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# The effects of oxytetracycline on the intestinal *Escherichia coli* flora of newly weaned pigs

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## SUMMARY

Four recently weaned pigs were dosed orally with oxytetracycline. This caused a rapid increase in the incidence of tetracycline resistance (TcR) among *Escherichia coli* isolates from the faecal flora. The isolates were differentiated further on the basis of O-serogroup, biotype and resistance pattern. There was no evidence that the administration of the antibiotic selected for a few TcR clones, but rather a relatively large number of TcR strains were identified during the dosing period. Using selective isolation media a proportion of these strains were demonstrated in the minority faecal *Esch. coli* flora before dosing, while the remainder were recognized for the first time after dosing commenced.

The incidence of TcR among *Esch. coli* isolates also increased after weaning in other pigs which were not dosed with oxytetracycline or any other antibacterial agent. In a proportion of these animals this increase was associated with the dominance of a TcR enteropathogenic serotype (0149:K 91, K 88a, c) in the faecal *Esch. coli* flora which was probably ingested in small numbers before weaning. The source of other TcR strains was probably the environment in which each pig was placed after weaning.

## INTRODUCTION

Tetracylines have been used widely in farm animals for therapeutic and growth promotion purposes and, since they rapidly select for resistant bacterial populations (Chopra *et al.* 1981), tetracycline resistance is prevalent among *Escherichia coli* isolates originating from pigs (Smith, 1975; Linton, 1977, 1981; Smith & Lovell, 1981; Jackson, 1981). The influence of subtherapeutic levels of tetracyclines on tetracycline resistance among faecal coliforms isolated from pigs has been studied (Smith & Crabb, 1957; Finlayson & Barnum, 1973; Linton, Howe & Osborne, 1975; Rollins *et al.* 1976; Langlois, Cromwell & Hay, 1978) but there is little detailed information on the effect these drugs have on *Esch. coli* strains present

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in the intestinal tract. Linton, Handley & Osborne (1978) used O-serogrouping and antibacterial drug resistogram typing to monitor the faecal *Esch. coli* flora of a single pig given a three-day oral course of oxytetracycline, at therapeutic levels, on two occasions during the second half of a 210-day rearing period. These authors concluded that the drug profoundly affected the proportion of tetracycline-sensitive and -resistant *Esch. coli* isolates and that there was a selection of a relatively few resistant O-serogroups, although not all of them persisted for long in the majority *Esch. coli* flora following the administration of the drug.

This paper considers the situation in a small number of young animals. Four newly weaned pigs were dosed orally with oxytetracycline over a five-day period and the intestinal *Esch. coli* flora studied using a biotyping technique in conjunction with O-serogrouping and resistogram typing to subdivide *Esch. coli* into strains. One pig from the same litter received no antibiotic and was housed separately as a control. In order to obtain additional information on the prevalence of tetracycline resistance among isolates of *Esch. coli* from other pigs in the same herd data from 27 pigs, receiving no antibacterial drug, were also analysed.

## MATERIALS AND METHODS

### *The animals and their treatment*

The animals studied were from a herd of enzootic-pneumonia-free Large White pigs. The principal litter studied, designated Litter T, was born on 19 October 1982. A commercially prepared 'creep' ration (Vitamealo, No. 4 Multiwean pellets) was offered *ad lib.* from the 10th day. The five pigs were weaned on different days of life (pig T 3 on the 20th day, pigs T 4 and T 5 on the 21st day and pigs T 6 and T 7 on the 22nd day), and moved to separate accommodation in the same room.

Four pigs (T 4, T 5, T 6 and T 7) were dosed orally with 150 mg oxytetracycline twice daily from weaning until slaughter. Oxytetracycline (110 mg/l) was also added to the drinking water from weaning. Pig T 3 did not receive any antibacterial drug and was kept separate from the other four pigs as an untreated control. A further 27 pigs were studied from three litters (designated F, H and S). These pigs were born between 18 August 1981 and 4 July 1983 and, together with 24 others, comprised the basis of a detailed study of the ecology of *Esch. coli* in healthy pigs after weaning (Hinton *et al.* in the Press).

