Sepsis is a frequent cause of morbidity and mortality in the neonatal period. It can be caused by a variety of microorganisms and often occurs in the absence of detectable pathogens [1]. In sepsis among adults, chills, fever, and other nonspecific signs and symptoms are commonly observed. However, in neonates, fever may be absent at the onset of the illness, resulting in a delay of diagnosis and treatment [2, 3]. In the sepsis syndrome, pathophysiologic events leading to multiple organ dysfunction, circulatory collapse, and death are poorly understood. Nonetheless, several studies have demonstrated an association between the development of clinical and laboratory manifestations of sepsis and the sepsis syndrome and endotoxin, a lipopolysaccharide found in the cell wall of gram-negative bacteria [4, 5]. Administration of endotoxin to experimental animals can elicit pathophysiologic symptoms similar to those seen in patients with sepsis [6].

In October 1996, 36 neonates developed fever and clinical sepsis and died in a 26-bed nursery at a 200-bed maternity hospital in Boa Vista, Roraima, Brazil. All spent their first days of life in the nursery, including premature and low–birth weight neonates born at the hospital. Limited advanced life support capabilities were available, including equipment and personnel to support 2 or 3 infants on ventilators and the ability to perform central (umbilical) vein catheterization for the administration of intravenous (iv) fluids. The affected neonates had fever, cyanosis, inadequate organ perfusion, and dyspnea beginning 24–72 h after birth. A local investigation traced the cause of the outbreak to unsanitary conditions, nursery overcrowding, and breaks in aseptic technique. Although recommendations to improve infection control were implemented at the hospital and there was a decrease in mortality, the increased incidence of fever and clinical sepsis persisted. As a result, in November 1996, the Roraima Secretary of Health requested the assistance of the US Centers for Disease Control and Prevention (CDC) in the investigation. Here, we summarize the results of this investigation.

Methods

Background mortality rates and demographics. To determine the background rate of deaths in the newborn nursery, we obtained the number of admissions to the nursery and the number of deaths from October 1995 through October 1996. To determine additional nursery demographics and to stratify the mortality rate by birth weight, we recorded from nursery logs the birth date, weight, discharge date, and outcome of all admissions during the outbreak period (October 1996) and the immediate preoutbreak period (June–September 1996).

Epidemiologic studies. A case-control study to identify risk factors for death due to clinical sepsis during the outbreak period
(October 1996) was performed. A case patient was defined as any neonate admitted to the hospital nursery during October 1996 who developed a temperature of \(\geq 38^\circ C\) without a recognized cause, treatment for sepsis by a physician, and subsequent death. Two randomly selected birth date–matched neonates were selected as control subjects.

A cohort study (October cohort) was performed to identify risk factors associated with developing fever during the outbreak period; these included neonates born on 4 representative days during the month of October 1996 (i.e., 1, 5, 15, and 25 October). A second cohort study (June cohort) was conducted among neonates born on the same 4 dates in June 1996 (i.e., 1, 5, 15, and 25 June), to determine whether risk factors for fever in the October cohort were associated with fever before the time of the increase in death rate (e.g., in the prepandemic period). A case patient for both cohort studies was defined as an infant born on a study date who developed a temperature of \(\geq 38^\circ C\) without a recognized cause, resulting in institution of therapy for sepsis, but not necessarily resulting in death.

To determine factors associated with the use of iv-administered fluids in the nursery both before and during the outbreak period, we performed an analysis of combined data from the 2 cohort studies. We also compared neonates in the combined cohort studies who received iv medications and developed fever with those who received iv medications but did not develop fever.

Procedure review. Procedures for delivery, routine care of the baby (e.g., cord care, bathing, and feeding), the pathway and storage of milk in the milk bank, routine infection control procedures (e.g., hand washing and use of gloves), insertion of iv catheters, and preparation and administration of iv medications were reviewed by members of the investigation team.

Laboratory studies. Laboratory studies included culture of neonate blood and iv medications, assays for endotoxin, and scanning electron microscopy (SEM) of the contents of unopened medication vials. Blood for culture (1–3 mL) was collected from neonates who had fever onset during 1–18 November 1996. We obtained blood by use of a butterfly needle and syringe, and the blood was injected into Vacutainer (Becton Dickinson) manual blood culture bottles. These were incubated at 36°C for 7 days and inspected daily for turbidity or other signs of growth. If a blood culture appeared to be positive, a Gram’s stain and subculture of the broth was performed. All blood cultures underwent blind terminal subcultures on sheep blood agar at the end of 7 days of incubation. Blood culture isolates were identified by standard microbiologic methods [7].

