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Effect of various oral dose levels of a trimethoprim/sulphadiazine mixture on *Bordetella bronchiseptica* infection and on the proliferation of trimethoprim-resistant faecal coliforms in pigs

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SUMMARY

When a 1:5 mixture of trimethoprim (TMP) and sulphadiazine was fed to pigs intra-nasally infected with *Bordetella bronchiseptica*, 10 mg/kg/day was shown to be highly effective in suppressing the organism. This dose level had little effect on numbers of TMP-resistant coliforms in faeces, but oral doses of 30 mg/kg/day eventually selected a resistant population. It is suggested that the proliferation of resistant coliforms would be minimized by administration of the lowest oral dose rates of antibacterial drugs compatible with efficacy.

INTRODUCTION

Administration of trimethoprim/sulphadiazine (TMP/SDZ) by daily subcutaneous injections has been shown to reduce upper respiratory colonization by *Bordetella bronchiseptica* and associated pathological changes in experimentally infected dogs (McCandlish & Thompson, 1979). In the young pig, Giles *et al.* (1981) found that while the temporary reduction in nasal colonization by *B. bronchiseptica* which followed each of several injections given at intervals of a few days was accompanied by a reduction in conchal atrophy, complete elimination of the infection could be achieved by continuous medication via drinking water for about 3 weeks. Because drinking water medication is not feasible in many large pig units with automatic watering systems, it was decided to investigate the efficacy against *B. bronchiseptica* of continuous oral medication via the feed. It is known that TMP-resistant coliforms are now widespread in the U.K. pig population (Smith & Lovell, 1981), and in view of concern that medication via feed may promote the dissemination of resistance, a study was simultaneously made of the relationship between medication at different dose levels and the rate of proliferation of TMP-resistant coliforms in faeces.

MATERIALS AND METHODS

Animals

In Expt. 1, 23 pigs aged 18 days were acquired from a herd known to be free from *B. bronchiseptica* infection, and housed in three well-isolated boxes containing 7 (Group 1), 8 (Group 2) and 8 (Group 3) pigs. Water and commercial weaner pellets were offered *ad libitum* throughout the experiment to all three groups.

In Expt. 2, three groups each of four pigs were similarly housed but an early weaning meal was fed throughout instead of pellets.

Infection

In Expt. 1, all pigs were infected with a strain of *B. bronchiseptica* (Bb212) of known *in vitro* sensitivity to both TMP and SDZ. The organism was harvested from a 24-h surface growth on tryptose agar, suspended in quarter strength Ringer's solution to a density equivalent to 3×10^8 organisms/ml, and 0·5 m was then instilled into each nostril. This procedure was carried out on 3 consecutive days, beginning when the pigs were 21 days old.

In Expt. 2 the pigs were not infected.

Medication

In Expt. 1, oral administration of a 1:5 mixture of TMP and SDZ was carried out by using extruded pellets containing 5% of the mixture in a lactose base. These pellets were added to about 2/3 of the daily intake of weaner pellets and later in the day, following consumption of the medicated pellets, further weaner pellets were offered to appetite. The daily doses of medicated pellets were calculated according to the group total bodyweights, Group 1 receiving 15 mg/kg bodyweight/day, Group 2, 10 mg/kg, while Group 3 were not dosed. (In this report, all dose levels of the 1:5 TMP/SDZ mixture refer to total active ingredients.) These medication rates continued for 28 consecutive days beginning (on Day 1) three days after the last infecting dose of *B. bronchiseptica*. On Day 28, four pigs from each of Groups 1 and 2 were removed and penned together, and were then fed medicated pellets for a further 14 days at an increased dose of 30 mg/kg/day. Doses of medicated pellets were adjusted weekly on the basis of group bodyweights.

In Expt. 2, medication was carried out by thorough mixing of a lactose-based powder containing 12% of a 1:5 TMP/SDZ mixture in the early weaning meal. Beginning on Day 1 when the pigs were 28 days old, meal was fed at the rate 7% of group bodyweight/day, drug inclusion rates in meal being calculated so that Groups 1, 2 and 3 received 30 mg/kg/day, 12·5 mg/kg/day, and no TMP/SDZ respectively. Medication at these rates was carried out for 21 consecutive days.

Bacteriological procedures

In Expt. 1 only, estimates of the extent of proliferation of *B. bronchiseptica* were carried out by bacteriological examination of nasal swabs from each pig twice weekly throughout the experiment. Nasal swabs were taken by inserting sterile wire-mounted paediatric grade swabs (MW142, Medical Wire & Equipment Co. Ltd) deep into the nasal passages of the piglets, and were processed within 2 h of collection. Swabs were cut off from the wire mount into bottles containing 0·5 ml of normal saline and agitated for 1 min on a Whirlemixer (Lablinc Instruments Inc). This saline suspension and decimal dilutions (10^{-1} and 10^{-2}) of it were spread in 0·1 ml volumes on modified MacConkey agar containing 1% glucose, 0·002% furaltadone, 0·001% crystal violet and 50 units/ml nystatin.

