implicated in the etiology of osteomyelitis, *Staphylococcus aureus* is the most frequently isolated organism and is encountered in 60 to 90% of cases (22). Despite the use of aggressive treatment regimens, including prolonged antibiotic therapy, surgery, and hyperbaric oxygen, osteomyelitis is still characterized by chronicity and resistance to therapy. Unlike other bacterial infections that can be completely eradicated with antibiotics, the treatment failure rates for chronic staphylococcal osteomyelitis have been reported to range from 30 to 80% (15, 21). This is not surprising in view of the fact that the pathogenesis of osteomyelitis involves the formation of sequestrae, secondary to vascular compromise and necrosis, which have been shown to impede the diffusion of antibiotics to the infective focus (7). Our knowledge regarding the pathogenesis of osteomyelitis is still minimal, particularly with respect to those predisposing factors that enable the infection to become established and achieve chronicity. For example, it has been reported that despite vigorous antibiotic therapy, 15 to 29% of patients with acute osteomyelitis progress to the chronic stage of the disease (1).

The role of humoral immunity in osteomyelitis has been the subject of considerable controversy in the literature. Total immunoglobulin levels in patients with osteomyelitis have been reported to be either decreased, elevated, or within normal limits (4, 20). Recent attempts to demonstrate the presence of antibodies to the teichoic acid antigen of *S. aureus* by gel diffusion (18), immunoelectrophoresis (8), or radioimmunoassay (23, 24), have met with only partial success. These studies did reveal, however, that teichoic acid antibodies are encountered more frequently in patients with acute osteomyelitis than in those with chronic osteo-

myelitis. Taylor et al. (17), using a hemolysin inhibition assay, reported the presence of antibodies to the gamma hemolysin of *S. aureus* in 80% of patients with staphylococcal osteomyelitis. This assay, however, lacked specificity, as evidenced by the fact that approximately 20% of patients with tuberculous osteomyelitis possessed cross-reacting antibodies to the gamma hemolysin of *S. aureus*.

At present, there is no serological test available to demonstrate a staphylococcal source of osteomyelitis. A serological test would be advantageous in diagnosis, especially in cases in which adequate cultures cannot be obtained, as well as in the selection of appropriate antibiotics for treatment. In the present study, we report on the development of an enzyme-linked immunosorbent assay (ELISA) and its application for the detection of anti-cell wall antibodies in an experimental chronic staphylococcal osteomyelitis model in rabbits.

MATERIALS AND METHODS

Bacterial strains. The Washington Hospital Center (WHC) strain of *S. aureus* was used throughout this study. This strain was originally isolated from a patient with mandibular osteomyelitis and was obtained through the courtesy of James Perry of the Naval Medical Research Institute, Bethesda, Md. Cultures for inoculation were prepared by growing the organisms at 37°C for 18 h in Trypticase soy broth (BBL Microbiology Systems, Cockeysville, Md.). The organisms were harvested by centrifugation at $8,000 \times g$ for 20 min, washed three times with sterile 0.85% NaCl, and resuspended to a final concentration of 5×10^7 CFU/ml.

Animals. New Zealand White rabbits (Dutchland Laboratories, Denver, Pa.), weighing between 3 and 4 kg each, were used. The animals were housed in individual cages and were maintained on a standard laboratory diet.

The experiments described in this paper were conducted according to the principles set forth in the current edition of the *Guide for the Care and Use of Laboratory Animals*,

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Osteomyelitis. The technique used to create osteomyelitis was that of Norden (14), with some minor modifications. Seventeen rabbits were anesthetized with a combination of ketamine hydrochloride (50 mg/kg) and xylazine (8.8 mg/kg). The right leg was shaved, and the area was cleaned with betadine solution. The cortex of the right mid-femur was exposed under sterile surgical conditions, and a duct was created in the medullary cavity with an activated sterile no. 8 round bur. An 18-gauge needle was used to inject 0.5 ml of a sclerosing agent, 3% sodium tetradecyl sulfate (Elkins-Sinn Inc., Cherry Hill, N.J.) and 0.1 ml of *S. aureus* cells (5×10^6 CFU) into the medullary cavity. The hole in the bone was then sealed with sterile bone wax, and the incision was closed in layers with interrupted 3-0 vicryl sutures. Two control rabbits received sclerosing agent without bacteria in the right femur, and a medullary duct was created in the left femur. The animals were observed for 10 weeks, during which time biweekly radiographs were obtained and serum samples were drawn for immunological studies. At the termination of the experimental period all surviving animals were sacrificed, and the femurs were harvested for bacteriological and histological studies as described below.

