Onset of diarrhea and pyrexia and time to detection of *Salmonella enterica* subsp *enterica* in feces in experimental studies of cattle, horses, goats, and sheep after infection per os

Helen Aceto, PhD, VMD; Stephanie A. Miller, VMD; Gary Smith, DVM

**Objective**—To determine time to first detection of *Salmonella* organisms in feces of animals after experimental infection PO and times to onset of diarrhea and pyrexia to evaluate a common method for identifying nosocomial infections on the basis of time of admission and onset of clinical signs (ie, the 3-day criterion).

**Design**—Meta-analysis.

**Sample Population**—Cattle, horses, goats, and sheep experimentally infected PO with *Salmonella enterica* subsp *enterica*.

**Procedures**—Online databases were searched for published reports describing results of experimental infection of cattle, horses, goats, and sheep PO with salmonellae. Time to detection of organisms in feces as well as to onset of diarrhea and pyrexia was noted. Analysis of covariance was used to examine relationships among these variables, host species and age, and *Salmonella* serovar and magnitude of infecting dose.

**Results**—Forty-three studies met the criteria for inclusion. Time to detection of salmonellae in feces ranged from 0.5 to 4 days. Times to onset of diarrhea and pyrexia ranged from 0.33 to 11 days and from 0.27 to 5 days, respectively. Time to onset of diarrhea was related to host age and *Salmonella* serovar. No other associations were identified.

**Conclusions and Clinical Relevance**—Time to detection of salmonellae in feces is unreliable for identifying hospital-acquired infections; a 3-day criterion will misidentify hospital- versus community-acquired infections. Relying on clinical indices such as times to onset of diarrhea and pyrexia to trigger fecal sampling for detection of *Salmonella* infection will increase the risk of environmental contamination and nosocomial spread because animals may begin shedding organisms in feces several days prior. (J Am Vet Med Assoc 2011;238:1333–1339)

Reports of nosocomial outbreaks of infectious disease in large animal veterinary hospitals are abundant in the literature. Among such outbreaks, salmonellosis is by far the most common.1–22 Outbreaks of salmonellosis have resulted in hospital closures, leading to disruption of clinical service, suspension of clinical rotations at teaching institutions, and loss of caseload and revenue, and sometimes require major facility remediation, culminating in substantial financial impact. Among such outbreaks, salmonellosis is a common reason for requiring such restrictions (77%).

Because fecal shedding of *Salmonella* organisms by hospitalized large animals is common23 and the risk of nosocomial infection can be high24,28 with concomitant potential for severe consequences, including possible human infections,17,30–34 there has been a great deal of interest in better defining criteria to indicate whether a given *Salmonella* infection is hospital-acquired. Unfortunately, classifying an infection as nosocomial can be challenging. Fecal cultures can be unreliable if not repeated several times for the same animal.13,20–34

In addition to identifying the serogroup of individual *Salmonella* isolates, further phenotypic and genotypic characterization, such as antimicrobial resistance profiling, serotyping, bacteriophage typing (where appropriate), pulsed-field gel electrophoresis, and plasmid profile analysis, can provide valuable insight in distinguishing nosocomial infections; however, results are not immediately available.17,22,23,35

Furthermore, stress, surgery, dietary changes, pharmacological treatment, and concurrent disease can exacerbate clinical signs of salmonellosis and can be associated with an increase in fecal excretion of *Salmonella* organ-

- **ABBREVIATIONS**
  - CI: Confidence interval
  - ICP: Infection control program

From the Department of Clinical Studies, New Bolton Center, School of Veterinary Medicine, University of Pennsylvania, Kennett Square, PA 19348. Dr. Miller’s present address is VCA Sinking Spring Animal Hospital, 21 Green Valley Rd, Sinking Spring, PA 19608. Address correspondence to Dr. Smith (garys@vet.upenn.edu).
isms. Such factors, associated with almost any hospitalization, introduce major difficulties in distinguishing nosocomial from community-acquired Salmonella infections. Shedding by subclinically infected carrier animals also contributes to the difficulty of identifying nosocomial Salmonella infections. A stringent ICP is fundamental in the prevention of hospital-acquired infections. In a number of veterinary hospitals, environmental cultures for Salmonella organisms are used to assess the adequacy of the ICP but accurate identification of nosocomial infections, coupled with results of bacteriologic culture of environmental samples, would allow for a more comprehensive appraisal of the program’s efficacy. Moreover, determining whether a Salmonella infection was acquired within the hospital has serious public relations and potentially legal implications for many veterinary hospitals. In salmonellosis in humans, the CDC defines the time from infection to shedding of organisms and onset of diarrhea to be typically 1 to 3 days, although it is sometimes longer. Little research has been done to measure the same parameters for salmonellosis in animals; nevertheless, tertiary referral veterinary hospitals (including our own) often assume that fecal shedding of Salmonella organisms 72 hours or later after admission is evidence of nosocomial infection (ie, the 3-day criterion). To test this criterion, information about the time to detection and the time to appearance of clinical signs relative to time of infection is required. These data are only available from experimental studies where the time of infection is known with certainty. Accordingly, in the present study, data were gathered from experimental studies involving infections PO of large animal species with Salmonella enterica subsp enterica. The objective was to examine whether the 3-day criterion was a satisfactory test and to identify factors that might inform conjectures about the positive predictive value of this criterion.

