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Further observations on the effect of feeding diets containing avoparcin on the excretion of salmonellas by experimentally infected chickens

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SUMMARY

Chickens which had been inoculated orally with a nalidixic acid-resistant strain of *Salmonella typhimurium* were reared on a diet containing different concentrations of avoparcin in an attempt to explain the variation in response to commercial levels of this antibiotic oberved by different workers. In one experiment small increases in faecal excretion of the inoculated salmonella occurred at 2·5 and 5·0 mg kg^{-1}, with greater increases between 7·5 and 20 mg kg^{-1}. In a second experiment there was a significant increase in excretion at 7·5 mg kg^{-1} and in a third experiment in which generally higher excretion rates were detected in all groups, significant increases were observed at 10 and 12·5 mg kg^{-1} only. In addition avoparcin significantly increased the faecal excretion of *S. cholerae-suis*, *S. dublin* and *S. arizonae*, serotypes not usually associated with poultry-derived food-poisoning in the United Kingdom. It did not increase faecal excretion of *S. pullorum*. Avoparcin at 10 mg kg^{-1} appeared to have little effect on the normal intestinal flora of *S. typhimurium* infected chickens despite the fact that when tested *in vitro* individual organisms were susceptible to this drug concentration. At 100 mg kg^{-1} viable counts of intestinal enterococci and Gram-negative anaerobic bacteria were considerably reduced while those of *S. typhimurium* and *Escherichia coli* increased. Antibiotic activity due to avoparcin was detectable in the alimentary tract and there was some increase in concentration of the antibiotic in the more distal regions.

INTRODUCTION

Smith & Tucker (1978, 1980) found that feeding diets containing the growth promoting antibiotic avoparcin to *Salmonella typhimurium* infected chickens increased the faecal excretion of this serotype. Salmonella organisms were excreted by avoparcin treated chickens in larger numbers and for longer periods than they were by birds fed an antibiotic free diet. Similar results were found under a variety of other conditions, such as using different breeds of chicken, different salmonella serotypes, different feeds and different rearing conditions. The work was extended to show that chickens infected by feeding unsterilized bonemeal in the diet or by providing drinking water containing a suspension of

salmonella-infected chicken faeces also showed increased faecal excretion of salmonella organisms when reared on diets containing avoparcin (Barrow, Smith & Tucker, 1984).

The commerically recommended range of concentrations of avoparcin for broiler chickens is 7·5–15 mg kg^{-1}. Smith & Tucker (1978, 1980) found that incorporation in the diet at 10 mg kg^{-1} increased faecal excretion of *S. typhimurium* in five out of seven replicated experiments while 100 mg kg^{-1} always did so. Using fewer replicates Mathes, Leuchtenberger & Loliger (1981) showed that 10 mg kg^{-1} produced an increase in faecal salmonella excretion by chickens whereas Hinton, Al-Chalaby & Linton (1986) suggested that it did not. In an unreplicated experiment Smith & Green (1980) found that 20 mg kg^{-1} did not increase excretion while Gustafson, Beck & Kobland (1982) found that 10 mg kg^{-1} promoted colonization of the chicken gut in one of three experiments in which chickens were either infected orally or via the drinking water by broth cultures of a strain of *S. typhimurium*.

The variable response to avoparcin at 10 mg kg^{-1} was thought by Smith & Tucker (1978) to be due to variations in the composition of the commensal microbial flora of the alimentary tract of different groups of chickens such that some chickens or groups of chickens may possess a flora which is more susceptible than that possessed by others. In this paper the results of experiments are reported in which attempts were made to identify the lowest concentrations of avoparcin which would increase faecal excretion. Additional experiments are also described on the effects of avoparcin on the faecal excretion of other serotypes not usually associated with human food-poisoning in the United Kingdom and on changes in the microflora of the alimentary tract of *S. typhimurium* infected chickens induced by different concentrations of avoparcin.

MATERIALS AND METHODS

Chickens

Specific pathogen-free Light Sussex chickens were used from a flock maintained at this Institute. Their management and diet have been described previously (Smith & Tucker, 1975). All chickens were kept on wire floors.

