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Septic Shock Due to *Vibrio vulnificus* Serogroup 04 Wound Infection Acquired from the Baltic Sea

Vibrio vulnificus was first isolated by the Centers for Disease Control in 1964 and given its name in 1979 (1). It belongs to the family of *Vibrionaceae*. *Vibrio vulnificus* is the most virulent species of the noncholera vibrios. It is a halophilic (salt-loving), gram-negative, motile, and curved rod, and ferments lactose (lactose-positive *Vibrio*). *Vibrio vulnificus* is found all over the world in seawater and shellfish. Several hundred infections have been reported from the United States, Japan, and other East Asian countries. In Europe, however, only a few cases have been described since 1979 (2, 3). We report here the first case of a *Vibrio vulnificus* infection in Germany which was acquired from the Baltic Sea off the island of Usedom.

A 71-year-old female was admitted to Benjamin Franklin Hospital of the Free University of Berlin in August 1994 for fever, distraction, mild diarrhea, and pain in the right leg. Five days previously, the patient had gone wading in the sea with a small traumatic wound on the lower right leg. Her medical history included only an uncomplicated cholecystectomy. On admission she was hypotensive (80/50 mm Hg), tachycardic (130/min), and showed slow reactions. The lower part of the right leg was red and swollen, showing a 15 x 5 cm hematoma in front and a small hemorrhagic bulla on the calf. On the second day of hospitalization, the patient showed two more hemorrhagic bullae: one on the lower part of the left leg and one on the head. They healed spontaneously during subsequent days. Laboratory findings on admission were normal. During the following 16 hours, the platelet count fell from 235/nl to 23/nl. The leucocyte count increased from 6.5/nl over 12.9/nl to 13.4/nl with a left shift. Echocardiography was negative for vegetations on the heart valves. Abdominal sonography showed free liquid perirenally, perivesically, and in the Douglas space. The working diagnosis was septic shock due to an infected hematoma on the right leg.

The hematoma was surgically cleaved and, under ampicillin and flucloxacillin i.v., the patient's condition initially improved. In spite of the decreasing temperature (37.8°C after 6 h), 11 hours after admission there was an acute deterioration with hypotension (60 mm Hg), respiratory distress, and peritonism. The patient was transferred to the intensive care unit for mechanical ventilation. The antibiotic regimen was changed to piperacillin/sulbactam and ciprofloxacin. During the following 24 hours she became anuric and developed dysfunction of the liver. The patient had to be ventilated for 16 days and hemodialyzed for 21 days; her total stay in the intensive care unit amounted to 5 weeks. The right leg showed large hemorrhagic bullae and a deep soft-tissue infection with necrosis. Necrotectomy was done and, under subsequent intensive local surgical therapy, normal function of the right leg was restored.

Three days after admission a gram-negative curved rod was isolated from 3 of 12 blood cultures (Septi Check, BBL, Microbiology Systems, USA) containing either brain heart infusion (1 bottle) or thioglycollate (2 bottles) medium. The organism grew on blood agar containing 0.5 % NaCl, forming greyish colonies surrounded by a zone of hemolysis. There was negative growth on McConkey agar. The oxidase and catalase reactions were positive. The organism was identified

as *Vibrio vulnificus* by using the API 20 E system (bioMérieux, France). T. Shimada, Tokyo, serotyped the isolated strain as belonging to serogroup 04 (4, 5), which may be interesting with regard to the source of infection. The strain was susceptible to all of the antibiotics employed: ampicillin (MIC 2.0 mg/l), piperacillin (MIC 2.0 mg/l), and ciprofloxacin (MIC 0.03 mg/l). The organism could be isolated neither from an initially taken specimen of the hemorrhagic bulla nor from different specimens taken from the wound of the right leg.

Vibrio vulnificus infections have two different major manifestations: 1) primary septicemia typically accompanied by metastatic bullous skin lesions following consumption of contaminated oysters or raw shellfish; 2) wound infection acquired after exposure to seawater or during fish preparation, which may result in tissue necrosis and secondary septicemia. Occasionally, gastroenteritis also develops, with stool cultures positive for *Vibrio vulnificus* (6). Predisposing factors for a complicated infection with *Vibrio vulnificus* are chronic liver diseases, elevated serum iron levels, low gastric acid, and a compromised immune system (7, 8).

Our case is unusual as the patient did not display any predisposing factors such as chronic diseases. According to her out-patient physician, her liver enzyme levels had never been elevated at previous investigations. During hospitalization her liver function and iron metabolism were normal, and hepatitis B and C serology was negative.