Cultures of bone. Specimens for culture were obtained from both bone fragments and medullary cavity contents as described previously (14). The specimens were immediately placed into peptone yeast glucose broth (Scott Laboratories, Inc., Fiskeville, R.I.), and then plated onto both Trypticase soy agar containing 5% sheep blood and mannitol salt agar (England Laboratories, Beltsville, Md.). The plates were incubated aerobically at 37°C overnight, and identification of *S. aureus* was performed on the basis of gram stain reaction, mannitol fermentation, and coagulase test.

Radiographic evaluation. Sequential biweekly radiographs were evaluated by two independent observers using similar criteria previously established by Norden (14). These included (i) periosteal elevation, (ii) increased medullary cavity density, (iii) bone destruction, and (iv) fracture. Animals were considered to have osteomyelitis if they displayed any or a combination of these radiographic features.

Histological analysis. Sections (2 cm) of the infected femur were obtained at the time of sacrifice. The specimens were fixed in 10% neutral buffered Formalin for 4 days, followed by decalcification in a solution containing 4% (vol/vol) hydrochloric acid and formic acid until a chemical endpoint indicated that the process was completed. This was accomplished by taking a 5-ml portion from the bottom of the container each day, adding 5 ml each of 5% ammonium hydroxide and 5% ammonium oxalate, and mixing. If, after 15 to 30 min, the cloudy solution became clear, the specimen was considered to be adequately decalcified. If it remained cloudy, the decalcification solution was discarded, replaced with fresh solution, and reexamined after 6 to 12 h. After decalcification, the tissues were processed in a Tissue Tek III (Sakura Ltd., Tokyo, Japan) and then cut to a thickness of 7 μ m. The specimens were stained with Mayer hematoxylin and eosin, and selected sections were subjected to a Brown and Hopps stain for bacteria (described in reference 11). Histological criteria used to establish the presence of osteomyelitis included either (i) presence of medullary inflammation, (ii) detection of bacteria within Haversian canals, (iii) loss of cortical integrity, or (iv) sequestration or active resorption.

Subcutaneous abscesses. Abscess formation was initiated in a separate group of 10 rabbits by the subcutaneous

injection of *S. aureus* cells (5×10^6 CFU in 0.1 ml of 0.85% NaCl) into the right posterior quadrant. Two animals received a subcutaneous injection of 0.1 ml of 0.85% NaCl without bacteria. The animals were observed for a total of 10 weeks, and the diameter of each abscess was measured with calipers at weekly intervals. Serum was obtained from each animal biweekly for immunological studies.

Antigen extraction. Cell walls were prepared from 18-h cultures of *S. aureus* by the method of Dziarski and Kwarecki (3). Cells were grown in Trypticase soy broth, suspended in distilled water, and heated in a water bath at 70°C for 1 h. The cells were then centrifuged at $8,000 \times g$, washed twice with distilled water and twice with acetone, and allowed to air dry. The cells were disrupted for 10 min in the presence of glass beads with a Bead Beater shaking apparatus (Biospec Products, Bartlesville, Okla.). The disrupted cells were passed through a coarse-sinter glass filter, and the filtrate was centrifuged at $13,000 \times g$ for 20 min. Cell walls were resuspended at a concentration of 10 mg/ml in 0.05 M phosphate buffer (pH 7.4) containing trypsin (0.2 mg/ml), ribonuclease (0.1 mg/ml), and deoxyribonuclease (0.05 mg/ml), and incubated at 37°C for 18 h. The cell walls were then washed three times with distilled water and lyophilized.

