ALTERNATIVE ANTIBIOTIC TREATMENT FOR CHLAMYDIOSIS

by

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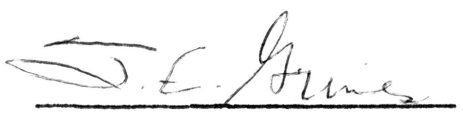

Dr. J. E. Grimes

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ALTERNATIVE ANTIBIOTIC TREATMENT OF CHLAMYDIOSIS

Chlamysiosis is a disease of many animals by a member of the genus Chlamydia. Tetracycline ond erythromycin are recommended for effective treatment of chlamydiosis. Since hypersensitivities for a given drug may exist, and also because bacteria are known to develop resistance to some drugs, alternative antibiotic treatment should be available. In this research, three drugs, Ampicillin, Amoxicillin, and Cephaloridine, were tested for effectiveness against C. psittaci organisms. Each was found to have a minimum inhibitory concentration (MIC) greater than 100 micrograms/ml. and are therefore not recommended as therapeutic agents for chlamydiosis.

OBJECTIVES:

1. Overall objective is to determine susceptibility of Chlamydia psittaci to various antibioties.
2. Specific aim is to find alternative antibiotic treatments for chlamydiosis.

INTRODUCTION:

Chlamydiosis is an infection of an organism by a member of the genus Chlamydia, of which there are two species, C. trachomatis and C. psittaci. The species C. psittaci has the widest host range of the chlamydia, causing disease in humans and a variety of lower animals. Primary animals involved are the psittacine birds, ie. parrots, budgerigars (parakeets), and the domestic fowl. Other animals in which chlamydiosis may occur include cattle, sheep, pigs, goats, horses, cats, rabbits, snowshoe hares, muskrats,

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The format for this paper generally follows the style for the Journal of Antimicrobial Chemotherapy.

guinea pigs, and mice. The most common diseases associated with an infection of C. psittaci are psittacosis, also called "parrot fever," (7) in humans, and psittacosis and ornithosis in man and wild and domestic birds. Other diseases include 1) pneumonitis in cats, sheep, cattle, goats, pigs, rabbits, horses, and mice; 2) polyarthritis in lambs and calves; 3) encephalomyelitis in calves and possibly dogs; 4) placentopathy in cattle, sheep, pigs, goats, rabbits, and mice; 5) conjunctivitis in sheep, cats, guinea pigs, cattle, and pigs; 6) a fatal enteritis in snowshoe hares and muskrats; and 7) an enteritis in cattle and sheep (5).

Psittacosis is a zoonotic disease which has increased recently in both psittacine birds and man (7). In 1979, 100 human cases were reported to the Center for Disease Control in Atlanta, Georgia. The clinical signs in man are malaise, headache, hyperthermia, and cough, with a mortality of possibly 20% in untreated cases. Dr. J. E. Grimes of the Department of Veterinary Microbiology and Parasitology, Texas A&M University-College of Veterinary Medicine feels this figure is misleadingly low probably because of lack of recognition, not difficulty of diagnosis.

Since bacteria may often develop resistance, selected antibiotics were used to test their effect. With the increase of reportings of this disease, there should be alternative antibiotic treatments available. Also, some humans may have a hypersensitivity to the two antibiotics presently recommended for treatment, tetracycline and erythromycin.

This study deals with susceptibility of C. psittaci to selected new antibiotics. The outcome of this study will be beneficial to both veterinary and human medicine.

The first half of the research was spent developing techniques and becoming familiar with the organism through a literature survey. Necessary techniques included 1) different staining technuques; 2) use of tissue cells and chicken embryos for culture; and 3) proper sterile technique.

The first half of the research was performed by inoculation of the tissue cells with C. psittaci followed by the actual antibiotic treatment. A control was used along with different dilutions of the different antibiotics to study the effects.

LITERATURE REVIEW:

The incidence of chlamydiosis is on the increase in the U.S. (7). Organisms have been known to develop resistance to certain antibiotics. For these reasons, new antibiotic therapy should always be sought. Table 1 is a partial list of tested antibiotics against C. trachomatis. One antibiotic, rosamycin, was found to be even more effective than either tetracycline or erythromycin, the two drugs currently recommended for chlamydiosis (16). Another antibiotic, rifampin, was also recommended as more effective (2).