Cultures of iv fluids and medications administered to the newborns were performed by passing fluids through a 0.45-\(\mu\)m filter and then by placing these filters aseptically on tryptic soy agar containing 5% sheep blood that was incubated at 36°C for \(\geq 48\) h. Endotoxin assays of iv fluids and medications were performed by the turbidimetric limulus amebocyte lysate-5000 assay (Associates of Cape Cod) [8].

SEM was performed as described elsewhere, with modification [9]. In brief, 10-mL samples were fixed with 2% glutaraldehyde in cacodylate buffer (0.067 mol [pH 6.2]) without ruthenium red for 24 h at room temperature. After fixation, each sample was filtered by using a 0.2-\(\mu\)m polycarbonate filter (Nuclepore). The filter was washed twice with Sorenson’s buffer (pH 6.0), dehydrated in a graded series of alcohol for 10 min each, and immersed in hexamethyldisilazan for 2 h at room temperature. The filters were placed in a desiccator containing anhydrous calcium sulfate overnight, mounted on aluminum stubs with silver paint, sputter coated with 20 nm of gold, and observed with a scanning electron microscope (Philips Electronic 515).

Statistical analysis. Data were collected on standardized forms, entered into a computer, and analyzed with Epi Info software (version 6.02; CDC). Categorical variables were compared by using the Fisher’s exact 2-tailed or Mantel-Haenszel \(\chi^2\) test. Medians and continuous variables were compared with the Student’s \(t\) or Kruskal-Wallis test. Odds ratios, risk ratios, and Cornfield 95% confidence intervals were calculated where appropriate.

Results

Background mortality rate. The death rate in the nursery during October 1996 was significantly higher than the median rate from October 1995 through September 1996 (6 vs. 1.7/100 births; \(P < .001\); figure 1). The overall median birth weight in the nursery from June through October 1996 was 3.25 kg (range, 0.55–5.51 kg; interquartile range, 2.94–3.56 kg). The median birth weight was unchanged between the preoutbreak (June–September) and outbreak (October) periods (3.24 vs. 3.23 kg; \(P = .6\)).

Birth weight–stratified mortality appeared to increase from the preoutbreak to outbreak period in the 3 lower birth weight quartiles (6.6–11, 0.8–2.7, and 0.4–0.7 deaths/100 births, respectively), whereas, in the uppermost quartile, the rate decreased slightly (1.5–1.2 deaths/100 births). However, none of the changes in mortality by birth weight quartile was statistically significant. Mortality was further stratified according to whether birth weight was \(\leq 1.5\) kg. Although mortality in this very low birth weight stratum (\(\leq 1.5\) kg) appeared to be unchanged or decreased slightly from preoutbreak to outbreak period (71.4–62.5 deaths/100 births; \(P = .7\)), mortality increased in the more normal birth weight stratum (1.6–3.1 deaths/100 births; \(P = .03\)). The overall median length of stay in the nursery was 2 days (range, 1–72 days) and was unchanged between the preoutbreak and outbreak periods (\(P = .8\)).

Figure 1. Secular trend in nursery mortality rate at hospital A, Brazil, during October 1995–October 1996.
Case-control study. Of 579 neonates admitted to the nursery during October 1996, 20 (3.5%) met the case definition. Case patients had a lower gestational age, lower birth weight, and lower APGAR (activity, pulse, grimace, appearance, and respiration) scores at 1 and 5 min than did control subjects (table 1). In contrast, the proportion of case patients who were delivered via caesarean section or were male was similar to control subjects. Although case patients were more likely than control subjects to have a lower birth weight, ~90% of case patients were full-term, normal birth weight newborns. Since all case patients had a peripheral iv before symptom onset, we focused our investigation on the fluids administered to the neonates. Several fluids were administered to case patients: calcium gluconate, penicillin, sodium chloride, potassium, sodium bicarbonate, glucose, aminophylline, and distilled water (used to reconstitute medications). Of these, only glucose, aminophylline, and distilled water were administered to all case patients. Seventeen (85%) of the case patients who received iv fluids did so on their birth date; all developed fever only after exposure to the parenteral medications. The median number of days between the start of iv medication and fever was 3 (range, 1–7 days). A review of case patients’ medical records suggested that many were given iv medications unnecessarily.

