In-vitro antibacterial properties of tilmicosin against Australian isolates of *Pasteurella multocida* and *Actinobacillus pleuropneumoniae* from pigs

Queensland Department of Primary Industries, Animal Research Institute, Yeerongpilly, Queensland 4105 — PJ BLACKALL, T ASAKAWA

Queensland Department of Primary Industries, Toowoomba Veterinary Laboratory, Toowoomba, Queensland 4350 — RJ GRAYDON

Department of Veterinary Clinical Studies, University of Sydney, Camden, New South Wales 2570 — M WHITE

Department of Agriculture, Benalla Regional Veterinary Laboratory, Benalla, Victoria 3672 — M ADAMSON

Elanco Animal Health, West Ryde, New South Wales 2114 — LK WADE, LB LOWE

Tilmicosin is a new macrolide antibiotic prepared by chemical modifications of desmycosin (Ose 1987; Debono *et al* 1989) and is the active ingredient of both Micotil 300® Injection* and Pulmotil® Premix*. While Micotil 300® should not be administered to swine, Pulmotil® Premix is an oral formulation of tilmicosin that has been developed for the treatment of porcine respiratory disease. Field trials evaluating Pulmotil® Premix are being conducted in at least ten countries around the world before applications for registration are lodged.

Studies performed overseas have established that tilmicosin has an antimicrobial spectrum characteristic of macrolide antibiotics. Ose (1987) and Debono *et al* (1989) have demonstrated that tilmicosin inhibited the growth of isolates of *Pasteurella multocida, Pasteurella haemolytica, Mycoplasma hyopneumoniae, Actinobacillus pleuropneumoniae, Streptococcus suis* and *Actinomyces pyogenes*, generally at concentrations of 6.25 μg/mL or less. Stephens *et al* (1993) have shown that tilmicosin is active *in vitro* against isolates of *P multocida* and *P haemolytica* obtained from Australian cattle, with all isolates being inhibited at 6.25 μg/mL or less. In this paper, we report on the in-vitro antibacterial activity of tilmicosin against 50 isolates of *A pleuropneumoniae* and 51 isolates of *P multocida*, all obtained from Australian pigs.

The 50 *A pleuropneumoniae* isolates used in this study have been described previously (Hampson *et al* 1993). They were obtained from cultures submitted to the national reference laboratory at the Animal Research Institute, Yeerongpilly, Queensland. The isolates represented the known genetic diversity of the species in Australia and included all 7 serovars known to occur in Australia — serovar 1 (11 isolates), serovar 2 (3 isolates), serovar 3 (4 isolates), serovar 5 (2 isolates), serovar 7 (6 isolates), serovar 11 (1 isolate) and serovar 12 (1 isolate) (Hampson *et al* 1993; Blackall and Pahoff 1994). The remaining 22 isolates cannot be confidently assigned to a serovar (Blackall and Pahoff 1994). The 51 *P multocida* isolates used in this study were obtained from piggeries in New South Wales (16 isolates), Queensland (27 isolates) and Victoria (8 isolates). The isolates were selected from as diverse a range of piggeries as possible and represented 16 farms in Queensland, 21 farms in New South Wales and 7 farms in Victoria. A reference strain of *P haemolytica* (strain 128k) was obtained from Dr T Shyrock (Eli Lilly, Indiana USA). Strain 128k is the recommended quality control strain for antimicrobial sensitivity testing involving tilmicosin (Anonymous 1992). The minimal inhibitory concentration (MIC) of tilmicosin against strain 128k is 1.56 μg/mL plus or minus one doubling dilution (Anonymous 1992).