Amino acid analysis. Amino acid analysis was performed after hydrolysis of cell walls with 6 N HCl in vacuo for 22 h. For determination of glucosamine and muramic acid, cell walls were hydrolyzed under similar conditions, but only for 4 h. Amino acid and amino sugar analysis were performed on an LKB 4400 amino acid analyzer (LKB Instruments, Inc., Rockville, Md.). In all cases, standards were treated identically to cell walls.

Preparation of hyperimmune serum. Hyperimmune anti-serum to *S. aureus* was prepared in rabbits with heat-killed cells by the method of Yoshida and Ekstedt (26). Hyperimmune rabbit antiserum to *Escherichia coli* M145C2 and *Bacteroides melaninogenicus* ATCC 25845 were kindly provided by B. D. Tall of the Center for Vaccine Development, University of Maryland.

ELISA. The microplate modification of the technique originally described by Engvall and Perlman (5) was used. Briefly, 0.2 ml of cell wall solution (0.1 mg/ml) in 0.05 M carbonate-bicarbonate buffer (pH 9.6) was added to the wells of polystyrene microtiter plates. Control plates received 0.2 ml of buffer without antigen. The plates were incubated at 37°C for 1 h and kept at 4°C overnight. The plates were then washed three times with phosphate-buffered saline (pH 7.4) containing 0.05% Tween 20. Serial twofold dilutions of sera ranging from 1:20 to 1:20,480 were prepared, and 0.2 ml of each dilution was added to the appropriate wells. The plates were incubated at 37°C for 1 h and washed as before, and 0.2 ml of a 1:3,000 dilution of goat anti-rabbit immunoglobulin G (IgG) conjugated with alkaline phosphatase (Miles Laboratories Inc., Elkhart, Ind.) was added. After a 1-h incubation at 37°C the plates were washed again, and the substrate solution (*p*-nitrophenylphosphate [1 mg/ml] in 10% diethanolamine buffer [pH 9.8]) was added. The plates were kept at 25°C for 30 min, and the reaction was terminated by adding 0.05 ml of 3 N NaOH. The results were recorded with a Titertek Multiscan Spectrophotometer (Flow Laboratories, Inc., McLean, Va.) at 405 nm. The titer was defined as the highest dilution of each serum resulting in an optical density of ≥ 0.20 after subtraction of the optical densities obtained with the non-antigen-coated wells. Hyperimmune rabbit antiserum to *S. aureus* was used as a positive control for the ELISA.

FIG. 1. Representative radiograph of the infected right femur of a rabbit at 59 days postinfection, showing periosteal reaction, increased medullary cavity density, and bone destruction. Arrow indicates the site of original inoculation.

Assay specificity. To evaluate the specificity of the ELISA, microtiter plates were coated with *S. aureus* cell wall antigen (100 µg/ml) and reacted against serial twofold dilutions (1:20 through 1:20,480) of hyperimmune rabbit antiserum to either *S. aureus* WHC, *E. coli* M145C2, or *B. melaninogenicus* ATCC 25845. The ELISA was performed as described above, and the titer was defined as the highest dilution of each serum that gave an optical density of ≥ 0.20 after subtraction of the optical density obtained with non-antigen-coated wells.

RESULTS

Osteomyelitis. Of the 17 animals that received sclerosing agent and *S. aureus*, 4 died early during the course of the studies. Of the 13 surviving animals, 10 (77%) developed osteomyelitis, as evidenced by radiographic changes that included periosteal elevation, increased medullary cavity density, or bone destruction (Fig. 1). *S. aureus* was recovered from the infected femurs of nine of these animals. Abscess formation was noted at or near the site of inoculation in 8 of 10 rabbits with osteomyelitis. In contrast, rabbits that received sclerosing agent alone did not develop radiographic evidence of bone infection.