Materials and Methods

Criteria for selection of cases—The literature was searched for journal articles, theses, and book chapters that contained descriptions of studies in which cattle, horses, goats, or sheep were experimentally infected PO with S enterica subsp enterica. Although there are a number of reports of experimental salmonellosis with pigs as the subjects, these were excluded from the present study because pigs represent a small fraction of the caseload at most veterinary referral hospitals and because they rely less on fermentative digestion than do the other species and we are not aware of any descriptions of nosocomial outbreaks of salmonellosis that include pigs. Articles were identified from reference lists in existing publications and by searching online databases. Searches were conducted in accordance with guidelines in the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement. Searches were made through PubMed and OVID. For OVID, the databases included in the search strategy were Ovid Medline 1930 to February 2010; Ovid Old Medline 1948 to 1965; Agriculture 1978 to February 1, 2010; Biosis Previews 1993 to 2008; and CAB Abstracts 1910 to 2010 week 6. Sixteen search terms were used individually as follows: experimental salmonellosis, experimental salmonellosis equine, experimental salmonellosis bovine, experimental salmonellosis ovine, experimental salmonellosis caprine, experimental salmonellosis horses, experimental salmonellosis cattle, experimental salmonellosis calves, experimental salmonellosis sheep, experimental salmonellosis lambs, experimental salmonellosis goats, equine salmonellosis, bovine salmonellosis, ovine salmonellosis, caprine salmonellosis, and nosocomial salmonellosis. In OVID, the term “and” was used to connect each keyword within the search term. A total of 3,937 and 3,200 citations were retrieved from PubMed and OVID. Once duplicates were removed, the remaining 3,437 citations were screened and excluded if they did not include the target species, if they did not involve experimental infection, or where experimental infection was used, if only endpoints other than fecal culture or clinical signs were reported. Most exclusions could be made on the basis of title alone, but approximately 20% required examination of the abstract to determine whether the study should be excluded or retained. Ultimately, 203 journal articles, including 21 foreign language articles, were reviewed in detail. In addition, information contained in 34 other sources was reviewed, including 29 books or book chapters, 3 theses, and 2 department of agriculture reports. Criteria for inclusion of studies in the present analysis were if the time course of the onset of diarrhea or pyrexia was described and if feces were sampled on a daily basis and the study recorded the time when Salmonella organisms were first detected in fecal culture via bacteriologic methods. Studies were rejected if they reported only the mean time of onset of the respective endpoints. Because interpretation of tables of results from papers in languages other than English was found to be error prone, with 1 exception where a good translation was readily available, such papers were excluded from the analysis. Although leukopenia and neutropenia are frequently associated with salmonellosis in horses, and less reliably in cattle, they were not included in the present analysis because very few of the retained experimental studies reported WBC counts. In studies in which antimicrobials, other treatments, or vaccines were evaluated, only data from control animals were considered.

Data analysis—The unit of analysis was the experimental group of animals rather than the study per se. This was because some of the retained studies included several experimental groups. These groups could be distinguished in terms of the age of the animals at the time of infection or the magnitude of the infecting dose. Endpoints of interest were the times to onset of fecal shedding, pyrexia, and diarrhea. There was little uniformity in the way in which the studies reported these endpoints, but there were some commonalities. For example, the first day that a new instance of fecal shedding was observed and last day that a new instance of fecal shedding was observed were more frequently reported than was the mean time to detection for each group. Almost no studies provided the data required to estimate these mean values if they were not reported. The same was true of the times to onset of pyrexia and diarrhea. We chose to use the range values (eg, the first
and last days on which a new instance of fecal shedding was detected) rather than the group means to increase
the number of studies that were included in the analysis, recognizing that for each endpoint, we were using
only 1 datum from each group.