Salmonella strains

Spontaneous nalidixic acid-resistant (Nal^r) mutants of *S. typhimurium* phage type 14 (Smith & Tucker, 1975; 1978; 1980; Impey, Mead & George, 1983; Barrow & Tucker, 1986; Barrow, Tucker & Simpson, 1987) and of *S. pullorum* strain 3, *S. cholerae-suis* strain 195, *S. dublin* strain 188 and *S. arizonae* strain 1100 were used. The parent strains were isolated from cases of systemic salmonellosis in chickens, pigs, cattle and turkeys respectively. All strains were maintained on Dorset's egg slopes at this Institute. Broth cultures were made in 10 ml volumes of nutrient broth (Oxoid CM 67) incubated for 24 h in a shaking water bath (100 strokes min^{-1}). They contained approximately 10^9 c.f.u. ml^{-1}.

Estimation of the effect of avoparcin on faecal excretion of salmonella strains

The methods of oral inoculation and for estimating the concentration of salmonella organisms in the faeces of experimentally infected chickens were those described by Smith & Tucker (1975). Groups of chickens were infected when they were 4 days old. Avoparcin was incorporated in the diet from 1 day of age. Differences in the excretion rate (calculated from number of chickens excreting salmonella organisms isolated by selenite enrichment and direct culture) at each time were analysed by Dr D. E. Walters Cambridge Research Station (IAPGR), Babraham Hall, Cambridge using Fisher's Exact Test for a contingency table. Comparisons in excretion rate were made between antibiotic-free control groups and those fed different concentrations of avoparcin.

Estimation of changes in the composition of the alimentary bacterial flora

A group of 15 chickens was reared on an antibiotic free diet for 3 weeks after hatching and was then divided into three groups of five chickens. In two of the groups the diet was changed to one containing avoparcin at 10 mg kg^{-1} or 100 mg kg^{-1} respectively. The third group was maintained on antibiotic free diet. Chickens were infected orally with *S. typhimurium* F98 Nal^r on the same day and after 5 days the birds were killed. A relatively short period of time for feeding avoparcin-containing diet was used to try to prevent the development of any resistance to the antibiotic. After killing samples were taken of the contents from the various parts of the alimentary tract.

The bacterial content of the samples was estimated using the method of Barrow, Fuller & Newport (1977) with some alterations. Coliform bacteria were counted on MacConkey agar (Oxoid CM7) since haemolytic organisms were unlikely to be encountered, *S. typhimurium* organisms were counted on Brilliant Green agar (Oxoid CM263) containing 20 μg ml^{-1} sodium nalidixate and 1 μg ml^{-1} novobiocin and obligate anaerobes were counted on the nutrient agar-blood-neomycin medium of Smith & Crabb (1961).

In vitro *sensitivity of bacterial strains to avoparcin*

Bacterial strains obtained from unmedicated chickens fed antibiotic-free feed were tested in the following manner. Minimum inhibitory concentrations (MIC) were estimated by incorporation of graded concentrations of avoparcin (Avotan 50, Cyanamid of Great Britain Ltd) into appropriate solid media: antibiotic sensitivity agar (Oxoid CM471) for coliforms, mitis-salivarius agar (Oxoid CM157) for streptococci, MRS agar (Oxoid CM361) for lactobacilli and the reinforced clostridial medium containing blood used by Barrow, Fuller & Newport (1977) for other anaerobes.

Detection of inhibiting activity due to avoparcin in the alimentary tract

The method used was essentially that of Smith (1970). Samples of gut contents were removed, moistened with water and heated in a water bath at 58 °C for 30 min. These and similarly treated food samples were spotted on to a lawn of a *Bacillus* sp. maintained in the Institute. After 24 h incubation at 37 °C the size of any zones of inhibition was measured.

Table 1. *Faecal excretion of* Salmonella typhimurium *by groups of 28 chickens fed diets containing avoparcin at different concentrations*

