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DETERMINATION OF ANTIBIOTIC SUSCEPTIBILITY OF RICKETTSIA USING THE PLAQUE ASSAY TECHNIQUE

Joseph E. McDade

JUNE 1969

DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland

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DETERMINATION OF ANTIBIOTIC SUSCEPTIBILITY OF RICKETTSIA USING THE PLAQUE ASSAY TECHNIQUE

Joseph E. McDade

Virus & Rickettsia Division
BIOLOGICAL SCIENCES LABORATORIES

Project 1B562602A059

June 1969

In conducting the research described in this report, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care," as promulgated by the Committee on the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Academy of Sciences-National Research Council.

ABSTRACT

The effect of antibiotic sensitivity discs on plaque formation by various rickettsiae was determined. Twenty-four-hour chick primary monolayers were infected with various rickettsiae and overlayed with agar, upon which commercial antibiotic discs were placed. Plaque formation was completely inhibited by discs impregnated with tetracycline, chloramphenicol, nitrofurantoin, or erythromycin; partial inhibition was observed around discs containing nalidixic acid or sulfisoxazole; no inhibition was seen around discs containing cephalothin, ampicillin, oxacillin, kanamycin, polymyxin B, streptomycin, or penicillin. This adaptation of the plaque technique should be useful in clinical and diagnostic microbiology and in the evaluation of new antibiotics.

DETERMINATION OF ANTIBIOTIC SUSCEPTIBILITY OF *RICKETTSIA* USING THE PLAQUE ASSAY TECHNIQUE*

A sensitive new plaque assay system for rickettsiae was recently reported.**,*** One of the advantages of a plaque system is that it is more versatile than the embryonated egg LD_{50} assay. An adaptation reported here describes how commercial antibiotic sensitivity discs can be used in the plaque assay system to screen for antibiotic sensitivity of *Rickettsia*.

Working seeds of *Rickettsia rickettsii* Bitter Root (R1) strain, *R. akari* MK, *R. mooseri* Wilmington strain, *R. prowazekii* Madrid E strain, and *R. prowazekii* Breinl strain were prepared by a modification of the procedure of Weiss, Rees, and Hayes.**** Infected yolk sacs were homogenized in a blender, partially purified by low- and high-speed centrifugation, suspended in sucrose-phosphate buffer (SP 25) pH 7.0, and stored at -65 C.

The plaque assay procedure used in these studies is essentially the same procedure described earlier.** Twenty-four-hour chick embryo primary cell monolayers in plastic tissue culture flasks were infected with 0.1 ml of a 10^{-3} dilution of working seeds and overlayed with medium 199 (5% calf serum) containing 0.5% agarose. Antibiotic sensitivity discs (Sensi-Discs, Baltimore Biological Laboratories) were then placed atop the agar overlay with sterile forceps. The infected monolayers were incubated in a closed system at 32 C until plaques were observed on controls (no discs added). Monolayers were stained for photography by adding a second overlay containing medium 199 (no calf serum), 0.5% agarose, and neutral red at a final concentration of 0.01%.

Figure 1 shows the effect of antibiotic sensitivity discs on *R. rickettsii*. Complete inhibition of plaque formation was observed with 30 µg tetracycline, 100 µg nitrofurantoin, 30 µg chloramphenicol, and 15 µg erythromycin (Fig. 1A); partial inhibition was observed with 30 µg nalidixic acid and 1.0 mg sulfisoxazole (Fig. 1B); no inhibition was seen around discs containing 30 µg cephalothin, 10 µg ampicillin, 1 µg oxacillin (Fig. 1C), 5 µg kanamycin, 300 units polymyxin B, 10 µg streptomycin (Fig. 1D), or 10 units penicillin (Fig. 1B). Infected and uninfected controls (no discs added) are shown for comparison in Figure 1E. Nearly identical results were found with the other species of *Rickettsia*. All were completely inhibited by the same concentrations of tetracycline,

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* This report should not be used as a literature citation in material to be published in the open literature. Readers interested in referencing the information contained herein should contact the author to ascertain when and where it may appear in citable form.

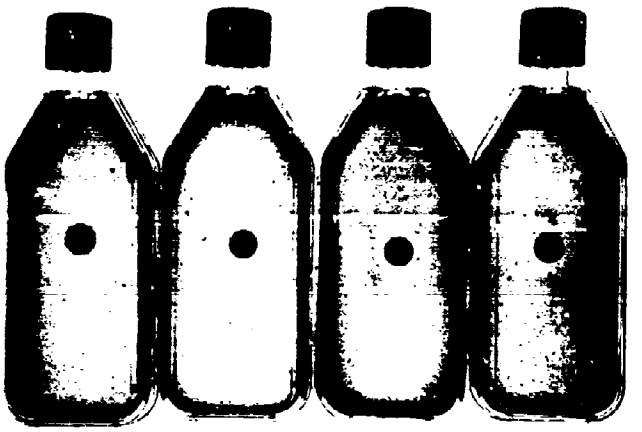
** Weinberg, E.H.; Stakebake, J.R.; Gerone, P.J. 1969. A plaque assay for *Rickettsia rickettsi*. J. Bacteriol. 98:398-402.