The MIC of tilmicosin for the isolates was determined by an agar dilution method as previously described (Stephens *et al* 1993). The antibiotic plates used with the *A pleuropneumoniae* isolates were supplemented with 0.0025% (w/v) reduced nicotinamide adenine dinucleotide (NADH) immediately before pouring. The agar plates contained a final concentration of tilmicosin that varied from 50 to 0.78 μg/mL. Preliminary work with a subset of *A pleuropneumoniae* and *P multocida* isolates established that overnight broth cultures of these organisms when diluted 10^{-3} yielded a viable count of about 5×10^5 colony forming units/mL. The test organisms were grown overnight in tryptose broth† for *P multocida* or tryptose broth† plus 0.0025% NADH for *A pleuropneumoniae*, and were diluted 10^{-3}. One μL of this dilution was then inoculated onto the plates. A plate that did not contain any antibiotic was included with each set of organisms. All organisms were also plated onto 5% sheep blood agar to confirm the purity of the inoculum. Each plate was also inoculated with the control strain of *P haemolytica* (128k). The inoculated plates were incubated aerobically at 37°C for 18 h. The MIC was taken as the lowest concentration to prevent growth. The MIC_{95} was defined as that concentration of tilmicosin that inhibited 95% of the strains examined.

The MIC value of the reference strain of *P haemolytica* was always 1.56 μg/mL plus or minus one doubling dilution. This value is the expected result for this strain (Anonymous 1992). The cumulative number of *A pleuropneumoniae* and *P multocida* isolates inhibited at the various concentrations of tilmicosin are presented in Table 1. The MIC_{95} of tilmicosin for the *A pleuropneumoniae* isolates was 6.25 μg/mL while for the *P multocida* isolates it was 12.5 μg/mL. The recommended interpretative criteria for tilmicosin are as follows: growth at 25 μg/mL - resistant; growth at 6.25 μg/mL but no higher - intermediate sensitivity; growth at less than 6.25 μg/mL - sensitive (Anonymous 1992). On the basis of these figures, 48 of the

* Elanco Animal Health, West Ryde, NSW

† Oxoid CM233, Oxoid Australia Pty Ltd, West Heidelberg, Vic

TABLE 1

Cumulative in-vitro antibacterial activity of tilmicosin against porcine isolates of *P multocida* and *A pleuropneumoniae*

Microorganism	Cumulative number (per cent) of isolates inhibited at the indicated concentration (μg/mL)					
	≤ 0.78	1.56	3.12	6.25	12.5	25
P multocida	5 (9.8)	23 (45.1)	42 (82.3)	48 (94.1)	50 (98)	51 (100)
A pleuropneumoniae	7 (14)	16 (32)	36 (72)	50 (100)	–	–

51 isolates of *P multocida* were sensitive to tilmicosin with the remaining three isolates showing intermediate sensitivity. By the same criteria, all 50 isolates of *A pleuropneumoniae* were sensitive to tilmicosin.

The results of this study are similar to those of a previous overseas study on the in-vitro activity of tilmicosin against porcine isolates of *P multocida* and *A pleuropneumoniae*. We found that the MIC_{95} of tilmicosin for the Australian porcine isolates of *P multocida* was 12.5 μg/mL while the study of Ose (1987) reported a slightly lower MIC_{95} of 6.25 μg/mL for tilmicosin for the 36 isolates they examined. We found that the MIC_{95} for the Australian isolates of *A pleuropneumoniae* was 6.25 μg/mL. The study of Ose (1987) did not report an MIC_{95} but did state that all 29 *A pleuropneumoniae* isolates were inhibited at 6.25 μg/mL and that "the most common MIC" was 1.56 μg/mL. Any differences between the Australian and overseas isolates cannot be regarded as significant because they differ by one doubling dilution only.

The results presented in this study provide evidence that the in-vitro activity of tilmicosin against Australian porcine isolates of *A pleuropneumoniae* and *P multocida* is very similar to that reported for isolates of the same bacterial species from pigs in North America.

References

Anon (1992) *Methods for Tilmicosin Antibacterial Susceptibility Testing*, Elanco Animal Health Publication A17489(25), Indianapolis

Blackall PJ and Pahoff J (1994) *Aust Vet J* **71**: 18

Debono M, Willard KE, Kirst HA, Wind JA, Crouse GD *et al* (1989) *J Antibiot* **42**: 1253

Hampson DJ, Blackall PJ, Woodward JM and Lymbery AJ (1993) *Zentralb Bakteriol* **279**: 83

Ose EE (1987) *J Antibiot* **40**: 190

Stephens CP, Blackall PJ, Wade LK and Lowe LB (1993) *Aust Vet J* **70**: 391

(Accepted for publication 19 June 1994)