Days* after infection	% of chickens whose faeces contained the following concentrations of *S. typhimurium* F98 Nal^r when fed diets containing avoparcin at (mg kg^{-1})																								Lowest concentration of avoparcin where excretion rate is significantly greater than that in control group (P)	
	0			2·5			5			7·5			10			15			20			40				
	> 50	D	T†	> 50	D	T	> 50	D	T	> 50	D	T	> 50	D	T	> 50	D	T	> 50	D	T	> 50	D	T		
1	21	61	86	32	64	82	32	82	100	46	79	88	50	86	100	71	93	100	79	89	100	93	100	100		
7	11	25	64	7	25	57	14	39	68	18	46	89	11	43	71	14	46	86	71	89	100	79	100	100	7·5	(< 0·01)
14	18	18	29	18	29	64	18	22	64	25	50	89	32	39	78	18	32	79	39	93	96	21	86	100	2·5	(< 0·01)
21	4	4	18	11	14	29	11	11	36	21	25	46	14	14	50	21	36	57	18	61	96	29	86	100	7·5	(< 0·01)
28	0	0	7	0	7	14	0	4	29	0	11	21	0	7	25	7	14	39	14	46	93	32	71	100	5	(< 0·01)
35	0	4	4	0	7	11	0	0	14	0	0	7	0	0	14	0	0	4	11	32	82	32	82	100	20	(< 0·01)
42	0	0	4	0	4	4	0	0	11	0	4	7	0	0	7	4	21	32	25	54	93	43	86	100	15	(< 0·01)
49	0	0	0	0	0	0	0	0	4	0	0	0	0	0	4	4	4	11	14	32	89	32	64	96	20	(< 0·01)
49 (Caeca)‡	0	0	0	0	0	4	0	0	4	0	0	0	4	4	4	25	36	46	100	100	100	100	100	100	15	(< 0·01)

* Chickens infected when 4 days old; antibiotic-containing food was fed from day 1.

† > 50 = greater than 50 salmonella organisms isolated on culture plate; D = salmonella organisms isolated by direct culture; T = salmonella organisms isolated by selenite enrichment or direct culture.

‡ Results of examining caecal contents when the chickens were killed at the end of the experiment

Table 2. *Faecal excretion of* Salmonella typhimurium *by groups of 28 chickens fed diets containing avoparcin at different concentrations*

Days after infection	% of chickens whose faeces contained the following concentrations of *S. typhimurium* F98 Nal^r when fed diets containing avoparcin at (mg/kg)																		Lowest concentration of avoparcin where excretion rate is significantly greater than that in control group (P)
	0			5			7·5			10			12·5			15			
	> 50	D	T	> 50	D	T	> 50	D	T	> 50	D	T	> 50	D	T	> 50	D	T	
7	37	87	97	40	87	100	73	87	100	60	97	100	67	90	100	63	93	100	—*
14	37	90	100	27	80	100	50	97	100	37	80	100	57	93	100	63	90	100	—
21	3	50	90	13	47	97	50	87	100	13	77	97	40	87	97	50	87	97	—
28	17	23	63	7	20	63	10	57	100	27	63	87	21	73	100	20	57	100	7·5 (< 0·01)
35	3	23	40	0	10	47	10	60	97	20	50	93	20	63	97	7	53	93	7·5 (< 0·01)
42	0	3	27	0	3	17	0	47	90	13	37	70	40	67	87	30	63	97	7·5 (< 0·01)
49	0	6	27	0	3	20	0	13	40	17	40	77	13	57	70	20	53	70	10·0 (< 0·01)
49 (Caeca)	6	20	40	6	6	10	50	90	97	80	90	93	73	100	100	100	100	100	7·5 (< 0·01)

* No statistically significant difference in excretion rate between chickens fed diet containing no avoparcin and diet containing 15 mg kg^{-1}.
For other details see Table 1.

Table 3. *Faecal excretion of* Salmonella typhimurium *by groups of 25 chickens fed diets containing avoparcin at different concentrations*

Days after infection	% of chickens whose faeces contained the following concentrations of *S. typhimurium* F98 Nal^r when fed diets containing avoparcin at (mg/kg)																		Lowest concentration of avoparcin where excretion rate is significantly greater than that in control group (*P*)
	0			5			7·5			10			12·5			15			
	> 50	D	T	> 50	D	T	> 50	D	T	> 50	D	T	> 50	D	T	> 50	D	T	
7	64	92	100	76	100	100	76	96	100	92	100	100	84	100	100	84	100	100	—
14	48	84	96	72	88	100	56	80	100	68	88	100	72	96	100	76	92	100	—
21	40	80	100	56	88	96	36	68	96	44	92	100	60	88	96	79	100	100	—
28	28	60	88	20	60	92	24	60	88	20	48	88	60	96	100	42	92	100	—
35	40	68	88	36	64	96	24	56	88	12	60	96	28	68	100	40	84	100	—
42	4	20	72	12	24	68	12	24	76	16	56	88	20	72	96	33	54	100	12·5 (< 0·01)
49	4	20	56	4	16	28	4	16	40	16	52	88	28	68	88	38	79	96	10·0 (< 0·01)
49 (Caeca)	4	24	60	28	48	52	40	76	84	84	92	92	96	100	100	100	100	100	10·0 (< 0·01)

For details see Tables 1 and 2.