*** McDade, J.E.; Stakebake, J.R.; Gerone, P.J. A plaque assay system for several species of rickettsiae. Manuscript in preparation.

**** Weiss, E.; Rees, H.B., Jr.; Hayes, J.R. 1967. Metabolic activity of purified suspensions of *Rickettsia rickettsi*. Nature 213:1020-1022.

4

A



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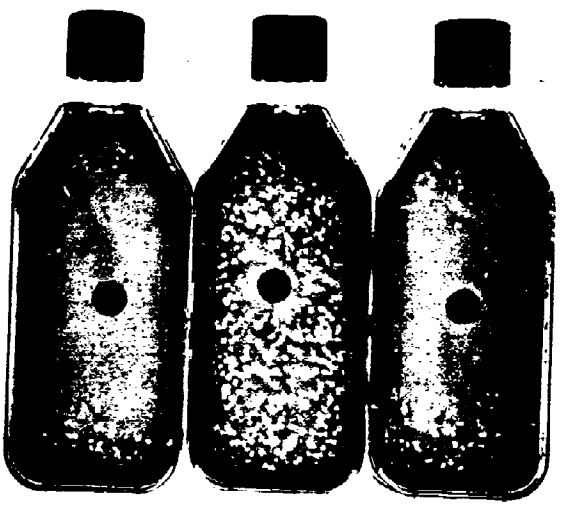

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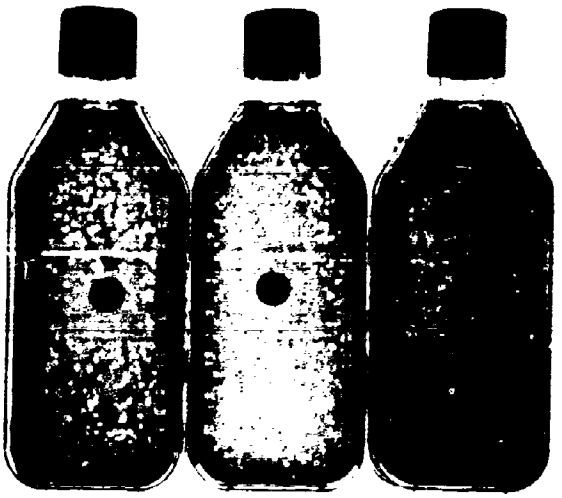


E

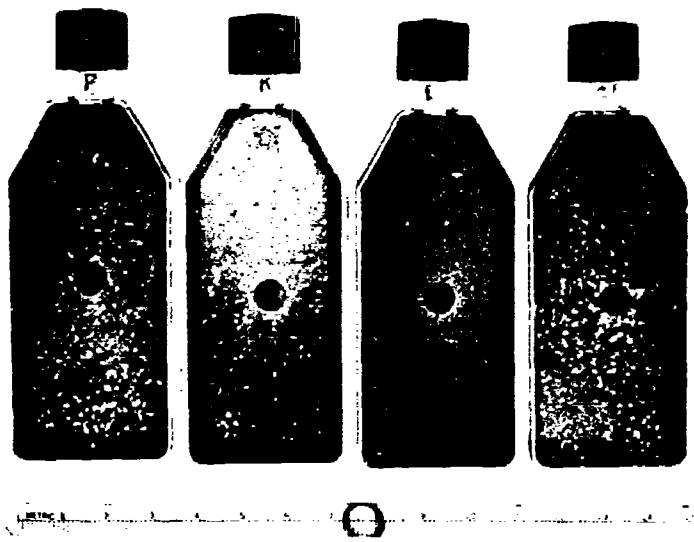
FIGURE 1. The Effect of Antibiotic Sensitivity Discs on Plaque Formation by the Bitter Root Strain of Rickettsia rickettsii. A. Left to right, 30 μg tetracycline, 100 μg nitrofurantoin, 30 μg chloramphenicol, 15 μg erythromycin. B. Left to right, 30 μg nalidixic acid, 10 units penicillin, 1.0 mg sulfisoxazole. C. Left to right, 30 μg cephalothin, 10 μg ampicillin, 1 μg oxacillin. D. Left to right, 5 μg kanamycin, 300 units polymyxin B, 10 μg streptomycin. E. Left to right, infected, uninfected, and infected controls.

nitrofurantoin, chloramphenicol, and erythromycin; each species was also partially inhibited by nalidixic acid, but no inhibition was apparent with the other antibiotics. Figure 2 shows the results obtained with R. prowazekii Breinl strain. The results of the other tests have been omitted because they were identical.