The methods used for detection of Salmonella organisms in studies41-50 in which culturing was performed
daily all included a selective enrichment step followed by differential plating to selective agar media.
Diarrhea and pyrexia were author defined in individual studies, but all definitions were within what would be
considered standard reference ranges for each species.

For each experimental group, we identified the species of host and the age at infection, the Salmonella
serovar (Salmonella ser Abortusovis, Salmonella ser Anatum, Salmonella ser Dublin, Salmonella ser Enteritidis,
Salmonella ser Heidelberg, Salmonella ser Infantis, and Salmonella ser Typhimurium), and the magnitude of the
infecting dose (the log dose used ranged between 10^6 and 10^9 organisms). To create conformity between the
studies, age at infection was represented as a categorical variable with 3 levels: neonatal (birth to weaning [0 to
2 months in cattle, sheep, and goats; 0 to 4 months in horses]), immature (weaning to production age or eligi-
bility for racing [2 to 24 months in cattle, 4 to 24 months in horses, and 2 to 12 months in sheep and goats]),
and mature (> 24 months in cattle and horses and > 12 months in sheep and goats). The magnitude of the in-
fected dose was recorded on a log scale and considered to be a continuous variable (range, 4 to 13).

Statistical analysis—Analysis of covariance4 was used to examine the relationships between the end-
points and the species and age of host, the Salmonella serovar used in the study, and the magnitude of the
infecting dose. The number of animals in each group varied considerably from one study to another (mean,
7,806; SE, 11,326); therefore, the number of animals in each group was used as a weighting factor in the anal-
ysis. We made no attempt to rate the quality of each study nor were we able to evaluate the possible effect of
clustering (ie, the use of several different groups from the same study). We initially attempted nested analy-
ses, but these were inconclusive or failed because of the unbalanced nature of the data.

Results

Forty-three studies33,42-43 met the criteria for in-
cclusion. Cattle and sheep studies were the most com-
mon and also had the greatest mean number of animals
per study (Table 1). There was only 1 study involving
goats. The data recorded in this study were not includ-
ed in the analysis. Fifteen of the studies were published
between 1970 and 1979, and there was a median of 4
studies from each of the other decades between 1940
and 2010. Two studies were from the 1920s.

Most of the animals that would go on to shed sal-
omonellae in their feces had begun shedding by 3 days
after infection (Figure 1). The 3-day criterion currently
used in our hospital would have incorrectly classified
these animals as having a community-acquired infec-
tion. A similar argument can be made for the onset of
pyrexia. The time to onset of diarrhea was much more
variable, but in > 60% of the studies, animals had de-
veloped diarrhea before day 3 and would also be incorrectly
classified as having a community-acquired infection.

It was of interest to identify factors that might in-
form any speculation about the positive predictive val-
ue of the 3-day criterion, so the effect of host species,

<table>
<thead>
<tr>
<th>Host species</th>
<th>Neonatal</th>
<th>Immature</th>
<th>Mature</th>
<th>Total</th>
<th>Mean No. of animals per group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cattle</td>
<td>23</td>
<td>0</td>
<td>2</td>
<td>25</td>
<td>8.9</td>
</tr>
<tr>
<td>Horses</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Sheep</td>
<td>0</td>
<td>2</td>
<td>10</td>
<td>12</td>
<td>7.5</td>
</tr>
<tr>
<td>Goats</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>23</td>
<td>4</td>
<td>16</td>
<td>43</td>
<td></td>
</tr>
</tbody>
</table>

Values presented are number of studies.
Age at infection was represented as a categorical variable with 3 levels: neonatal (birth to weaning [0 to 2 months in cattle, sheep, and goats; 0 to 4 months in horses]), immature (weaning to production age or eligibility for racing [2 to 24 months in cattle, 4 to 24 months in horses, and 2 to 12 months in sheep and goats]), and mature (> 24 months in cattle and horses and > 12 months in sheep and goats).

— = No data available.
host age, *Salmonella* serovar, and log dose on the onset of fecal shedding, pyrexia, and diarrhea were examined.