October cohort. Of 66 neonates admitted to the nursery during the 4-day October 1996 study period, 6 (9%) met the cohort study case definition of unexplained fever. Case patients had lower APGAR scores at 1 and 5 min and were more likely to have had a peripheral iv catheter and to have received iv fluids than control subjects (table 2). In contrast, case patients and control subjects were similar with regard to sex, cesarean section delivery, birth weight, and gestational age.

June cohort. Of the 55 neonates admitted to the nursery during the 4-day June study period, including 11 who were exposed to iv medications, none developed fever.

Combined cohort analysis. Of the 120 neonates in the combined cohort studies, 20 (17%) received iv medications. Neonates who received iv medication had a lower median birth weight (2.9 vs. 3.3 kg, \(P = .02\)) and lower median APGAR scores at 1 (7.0 vs. 8.0; \(P < .0001\)) and 5 min (8.5 vs. 9.0; \(P = .0001\)). In contrast, neonates who received iv fluids were similar to those who did not receive any with regard to sex, type of delivery, and gestational age (data not shown).

The 6 neonates in the combined cohort studies who received iv fluid and subsequently developed fever were similar to the 14 who received iv fluid but did not develop fever with respect to birth weight, 1- and 5-min APGAR scores, sex, type of delivery, age when iv fluid was started, and gestational age (data not shown).

Procedure review. Confined space, overcrowding, and understaffing characterized conditions prior to and at the time of the outbreak onset when all infants delivered were taken to the unit. Several changes in infection control procedures occurred before the beginning of our investigation. The nursery had been moved to a new location, and the number of admissions to the nursery had decreased, with the healthiest newborns being cared for on the maternity ward. Infection control techniques had improved, and, during our investigation, there were no lapses in aseptic technique observed that could account for episodes of clinical sepsis and death.

Laboratory studies. After ≥48 h of incubation, all cultures of parenteral fluids and medications used in the nursery were negative for bacterial growth. Of 14 blood cultures of newborns done ≤2 days after the onset of fever, only 1 grew a microorganism (Klebsiella pneumoniae). Six (46%) of 13 unopened vials of bidistilled water for injection and 12 (80%) of 15 unopened vials of 25% glucose had elevated endotoxin levels (table 3); both fluids were manufactured by the same company (Hi-polar Farmaceutica). Samples of bidistilled water containing and not containing elevated levels of endotoxin were examined by SEM. Samples containing elevated levels of endotoxin showed the presence of a caked amorphous-like material and few bacterial cells (figure 2). A brownish gelatinous mass formed in the filtrate after passage of the sample through the filter. The same was not observed in samples negative for endotoxin.

Discussion

Our investigation documented an outbreak of clinical sepsis and death of neonates during October 1996 associated with iv fluids intrinsically contaminated with endotoxin. Endotoxin has many different biologic effects, with fever as its hallmark. The release of endotoxin into the circulating system is the initial event of sepsis due to gram-negative infection, and reactions may range from no detectable response to profound shock [1]. Such reactions are highly dependent on the patient’s body weight [10]. Because the minimal pyrogenic dose of endotoxin is 5 endotoxin units (EU)/kg [11], <2 mL of the contaminated bidistilled water (median level of contamination, 3.7 EU/mL) would be sufficient to evoke pyrogenic reactions in an average 2000-g infant and also may have been sufficient to cause death [12, 13]. In addition, it is possible that contaminants other than endotoxin, accounting for the amorphous material seen on the

<table>
<thead>
<tr>
<th>Variable</th>
<th>Case patients (n = 20)</th>
<th>Control subjects (n = 40)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Categorical, no. (%) of subjects</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male sex</td>
<td>13 (65)</td>
<td>20 (50)</td>
<td>.4</td>
</tr>
<tr>
<td>Cesarean section delivery</td>
<td>6 (30)</td>
<td>9 (23)</td>
<td>.5</td>
</tr>
<tr>
<td>Peripheral intravenous catheter</td>
<td>20 (100)</td>
<td>2 (5)</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Continuous, median (range)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Birth weight, kg</td>
<td>2.1 (0.8–4.9)</td>
<td>3.3 (2.4–4.1)</td>
<td>.0007</td>
</tr>
<tr>
<td>APGAR score at 1 min</td>
<td>7 (1–9)</td>
<td>8 (3–8)</td>
<td>.011</td>
</tr>
<tr>
<td>APGAR score at 5 min</td>
<td>8 (7–10)</td>
<td>9 (7–10)</td>
<td>.003</td>
</tr>
<tr>
<td>Gestational age, weeks</td>
<td>32 (22–41)</td>
<td>39 (32–42)</td>
<td>.001</td>
</tr>
</tbody>
</table>

NOTE. APGAR, activity, pulse, grimace, appearance, and respiration.