RESULTS

The faecal excretion of salmonella typhimurium *by chickens fed diets containing different concentrations of avoparcin*

The effect on the faecal excretion of *S. typhimurium* F98 Nal^r of feeding diets containing different concentrations of avoparcin to groups of 25 or 28 orally inoculated chickens are shown in Tables 1, 2 and 3.

In the first experiment (Table 1) the excretion rates were initially high in all groups and gradually decreased with time, the reduction being greatest in the avoparcin-free group. Very small increases in faecal excretion were seen in chickens fed avoparcin at 2·5 mg kg^{-1} but those increases were statistically significant ($P < 0{\cdot}01$) only at day 14 post-infection (p.i.). Higher excretion rates were seen in chickens fed avoparcin at 5·0, 7·5 and 10·0 mg kg^{-1}, these differences being most significant ($P < 0{\cdot}01$) between 7 and 28 days. The excretion rate increased with higher concentrations, statistically significant increases occurring between the concentrations of 10 and 15 mg kg^{-1} and between 15 and 20 mg kg^{-1} avoparcin.

In subsequent experiments the range of concentrations of avoparcin was reduced to between 5 and 15 mg kg^{-1} since most of the increases in the first experiment had taken place between these two levels. In the second experiment (Table 2) no significant increases in the excretion rate were induced by the 5 mg kg^{-1} concentration. Statistically significant increases in excretion were observed in the group fed avoparcin at 7·5 mg kg^{-1} between 28 and 42 days p.i. and were also found in the chicken caeca at slaughter. The increase produced at 49 days by this concentration was not statistically significant but that produced by 10 mg kg^{-1} at this time was. Additional increases in the concentration of avoparcin did not appear to induce further increases in faecal excretion. In the third experiment (Table 3) a high excretion rate was observed in all groups throughout the experiment which made comparisons difficult. However, it was apparent that consistent small increases in faecal excretion were produced in the later stages of the experiment by 10 mg kg^{-1} (day 49 and in the caeca at slaughter) and by 12·5 mg kg^{-1} (day 42). Avoparcin at 5 and 7·5 mg kg^{-1} produced no detectable increase in faecal excretion in this experiment.

The effect of avoparcin on the faecal excretion of salmonella serotypes not usually associated with food poisoning in the United Kingdom

The effects of feeding diets containing avoparcin at 10 and 100 mg kg^{-1} on the faecal excretion of some salmonella serotypes which are not usually associated with human food-poisoning in the United Kingdom are shown in Table 4.

In the experiment involving the *S. cholarae-suis* strain sodium selenite enrichment was not used because Smith (1952) showed that this was an unsuitable medium for the growth of this serotype. As found by Smith & Tucker (1980) *S. cholerae-suis* was only excreted in the faeces of avoparcin-free chickens for a short period. This was also the case for chickens fed a diet containing 10 mg kg^{-1} avoparcin. At the higher concentration, however, a much higher excretion rate was seen and *S. cholerae-suis* was shed in the faeces throughout the experiment and was cultured from the caecal contents at its termination. This culture was used to

Table 4. *Faecal excretion of* Salmonella cholerae-suis, S. dublin, S. arizonae, *and* S. pullorum *by groups of 20 chickens fed diets containing avoparcin at different concentrations*