Very few of the antibiotics listed in Table 1 have been tested against C. psittaci. Gentamicin has been tested against C. psittaci with the same negative results as when tested

against C. trachomatis (19). Less research has been done on C. psittaci than on C. trachomatis because C. psittaci is mainly more of veterinary importance whereas C. trachomatis causes more human problems. There is a chance of an increase of psittacosis especially in pet bird owners or pet bird shop workers. The organism can be spread by inhalation of infective fecal dust from cleaning an infected bird's cage. For these reasons, we felt we should test a range of drugs for possible therapeutic efficacy.

MATERIALS AND METHODS

ORGANISMS:

Two strains of C. psittaci were originally used in the research, cases 78105 and 79035. These were the original case numbers of birds proven to have the disease. Each was cultured in yolk sac and harvested in about one week. 78105 was found to kill the embryo much faster. The pool was divided into aliquots of 3 to 4 ml. each and frozen at -70 degrees C until required. A concentration of 1 part Chlamydia yolk sac suspension to 1000 parts MEM was found to produce about one inclusion per oil immersion field for 78105, while a 1:100 dilution was necessary to achieve the same results for 79035.

CELLS:

L929 mouse fibroblasts were grown in 25 ml. tissue culture flasks in 5 ml. of growth medium to porvide a confluent monolayer. These cells were diluted with the necessary amount

of medium so that 1 ml. was added to each of 24 wells in a culture plate, each well containing a glass coverslip (see Fig. 1). This was incubated 3 to 4 days until a confluent monolayer was again achieved.

ANTIBIOTICS:

The antibiotics tested were amoxicillin (Amoxi-drop), ampicillin (Polyflex), and cephaloridine (Keflodin).

INOCULATION OF CELLS:

Double concentrations of the antibiotic and the inoculum were prepared separately so that when the MEM was drawn off, 0.5 ml. of each one was placed in each well to provide the necessary dilution. This mixture was left on the cells approximately 48 hours at 37 degrees C. The coverslips were then removed, mounted on slides, and stained using the Giemsa staining technique. The slides were then observed under the oil immersion objective.

RESULTS AND DISCUSSION

An initial experiment determined what concentration of the two inoculums would provide about one inclusion per oil immersion field. For 78105, a dilution of 1:1000 was necessary, while for 79035, a dilution of 1:100 was necessary.

The first antibiotic was tested with the lowest minimum inhibitory concentration (MIC) being 1 microgram/ml. Further dilutions of the antibiotic were made and tested with an MIC of 0.001 micrograms/ml. This seemed highly questionable since the best MIC of any drugs is about 0.03 micrograms/ml.

The test was redevised. Dilutions of 1:100, 1:1000, and 1:10,000 of the inoculum were tested against each of the dilutions of the antibiotic.. Each of the antibiotics was tested to as low a dilution as one microgram/ml., the suggested therapeutic dose. None of the three tested antibiotics were therapeutically beneficial.

CONCLUSION:

Negative results such as the ones reported are important for future research in this area. Even though positive results are more desirable, negative results aid other investigators. For these reasons, the experiment was not a failure. Given more time and the desired drugs which were not available, more positive results may have been attained.

TABLE 1

A Partial List of Antibiotics Tested for *C. trachomatis*

Doxycycline (3)
Gentamicin (15)
Oxytetracycline (15)
Erythromycin (15)
Penicillin G (15)
Ampicillin (15)
Sulphamethoxazole (15)
Spiramycin (6)
Minocycline (3)
Trimethoprim (15)
Spectinomycin (12)
Chloramphenicol (8)
Sulfonamides (10)
Rifampin (2)
Rosamicin (16)

Table 2

Antibiotics Used In This Experiment with Results

Ampicillin	greater than 100 micrograms/ml.
Amoxicillin	greater than 100 micrograms/ml.
Cephaloridine	greater than 100 micrograms/ml.

Table 3

Growth Medium Recipe

100 ml. MEM
10 ml. Fetal Beef Serum
1 ml. L-Glutamine
0.2 m. Streptomycin-Kanamycin-Vancomycin Mixture (omitted during actual antibiotic testing)