The susceptibility determinations reported here are in agreement with what is known about antibiotic susceptibility of rickettsiae. These results contain no new information on the susceptibility of rickettsiae to antibiotics, but they do indicate that this method may be more convenient and possibly more sensitive than egg assays for qualitative and quantitative determinations of antibiotic susceptibility in rickettsiae. This adaptation of the plaque technique should be useful in clinical and diagnostic microbiology and in the evaluation of new antibiotics, and may also provide a tool for genetic studies with rickettsiae.

A

B



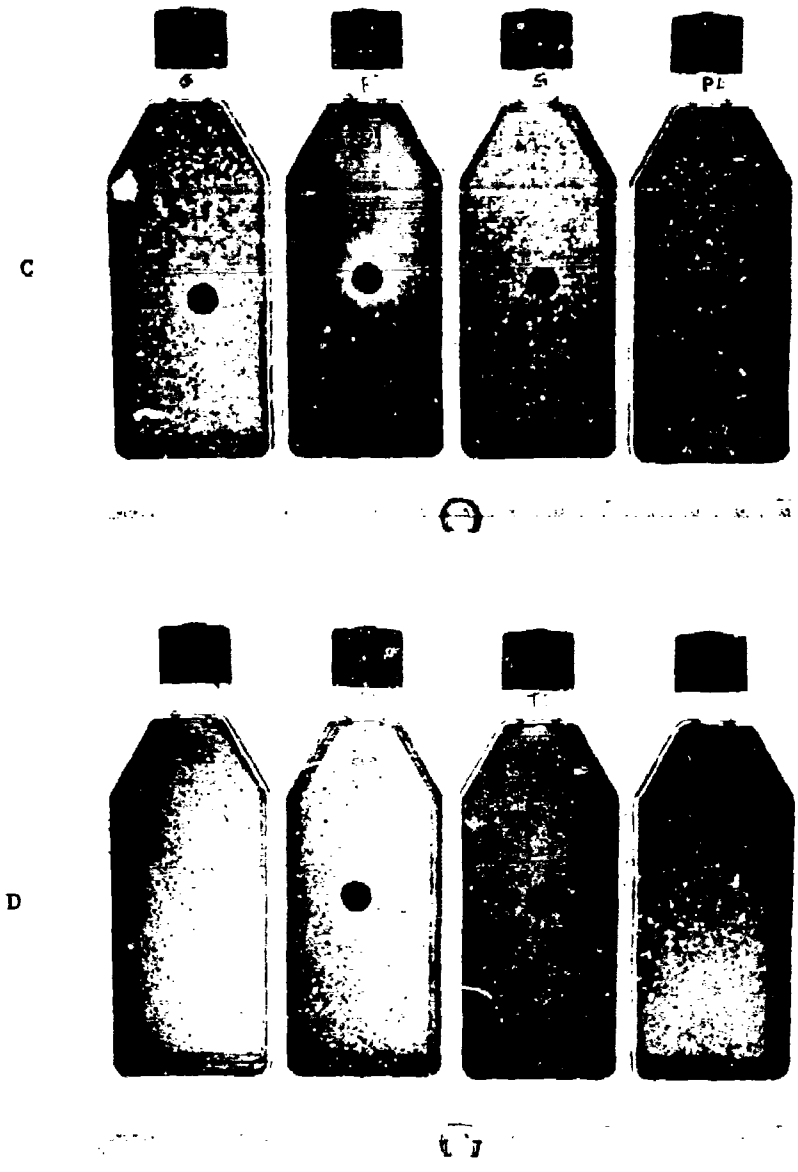


FIGURE 2. The Effect of Antibiotic Sensitivity Discs on Plaque Formation by the Breinl Strain of Rickettsia prowazekii. A. Left to right, 30 µg chloramphenicol, 30 µg nalidixic acid, 1 µg oxacillin. B. Left to right, 10 units penicillin, 5 µg kanamycin, 15 µg erythromycin, 30 µg cephalothin. C. Left to right, 1.0 mg sulfisoxazole, 100 µg nitrofurantoin, 10 µg streptomycin, 300 units polymyxin B. D. Left to right, uninfected control, 10 µg ampicillin, 30 µg tetracycline, infected control.

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13. ABSTRACT

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14. Key Words

* Rickettsia
* Plaques
* Assaying
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