Strain	Avoparcin conc. (mg kg^{-1})	% of chickens whose faeces contained salmonella organisms at the following days after infection: 1			7			14			21			28			35			42			49			49 (caeca)		
		> 50	D	T	> 50	D	T	> 50	D	T	> 50	D	T	> 50	D	T	> 50	D	T	> 50	D	T	> 50	D	T	> 50	D	T
S. cholerae-suis 195 Nal^r	0	0	15	...*	0	0	...	0	5	...	0	0	...	0	0	...	0	0	...	0	0	...	0	0	...	0	0	...
	10	0	3	...	0	0	...	0	0	...	0	0	...	0	0	...	0	0	...	0	0	...	0	0	...	0	0	...
	100	10	25	...	50	100	...	50	80	...	45	70	...	25	50	...	25	75	...	30	30	...	10	20	...	55	65	...
	P†	100	(< 0·01)		100	(< 0·01)		100	(< 0·01)		100	(< 0·01)		100	(< 0·01)		100	(< 0·01)		100	(< 0·01)		100	(< 0·01)		100	(< 0·01)	
S. dublin 188 Nal^r	0	5	20	65	0	35	37	0	15	37	0	10	25	0	0	15	0	0	5	0	0	5	0	0	0	0	0	0
	10	0	45	85	20	60	89	15	45	85	0	30	60	0	5	30	0	0	15	5	5	5	0	0	15	0	5	40
	100	85	100	100	60	100	100	40	65	100	30	60	90	60	65	90	40	50	70	15	30	65	0	10	20	10	15	25
	P	100	(< 0·05)		10	(< 0·01)		10	(< 0·01)		10	(< 0·05)		100	(< 0·01)		100	(< 0·01)		100	(< 0·01)		100	(< 0·01)		10	(< 0·01)	
S. arizonae 1100 Nal^r	0	5	5	42	10	10	26	0	11	26	0	11	16	0	0	16	0	5	11	5	5	5	0	0	0	0	0	5
	10	0	10	55	5	10	47	0	21	32	5	21	42	11	21	26	21	53	79	11	11	42	0	5	5	5	5	5
	100	40	85	100	65	90	100	60	85	100	50	85	89	60	85	100	55	85	95	45	80	100	60	85	100	100	100	100
	P	100	(< 0·01)		100	(< 0·01)		100	(< 0·01)		100	(< 0·01)		100	(< 0·01)		10	(< 0·01)		10	(< 0·01)		100	(< 0·01)		100	(< 0·01)	
S. pullorum 3 Nal^r	0	0	0	22	0	6	17	0	6	24	0	0	12	0	0	0	0	0	6	§						0	0	6
	10	0	0	10	0	15	30	0	15	40	0	0	15	0	0	0	0	0	0							0	0	0
	100	0	0	35	10	15	25	5	25	40	10	10	15	5	5	10	5	5	5							5	5	5
	P		—‡			—			—			—			—			—								—	

* ..., *S. cholerae-suis* not cultured in selenite broth.

† Lowest concentration of avoparcin producing an excretion rate significantly greater than that observed in control group. *P* value in parentheses.

‡ —, No statistically significant difference between excretion rates in any of the groups.

§ –, Chickens killed and caeca cultured at 42 days.

For other details see Table 1.

inoculate a second group of unmedicated chickens fed avoparcin-free diet. The excretion rate in this group of chickens was again very low and of short duration indicating that the presence of the antibiotic had not selected for variants which were better able to colonize the alimentary tract. The experiment was repeated with very similar results. The one difference was that one chicken fed avoparcin-free diet excreted large numbers of *S. cholerae-suis* throughout the experiment. The organism excreted was rough, as shown by its agglutination by acriflavine (Smith, 1965). When this chicken was killed at the end of the experiment profuse growth was obtained by culture of the contents of the caeca, small intestine and gall bladder.

The *S. dublin* and *S. arizonae* strains were each excreted by unmedicated chickens for several weeks. The incorporation of avoparcin in the diet at both concentrations increased faecal excretion. Excretion persisted for longer in the 10 mg kg^{-1} group than in the control group and as a consequence the excretion rate was significantly higher, in the case of *S. dublin* at 7–21 days and in the caeca at slaughter and in the case of *S. arizonae* at 35 and 42 days. *S. pullorum* was excreted in low numbers by both avoparcin-fed and avoparcin-free chickens and none of the differences in faecal excretion rates increases was statistically significant.

The effect of avoparcin on the bacterial flora of the alimentary tract of the chicken

The bacterial flora of the alimentary tract of 3-week-old chickens was examined 5 days after incorporating avoparcin at 10 or 100 mg kg^{-1} in the feed and infection with *S. typhimurium* F98 Nal^r. The chickens were not examined later since any changes may have become obscured by colonization with avoparcin resistant bacterial mutants. The results are shown in Table 5.

