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Salmonella infection in chicks following the consumption of artificially contaminated feed

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SUMMARY

Poultry feed was contaminated artificially with either *Salmonella kedougou* or *S. livingstone* using a two-stage mixing process. Intestinal infection became established in a small proportion of birds when feed containing between 0·1 and 0·3 salmonellas/g was given continuously for 2 or 3 weeks from the day of their purchase as 'day-olds' while nearly all birds became infected when the feed contained 100–300 salmonellas/g. Between these limits a dose response was demonstrated in that the proportion of birds becoming colonized with salmonellas increased as the numbers of salmonellas added to feed was increased.

Nalidixic acid-resistant strains of both serotypes were used to facilitate the recovery of organisms. The isolation rate was higher from dilutions of caecal contents inoculated directly onto brilliant green agar supplemented with nalidixic acid than it was from swabs of cloacal faeces, even when an enrichment technique was used. This observation confirms that the incidence of salmonella carriage in flocks will be under-estimated if only cloacal faeces are cultured.

Of the two serotypes used *S. kedougou* proved the more efficient colonizer although for both serotypes variation in infection rates was demonstrated in different groups of birds given feed containing comparable numbers of salmonellas.

INTRODUCTION

Feed contaminated with salmonellas may act as a source of infection for poultry (Williams, 1981) and, although these infections are rarely associated with salmonellosis in the birds, they are of importance since people who either handle or consume salmonella-contaminated carcase meat may subsequently develop food-poisoning.

The course of feed-borne infections in poultry has been studied using 'naturally' contaminated feed although on occasions inconsistencies between the results obtained from replicated experiments have made interpretation difficult (Linton, Al-Chalaby & Hinton, 1985; Hinton, Al-Chalaby & Linton, 1986). It is possible that those variations were due to differences in the numbers of salmonellas present in the feed. There is clearly no control over this factor when 'naturally' infected ingredients are used to compound the ration. Consequently, a method for contaminating the feed artificially was developed (Hinton, 1986) with the hope

that this problem might be overcome. In these initial investigations the feed was contaminated with relatively large numbers of salmonellas ($> 10^3$/g) so that the chance of infecting the birds was maximized. This level of contamination exceeds considerably that likely to be encountered in commercially prepared feed. This paper describes a series of experiments in which feed containing between 0·1 and 300 salmonellas/g was given *ad libitum* to young chickens so that the minimum number required to initiate infection could be determined. In addition, because culture of cloacal faeces underestimates the incidence of intestinal carriage with salmonellas (Snoyenbos *et al.* 1969), the relation between the results of examining both cloacal faeces and caecal contents was explored in detail.

MATERIALS AND METHODS

Birds and their management

A total of 936 birds were purchased as day-olds from a commercial hatchery in batches of 20–160 on 17 occasions between December 1985 and November 1986 (Table 1). They were kept in groups of 6, 10 or 13 in either fibre-glass tubs or cardboard boxes. Wood shavings were provided as bedding material and commercially-prepared chick starter feed and water were available *ad libitum* (Hinton, 1986).

Inoculation of the food

This involved a two-stage mixing process (Hinton, 1986). Briefly decimal dilutions of overnight broth cultures of nalidixic acid-resistant strains of either *Salmonella kedougou* or *S. livingstone* were mixed initially with desiccated coconut (1:100 v/w) with the contaminated coconut then being added to the feed (1:50 w/w). The dilutions of broth used for the two serotypes were 10^{-3}–10^{-7} and 10^{-3}–10^{-6} respectively and the contaminated diets prepared with them designated K3–K7 and L3–L6 (Table 1). In all 5 ml of diluted culture was mixed into 25·5 kg food with coconut. It was not possible to determine the actual numbers of salmonellas in the feed precisely since the numbers were low. However, the estimated numbers/g were 10^2 (range 100–299), 10^1 (10–29), 10^0 (1–2·9) and 10^{-1}(0·1–0·29) for diets K3 and L3, K4 and L4, K5 and L5 and K6 and L6 respectively, and 10^{-2} (0·01–0·03) c.f.u./g for diet K7.