Of the rabbits that displayed radiographic changes consistent with osteomyelitis, histological analysis confirmed the diagnosis in 8 of 10 of these animals. The right femurs of these animals exhibited both an acute and a chronic inflammatory infiltrate that was composed of neutrophils, plasma cells, and lymphocytes as well as bony sequestrae paved with osteoclasts (Fig. 2). Evolving from the original inoculation site there were multiple microabscesses which appeared to spread contiguously within the marrow and by extension into the medullary or nutrient vessels or both. Surrounding the abscesses was an intense infiltrate of polymorphonuclear leukocytes, which was gradually replaced with chronic inflammatory cells. The centers of the abscesses were necrotic and contained cellular debris or

involucra. Peripherally there was an advancing zone of eosinophilic acellular edema and a rich matlike layering of intramedullary fibrosis. Osteomyelitis was not observed by histological analysis in animals that received sclerosing agent without bacteria, although medullary cortical cavitation and aseptic necrosis were readily evident.

Abscess. All of the 10 rabbits that were injected subcutaneously with *S. aureus* cells developed abscesses. Abscess formation was usually first noted between 2 and 7 days postinoculation and was characterized by an indurated nodule that was circumscribed by a zone of erythema. The maximum diameter of individual abscesses ranged from small (<10 by 10 mm) to large (>30 by 20 mm). In 9 of 10 animals, the abscesses resolved spontaneously within 3 to 5 weeks; suppuration was only observed in 3 of 10 of these rabbits. Abscess formation was not observed in rabbits that received a subcutaneous injection of 0.85% NaCl without bacteria.

Amino acid analysis. The results of the cell wall amino acid and amino sugar analysis are presented in Table 1. The molar ratios of the various constituents detected are typical for cell walls of gram-positive bacteria and compare favorably with those reported for other *S. aureus* strains (9).

IgG antibody response in osteomyelitis and subcutaneous abscesses. The results of the IgG antibody response of rabbits with either osteomyelitis or subcutaneous abscesses to the cell wall antigen of *S. aureus* are presented in Tables 2 and 3. Of the 20 total animals in both groups, only rabbit 65-G (Table 2) had an elevated preinfection antibody titer to the cell wall antigen. Significantly elevated levels of IgG antibodies were detected in 9 of 10 rabbits with radiographic evidence of osteomyelitis at various time intervals postinfection. In contrast, only 1 of 10 rabbits with subcutaneous abscesses (rabbit 66-T) displayed a significant rise in titer postinfection (Table 3). In the animals with subcutaneous abscesses, there was no apparent correlation between the size of the individual abscesses or the presence of suppura-

FIG. 2. Photomicrograph of the femur of a rabbit with clinical and radiographic evidence of osteomyelitis, showing a bony sequestrum undergoing resorption by osteoclasts. The sequestrum is surrounded by an intense inflammatory infiltrate within which there are multifocal abscesses and macrophages containing bacteria. (Hematoxylin and eosin stain, $\times 125$.)

tion and the extent of the antibody response observed. An analysis of the antibody response of rabbits with osteomyelitis at various time intervals during the course of the infection revealed that at 10 days postinfection only 1 of 10 animals displayed a significant rise in titer. By 21 days, however, significantly elevated levels of IgG antibodies were detected in 7 of 10 rabbits, and antibody titers continued to increase in these animals even at 59 days postinfection.

Three animals (67-G, 69-G, and 14-V) that received an i.m. application of sclerosing agent and *S. aureus* cells did not develop osteomyelitis (Table 2). In two of these rabbits, the preinfection titer was $<1:20$ and remained at this level at all time intervals tested. In the third rabbit, the preinfection

titer was also $<1:20$, but by 59 days it had increased to 1:2,560. Rabbits that received sclerosing agent alone did not develop osteomyelitis and did not exhibit an antibody response to the cell wall antigen.

Assay specificity. The ELISA IgG antibody titer of the hyperimmune rabbit anti-*S. aureus* WHC serum was $>1:20,480$. No cross-reactivity was detected by ELISA with either the hyperimmune rabbit anti-*E. coli* M145C2 or the anti-*B. melaninogenicus* ATCC 25845 sera. The ELISA IgG antibody titer to the *S. aureus* cell wall antigen was $<1:20$ for both of these sera.