No salmonella organisms ($\log_{10} < 2{\cdot}0$) were isolated from the avoparcin-free control chickens. Small numbers ($\log_{10}$ 2·6) were isolated from the caeca and cloaca of chickens fed the antibiotic at 10 mg kg^{-1} and much higher numbers ($\log_{10}$ 7·7) from the caeca of chickens fed the drug at 100 mg kg^{-1}. *E. coli* was found throughout the alimentary tract of avoparcin-free birds. Avoparcin at 10 mg kg^{-1} had little effect on the viable counts of *E. coli* but the higher concentration produced an increase of greater than one log in most parts of the gut with the greatest increases occurring in the caeca and cloaca. Avoparcin at 10 mg kg^{-1} produced little quantitative effect on the enterococcal counts but 100 mg kg^{-1} of the antibiotic eliminated detectable streptococci from the alimentary tract with the exception of the crop where some organisms persisted, possibly as a result of ingestion of faeces. The viable counts of lactobacilli were unaffected by either concentration of avoparcin. Viable counts of anaerobic bacteria growing on the nutrient agar–blood–neomycin medium were unaffected by 10 mg kg^{-1} avoparcin but were greatly reduced by the higher concentration. In the cases where no quantitative changes were detectable there were no obvious qualitative changes amongst the numerically dominant microorganisms.

Bacterial isolates from the alimentary tract of healthy unmedicated chickens were tested for their sensitivity to avoparcin. The MIC values of five isolates of *E. coli* were > 200 μg ml^{-1}. The MIC values of 20 isolates of enterococci were > 30 μg ml^{-1} (1 isolate), 25 μg ml^{-1} (7 isolates), 10 μg ml^{-1} (7 isolates), and 5 μg ml^{-1}

Table 5. *The bacterial content of the alimentary tract of groups of five chickens infected with* Salmonella typhimurium *F98 Nal^r and fed diets containing different concentrations of avoparcin*

Concentration of avoparcin in feed (mg kg^{-1})	Type of organism	Log_{10} numbers of viable bacteria per gram of contents* in							
				Small intestine					
		Crop	Proventriculus	Gizzard	Duodenum	Jejunum	Ileum	Caeca	Cloaca
None	Salmonella	< 2·0	< 2·0	< 2·0	< 2·0	< 2·0	< 2·0	< 2·0	< 2·0
	E. coli	4·9	2·7	2·5	2·0	4·2	5·9	7·8	5·8
	Streptococci	6·8	4·5	2·3	3·9	5·5	6·7	7·3	6·8
	Lactobacilli	7·5	7·1	3·9	5·7	6·9	6·9	8·8	4·8
	Anaerobes	5·9	3·1	< 2·0	3·2	5·2	5·9	8·7	6·0
10	Salmonella	< 2·0	< 2·0	< 2·0	< 2·0	< 2·0	< 2·0	2·6	2·3
	E. coli	4·5	5·5	3·6	3·9	5·2	6·0	7·2	5·2
	Streptococci	4·8	6·0	6·5	6·5	5·8	6·2	6·9	6·2
	Lactobacilli	5·1	7·3	5·7	5·8	5·9	6·2	7·6	5·6
	Anaerobes	2·8	4·7	4·2	4·2	4·3	5·5	8·7	4·5
100	Salmonella	< 2·0	< 2·0	< 2·0	< 2·0	< 2·0	< 2·0	7·7	< 2·0
	E. coli	5·6	5·7	4·4	5·2	6·1	7·6	9·1	7·9
	Streptococci	4·0	< 2·0	< 2·0	< 2·0	< 2·0	< 2·0	< 2·0	< 2·0
	Lactobacilli	5·6	7·9	6·3	7·3	7·0	6·6	8·0	6·8
	Anaerobes	3·3	3·2	< 2·0	2·3	2·9	3·6	3·3	< 2·0

Median count of five birds killed 5 days after infection.