The plates were incubated aerobically at 37 °C for 48–72 h before counting the colonies morphologically characteristic of *B. bronchiseptica – greyish-tan colonies* surrounded by an alkaline zone. The counts were carried out on a plate in the

dilution series showing approximately 100–200 colonies and were expressed in terms of viable bordetellae/ml of the original nasal-swab suspension.

In both experiments faecal coliforms sensitive and resistant to TMP were counted twice weekly. Rectal faecal samples were weighed in plastic envelopes and dispersed in 9 vol. of quarter-strength Ringer's solution in a 'Colworth Stomacher 80' for 15 s. Decimal dilutions were made up to 10^{-5} and 0·1 ml aliquots were spread on Oxoid Isosensitest agar containing 1 % glucose, 0·05 % sodium deoxycholate, 0·003 % neutral red and 50 units/ml nystatin and also on this medium containing 100 μg TMP/ml in addition. Following 24 h aerobic incubation at 37 °C, typical coliform colonies were counted on both media and expressed in terms of total and TMP-resistant coliforms per g faeces.

On the 6th and the 16th day of medication in Expt. 2, TMP-resistant *Escherichia coli* from all pigs from which they were isolated were tested for the presence of plasmids by the method described by Datta & Hedges (1972), using a selective medium comprising Isosensitest agar with neutral red, 1 % lactose, 10 μg/ml TMP and 30 μg/ml nalidixic acid for the isolation of recombinants.

RESULTS

The results of *B. bronchiseptica* counts on nasal swab suspensions are presented graphically in Figure 1, in which each line represents the geometric mean result from all seven or eight pigs in each group. The counts in Group 3 (untreated) remained consistently high at about 10^5 per swab for the first 14 days, and thereafter fell slightly, but in both treated groups (1 and 2), counts had fallen to less than 10 % of the control numbers after 3 days of medication, to less than 1 % by the 6th day, with further progressive falls. The bordetellae counts in Groups

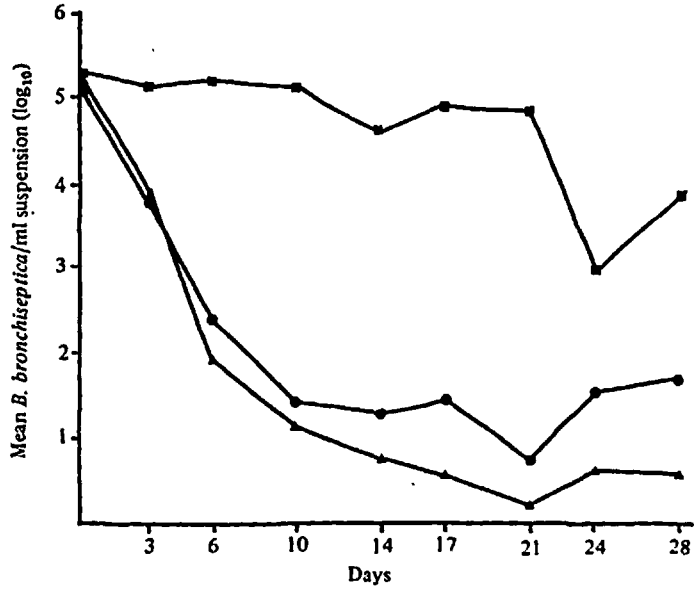

Fig. 1. Expt. 1. Isolation of *B. bronchiseptica* from nasal swab suspensions (group geometric mean values). ▲ = Group 1, 15 mg/kg; ● = group 2, 10 mg/kg; ■ = group 3, unmedicated.

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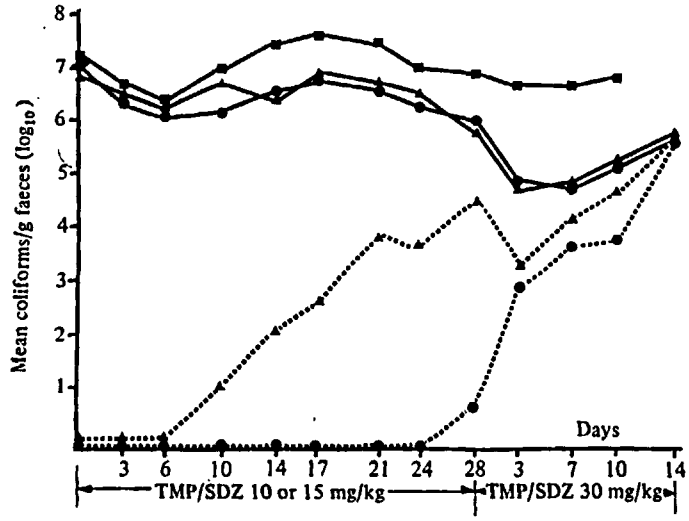

