Control of Contamination in Psittacosis Specimens by Antibiotics

VESTER J. LEWIS AND HELEN M. ENGELMAN

Viral Immunoserology Unit, Center for Disease Control, Atlanta, Georgia 30333

Received for publication 13 May 1971

Diluent containing a combination of vancomycin, kanamycin, and streptomycin is shown to be more effective than diluent previously used for isolating Chlamydia psittaci from bird tissue specimens.

Attempts to isolate Chlamydia psittaci are frequently thwarted by bacterial contamination. For example, tissue suspensions from 32 of 100 birds suspected of psittacosis that were submitted to the Center for Disease Control from diverse localities during a recent 3-month period yielded only bacteria. These suspensions contained 2 mg of streptomycin per ml, 20 times that suggested for purified specimens (1). The present study was undertaken to determine whether adding vancomycin and kanamycin would control contamination more effectively.

Table 1. Survival of Chlamydia psittaci in the presence of antibiotic diluents

| Strain of C. psittaci | Time (hr) of incubation at 4 \( ^\circ \)C | Log10 mouse LD50 in | Diluent S | Diluent SKV |
|----------------------|------------------------------------------|-------------------|-----------|-------------|
| 606                  | 0                                        | 7.0               | 6.6       |
| 641                  | 0                                        | 6.8               | 7.0       |
|                      | 18                                       | 6.5               | 6.7       |
| 849                  | 0                                        | 5.6               | 5.6       |
|                      | 18                                       | 6.5               | 6.7       |

The basic diluent used was 0.01 m phosphate-buffered distilled water (pH 7.6) containing 10% egg yolk. Diluent S was prepared by adding 2 mg of streptomycin SO4 (Squibb) per ml; diluent SKV was prepared by adding 1 mg of streptomycin SO4 and 0.5 mg each of vancomycin HCl (Lilly) and kanamycin SO4 (Bristol) per ml. Neither diluent had observable ill effects upon 3-week-old mice (ICR) when inoculated intracerebrally in 0.03-ml amounts, the method used throughout the study.

To test susceptibility of the psittacosis organism to the antibiotics, serial 10-fold dilutions of mouse brain pools of three C. psittaci strains were prepared in each diluent. Each dilution was inoculated into five mice immediately and into another group of five after overnight incubation at 4 \( ^\circ \)C. LD50 values (2) after 21 days of observation were essentially the same regardless of diluent or overnight incubation (Table 1).

The diluents were compared for control of bacterial contamination in kidney-spleen-liver tissue pools from 21 birds received from diverse areas. Two 10% suspensions were made from each of the 21 pools, one in diluent S and the other in diluent SKV. Five mice were inoculated with each suspension, and Macchiavello-stained impressions were made from the dura of those which died during a 21-day observation period. Bacteria were seen in all impressions; chlamydiae were seen in none. The survival ratios presented in Table 2 show superiority of diluent SKV. No diluent S mice survived with nine specimens, whereas 20 to 100% of the diluent SKV counterparts survived. With none of the 21 pools tested was the mouse survival rate greater with diluent S than with diluent SKV.

The two diluents were then compared for effectiveness with bacterially contaminated tissue...
TABLE 3. Comparison of antibiotic diluents for demonstration of Chlamydia in bacterially contaminated specimens

| Specimen | Diluent | C. psittaci strain 606 | C. psittaci strain 641 |
|----------|---------|------------------------|------------------------|
|          |         | 1,000 LD₅₀ | 10 LD₅₀ | 1,000 LD₅₀ | 10 LD₅₀ |
| 884      | S       |            | ±        |            | ±        |
|          | SKV     |            | ±        |            | ±        |
| 890      | S       |            | +        |            | +        |
|          | SKV     |            | +        |            | +        |
| 896      | S       |            | +        |            | +        |
|          | SKV     |            | +        |            | +        |
| 1138     | S       |            | +        |            | +        |
|          | SKV     |            | +        |            | +        |
| 1156     | S       |            | ±        |            | ±        |
|          | SKV     |            | +        |            | +        |

* Demonstrated in inoculated mice by Macchiavello staining of dural impressions.

With 10 LD₅₀ of C. psittaci strain 606. The other two diluent S suspensions were seeded with 1,000 or 10 LD₅₀, respectively, of strain 641. The diluent SKV suspensions were seeded as the diluent S suspensions. After overnight incubation at 4°C, each suspension was inoculated into five mice. Macchiavello-stained dural impressions of mice dying within 21 days were examined for chlamydiae.

Eleven samples negative for C. psittaci in diluent S were positive in diluent SKV (Table 3). All samples positive in diluent S were also positive in diluent SKV.

To date, we have used diluent SKV in attempted isolation of C. psittaci from 133 birds of several species submitted from various localities. Only 12 were unsatisfactory because of bacterial contamination.

In contrast, 39 of the 100 immediately preceding similar specimens, suspended in diluent S, were unsatisfactory because of bacterial contamination.

Specimens known to contain C. psittaci. A pool of kidney, spleen, and liver tissue was made from each of five grossly contaminated birds. From each of the five pools, four suspensions (10%) were prepared in diluent S and four in diluent SKV. One of the four diluent S suspensions was seeded with 1,000 LD₅₀ and another was seeded with 10 LD₅₀ of C. psittaci strain 606. The other two diluent S suspensions were seeded with 1,000 or 10 LD₅₀, respectively, of strain 641. The diluent SKV suspensions were seeded as the diluent S suspensions. After overnight incubation at 4°C, each suspension was inoculated into five mice. Macchiavello-stained dural impressions of mice dying within 21 days were examined for chlamydiae.

Eleven samples negative for C. psittaci in diluent S were positive in diluent SKV (Table 3). All samples positive in diluent S were also positive in diluent SKV.

To date, we have used diluent SKV in attempted isolation of C. psittaci from 133 birds of several species submitted from various localities. Only 12 were unsatisfactory because of bacterial contamination.

In contrast, 39 of the 100 immediately preceding similar specimens, suspended in diluent S, were unsatisfactory because of bacterial contamination.

We thank Leslie A. Page, National Animal Diseases Laboratory, Ames, Iowa, for suggesting the possible effectiveness of vancomycin and kanamycin in the described context.

LITERATURE CITED

1. Meyer, K. F., B. Eddie, and J. Schachter. 1969. Psittacosis-lymphogranuloma venereum agents, p. 888. In E. H. Lennette and N. J. Schmidt (ed.), Diagnostic procedures for viral and rickettsial diseases, 4th ed. American Public Health Association, New York.

2. Reed, L. J., and H. Muench. 1938. A simple method of estimating fifty percent end points. Amer. J. Hyg. 27:493.