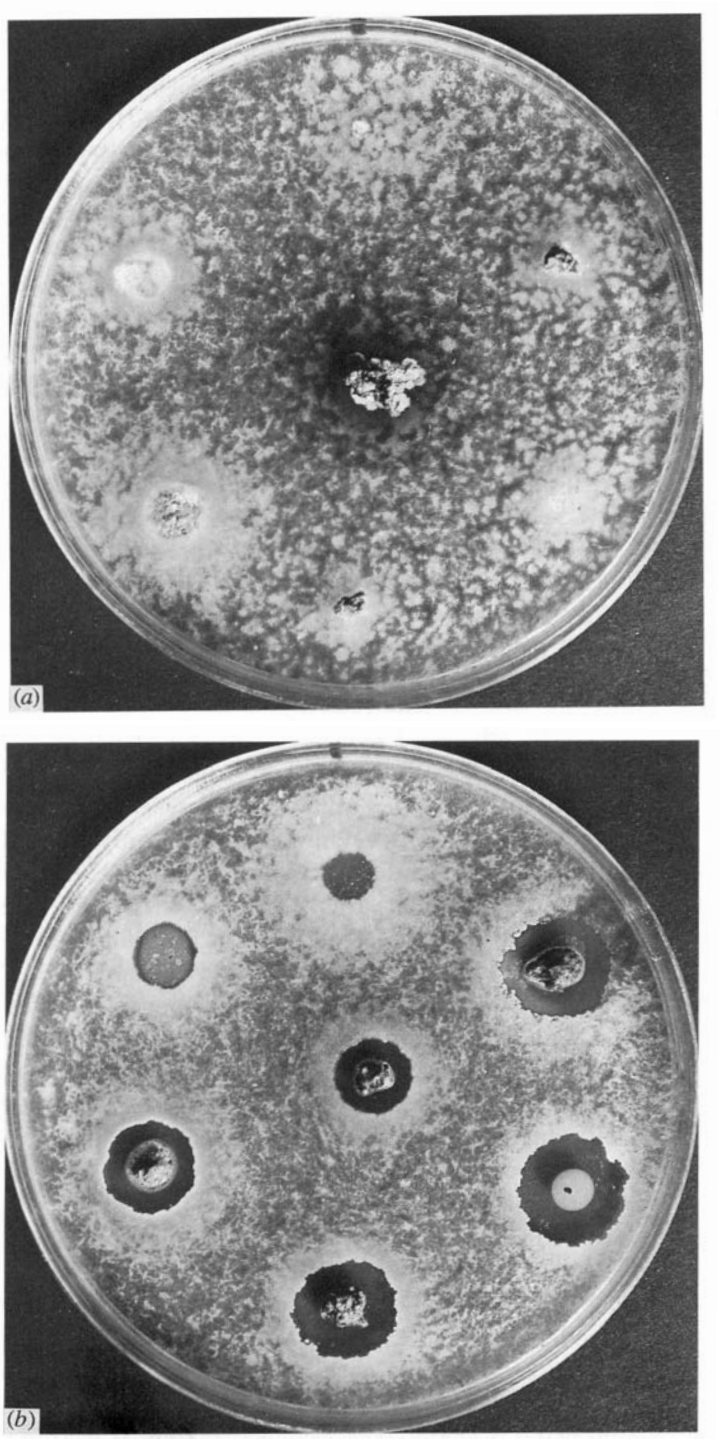

Fig. 1. *In vitro* inhibition of *Bacillus* sp. by food and intestinal contents of chickens fed avoparcin. (*a*) Chicken fed unmedicated diet. (*b*) Chicken fed diet containing avoparcin at 100 ppm. Centre sample, food; top sample, crop; then running anti-clockwise duodenum, jejunum, ileum, caecum and cloaca.

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(5 isolates). Two types of lactobacilli were found, a smooth, white colony, characteristic of *Lactobacillus fermentum* (Barrow *et al.* 1977) and a rough, grey colony, characteristic of *L. acidophilus*. The MIC values of four smooth isolates were $> 100\ \mu g\ ml^{-1}$. For 8 rough isolates the values were $10\ \mu g\ ml^{-1}$ (5 isolates) and $20\ \mu g\ ml^{-1}$ (3 isolates). Obligately anaerobic bacteria were difficult to passage using anaerobic jars. However, the MIC values of six miscellaneous isolates were $> 30\ \mu g\ ml^{-1}$. *Clostridium perfringens* was not isolated from the chickens. Five laboratory strains had MIC values of $< 10\ \mu g\ ml^{-1}$.

The zones of inhibition produced on a lawn of a *Bacillus* sp. by moistened food or intestinal contents from avoparcin-free chickens and from chickens fed 100 mg kg^{-1} avoparcin are shown in Fig. 1. No zones of inhibition were observed from testing samples from avoparcin-free chickens. In fact stimulation of growth around the intestinal contents was observed. The food containing 100 mg kg^{-1} avoparcin produced a zone of inhibition with a radius of 3 mm. Smaller zones were produced by the more liquid crop and duodenal contents. The zone sizes increased from the jejunum to the caecum so that the zones for the ileum, caeca and cloaca were larger than that produced by the food itself. Similar results were obtained for chickens fed a diet containing avoparcin at 10 mg kg^{-1} except that the zones were much smaller, 1 mm in the case of the undigested food.