DISCUSSION

This study evaluated the IgG antibody response of rabbits with chronic staphylococcal osteomyelitis to the cell wall antigen of *S. aureus* by an ELISA. The results indicated that at 10 days postinfection only 1 of 10 rabbits with radiographic evidence of osteomyelitis had a significantly elevated titer, but that by 21 days elevated levels of antibodies were detected in 7 of these 10 animals. It is of importance that radiographic changes indicative of osteomyelitis were not usually apparent until 21 to 28 days postinfection, which corresponded to the approximate time interval at which increased antibody levels were first detected in the majority of these animals. In contrast, of the 10 animals that received a subcutaneous injection of *S. aureus* and subsequently developed abscesses, only 1 displayed a rise in titer postinfection. This observation is in agreement with the findings of

TABLE 1. Results of amino acid and amino sugar analysis of cell walls of *S. aureus* WHC

Amino acids and amino sugars	Amt (mol) ^a	Ratio ^b
Glutamic acid	0.240	1.00
Lysine	0.223	0.93
Alanine	0.530	2.21
Glycine	0.966	4.03
Muramic acid	0.316	1.32
Glucosamine	0.393	1.64

^a Moles per gram of cell walls.

^b Ratio with respect to glutamic acid.

Verbrugh et al. (19), who reported that only 2 of 22 (9%) patients with superficial staphylococcal infections possessed significantly elevated IgG antibodies to the cell wall peptidoglycan of *S. aureus*.

Although this experimental model differs from the natural pathogenesis of osteomyelitis because it involves the application of a sclerosing agent to create vascular occlusion, it closely resembles the clinical, histological, and radiographic features associated with osteomyelitis in humans. A major difference noted between this experimental model and human osteomyelitis, however, lies in the extent of the antibody response observed. Although in the present study osteomyelitis was associated with an enhanced humoral immune response, an analysis of the combined results of several previous studies (6, 8, 12, 18, 23) that measured the levels of teichoic acid antibodies in a total of 86 patients with chronic staphylococcal osteomyelitis revealed that such antibodies were absent in about 70% of these patients. More recently, Wheat et al. (24), using a solid-phase radioimmunoassay, detected elevated levels of teichoic acid antibodies in 22 of 47 (47%) patients with chronic *S. aureus* osteomyelitis. This contrasts sharply with other serious staphylococcal infections, such as bacterial endocarditis, in which teichoic acid antibodies were reported to be elevated in nearly all such patients (8, 18, 23). These observations led to the conclusion that the poor antibody response in chronic osteomyelitis in humans may be related to the avascularity of the lesion and the isolation of the infective focus within the sequestrae, thereby preventing bacterial antigens from leaching out to stimulate the host immune system (4, 18).

Our findings, however, as well as those of several previous studies, do not support this conclusion. For example, Taylor et al. (17) have shown that approximately 80% of patients with staphylococcal osteomyelitis produced high levels of antibodies to the gamma hemolysin of *S. aureus*, and Williams et al. (25) reported that some patients with chronic staphylococcal osteomyelitis possessed elevated levels of agglutinating, opsonic, as well as complement fixing antibodies. Rahn et al. (16), using a Clq-binding assay, detected a 40-fold increase in the levels of circulating immune complexes in a patient with *Pseudomonas aeruginosa* osteomy-

TABLE 3. IgG antibody response of rabbits with subcutaneous abscesses to the cell wall antigen of *S. aureus* by ELISA

Rabbit no.	Abscess diam (mm) at various days postinfection			ELISA titer ^a at various days postinfection			
	10	21	35	Pre	10	21	59
50-T	34 × 20	10 × 15	R ^b	<20	<20	<20	<20
52-T	17 × 10	20 × 13	R	<20	<20	<20	<20
54-T	23 × 18	10 × 10 ^c	R	<20	<20	<20	<20
56-T	7 × 7	9 × 5 ^c	R	<20	<20	<20	<20
60-T	7 × 5	10 × 10	R	<20	<20	<20	<20
62-T	7 × 5	20 × 18	15 × 12	<20	<20	<20	<20
64-T	12 × 10	10 × 15	R	<20	<20	<20	<20
66-T		15 × 15	R	<20	<20	<20	160
68-T	28 × 20 ^c	20 × 15 ^c	R	<20	<20	<20	<20
30-V	10 × 10	R	R	<20	<20	<20	<20

^a Reciprocal of highest serum dilution resulting in an optical density at 405 nm of ≥ 0.20 .