Fig. 2. Expt. 1. Comparison of total (——) and TMP-resistant (--------) coliform counts (group geometric mean values). ▲ = Group 1, 15 mg/kg followed by 30 mg/kg; ● = group 2, 10 mg/kg followed by 30 mg/kg; ■ = group 3, unmedicated.

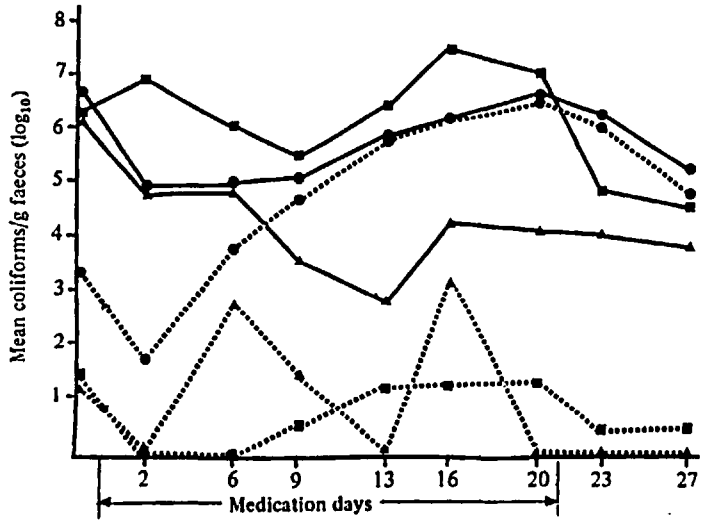

Fig. 3. Expt. 2. Comparison of total (——) and TMP-resistant (--------) coliform counts (group geometric mean values). ● = Group 1, 30 mg/kg; ▲ = group 2, 12·5 mg/kg; ■ = group 3, unmedicated.

1 and 2 at the end of the medication period were mainly accounted for by one pig in each group.

The total and TMP-resistant geometric mean faecal coliform counts from the same four pigs in each group in Expt. 1 are shown in Figure 2. The total counts in Group 3 (unmedicated) remained at about 10^7 throughout, and TMP-resistant coliforms were never isolated from this group. In comparison with the Group 3

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counts, the mean total counts of the two treated groups were reduced by about 90% after about the first 10 days of medication, but TMP-resistant coliforms were not isolated from Group 2 (10 mg/kg) until the final sampling at this dose level on day 28, one pig only yielding resistant organisms. In Group 1 (15 mg/kg), resistant organisms were first isolated after 10 days of medication, and numbers gradually increased, with all four pigs excreting resistant coliforms at 28 days, comprising about 3% of the total count. On increasing the dose rate to 30 mg/kg in all eight treated sampled pigs, the total and resistant counts converged until the total population had become resistant after 14 days medication at this dose level.

In Expt. 2, the total and resistant coliform counts are similarly presented in Figure 3. In the prior experiment, TMP-resistant coliforms were not isolated before medication, but in this experiment resistant organisms were initially present in all three groups, and even in the untreated Group 3 pigs low numbers of TMP-resistant coliforms were intermittently isolated throughout the experiment. In Group 1 (30 mg/kg), all four pigs were excreting resistant coliforms by the 6th medication day, with a slight drop in the total count, and from the 9th day onwards the resistant and the total counts in all four pigs were almost identical. After withdrawal of TMP/SDZ, the proportion of TMP-resistant coliforms tended to fall over the next 7 days, being absent from the faeces of one pig at the last sampling. In Group 2 (12·5 mg/kg), two of the four pigs were excreting considerable numbers of resistant coliforms on day 6, but, thereafter, only low numbers were intermittently excreted, none being isolated from any pig on the last day of medication and at the two subsequent samplings. A marked fall in total coliform counts was apparent in this group throughout medication.

Of the 17 TMP-resistant strains (6 and 11 strains isolated on the 6th and 16th day of medication respectively) tested for the presence of plasmids conferring TMP resistance, five strains were found to transfer resistance to the recipient K12 strain of *E. coli*. All 17 original isolates were shown to be resistant to more than 1000 μg/ml TMP.