DISCUSSION

Previous studies on the effects of avoparcin on the faecal excretion of food-poisoning salmonella serotypes have resulted in some confusion over the lowest concentration of the antibiotic at which an increase in faecal excretion occurs. The commercially recommended concentration most commonly chosen for experimental work (10 mg kg^{-1}) does produce increases in faecal excretion but not on every occasion (Smith & Tucker, 1978; Smith & Green, 1980; Mathes, Leuchtenberger & Loliger, 1981; Gustafson, Beck & Kobland, 1982; Hinton, Al-Chalaby & Linton, 1986). In the present paper graded concentrations of avoparcin were incorporated in the feed of chickens experimentally infected with *S. typhimurium*. In the first experiment statistically significant increases in faecal excretion of *S. typhimurium* occurred early in the experiment in chickens fed avoparcin at between 2·5 and 7·5 mg kg^{-1} and later in the experiment in the groups fed 15 and 20 mg kg^{-1}. In the second and third experiments similar significant increases occurred at between 7·5 and 10 mg kg^{-1} and 10 and 12·5 mg kg^{-1} respectively. It is clear that by using avoparcin at 10 mg kg^{-1} equivocal results could be obtained, particularly if faecal shedding was monitored on only one occasion. The lowest concentration which increases faecal excretion obviously varies but it is close to 10 mg kg^{-1}. The commercially recommended range of concentrations for broilers is 7·5 to 15 mg kg^{-1}. Because the 'cut off point' for an increase in salmonella excretion falls within this range, use of avoparcin at the highest recommended concentration would be more likely to adversely affect excretion than would using it at the lowest concentration. It is apparent from the results presented here that use at 5 mg kg^{-1} or, if this does not cause growth enhancement, at 7·5 mg kg^{-1} ought to reduce the risk of increased salmonella shedding. Reduced weight gains would be an additional consequence of using a lower concentration.

Avoparcin was also shown to increase the faecal excretion of salmonella serotypes which are not usually associated with human food-poisoning in the United Kingdom: *S. cholerae-suis* and *S. dublin*, serotypes isolated from systemic disease in pigs and cattle respectively and *S. arizonae*, an organism more frequently associated with food-poisoning in North America (Taylor & McCoy, 1969). The reason why *S. pullorum* was unaffected in this way is unclear. It is an avian pathogen which, unlike the food-poisoning serotypes, does not persist for long periods in chicken faeces following experimental oral inoculation (Smith & Tucker, 1980). It may be that its presence in the faeces is a result of a more generalized infection resulting in invasion of the alimentary tract from the tissues. Similarly, the *S. cholerae-suis* strain did not persist in the faeces unless high concentrations of avoparcin were present. The exception was one avoparcin-free chicken which excreted large numbers of a rough mutant of this organism from the gall bladder.

An interestingly corollary of the ability to isolate large numbers of salmonella organisms, including some non-enteric serotypes, from the alimentary tract of chickens fed avoparcin at 100 mg kg^{-1} is that it might be used at this concentration to assist culturing salmonella strains from feed and other substances. This research institute monitors salmonella contamination of the feed administered to its SPF flock by culturing faeces obtained from chickens fed new batches of food; rearing the chicken on avoparcin at 100 mg kg^{-1} might increase the frequency of isolation.

When avoparcin was fed at 10 mg kg^{-1} to *S. typhimurium* infected chickens no differences were observed in the alimentary microflora. At 100 mg kg^{-1} the numbers of *E. coli*, as well as *S. typhimurium*, were increased. These two organisms were relatively resistant to avoparcin *in vitro*. At 100 mg kg^{-1} the numbers of streptococci and obligate anaerobes were reduced. No quantitative or qualitative differences were observed in the numbers of lactobacilli, despite the fact that the growth of one group of lactobacilli, morphologically resembling *L. acidophilus*, was inhibited *in vitro* by 10 mg kg^{-1} avoparcin. The MIC values obtained *in vitro* for the streptococci and lactobacilli were similar to those obtained by Dutta & Devriese (1981, 1982).

The highest number of salmonella and *E. coli* organisms appeared in the caeca and corresponded with a greater concentration of avoparcin in this organ, when compared with other regions of the gut. Whether this apparent increase in concentration is the result of water absorption is unclear. It would be interesting to see whether other growth promoting antibiotics behave in a similar manner.

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