Table 1. Case-control study of risk factors for clinical sepsis and death at hospital A, Brazil, in October 1996.
SEM, may have had a role in causing the death of some neonates. Although endotoxin and other contaminants alone could have caused these deaths, other factors probably contributed to the evolution of fatal outcome in so many neonates. Exposure to endotoxins may have increased bloodstream infections and death by causing debilitation, prolonging exposure to peripherally inserted catheters, or by prolonging exposure to breaks in aseptic technique during the catheter manipulation. In support of these last 2 hypotheses, after improvement of infection control, there was a marked decrease in mortality during November 1996, although episodes of fever persisted.

Many neonates in this investigation were full-term and of normal or near-normal weight and had few clear indications for iv therapy. Although the infants who received iv therapy and became case patients had lower birth weights, lower APGAR scores, and were more premature (table 1), most still weighed >2 kg at birth and had a gestational age of >30 weeks—yet received iv medications. This contrasts with most nurseries, where only the most premature, lowest birth weight neonates require iv therapy. During the study period, neonates were routinely given iv glucose, aminophylline, and bidistilled water to assist in the management of mild respiratory distress syndrome (surfactant was not in use at this time). However, no data support the use of aminophylline for this purpose [14]. Although iv fluid therapy is an essential component of modern medical care, it has the potential to cause numerous health hazards and complications [15], and overuse therefore should be avoided.

The results of this investigation should focus attention on the importance of 2 critical issues in public health and the prevention of medical errors: the value of local and national surveillance of health care–associated infections and the need for proper quality control of medication manufacturing. The routine surveillance of health care–associated infections at the hospital level is essential for the early detection and control of these medical errors, including epidemics. Clusters of pyrogenic reactions always should lead to an evaluation of possible product contamination. Active surveillance for health care–associated infections would have facilitated earlier recognition of this outbreak. Unfortunately, because there was no such surveillance or active infection control program, this outbreak was detected only after a substantial number of deaths occurred.

Surveillance also is important at the national level. In this outbreak, contaminated medications (manufactured by Hipolabor Farmaceutica) were widely distributed throughout Brazil, and neonatologists and the media reported similar outbreaks of sepsis and death among newborns in nurseries around the country, suggesting a nationwide outbreak. During our investigation, we contacted personnel at some of these nurseries to communicate our findings and to suggest the possibility that these outbreaks could be related. We requested a list of iv medications being administered to the newborns; only one hospital complied with our request. That list included medications from the manufacturer that produced the contaminated iv fluids. Unfortunately, multiple on-site investigations and microbiologic studies were not conducted, and these outbreaks usually were attributed to the low birth weight of newborns, nursery overcrowding, and lack of nursery personnel (all conditions originally thought to be responsible for the outbreak at the hospital studied). Although the connection between the nursery outbreaks may have been overlooked, there has been one other report of an outbreak associated with contaminated medications from the manufacturer implicated in our investigation [16]. In that outbreak, 39 oncology patients developed Bacillus species bloodstream infections over a 14-week period. These infections were associated with parenteral calcium gluconate solution (Hipolabor Farmaceutica).

Regarding quality control of medications, it is well recognized that, unless strict standard operating procedures are followed, there is risk of intrinsic endotoxin or bacterial contamination of iv medications. Endotoxin is ubiquitous and can