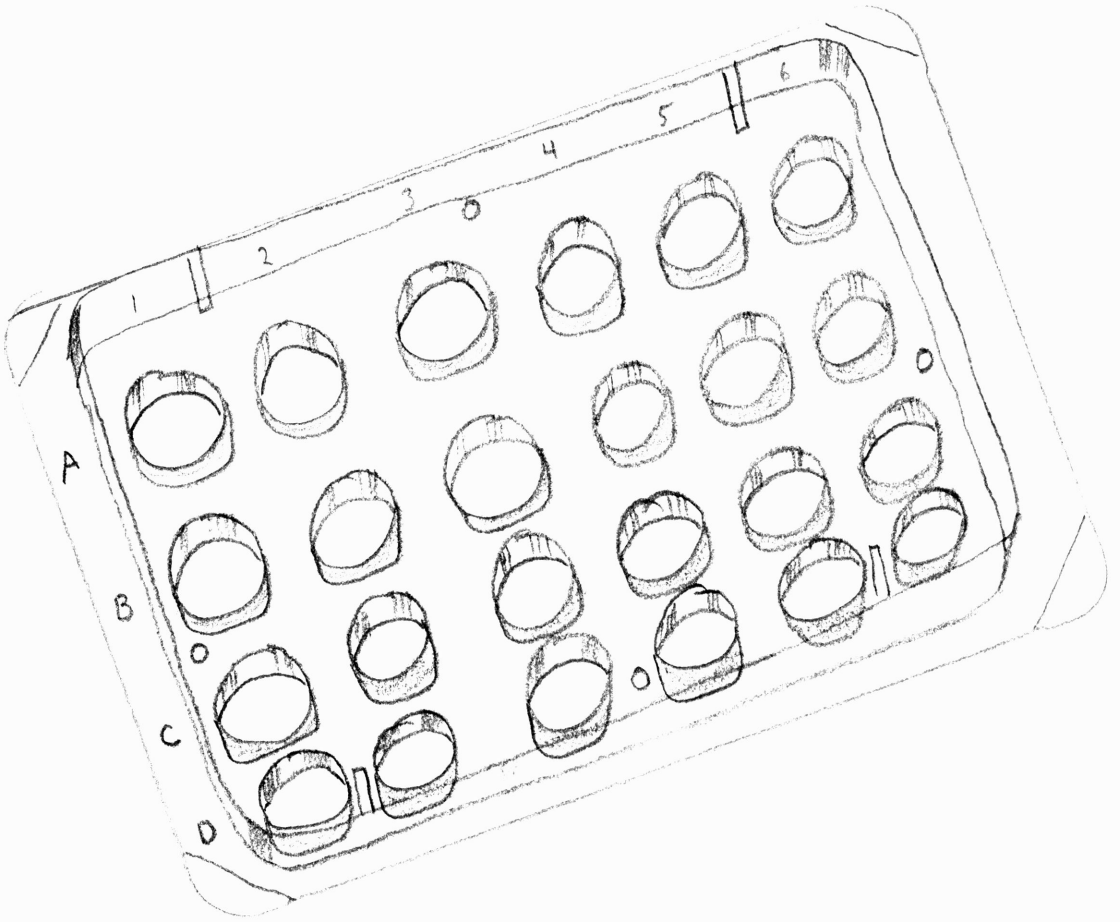


Fig. 1
The Culture Tray

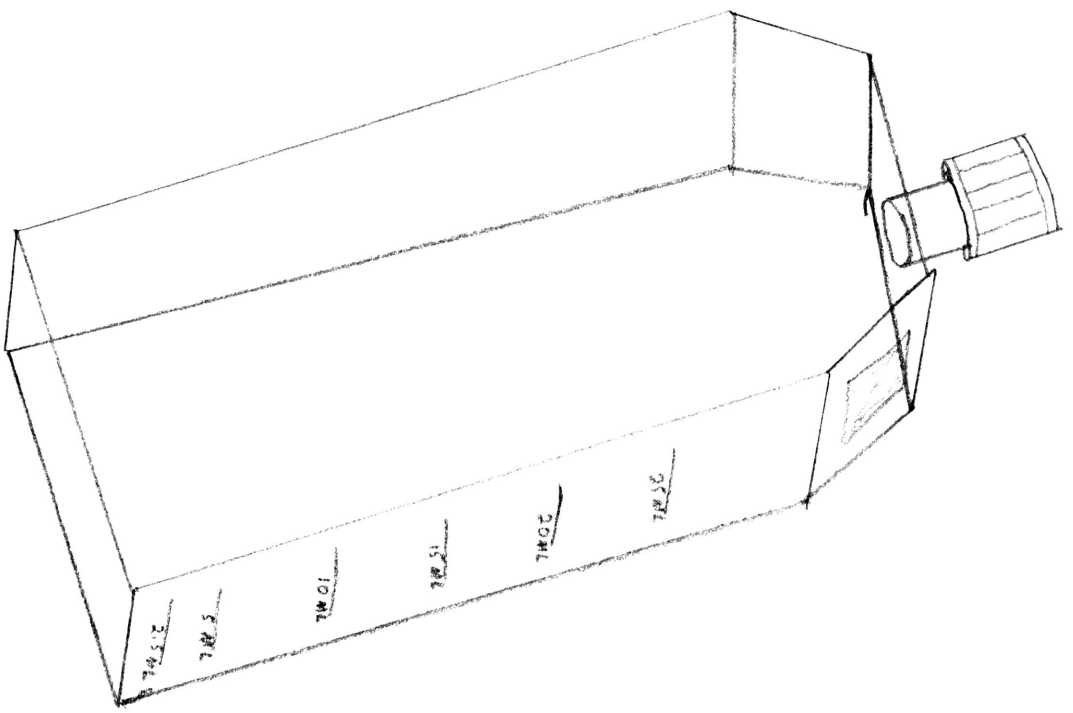


Fig.2
The Tissue Culture Flask

Fig. 3

A

B

