

In Vitro Activities of Doxycycline and Enrofloxacin against European *Chlamydia psittaci* Strains from Turkeys

P. BUTAYE,^{1*} R. DUCATELLE,¹ P. DE BACKER,² H. VERMEERSCH,³
J. P. REMON,⁴ AND F. HAESEBROUCK¹

Department of Pathology, Bacteriology and Avian Diseases,¹ and Department of Pharmacology and Toxicology,² Faculty of Veterinary Medicine, and Department of Pharmaceutical Technology, Faculty of Pharmaceutical Science,⁴ University of Ghent, Ghent, and N.V. OROPHARMA, Temse,³ Belgium

Received 15 May 1997/Returned for modification 6 August 1997/Accepted 6 October 1997

The in vitro susceptibility of 14 European *Chlamydia psittaci* strains from turkeys to the antibiotics doxycycline and enrofloxacin was tested. For doxycycline the MIC ranged from 0.05 to 0.2 µg/ml, with an average of 0.1 µg/ml. For enrofloxacin the MIC was 0.25 µg/ml. Acquired resistance was not detected against doxycycline and enrofloxacin.

Chlamydia psittaci infections in turkeys are frequently diagnosed (3, 14). *C. psittaci* is a well-known zoonotic agent which has been shown to spread from turkeys to humans (3). In human medicine, doxycycline and some fluoroquinolones are recommended for the treatment of *Chlamydia trachomatis* infections (15). For the treatment of *Chlamydia pneumoniae* infections in humans, doxycycline is effective (4). The antibiotics of choice in veterinary medicine for the treatment of *C. psittaci* infections are doxycycline or other tetracyclines and the fluoroquinolone enrofloxacin (2). Resistance of *Chlamydia* species to tetracyclines has been reported in human and veterinary medicine in only a few cases (8, 9).

To our knowledge, the in vitro susceptibilities of *C. psittaci* strains from turkeys to doxycycline and enrofloxacin have not been reported previously. It was the purpose of the present study to determine the MICs of doxycycline and enrofloxacin against a series of *C. psittaci* isolates collected from field infections in turkeys in Europe.

Buffalo Green monkey cells (kidney cells from the Buffalo Green monkey) were used for in vitro culturing of *C. psittaci* and for MIC determinations. Cells were grown as described before (13). The cells were subcultured for at least two passages in medium without antibiotics prior to use in determining the MICs of the antibiotics, as described below. The 14 *C. psittaci* strains (1 Dutch, 2 Italian, and 11 Belgian) were obtained from field infections in commercial turkey flocks by using a sampling and isolation technique described elsewhere (13). Doxycycline (Alpha Pharma, Zwevegem, Belgium) and enrofloxacin (Bayer, Leverkusen, Germany) solutions were prepared freshly on each testing occasion. The tested dilutions of doxycycline and enrofloxacin ranged from 0.2 to 0.00625 µg/ml and from 1 to 0.03125 µg/ml, respectively. Titration of the *C. psittaci* strains was done as described by Vanrompay et al. (13). MIC determination assays were performed according to the method of Henning and Krauss (6).

Both doxycycline and enrofloxacin were effective antimicrobial agents for in vitro inhibition of *C. psittaci* strains from

turkeys. The MIC range for doxycycline was between 0.05 and 0.2 µg/ml, with an average of 0.1 µg/ml. For enrofloxacin, the MIC for all strains was 0.25 µg/ml.

Determination of MICs against obligate intracellular bacteria such as *C. psittaci* is more difficult than for extracellular bacteria. The methods used are not yet standardized, and results can be influenced by technical differences in performance of the assays (4). One such difference is the inoculum size. However, when inoculum sizes causing no immediate cytopathic effects are tested, no differences in MICs are found for *C. trachomatis* isolates (12). The time between inoculation and addition of the antibiotic to the medium is only of importance when doxycycline is added more than 3 h after inoculation of *C. psittaci* (5). The use of different *Chlamydia* detection methods gave only slightly different results (7). MIC results are not influenced by differences in cell cultures, duration of contact with the antibiotics tested, or cell treatments (6).

In the present studies the method of Henning and Krauss (6) was used for determination of the MICs. In this method, the interval between inoculation of the *Chlamydia* strains and addition of the antibiotic is less than 3 h, and an inoculum size that does not cause immediate cell lysis is used. The results obtained with doxycycline against *C. psittaci* strains isolated from turkeys are in accordance with those reported by Henning and Krauss (6) and Kimura et al. (10), who used a method proposed by the Japanese Society of Chemotherapy for *C. psittaci* isolates from different birds (other than turkeys) and mammals (0.01 to 0.05 µg/ml). They are also comparable to those of most other studies of MICs against *C. trachomatis* and *C. pneumoniae*, with MICs for doxycycline ranging from 0.01 to 0.5 µg/ml (4). To our knowledge, only four *Chlamydia* strains have been tested for in vitro susceptibility to enrofloxacin (11). The results were similar to those reported here.

In the present studies, acquired resistance to doxycycline and enrofloxacin was not detected. This finding is similar to the results of Henning and Krauss (5), who did not detect acquired resistance against doxycycline in 31 avian strains or in 30 mammalian strains of *C. psittaci*. Only one avian *C. psittaci* strain, originating from a duck and designated JSD, has been reported as having acquired resistance to multiple antibiotics (8). Acquired resistance has not been reported for *C. pneumoniae* and seems to be rare in *C. trachomatis* (9).