### *Collection of samples*

#### *Pigs in litter T*

Swabs of rectal faeces were collected daily from each animal on seven occasions, commencing 2 days before weaning and continuing until the fourth day afterwards. The third swab was taken at the time of weaning but before dosing with tetracycline commenced. On the fifth day after weaning (the eighth sampling day) the animals were killed and homogenates of duodenal, jejunal, ileal, caecal and colonic mucosa prepared as described elsewhere (Hampson, Hinton & Kidder, in the Press).

#### *Pigs in litters F, H and S*

A rectal swab was obtained from each of the 27 pigs immediately before weaning. The pigs were then killed 2 to 11 days after weaning and samples of mucosal homogenates obtained from the duodenum, jejunum, ileum, caecum and colon.

### *Microbiological investigations*

*Pigs in litter T*

All rectal swabs and mucosal homogenates were inoculated on to bile lactose agar without salt (BLA) (Oxoid CM 7 b). In order to detect tetracycline-resistant (TcR) strains in the minority flora before dosing with oxytetracycline was commenced, the first three rectal swabs from each pig were also inoculated on to BLA supplemented with tetracycline (50 μg/ml) (BLA + Tc). After overnight incubation at 37 °C in air, up to 20 lactose-fermenting colonies typical of *Esch. coli* were picked from each plate and their identity confirmed as *Esch. coli* by positive Eijkman and indole tests (Hartley *et al.* 1975).

All identified *Esch. coli* were biotyped on the basis of 13 tests. These included the ability to ferment adonitol, dulcitol, D-raffinose, L-rhamnose, L-sorbose and sucrose in solid media (Hinton, Allen & Linton, 1982) and to utilise L(-)fucose, DL-galactonic acid, DL-glyceric acid, mucic acid and DL-saccharic acid as a sole carbon and energy source. These five compounds were included (0·2 % w/v) in a minimal salts solution with trace elements (Cruickshank *et al.* 1980) solidified with 2 % (w/v) agar. The inclusion of 2,3,5-triphenyl-tetrazolium chloride (0·5 g/l) in the minimal salts agar facilitated examination for growth, since the colonies appeared red in colour. In addition, ornithine decarboxylation was assessed using 'Replipack' media (Cathra International, Formby, Lancs), made up according to the manufacturer's instructions, and haemolysin production was detected on layered 4 % sheep blood agar plates.

The resistance of all isolates to 13 antibacterial agents was determined. The agents were incorporated separately into Isosensitest agar (Oxoid CM 471) to which 1·2 % (w/v) lysed horse blood was added. The concentrations (μg/ml) used were ampicillin (50), carbenicillin (50), cephalothin (50), chloramphenicol (50), colistin sulphate (50), furazolidone (35), gentamicin (10), kanamycin (30), spectinomycin (25), streptomycin (10), sulphadiazine (250), tetracycline (50) and trimethoprim (1·25).

*Pigs in litters F, H and S*

The *Esch. coli* were isolated on BLA only. Up to 10 lactose-fermenting colonies were picked from each plate and, after their identity had been confirmed as *Esch. coli*, their resistance to tetracycline was determined using the method described for litter T.

### *Analysis of the results*

The data were analysed with the aid of 'Minitab' (Ryan, Joiner & Ryan, 1976) and 'SPSS' (Nie *et al.* 1975; Hull & Nie, 1981).

## RESULTS

### *Pigs in litter T*

None of the five pigs developed any sign of clinical illness during the survey period. The faeces of all animals appeared slightly 'loose' after weaning although in no case did the faecal water exceed 80 %, a value suggested by Kenworthy & Allen (1966) as indicative of diarrhoea.

Table 1. *Cumulative number of* Escherichia coli *strains* isolated on unsupplemented bile lactose agar from rectal faeces, from five pigs in litter T according to their sensitivity (TcS) or resistance (TcR) to tetracycline*