Bacteriological investigations

All batches of chicks were screened for the absence of salmonellas by either examining cloacal swabs collected from each bird on the day of arrival (expts 1–11, Table 1) or the paper liners from the boxes used to transport the chicks from the hatchery (expts 12–17).

Swabs of cloacal faeces were collected at the end of the first, second or third week of the rearing period, excepting experiment 1 when swabs were only obtained in the third week. Each swab was used to inoculate $\frac{1}{4}$ of the surface of a brilliant green agar plate (LabM 34) supplemented with 30 μg/ml nalidixic acid (BGNA). The swabs collected during the third week were placed into selenite F broth (LabM 44a) after inoculation of the BGNA plate and this was subcultured onto BGNA after overnight incubation at 43 °C in air.

Table 1. *Summary of the protocols for 17 experiments in which feed contaminated with either* Salmonella kedougou *or* S. livingstone *was fed to young chicks*

Exp no.	Serotype*	No. of birds purchased	No. of birds per box	Salmonella counts ($\times 10^8$) in the broths used to contaminate the feed	Diet code†	Age when examined (weeks)
1	K	60	10	7·4	K3, K4, K5	3
	L	60	10	5·1	L3, L4, L5	3
2	L	60	10	9·9	L3, L4, L5	3
3	K	48	6	7·2	K5, K6	2, 3
4	K	60	10	14·6	K5, K6, K7	3
5	K	36	6	12·5	K4, K5, K6	2, 3
	L	18	6	9·5	L4, L5, L6	3
6	K	20	10	8·5	K4, K5	3
7	K	40	10	9·2	K4, K5	3
8	K	60	10	8·6	K4, K5, K6	2, 3
	L	30	10	5·2	L4, L5, L6	3
9	K	160	10	11·9	K4, K5	3
10	K	104	13	7·5	K4, K5	3
11	K	60	10	8·2	K4	2, 3
12	K	20	10	8·8	K3, K4	2
13	K	20	10	11·5	K3, K4	2
14	K	20	10	5·9	K4, K5	2, 3
15	K	20	10	9·9	K3, K4	2
16	K	20	10	14·8	K4, K5	2, 3
17	K	20	10	11·4	K4, K5	2, 3

* K, *S. kedougou*; L, *S. livingstone*.

† Diets with codes 3, 4, 5, 6 and 7 had 10^2, 10^1, 10^0, 10^{-1} and 10^{-2} salmonellas/g respectively (see text for details).

The birds were killed humanely when either 2 or 3 weeks of age (Table 1) and the caecal contents collected aseptically for the determination of the number of salmonella organisms/g using BGNA as the plating medium. In order to obtain an estimate of low numbers of salmonellas 0·02 ml of the first dilution (10^{-1}) of caecal contents, which was made in selenite F broth, was spread onto the surface of each of two BGNA agar plates. A single colony growing on one of these was equivalent to 25 c.f.u./g. If all 'count' plates were negative the selenite F broth, which had been incubated overnight at 43 °C in air, was subcultured onto BGNA agar. If salmonellas were isolated the count was recorded arbitrarily at 10 c.f.u./g. All negative cultures were scored as 1 c.f.u./g to allow log transformation of the values.

The identity of representative isolates of each serotype was checked from time to time during each experiment using the slide agglutination test.

RESULTS

No salmonellas were recovered from the day-old birds and, apart from 10 (1·1 %) which died during the first 10 days of the rearing period, all birds remained healthy.

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Table 2. *The establishment of* Salmonella kedougou *infection in groups of 10 and 13 birds*

Serotype added to feed	No. of salmonellas/g feed*	No. of groups	The number of groups of birds in which the proportion (%) of infected birds was†					Proportion (%) of groups with positive birds	No. of 'positive' groups missed if only caecal swabs examined at 2 weeks
			0	10–39	40–69	70–99	100		
S. kedougou‡	10^2	3	0	0	0	0	3	100	0
	10^1	29	2	3	2	6	16	93	2
	10^0	22	6	3	4	3	6	73	4
	10^{-1}	4	3	0	0	1	0	25	0
	10^{-2}	2	2	0	0	0	0	—	0

* See text. 10^2–10^{-2} correspond to diet codes K3–K7 respectively.
† > 10 salmonellas/g of caecal contents at 2 or 3 weeks of age.
‡ Experiments 4 and 6–17 (Table 1).