Although doxycycline has high in vitro activity against *C.*

* Corresponding author. Mailing address: University of Ghent, Faculty of Veterinary Medicine, Department of Pathology, Bacteriology and Avian Diseases, Salisburylaan 133, B-9820 Merelbeke, Belgium. Phone: 32 9 264 74 48. Fax: 32 9 264 74 94. E-mail: pbutaye@allserv.rug.ac.be.

psittaci, in vivo experiments in birds indicate that treatment periods of 30 to 45 days are necessary (2). This long period of treatment is not surprising, since clearance of *Chlamydia* from the host is highly dependent on host responses, and reinfection from the environment is possible (1). In contrast, for the treatment of *C. trachomatis* infections in humans, a 7-day treatment with doxycycline is recommended. Failure rates in clinical trials have ranged from 0 to 3% in men and 0 to 8% in women (15). It seems that doxycycline is more effective in the treatment of human *C. trachomatis* infections than it is in the treatment of *C. psittaci* infections in birds, although the in vitro susceptibility is within the same range. A possible explanation for the better results in humans might be a lower reinfection rate.

In conclusion, both doxycycline and enrofloxacin have strong in vitro antimicrobial activities against *C. psittaci* strains isolated from turkeys in Europe. Acquired resistance was not detected in the strains tested against doxycycline and enrofloxacin.

The IWT (Institute for Encouragement of Scientific and Technological Research in Industry, Brussels, Belgium) is gratefully acknowledged for financial support.

We also thank A. Verleye and G. Massaer for skilled technical assistance.

REFERENCES

1. **Flammer, K.** 1987. Avian chlamydiosis—observations on diagnostic techniques and use of oral doxycycline for treatment, p. 149–157. Proceedings of the First International Conference on Zoological and Avian Medicine, Hawaii.
2. **Flammer, K.** 1989. Potential new methods for treating chlamydiosis in psittacine birds, p. 35–37. Proceedings of the 1989 Annual Conference of the Association of Avian Veterinarians, Seattle, Washington. (Abstract.)
3. **Grimes, J. E., and P. B. Wyrick.** 1991. Chlamydiosis (ornithosis), p. 311–325. In B. W. Calneck (ed.), Diseases of poultry, 9th ed. Iowa State University Press, Ames, Iowa.
4. **Hammerschlag, M. R.** 1994. Antimicrobial susceptibility and therapy of infections caused by *Chlamydia pneumoniae*. Antimicrob. Agents Chemother. **38**:1873–1878.
5. **Henning, K., and H. Krauss.** 1986. Felduntersuchung zur Frage einer Resistenzbildung von *Chlamydia psittaci* gegen Tetrazykline. Berl. Muench. Tierärztl. Wochenschr. **99**:381–382.
6. **Henning, K., and H. Krauss.** 1986. Zur Methodik der Bestimmung der Antibiotikumempfindlichkeit von Chlamydien in vitro. J. Vet. Med. Ser. B **33**:447–461.
7. **How, S. J., D. Hobson, A. Hart, and E. Qualey.** 1985. A comparison of the in vitro activity of antimicrobials against *Chlamydia trachomatis* examined by Giemsa and a fluorescent antibody stain. J. Antimicrob. Chemother. **15**:399–404.
8. **Johnson, F. W. A., and W. N. Spencer.** 1983. Multiantibiotic resistance in *Chlamydia psittaci* from ducks. Vet. Rec. **112**:208.
9. **Jones, R. B., B. Van Der Pol, D. H. Martin, and M. K. Shepard.** 1990. Partial characterization of *Chlamydia trachomatis* isolates resistant to multiple antibiotics. J. Infect. Dis. **162**:1309–1315.
10. **Kimura, M., T. Kishimoto, Y. Niki, and R. Soejima.** 1993. In vitro and in vivo antichlamydial activities of newly developed quinolone antimicrobial agents. Antimicrob. Agents Chemother. **37**:801–803.
11. **Lindenstruth, H., and J. W. Frost.** 1993. Enrofloxacin (Baytril®)—eine Alternative in der Psittakoseprophylaxie und-therapie bei importierten Psittaciden. Dtsch. Tierärztl. Wochenschr. **100**:364–368.
12. **Nagayama, A., T. Nakao, and H. Tean.** 1988. In vitro activities of ofloxacin and four other new quinoline-carboxylic acids against *Chlamydia trachomatis*. Antimicrob. Agents Chemother. **32**:1735–1736.
13. **Vanrompay, D., R. Ducatelle, and F. Haesebrouck.** 1992. Diagnosis of avian chlamydiosis: specificity of the modified Giménez staining on smears and comparison of the sensitivity of isolation in eggs and three different cell cultures. J. Vet. Med. Ser. B **39**:105–112.
14. **Vanrompay, D., P. Butaye, A. Van Nerom, R. Ducatelle, and F. Haesebrouck.** 1997. Prevalence of *Chlamydia psittaci* infections in Belgian commercial turkey poult. Vet. Microbiol. **54**:85–93.
15. **Weber, J. T., and R. E. Johnson.** 1995. New treatments for Chlamydia trachomatis genital infection. Clin. Infect. Dis. **20**(suppl. 1):966–971.