Microscopic Examination of Stools from Nonhuman Primates as a Way of Predicting the Presence of Shigella

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Microscopic examination of stool samples from captive nonhuman primates with diarrhea for the presence or absence of leukocytes or erythrocytes or both as a means of predicting the presence of Shigella spp. was performed. Analysis of the data multivariately by means of a log linear model did not show a three-way association between bowel, Shigella, and the presence or absence of cells.

Species of the genus Shigella produce disease by invasion and subsequent production of a toxin (5, 10, 11, 13, 14, 16). The tissues invaded are primarily the cells of the colonic epithelium (6, 11, 14, 17). Typically, after invasion and progression of the disease, erythrocytes, leukocytes, cellular debris, and mucus appear in the lumen of the bowel (2, 6). A stool containing such elements is defined as being dysenteric.

Shigellosis is a major intestinal disease of captive nonhuman primates (7, 12, 15). Indeed, some have ascribed almost all diarrheal disease in such animals to infection and invasion by Shigella spp. (12). Correct diagnosis of this condition is dependent upon the results of stool cultures received in some instances 48 h after collection of the sample. Assumption that all animals with grossly dysenteric stools or with diarrhea are experiencing disease due to Shigella is unwarranted. Such assumptions promote antimicrobial usage that is not only irrational, but counterproductive as well, due to R-plasmid selection in a closed population of animals.

In human beings, microscopic examination of the stool is helpful in making the correct diagnosis for enteric disease caused by Salmonella, Shigella, and invasive Escherichia coli (8). Individuals with disease caused by one of the above characteristically shed erythrocytes and leukocytes in their stool. Armed with this knowledge, the clinician can instigate rational therapy 24 to 48 h before the results of culture.

In the nonhuman primate, information concerning the relationship between the presence of Shigella spp., the presence or absence of cells, and an abnormal stool is lacking. Veterinary clinicians must assume shigellosis by clinical signs and instigate appropriate therapy or wait 24 to 48 h for the results of culture. For this reason, we undertook a study to determine whether a relationship existed between the presence of cells in an abnormally formed stool and the subsequent isolation of Shigella spp.

MATERIALS AND METHODS

Source of specimens. Rectal swabs were taken from a total of 806 nonhuman primates (Macaca spp.) housed at the California Primate Research Center, Davis. Of these samples, 398 were taken from animals with diarrhea, and 408 were taken from monkeys with clinically normal stools. Animals sampled more than once were not included in this study.

Sample processing. Two rectal swabs were obtained simultaneously from each animal in the study. One swab was rolled over the surface of a glass microscope slide, and the other was used for bacteriological culture. The microscope slide was stained with Wright-Giemsa stain and then examined under the microscope. The presence or absence of leukocytes or erythrocytes or both was noted before knowledge of culture results.

The swab reserved for culture was streaked over the surface of a xylose-lysine-Desoxycholate agar plate (XLD; BBL Microbiology Systems, Cockeysville, Md.) and a MacConkey agar plate (BBL Microbiology Systems). The swab was then placed in a tube containing gram-negative broth (BBL Microbiology Systems). The plates and the broth were incubated at 37°C in an atmosphere of air. Twenty-four hours after inoculation, a portion of the gram-negative broth was streaked onto a plate of XLD agar that was incubated as above.

After incubation for 24 h, XLD and MacConkey agar plates were examined for colonies exhibiting the correct fermentation pattern for shigellae. If such colonies were present, three to five were picked and identified by established criteria. Species and subtype were determined by means of a slide agglutination test with group- and type-specific antisera.

Statistical analysis. The data were analyzed by a two-way and a multiway frequency table with a log linear model (1, 4). A Burroughs 6700 computer was used as an aid to these calculations.

RESULTS

During the course of this study, no Salmo-
nella spp. were isolated from any of the monkeys cultured. Shigella flexneri 4 was the only Shigella isolated.

The results of examining the stools of monkeys with and without diarrhea for shigellae and cells are shown in Table 1.

Table 2 presents the data arranged in a 2 × 2 frequency table. The simplest model that best described the association between the three factors, cells (C), diarrhea (D), and Shigella (S), was (CD, CS, DS), Table 3. This means that with two factors (K-factor = 2) such as (CD), the Pearson chi-square was significantly high (336.70), so that the probability of not having an association between the two factors was quite low ($P = 0.000$). In other words, there was a significant association between the presence of (C) and (D), the presence of (C) and (S), or the presence of (D) and (S). However, when the three factors were analyzed simultaneously (K-factor = 3), the Pearson chi-square value (0.19) was significantly low and the probability of not having a three-way association (C, D, S) was quite high ($P = 0.662$). Although there was an association between two of the factors, it was independent of the third; e.g., there was a significant association between the presence of cells and diarrhea, but this was independent of the presence of Shigella.

**DISCUSSION**

The data show that the presence of cells in the stool of a monkey with diarrhea does not act as a predictor of whether or not Shigella will be isolated from the stool. From analysis of these data with multiple 2 × 2 frequency tables, there appeared to be a significant association between the three factors. This association does not hold up when all three variables are analyzed multivariately.

A number of explanations might be proposed to explain the lack of correlation between diarrhea, inflammatory cells, and Shigella. One possibility is that not all naturally occurring cases of shigellosis in monkeys manifest with a loose stool containing inflammatory cells. Monkeys experimentally inoculated with Shigella flexneri manifest disease with either a diarrheal stool, a dysenteric stool, or both characteristics (6). Whether inflammatory cells would have been visualized microscopically in the diarrheal stool described above was not reported. Perhaps in the naturally occurring disease, a loose stool without demonstrable inflammatory cells occurs with high enough frequency to negate any positive correlation that may exist between the presence of inflammatory cells, Shigella, and diarrhea.

A second possibility is that there are many other causes of diarrhea and dysentery in monkeys, causes as common as Shigella. Salmonella is a possibility, but none was isolated. Other causes would include diarrhea due to entero-toxigenic strains of E. coli, dysentery caused by invasive E. coli or parasites (e.g., amoebae), or simply psychogenic causes. No studies have been conducted to assess the role of these other causes of dysentery and diarrhea in monkey colonies, but one would predict that they might play a significant role.

And finally, there is the possibility that our cultural procedures were inadequate to detect Shigella in each Shigella-containing stool sample. This could be due to sample size, frequency of culture, or biological variation. It would seem, however, that if Shigella spp. were the cause of the abnormal clinical signs, especially during the acute stages of the disease, sufficient numbers should be present to be demonstrated (9). Such has been the experience with human beings (2). However, some human subjects, although showing signs of shigellosis after oral inoculation, did not shed detectable numbers of shigellae in their stools (3).
It would seem, therefore, that the examination of a direct smear of stool for the presence or absence of cells is not useful for early diagnosis of shigellosis in the monkey.

LITERATURE CITED