| Time before or after weaning in days† | Undosed pig T 3 No. strains | | Dosed with oxytetracycline: Pig T 4 No. strains | | Pig T 5 No. strains | | Pig T 6 No. strains | | Pig T 7 No. strains | |
|---|---|---|---|---|---|---|---|---|---|---|
| | TcS | TcR | TcS | TcR | TcS | TcR | TcS | TcR | TcS | TcR |
| −2 | 7 | 2 | 12 | 1 | 1 | 0 | 5 | 1 | 6 | 2 |
| −1 | 14 | 5 | 17 | 2 | 1 | 0 | 15 | 4 | 7 | 2 |
| 0 | 18 | 5 | 17 | 3 | 2 | 0 | 18 | 4 | 10 | 4 |
| 1 | 21 | 7 | 20 | 4 | 5 | 7 | 18 | 6 | 10 | 10 |
| 2 | 25 | 12 | 20 | 8 | 6 | 8 | 19 | 13 | 10 | 17 |
| 3 | 25 | 14 | 20 | 12 | 6 | 11 | 19 | 18 | 10 | 20 |
| 4 | 27 | 14 | 21 | 20 | 6 | 21 | 19 | 21 | 10 | 27 |
| 5 | 31 | 16 | 21 | 27 | 6 | 28 | 21 | 25 | 11 | 29 |
| Total strains | 47 | | 48 | | 34 | | 46 | | 40 | |
| Incidence (%) of TcR among strains isolated | | | | | | | | | | |
| Before weaning | 22 | | 15 | | — | | 18 | | 28·5 | |
| After weaning | 46 | | 86 | | 87·5 | | 87·5 | | 96 | |

* Differentiated on the basis of O-serogroup, biotype and resistance pattern. † O = day of weaning.

### Esch. coli *isolates on unsupplemented bile lactose agar*

Tetracycline-resistant (TcR) *Esch. coli* isolates were less prevalent before weaning in all pigs (Table 1). The proportion of isolates resistant to the drug increased after weaning in both the 'undosed' (T 3) and the four 'dosed' pigs (T 4, T 5, T 6 and T 7). This increase was greater in the 'dosed' pigs reaching 100% 3 days after dosing commenced (Fig. 1).

The average numbers of *Esch. coli* strains, differentiated on the basis of O-serogroup, biotype and resistance pattern, isolated from each rectal swab obtained from pigs T 4 to T 6, before and after dosing with oxytetracycline was commenced, were 6·1 (range 1–14) and 7·6 (range 4–14) strains respectively. This difference was not significant ($t = 1{\cdot}08$; $P = 0{\cdot}3$). The comparable data for the 'control' pig (T 3) were 9 (range 5–13) and 6·75 (range 5–11) strains respectively.

'New' strains (i.e. those isolated for the first time) were identified continually during the sampling period, a total of 34–48 strains being isolated from each pig (Table 1). Strains resistant to tetracycline isolated for the first time before weaning were in the minority (< 30%) while after weaning the converse was true, with the proportion of resistant strains being 46% and > 85% for the 'control' pig and the four 'dosed' pigs respectively. The isolation rate of 'new' strains per swab did not appear to be influenced significantly by dosing with the antibiotic. The average numbers of 'new' strains per swab before and after dosing commenced were 4·8 and 5·4 strains respectively ($t = 0{\cdot}41$; $P = 0{\cdot}69$) (Table 1).

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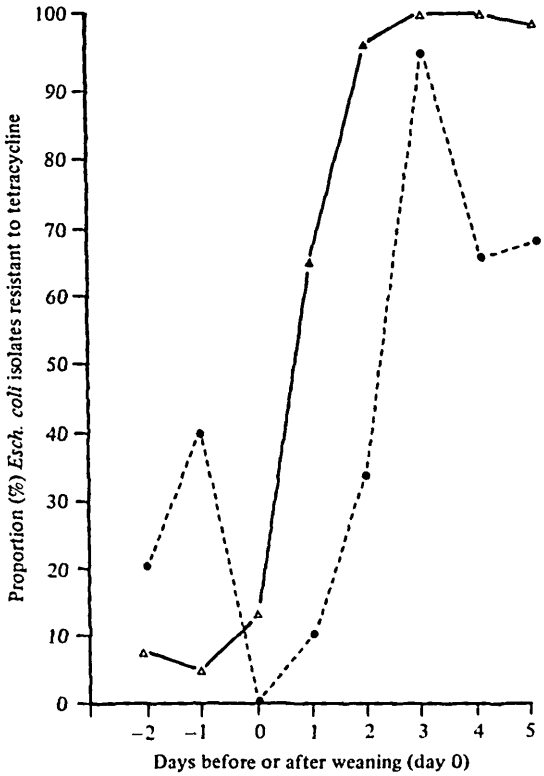


Fig. 1. The incidence of tetracycline resistance among *Escherichia coli* isolates from four pigs dosed with oxytetracycline after weaning (△), and the 'control' pig which was not dosed (●).