Table 3. *The isolation of* Salmonella kedougou *and* S. livingstone *from swabs of cloacal faeces used to inoculate directly brilliant green phenol red agar supplemented with 30 μg/ml nalidixic acid*

Serotype added to feed	No. of birds sampled*	Age (weeks)	Number of salmonellas/g of feed†				
			10^2	10^1	10^0	10^{-1}	10^{-2}
S. kedougou‡	699	1	28/29 (97)	172/309 (56)	43/266 (16)	0/75	0/20
	699	2	27/29 (93)	195/309 (63)	68/266 (26)	2/75 (3)	0/20
	509	3	—	136/219 (62)	63/223 (28)	1/47 (2)	0/20
S. livingstone§	108	1	8/20 (40)	11/36 (31)	7/36 (19)	0/16	—
	108	2	9/20 (45)	12/36 (33)	11/36 (31)	1/16 (6)	—
	108	3	7/20 (35)	11/36 (31)	8/36 (22)	1/16 (6)	—

Numerator, no. of birds positive; denominator, no. of birds examined. Proportion positive (%) in parentheses.

* Experiment 1 omitted as cloacal swabs only collected when the birds were 3 weeks old.

† See text. 10^2–10^{-2} correspond to diet codes K3–K7 and 10^2–10^{-1} to L3–L6 respectively.

‡ Experiments 3–17 (Table 1). § Experiments 2, 5 and 8 (Table 1).

Isolation of salmonellas from groups of birds

The number of groups of 10–13 birds in which *S. kedougou* infections established was determined (Table 2). Twenty-nine groups were given feed containing 10^1 *S. kedougou*/g (diet code K4) and infection became established in 27 (93%) although in four groups less than half the birds were infected.

When the number of *S. kedougou* in the feed was 10^0, 10^{-1} and 10^{-2}/g infection became established in progressively fewer groups (16/22, 1/4 and 0/2 respectively).

S. livingstone was given to only 24 groups and the results obtained were comparable to those obtained with *S. kedougou*. Infection was demonstrated in 4/4, 5/8 5/8 and 0/4 groups given diets L3, L4, L5 and L6 respectively.

Isolation of salmonellas from swabs of cloacal faeces

The proportion of positive birds identified decreased as fewer *S. kedougou* were added to the feed (Table 3). Nearly all the birds given the K3 diets were positive after 7 days of exposure. The infection rates increased from the first to the second week for those birds given diets K4, K5 and K6 although the increase was only significant ($\chi^2_{(1)} = 7{\cdot}1$, $P < 0{\cdot}01$) for the K5 birds.

For the birds given *S. livingstone* the results were generally similar to those obtained with *S. kedougou* excepting that at a given rate of salmonella contamination the incidence of infection was lower (Table 3).

The numbers of salmonellas in the caecal contents

As the challenge dose of *S. kedougou* in the feed increased the proportion of birds with $> 10^6$ *S. kedougou*/g of caecal contents also increased while there was a corresponding decrease in the proportion of birds which remained apparently

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Table 4. *The distribution of birds according to the number of salmonellas in their caecal contents*

Serotype added to feed	Age (weeks)	No. of salmonellas/g of feed*	No. of birds	No. of birds with caecal counts (log 10) of				Median count	Proportion positive (%)
				0	1–2	3–5	6–8		
S. kedougou†	2	10^2	29	0	0	1	28	6·98	100
		10^1	90	18	7	2	63	6·63	80
		10^0	43	30	3	10	10	0·00	30
		10^{-1}	38	34	4	0	0	0·00	11
	3	10^2	20	0	2	9	9	5·74	100
		10^{-1}	239	56	38	56	89	4·47	77
		10^0	243	116	39	61	27	1·00	52
		10^{-1}	47	42	5	0	0	0·00	11
		10^{-2}	20	20	0	0	0	0·00	—
S. livingstone‡	3	10^2	40	2	14	23	1	3·42	95
		10^1	55	26	16	12	1	1·00	53
		10^0	56	25	14	16	1	1·00	55
		10^{-1}	16	15	1	0	0	0·00	6

* See text. 10^2–10^{-1} correspond to diet codes K3–K6 and L3–L6 respectively. † Experiments 1, 3–17. ‡ Experiments 1, 2, 5 and 8.