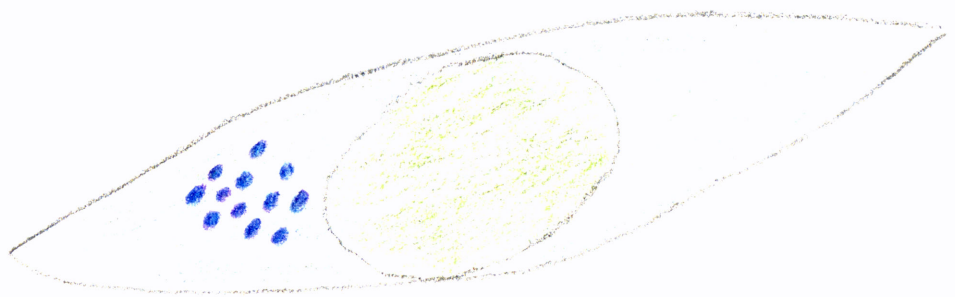
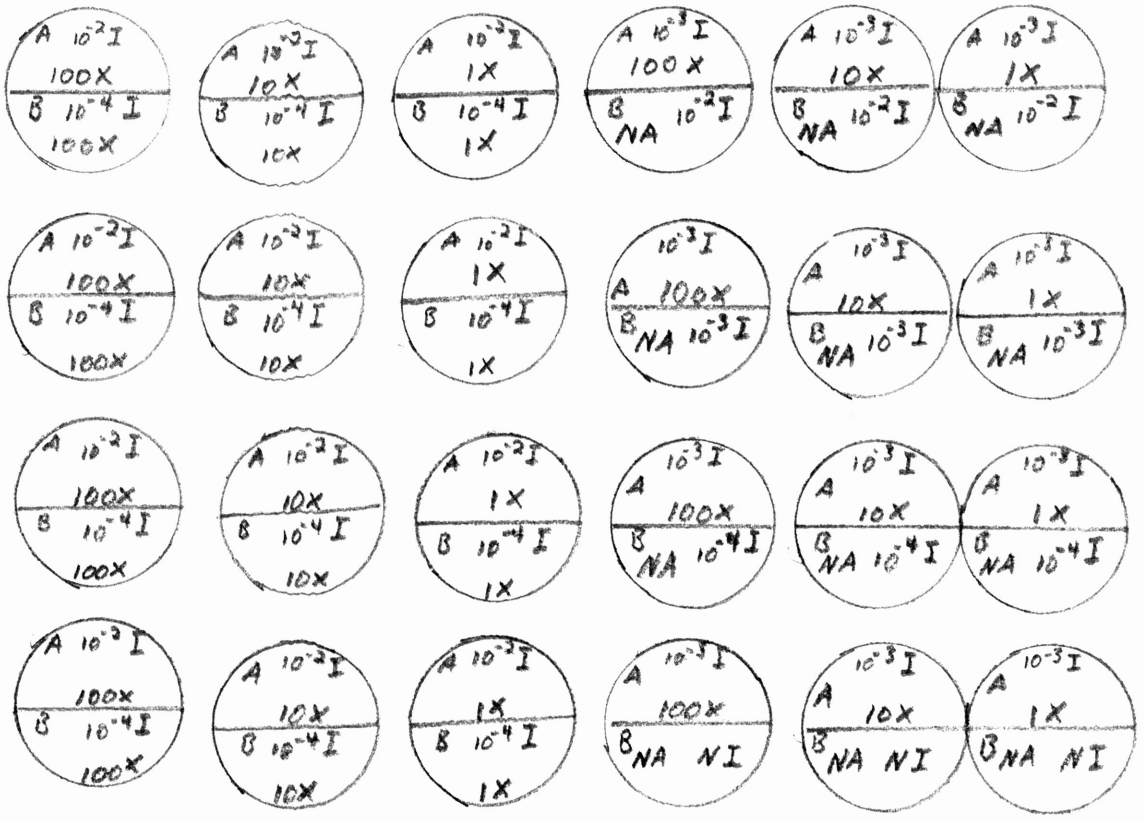


Fig. A shows a normal mouse fibroblast cell.
Fig. B shows a mouse fibroblast cell with a Chlamydial inclusion.