The time to first instance of fecal shedding was recorded in 28 groups of animals (14 studies). The time to last instance of fecal shedding could only be determined with certainty in 18 groups. Methods used for detection of *Salmonella* organisms in studies in which culturing was performed daily all included a selective enrichment step in either selenite, tetrathionate, Rappoport-Vassiliadis, or brilliant green broths, followed by plating to one of several selective agar media (brilliant green, MacConkey, desoxycholate, or xylose-lysine-deoxycholate). Three studies used a combination of agars and broths: tetrathionate or selenite broth with MacConkey, desoxycholate, or xylose-lysine-deoxycholate. Pyrexia was observed in 60 groups of animals (33 studies). Summarized over all studies, the first instance of pyrexia in a group of animals infected PO with *Salmonella* occurred a mean of 1.50 days after infection (95% CI, 1.47 to 1.53 days; range, 0.27 to 3 days). Similarly, the last occasion on which a new instance of pyrexia was observed in a group of animals infected PO with *Salmonella* was 2.47 days after infection (95% CI, 2.36 to 2.57 days; range, 0.27 to 5 days). There were no systematic associations between the weighted mean times to first or last onset of pyrexia and the host species, host age, serovar, or log dose.

The time to onset of diarrhea was recorded in 50 groups of animals (32 studies). Summarized over all studies, the first case of diarrhea in a group of animals infected PO with *Salmonella* was first detected a mean of 1.31 days after infection (95% CI, 1.22 to 1.39; range, 0.75 to 3 days). In studies that used cattle hosts, there were no significant systematic associations between the time at which fecal shedding was first observed in the group and the age of the hosts, the *Salmonella* serovar used, or the log dose. Too few studies involving horse or sheep hosts recorded fecal shedding in these species to permit similar analysis (Table 2); the same was true for all species in the case of the last day of a new instance of fecal shedding.

Discussion

At veterinary teaching hospitals, onset of clinical signs, for example, or fecal shedding at 72 hours (3 days) after admission or later are currently the most frequently used criteria for evaluating the likelihood that a given *Salmonella* infection is nosocomial. Many studies have investigated risk factors associated with salmonellosis and shedding of *Salmonella* organisms in large animal species. However, little information exists detailing the temporal aspects of the disease and the effect of variables such as age, host species, and dose on the progression of salmonellosis. In the present study, we used data from the published literature to investigate

---

**Table 2**—The time interval between infection and the first detection of *Salmonella* organisms in the feces for the studies in Table 1.

<table>
<thead>
<tr>
<th>Host species</th>
<th>Interval between infection and first instance of fecal shedding (d)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.0–0.5</td>
</tr>
<tr>
<td>Cattle</td>
<td>1</td>
</tr>
<tr>
<td>Horses</td>
<td>—</td>
</tr>
<tr>
<td>Sheep</td>
<td>—</td>
</tr>
</tbody>
</table>

Values presented are number of studies reporting a given interval. — = No data available.

**Table 3**—Weighted mean first and last times to detection of a new case of diarrhea for animals in the studies in Table 1.

<table>
<thead>
<tr>
<th>Host age group</th>
<th><em>Anatum</em></th>
<th><em>Dublin</em></th>
<th><em>Enteritidis</em></th>
<th><em>Infantis</em></th>
<th><em>Typhimurium</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Neonatal</td>
<td>2.12</td>
<td>1.49</td>
<td>2.12</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Immature</td>
<td>1.81</td>
<td>2.52</td>
<td>2.07</td>
<td>2.92</td>
<td>2</td>
</tr>
<tr>
<td>Mature</td>
<td>1.93</td>
<td>5.43</td>
<td>5.6</td>
<td>9.93</td>
<td>—</td>
</tr>
</tbody>
</table>

Values presented are mean number of days. See Table 1 for remainder of key.
both the time to positive results of *Salmonella* culture and time to onset of diarrhea and pyrexia to establish a range of time within which clinical signs and positive results for detection of *Salmonella* organisms in bacteriologic cultures of feces can be expected after infection. Our goal was to examine whether the 3-day criterion commonly used in tertiary referral veterinary hospitals was a satisfactory test for a nosocomial infection and to identify factors that might inform conjectures about the positive predictive value of the criterion.