Table 2. *The number of* Escherichia coli *strains* isolated from rectal faeces from five pigs in litter T*

| Pig | Dosed with oxytetracycline after weaning | No. of *Esch. coli* isolated on BLA+Tc (samples 1–3) | No. (%) of *Esch. coli* isolated on BLA+Tc that were isolated on | | |
|---|---|---|---|---|---|
| | | | BLA+Tc only | BLA before weaning (samples 1–3) | BLA after weaning (samples 4–7) |
| T 3 | No | 10 | 3 (30) | 0 | 7 (70) |
| T 4 | Yes | 12 | 7 (58) | 2 (17) | 3 (25) |
| T 5 | Yes | 11 | 5 (45) | 1 (9) | 5 (45) |
| T 6 | Yes | 13 | 7 (54) | 1 (8) | 5 (38) |
| T 7 | Yes | 9 | 5 (55) | 2 (22) | 2 (22) |

* Differentiated on the basis of O-serogroup, biotype and resistance pattern.

*Isolates from bile lactose agar supplemented with tetracycline*

Tetracycline-resistant *Esch. coli* strains were present in the minority flora of all pigs before weaning, since they could be isolated on selective media (BLA+Tc) but not on unsupplemented medium (BLA) inoculated at the same time (Table 2). About half the strains (45–58%), isolated on the BLA+Tc from the

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Table 3. *The number of resistance (R) determinants carried by tetracycline-resistant strains of* Escherichia coli *isolated from rectal faeces from five pigs in litter T*

| Time of isolation of strain in relation to dosing | Pigs sampled | Isolation media* | No. of *R* determinants† | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | 1 | 2 | 3 | 4 | 5 | 6 | Mean | S.E. |
| Before | T 3–7 | BLA | 8 | 8 | 10 | 1 | 0 | 0 | 2·15 | 0·17 |
| Before | T 3–7 | BLA+Tc | 9 | 9 | 27 | 8 | 0 | 1 | 2·70[a] | 0·14 |
| After | T 4–7 | BLA | 12 | 23 | 44 | 14 | 5 | 0 | 2·77[a] | 0·10 |

* See text. † Means with same superscript do not differ significantly ($P = > 0{\cdot}05$); S.E. = standard error.

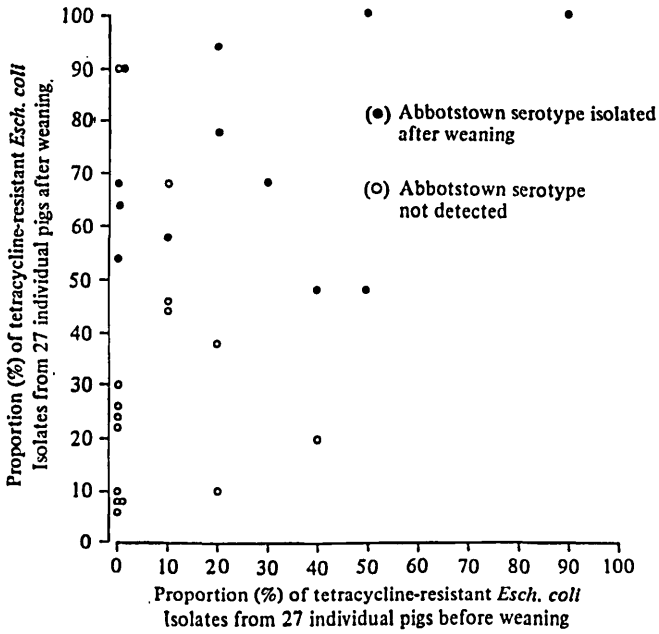


Fig. 2. The incidence of tetracycline resistance among *Escherichia coli* isolates from 27 individual pigs before and after weaning.

four pigs dosed with oxytetracycline were never isolated on BLA following dosing (Table 2). The majority of strains isolated on both media were isolated on unsupplemented BLA only after dosing commenced (i.e. after weaning). A similar situation was also observed in pig T 3, which was not dosed. In this animal 7 of the 10 strains isolated originally on BLA + Tc were subsequently isolated after weaning (Table 2).