^b Resolution of abscess.

^c Suppuration and drainage.

elitis during the initial 5 weeks of treatment. This patient also had a significantly elevated IgG antibody titer to a sonicated antigen preparation of this organism. Similarly, Verbrugh et al. (19) reported the detection by ELISA of elevated levels of antibodies to the peptidoglycan of *S. aureus* in 80% of patients with serious staphylococcal infections, including 11 of 14 patients with staphylococcal osteomyelitis. Studies of the immune response of rabbits with experimentally induced staphylococcal osteomyelitis have also shown that these animals produced high levels of agglutinating (14), opsonic (13), and anti-staphylococcal gamma hemolysin (10) antibodies during the course of the infection. It is evident, therefore, that even though the bacteria may be localized within the bone, antigenic fragments are released from the focus of infection and interact with immunocompetent cells to stimulate humoral immunity.

The lack of an elevated antibody response to teichoic acid in patients with chronic staphylococcal osteomyelitis is in direct contrast to the situation observed in bacterial endocarditis. One possible explanation for this difference may be deduced from the studies of Dziarski and Kwarecki (3), who showed that injection of mice with either crude cell wall preparations of *S. aureus* or peptidoglycan-teichoic acid fragments, but not purified teichoic acid alone, resulted in the elicitation of an immune response. Similarly, Burger (2) has shown that purified teichoic acids are not antigenic in rabbits; however, immunogenicity may be restored by coupling the teichoic acid to an appropriate carrier such as methylated bovine serum albumin. It is therefore tempting to speculate that the poor antibody response to teichoic acid in osteomyelitis, as opposed to bacterial endocarditis, may be due to differences in either antigen processing or presentation by macrophages, or both, in these two types of staphylococcal infections. The findings of elevated anti-cell-wall antibodies in experimental staphylococcal osteomyelitis in the present study, as well as antibodies to purified peptidoglycan in patients with staphylococcal bone infections (19), suggests that these antigens may be more suitable for serodiagnosis than are purified teichoic acids.

In conclusion, we have used a rapid and sensitive ELISA to measure the IgG antibody response to *S. aureus* cell walls in experimental staphylococcal osteomyelitis. The results indicated that the establishment and progression of osteomyelitis was associated with a significant increase in anti-cell-wall antibodies and that the ELISA accurately

TABLE 2. IgG antibody response of rabbits with osteomyelitis to the cell wall antigen of *S. aureus* by ELISA

Rabbit no.	Clinical findings by:			ELISA titer ^a at various days postinfection			
	Radiology	Histology	Culture ^b	Pre	10	21	59
51-G	+ ^c	+	+	<20	<20	320	640
53-G	+	+	+	<20	<20	80	640
55-G	+	+	+	<20	<20	1,280	5,120
59-G	+	+	+	<20	<20	<20	1,280
61-G	+	-	+	<20	<20	80	320
63-G	+ ^c	+	+	<20	<20	80	20,480
65-G	+	+	+	320	320	640	640
8-V	+	+	+	<20	80	<20	<20
10-V	+	+	+	<20	<20	80	<20
12-V	+	-	-	<20	<20	10,240	20,480
67-G	-	-	-	<20	<20	<20	ND ^d
69-G	-	-	-	<20	<20	<20	2,560
14-V	-	-	-	<20	<20	<20	<20

^a Reciprocal of highest serum dilution resulting in an optical density at 405 nm of ≥ 0.20 .

^b Isolation of *S. aureus* from infected femur.

^c Compound fracture of infected femur.

^d ND, Not determined.

discriminated between animals with deep-seated staphylococcal bone infections and those with only superficial skin abscesses. These findings suggest that the cell wall ELISA may be a useful serological test for the diagnosis of staphylococcal osteomyelitis in humans and would be especially beneficial in cases in which adequate cultures cannot be obtained and the infecting organisms cannot be identified.

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