### Table 2. Risk factors for fever at hospital A, Brazil, for October 1996 cohort.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Exposed</th>
<th>Unexposed</th>
<th>Risk ratio</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Categorical, no./total (%) of subjects</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male sex</td>
<td>2/31 (7)</td>
<td>4/35 (11)</td>
<td>0.6 (0.1–2.9)</td>
<td>.7</td>
</tr>
<tr>
<td>Cesarean section delivery</td>
<td>2/25 (8)</td>
<td>4/41 (10)</td>
<td>0.8 (0.2–4.2)</td>
<td>1.0</td>
</tr>
<tr>
<td>Peripheral intravenous catheter</td>
<td>6/9 (67)</td>
<td>0/57 Undefined</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Continuous, median (range)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Birth weight, kg</td>
<td>3.1 (2.2–4.8)</td>
<td>3.3 (2.1–4.4)</td>
<td>.7</td>
<td></td>
</tr>
<tr>
<td>APGAR score at 1 min</td>
<td>4.5 (1–8)</td>
<td>8.0 (1–9)</td>
<td>.002</td>
<td></td>
</tr>
<tr>
<td>APGAR score at 5 min</td>
<td>7.5 (5–9)</td>
<td>9.0 (6–10)</td>
<td>.009</td>
<td></td>
</tr>
<tr>
<td>Gestational age, weeks</td>
<td>39 (38–46)</td>
<td>39 (27–44)</td>
<td>.9</td>
<td></td>
</tr>
</tbody>
</table>

**NOTE:** APGAR, activity, pulse, grimace, appearance, and respiration.

### Table 3. Bidistilled water and glucose endotoxin levels and United States Pharmacopeia (USP) endotoxin limits at hospital A, Brazil, during October 1996.

<table>
<thead>
<tr>
<th>Fluid</th>
<th>No. of vials</th>
<th>Endotoxin level, median EU/mL (range)</th>
<th>USP limit, EU/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bidistilled water</td>
<td>6</td>
<td>3.3 (0.86–5.8)</td>
<td>0.25</td>
</tr>
<tr>
<td>Glucose</td>
<td>12</td>
<td>1.2 (0.8–1.9)</td>
<td>0.5</td>
</tr>
</tbody>
</table>

**NOTE:** EU, endotoxin unit.
easily contaminate parenterals [17]. Many gram-negative organisms that are a source of endotoxin require few nutrients and can even grow in distilled water at 4°C. In addition, endotoxin is not destroyed by steam autoclaving, organic solvents, acids, ethanol, or sterilizing liquids. Thus, product sterility does not ensure the absence of endotoxin. Only dry heat (≥250°C for 30 min or ≥180°C for 3 h) can ensure the elimination of endotoxin [18]. Therefore, strict efforts to avoid bacterial or endotoxin contamination during the manufacturing process of medications is essential, particularly if a sterilization method other than dry heat is used.

Unopened vials of contaminated medication in this outbreak were undamaged and had no evidence of tampering, suggesting that contamination most likely occurred during the manufacturing process. After our investigation, the Secretary of Health of Minas Gerais closed the manufacturing plant where the implicated medications were made; an on-site investigation revealed inadequate quality control testing. All Brazilian state secretaries of health were notified of the closure, but a nationally coordinated product recall was not performed. In part on the basis of the findings of our investigation, the Secretary of Health of Minas Gerais decided not to allow the facility where the implicated products were manufactured to reopen until quality control measures were improved.

As recently reported by the Institute of Medicine, creating a safer health care environment by reducing medical errors needs to be a focus of clinicians and public health personnel at the local, state, and country level [19]. The high death toll and adverse consequences of this outbreak should lead to widespread changes at industry, hospital, and federal levels in Brazil. At the industry level, manufacturers of parenterals (and other medications) should institute strict quality control measures to ensure that standard operating procedures (e.g., Good Manufacturing Practices or ISO9000) are sufficient to prevent intrinsic bacterial or endotoxin contamination. Monitoring of final products should be enforced to ensure that current United States Pharmacopeia (USP) standards are met.

At the hospital and federal levels, this outbreak should result in the creation and strengthening of existing health care–associated infection surveillance and control programs. In addition, the existence of a federal agency with the mandate to regulate and supervise drug manufacturing at the national level, similar to the US Food and Drug Administration, would enhance the quality of parenterals and medications, facilitate rapid removal of parenterals not meeting USP standards, and reduce the risk of similar outbreaks. It is imperative that lessons be learned from this tragic outbreak so that similar epidemics in Brazil and other countries can be avoided in the future.

Improving the quality of care of neonates in health care facilities worldwide will require the adoption of systems, such as national surveillance systems for health care–associated infections, public health outbreak investigative teams, and public health prod-
uct monitoring systems, which have proven successful in other countries or settings.

References