The management of *Vibrio vulnificus* infections includes intensive-care monitoring, aggressive wound care, and prompt initiation of antibiotic therapy. Tetracycline is currently recommended as the drug of choice, while in vitro, the organism is susceptible to nearly all antibiotics (7). In our case, because the infecting organism was unknown at the time of the patient's acute deterioration, piperacillin/sulbactam was chosen in order to cover an extended spectrum including gram-negative organisms, staphylococci, and anaerobes. Because of the severe local infection of the right leg, ciprofloxacin was included owing to its excellent tissue penetration. The patient overcame this infection probably because of her excellent physical condition, the prompt initiation of antibiotic and aggressive surgical therapy, and the high standard of intensive care.

Vibrios belong to the most common organisms in surface waters worldwide. Optimal growing conditions are salinity of water between 0.7 % to 1.6 % and temperatures not below 17°C (7). In August 1994, the water in the German part of the Baltic Sea reached unusually high temperatures of more than 20°C, no doubt allowing the survival of this *Vibrio* species. Most previously reported infections in Europe were acquired from the North Sea. Veenstra et al. (9) isolated *Vibrio vulnificus* strains along the Dutch North Sea coast in August, when the water temperature is highest. Thus far, Baltic seawater has not been investigated for *Vibrio* species. We did not initiate an investigation of the seawater off Usedom island for *Vibrio vulnificus* in this case, since the hottest period had just ended when the patient was admitted to hospital. However, it may be worth discussing whether the quality control of European seawater should be extended to the investigation of *Vibrio* species in hot summer periods. Remarkably, so far no infections have been reported from southern Europe, which is probably because the salinity of the Mediterranean Sea is too high for the survival of *Vibrio vulnificus*.

In light of the reported case, we would like to emphasize that even in Europe, the presence of *Vibrio vulnificus* must be considered if painful, extended wound infections or severe septicemia, especially associated with hemorrhagic bullae, are seen and the consumption of raw seafood or wound contact with seawater are reported.

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Evaluation of Direct Immunofluorescence, Dot-Blot Enzyme Immunoassay, and Shell-Vial Culture for Detection of Respiratory Syncytial Virus in Patients with Bronchiolitis

Respiratory syncytial virus (RSV) is recognized as the principal causative agent of acute lower respiratory tract infections, affecting mainly infants and young children. The majority of these infections occur in annual epidemic outbreaks in winter and early spring. It is, therefore, important to obtain a rapid, early diagnosis that permits identification of infected children (1, 2).

There are two rapid techniques for the diagnosis of infection by RSV, both based on viral antigen detection in respiratory secretions: enzyme immunoassay (EIA) and direct immunofluorescence. Isolation of the virus in cell culture has been considered the gold standard with which all new techniques of detection and isolation of RSV should be compared (3, 4). However, shell-vial culture has been shown to be more rapid and more sensitive than classic cell culture, suggesting that it may be used as an alternative comparative method for the study of epidemic outbreaks of bronchiolitis by RSV (5, 6).

We performed a prospective study of the efficacy of two rapid methods of antigen detection, a dot-blot (DB) EIA (Directigen RSV, Becton Dickinson, USA) and direct immunofluorescence (Monofluokit RSV, Pasteur Diagnostics, France), compared with the shell-vial method of isolation in culture for the detection of RSV in 229 nasopharyngeal aspirates obtained from children soon after the onset of bronchiolitis. The DB-EIA technique was performed using 250 µl of diluted sample. The sample was prepared and the results read in accordance with the manufacturer's instructions. For the direct immunofluorescence technique, 200 µl of the sample was centrifuged in a cytospin (Cytospin 3, Shandon, UK) at 700 x *g* for 10 min. After drying, the smears were fixed with acetone for 10 min at -20°C and then stained with a direct RSV fluorescent antibody stain (Monofluokit RSV). The slides were incubated at 36°C in a humidity chamber for 30 min, after which they were rinsed in phosphate-buffered saline. The slides were viewed at x 400 on a fluorescence microscope.

For the shell-vial technique 200 µl of the sample was inoculated into two Hep-2 vials (Vircell, Ingelheim Diagnostica, Spain). The vials were then centrifuged at 700 x *g* for 45 min. They were allowed to rest at 36°C for 60 min and the supernatant was discarded. One ml of maintenance medium, MEM with 1 % fetal bovine serum, was added to each sample. The vials were incubated at 36°C for two days if both rapid tests were positive and for three days if only one test was positive or if both were negative. After incubation the monolayers were stained with anti-RSV (Monofluokit RSV). The monolayers were viewed at x 200 and x 400 on a fluorescence microscope.

Sensitivity, specificity, and positive and negative predictive values for the DB-EIA and the direct immunofluorescence technique were calculated by comparison with isolation of RSV in the shell-vial culture.

Of the 229 samples studied, 130 (56.8 %) were considered positive for RSV. In this group of 130 samples, we detected 116 cases of RSV infection (89.2 %) and 14 cases of infection by other viruses. Of the total samples studied, RSV accounted for 50.6 % of the viral infections. The results obtained with the different techniques are shown in Table 1. The DB-EIA detected 74 cases (63.7 %) of RSV infection, direct immuno-