X = μg/ml of antibiotic
I = Inoculum
A = Plate 1
B = Plate 2
NI = No inoculum
NA = No antibiotic

Fig. 4
The Inoculation Scheme

BIBLIOGRAPHY

1. Beem, M., E. Saxon, and M. Tipple. 1979. Treatment of Chlamydial Pneumonia in Infancy. Pediatrics. 63:192-197.

2. Blackman, H., C. Yoneda, C. Dawson, and J. Schachter. 1977. Antibiotic Susceptibility of Chlamydia trachomatis. Antimicob. Agents Chemther. 12:673-677.

3. Bowie, W., E. Alexander, J. Floyd, J. Holmes, Y. Miller, and K. Holmes. 1976. Differential Response of Chlamydial and Ureaplasma-Associated Urethritis to Sulphafurazole (Sulfisoxazole) and Aminocyclitols. Lancet. 2:1276-1278.

4. Bowie, W. R., C. K. Lee, and E. R. Alexander. 1978. Prediction of Efficacy of Antimicrobial Agents in Treatment of Infections Due to Chlamydia trachomatis. J. Infect. Dis. 138:655-659.

5. Cottral, G. E. 1978. Manual of Standardized Methods of Veterinary Microbiology, Cornell University Press.

6. Dunlop, E. M. 1977. Treatment of Patients Suffering from Chlamydial Infections. J. Antimicob. Chemother. 3:377-383.

7. Grimes, J. E., and T. D. Miller. September, 1978. Recent Increased Incidence of Chlamydiosis (Psittacosis) in Psittacine Birds in Texas. The Southwestern Veterinarian.

Vol. 31, No. 3:189-192.

8. Johnson, F. W. and D. Hobson. 1977. The Effect of Penicillin on Genital Strains of Chlamydia trachomatis in Tissue Culture. J. Antimicrob. Chemother. 3:49-56.

9. Kramer, M., R. Cleeland, and E. Grunberg. 1979. Activity of Oral Amoxicillin, Ampicillin, and Oxytetracycline Against Infection with Chlamydia trachomatis in Mice. J. Infect. 139:717-719.

10. Kuo, C., S. Wang, and J. Grayson. 1977. Antimicrobial Activity of Several Antibiotics and a Sulfonamide Against Chlamydia trachomatis Organisms in Cell Culture. Antimicob. Agents Chemother. 12:80-83.

11. Oriel, J. G. Ridgway, P. Reeve, D. Beckingham, and J. Owen. 1976. The Lack of Effect of Ampicillin plus Probenecid Given for Genital Infections with Neissaria gonorrheae on Associated Infections with Chlamydia trachomatis. J. Infect. 133:568-571.

12. Oriel, J., G. Ridgway, S. Tchamouroff, and J. Owen. 1977. Spectinomycin Hydrochloride in the Treatment of Gonorrhoea: Its Effect on Associated Chlamydia trachomatis Infections. Br. J. Vener. Dis. 53:226-229.

13. Reeve, P. 1976. The Inactivation of Chlamydia trachomatis by Povidone-Iodine. J. Antimicrob. Chemother. 2:77-80.

14. Ridgway, G., J. Owen, and J. Oriel. 1976. A Method for Testing the Antibiotic Susceptibility of Chlamyia trachomatis in a Cell Culture System. J. Antimicob. Chemother. 2:71-76.

15. Smith, T. and H. Washton. 1978. In Vitro Susceptibility of 30 Strains of Chlamydia trachomatis to Rosamicin. Antimicob. Agents Chemother. 14:493-494.

16. Storz, J. Chlamydia and Chlamydia Induced Diseases. Thomas, Springfield, Ill., 1971.

17. Waugh, M. A. and K. C. Nayyar. 1977. Triple Tetracycline (Deteclo) in the Treatment of Chlamydial Infection of the Female Genital Tract. Br. J. Vener. Dis. 53:96-97.

18. White, L., H. Hall, T. Tzianabos, and W. Chappell. 1976. Effect of Gentamicin on Growth of Viral, Chlamydial, and Rickettsial Agents in Mice and Embryonated Eggs. Antimirob. Agents Chemother. 10:344-346.