Forty-three studies met the criteria for inclusion, and some of them were ≥ 50 years old. Publication years of retained studies ranged from 1920 to 2008, with 31% published in the 1970s (n = 15) and 1980s (7). Only 3 of the 43 were published after 2000. Several possible problems arise. A difficulty was that not all of the studies reported on prognostic indicators now known to be important. For example, few of the studies6,12,13,23 reported WBC counts, which are now known to be prognostic of salmonellosis in cattle and particularly in horses. On the other hand, pyrexia was often reported and, despite the fact that pyrexia on its own is not a clinically useful indicator of salmonellosis (because a great portion of hospitalized animals are pyrexic for other causes), it is frequently used in combination with other clinical endpoints in algorithms designed to trigger testing for salmonellae in hospitalized patients and was included in the analysis. Another difficulty was the extent to which the studies were comparable with respect to their ability to detect *Salmonella* organisms in the feces. All of the studies that reported the results of fecal culture used bacteriologic methods that included a selective enrichment step followed by differential plating to one of several selective agar media and so are equivalent to methods still commonly used in most clinical microbiology laboratories. Although such methods are rather specific for salmonellae, they are generally recognized as lacking sensitivity, compared with culture techniques proposed by the International Standards Organization66 or modifications thereof.41-46 As a result, even though the results reported by the experimental studies are likely to be similar to those which would be detected at most veterinary institutions, the general lack of sensitivity of these methods means that some caution must be applied in interpreting the fecal excretion data as a true reflection of the time to fecal shedding. The increasing recognition that both the matrix (e.g., horse vs cattle vs sheep feces) and the *Salmonella* serovar involved can greatly influence the performance characteristics of various culture methods66 only adds to the need for caution. With the preceding caveats in mind, despite the age of some of the publications retained for analysis, the available endpoints are straightforward (diarrhea and pyrexia), requiring no advanced technology to measure them, and the culture methods used are compatible with those routinely performed in today’s clinical microbiology laboratories, so it is reasonable to assume that the results from these studies are applicable to those that might be obtained today. That does not, however, obviate the need to consider additional studies in this subject area, particularly those which use more sensitive detection methods, as many questions remain to be properly addressed.

With respect to whether the 3-day criterion was a satisfactory method of distinguishing hospital-acquired *Salmonella* infections from community-acquired *Salmonella* infections, the results of the present study do not permit any quantitative evaluation of the criterion’s sensitivity or specificity. However, qualitatively, we note that the 3-day criterion currently used in our hospital would have incorrectly classified most of the animals infected within 24 hours after admission as having a community-acquired infection. This has important implications for ICPs.

We were also interested in identifying factors that might inform conjectures about the positive predictive value of the criterion. The unbalanced nature of the data compromised this analysis, and although it seems clear that the onset of diarrhea is earlier in younger hosts and is influenced by the serovar used to cause the infection (Table 3), we could make no statement about the effect of host species or log dose on any of the endpoints examined. *Salmonella* Typhimurium is the most frequent clinical isolate identified in samples submitted for serotyping to the National Veterinary Services Laboratory.86 Standardized over all studies, the first case of diarrhea in a group of animals infected PO with *Salmonella* Typhimurium typically occurred earlier than when other serovars were used, whereas the last case of diarrhea in a group of animals infected PO with *Salmonella* Typhimurium occurred later than when other serovars were used.

The scope of the present study was limited to experimental infections in previously healthy animals. Unfortunately, the population in a large animal hospital does not mirror the health status of the experimental animals from which the study data were obtained. As a result, the extent to which our findings are applicable to animals in the hospital setting is unknown. Nonetheless, given that many hospitalized patients have disturbed gastrointestinal function, it is perhaps not surprising that in the clinical setting, multiple fecal cultures are often required to isolate *Salmonella* organisms7,12,29,79 and that the time to detection of positive culture results is usually at least 3 days after admission and often exceeds that period.7,12,15,71 On the basis of the time course of fecal shedding in experimental studies, it is far from certain that detection of *Salmonella*-positive samples at ≥ 3 days is indicative of a hospital-acquired infection in large animal patients and a much longer period should be considered before a community-acquired, hospital-expressed infection can be ruled less likely. Just as importantly, even when *Salmonella*-positive fecal samples are collected between 12 and 72 hours after admission, the time course data from experimental studies indicate that these cases could be hospital acquired, an example of which can be seen in a 1996 report73 of *Salmonella* Krefeld infection in a veterinary intensive care unit in which 23% of infected horses had been in the unit for only 1 day when detected as infected. As a consequence, when studying *Salmonella* infections in large animal patients, great caution should be exercised in excluding the possibility of nosocomial infection in patients with positive samples detected during the first 72 hours, with the rationale that these animals are unlikely to have hospital-acquired infections.
The present study demonstrates that fecal shedding of Salmonella organisms can occur well in advance of the appearance of diarrhea and pyrexia. As a result, reliance on clinical indices such as the onset of diarrhea and pyrexia alone to trigger fecal sampling for salmonellae will increase the risk of environmental contamination and nosocomial spread because animals may begin shedding organisms in their feces several days before the appearance of clinical signs.

References