DISCUSSION

In Expt. 1, the rate and extent of disappearance of *B. bronchiseptica* from the nasal cavities of the treated pigs given TMP/SDZ via the feed, as judged by the counts on suspensions of swabs, was similar to the findings of Giles *et al.* (1981) following *ad lib.* consumption of medicated drinking water by experimentally infected pigs. These authors found that 80 and 40 mg/l of TMP/SDZ in drinking water were highly effective but half the latter concentration had little effect. Assuming a water intake of 200 ml/kg bodyweight for pigs on dry food, the resulting dose rates of TMP/SDZ would have been 12, 6 and 3 mg/kg/day, with the minimum effective rate being about 6 mg/kg. The results in Expt. 1 reported above, in which 15 and 10 mg/kg/day in food were effective, are therefore consistent with the reported efficacy of water medication.

The results in Figure 2 indicate that 10 mg/kg/day of TMP/SDZ given in food for 28 days did not result in the proliferation of TMP-resistant coliforms in the alimentary tract, but a progressive increase in the proportion of resistant organisms occurred at the higher dose level, and further increase in dose level to

30 mg/kg/day in both groups rapidly produced a completely resistant population. The results of similar observations in Expt. 2 appeared to confirm that 30 mg/kg/day of TMP/SDZ in food resulted in a totally resistant coliform population, but 12·5 mg/kg/day did not have this effect.

Transferable TMP resistance was demonstrated in only five out of 17 resistant strains isolated in the second experiment but only one transfer attempt was made with each strain. Several of the strains yielded only very few recombinant colonies, and it is therefore likely that further transfer attempts would have been more successful. In their survey of antibacterial resistance in pigs, Smith & Lovell (1981) reported the direct transfer of TMP resistance by 21 (30 %) of 69 TMP-resistant isolates, but TMP resistance was mobilized by use of a conjugative plasmid in a further 39 of these strains. No such attempt at mobilization of resistance factors was made with the strains from Expt. 2, but it is probable that this would have increased the proportion with demonstrable TMP-resistance plasmids. Although these experiments were carried out with small numbers of pigs, the proliferation under selective pressure of strains carrying plasmid-mediated TMP resistance emphasizes the importance of using veterinary TMP products at dose rates which exert minimal selective pressure.

Few reports appear to have been made of the relationship between dose level of antibacterial drugs and proliferation of resistant enteric coliforms, but Rollins *et al.* (1975) found that the feeding of 10 μg oxytetracycline/g of dry meal to beagles resulted in the appearance of a high proportion of resistant coliforms, whereas 2 μg/g had no such effect. These observations are similar to those reported above for TMP/SDZ, suggesting that high oral doses of an antibacterial drug are more likely to induce resistance in enteric coliforms than lower doses, presumably because of the exertion of greater selective pressure in favour of any resistant organisms present. The greater effect of high doses in this respect is contrary to the widely held opinion that low doses of antibacterials pose a greater threat than high doses in generating resistant bacterial populations, a view embodied in the report of the Joint Committee on the Use of Antibiotics in Animal Husbandry and Veterinary Medicine (1969). In this report it is stated (para 5.5) that 'The development of resistance is encouraged by the use of low concentrations of antibiotic...', but this conclusion appears to have been derived from the established *in vitro* phenomenon of the stepwise increases in resistance to streptomycin exhibited by coliforms exposed to sub-inhibitory but increasing concentrations. These circumstances, involving only a single strain of bacterium, are not, however, analogous to conditions in the alimentary tract, in which many strains of coliforms of varying levels of sensitivity are always present. The proliferation of any highly resistant organisms in the gut probably depends on the suppression of the sensitive populations, and it seems rational to expect that high doses would be more effective in this respect than lower doses.

In considering the strategy of oral medication of a group of animals for controlling a bacterial disease such as atrophic rhinitis, the results reported above suggest that to minimize the proliferation of resistant faecal coliforms, the dose rate selected should be the lowest which is known to be effective. The duration of treatment is also important, as illustrated by the fact that the development of a totally TMP-resistant coliform population in Expt. 2 took place between the 6th

and the 9th day of medication at 30 mg/kg, and therefore the selected period of medication should also be the minimum consistent with efficacy. In the case of TMP/SDZ, a daily rate of about 10 mg/kg, given either in food or drinking water appears to be adequate for the suppression of colonization of the nasal cavities of pigs by sensitive strains of *B. bronchiseptica*, and this dose level also seems to exert a very low selective pressure in the alimentary tract in favour of TMP-resistant coliforms. The period of medication under farm conditions would depend on the period of risk, but in most circumstances, taking into account the known immunity to *B. bronchiseptica* which develops following exposure, a medication period of not more than 14 days would probably be adequate.

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