*Antibacterial drug resistance among* Esch. coli *isolates*

All isolates examined were sensitive to chloramphenicol, colistin sulphate, gentamicin and trimethoprim, while a small proportion of strains were resistant to ampicillin, carbenicillin, cephalothin, furazolidone and spectinomycin.

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The number of *R* determinants carried by TcR *Esch. coli* strains isolated from each of the five pigs was calculated. The average for the 27 strains isolated on BLA before dosing with oxytetracycline was 2·15, with only one (3·7 %) strain having > 3 *R* determinants (Table 3). Isolation of strains either by the use of bile lactose agar supplemented with Tc (BLA+Tc) before dosing, or following the oral administration of oxytetracycline itself, was associated with a significant increase in the average number of *R* determinants carried per TcR strain (2·70 and 2·77 respectively). The proportion of strains with > 3 *R* determinants also increased to 17 % and 19 % respectively (Table 3).

### *Pigs in litters F, H and S*

The incidence of TcR among *Esch. coli* isolates from swabs of rectal faeces collected from 27 pigs in litters F, H and S before weaning ranged between zero and 90 %, but was < 30 % in 21 (78 %) of the animals (Fig. 2). After weaning, the incidence of TcR among isolates rose in all but three animals and exceeded 40 % in 16 cases. The Abbotstown serotype of *Esch. coli* (0149:K 88a, c) was isolated from 12 of the 27 pigs after weaning. This strain was TcR and, when present, tended to dominate the intestinal *Esch. coli* flora (Hinton *et al.* in the Press). As expected, the incidence of TcR among the *Esch. coli* isolates from these animals tended to be higher than it was in the other weaners. The incidence of resistance exceeded 40 % in all 12 pigs, being 90 % or more in four of them (Fig. 2).

## DISCUSSION

The administration of oxytetracycline to four pigs was followed by a rapid and progressive increase in the incidence of TcR among *Esch. coli* present in their faeces. This increase was not associated with the selection of a few clones of *Esch. coli* in the intestinal flora but rather the appearance of a relatively large number of resistant strains; from 21 to 28 strains were identified per pig over 5 days (Table 1). A proportion of these TcR strains were demonstrated in the minority *Esch. coli* flora before dosing by the use of selective isolation medium containing Tc, while the majority were only isolated after dosing commenced.

The prevalence of TcR also increased after weaning in both the control 'undosed' pig (T 3) and in the majority of other 'untreated' pigs from litters F, H and S. This apparently paradoxical observation is of interest, since it demonstrates that the incidence of drug resistance among bacterial populations can change dramatically in the absence of any direct antibiotic selection pressure. It is possible that these changes were a reflexion of the distribution of sensitive and resistant strains within this group of animals. However, in about half of the weaners from litters F, H and S the increase in incidence of TcR was associated with the dominance of a single enterotoxigenic serotype (0149:K 91, K 88a, c) which was tetracycline-resistant. Experimental studies have suggested that young pigs probably become infected with small numbers of this enteropathogen during the suckling period (Miller *et al.* 1984). It then remained undetectable in the faecal flora of most pigs until after weaning when it proliferated to dominate the majority intestinal *Esch. coli* flora. The reason why this occurred has yet to be fully explained (Newby *et al.* 1984).

On the other hand, in the 'undosed' control pig (T 3), the increased incidence of TcR *Esch. coli* isolates was not associated with the dominance of a single clone since 11 TcR strains were isolated after weaning (Table 1). However, this animal was moved from the sow after weaning and it is possible that these resistant strains were derived from the environment into which the animal was placed, since a similar phenomenon has been demonstrated in young calves reared as dairy cow replacements (Hinton, Linton & Hedges, 1985). In those animals the incidence of resistance among the faecal *Esch. coli* flora, as measured by an Antibiotic Resistance Index (Hinton & Linton, 1983), increased following the removal of the animals from the calving boxes to the nursery pens. The source of these resistant strains was shown in later experiments to be the environment (unpublished observations). It was assumed that drug resistance among the *Esch. coli* strains was selected for by the therapeutic use of antibacterial drugs during the rearing of several generations of calves in the same building. It is probable that this was also the explanation for the relatively high incidence for TcR among *Esch. coli* strains present on this occasion, particularly since Langlois *et al.* (1983) found that tetracycline resistance persisted in coliforms for over 10 years after the complete cessation of antibiotic use for both therapy and growth promotion in a pig-rearing unit.

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