Table 5. *Distribution of 3-week-old birds according to (1) recovery of salmonellas from their cloacal faeces and (2) the numbers of salmonellas in their caecal contents*

Serotype	No. of salmonellas/g of feed*	Isolation from cloacal faeces: Direct culture	Isolation from cloacal faeces: After enrichment	No. of birds	Salmonella count (log 10): 0	1–2	3–5	6–8	Median count	Proportion positive (%)
S. kedougou†	10^2	−	−	2	0	1	1	0	4·01	100
		−	+	4	0	1	1	2	5·60	100
		+	−	2	0	0	1	1	5·54	100
		+	+	12	0	0	6	6	6·08	100
	10^1	−	−	75	53	15	4	3	0·00	29
		−	+	23	2	12	5	4	2·30	91
		+	−	9	0	1	5	3	4·03	100
		+	+	117	1	10	40	66	6·28	99
	10^0	−	−	126	104	19	3	0	0·00	17
		−	+	21	1	8	10	2	3·63	95
		+	−	2	0	0	2	0	5·04	100
		+	+	79	0	8	46	25	5·14	100
S. livingstone‡	10^2	−	−	17	2	7	8	0	2·85	88
		−	+	11	0	5	6	0	3·16	100
		+	−	5	0	1	4	0	3·74	100
		+	+	7	0	1	5	1	3·79	100
	10^1	−	−	32	22	8	2	0	0·00	31
		−	+	7	2	4	1	0	1·00	71
		+	−	1	0	0	1	0	3·00	100
		+	+	9	0	1	8	0	3·35	100
	10^0	−	−	30	18	9	3	0	0·00	40
		−	+	11	2	3	6	0	3·28	82
		+	−	2	0	0	1	1	5·12	100
		+	+	7	0	2	5	0	3·26	100

* See text. † Experiments 1 and 3–11 (Table 1). ‡ Experiments 1, 2 and 8 (Table 1).

uninfected (Table 4). For birds fed the K3 and K4 diets respectively the median counts were higher at 2 weeks than 3 weeks. For both inclusion rates the difference between the median counts was statistically significant ($P < 0{\cdot}001$ for both using the Kruskal–Wallis H test).

The birds challenged with *S. livingstone* were killed when 3 weeks of age. The median *S. livingstone* counts/g contents for the birds given feed containing 10^1 or 10^2 *S. livingstone*/g were significantly lower ($P < 0{\cdot}001$ for both) than the comparable median count for birds given feed containing *S. kedougou* (Table 4).

Isolation of salmonellas from cloacal faeces and caecal contents

In expts 1 and 3–11 the cloacal faeces of birds aged 3 weeks were cultured for *S. kedougou* and the numbers of salmonellas in their caecal contents determined. The data for birds given diets K3, K4 and K5 are summarized in Table 5.

Nearly a quarter (23%) of the 203 given these diets had negative cloacal faeces after both direct culture and enrichment but had countable numbers (> 25/g) *S. kedougou* in the caecal contents. On the other hand only 1 (0·5%) of the 221 birds whose cloacal faeces were positive on direct culture had no salmonellas in the caecal contents.

Over 200 birds were given both the K4 and K5 diets and in both categories the median salmonella counts in the caecal contents were higher in birds with positive cloacal faeces on direct culture than those whose faeces were negative for *S. kedougou* (Table 5).

The results obtained for *S. livingstone* were broadly similar to those of *S. kedougou* and confirm that this serotype was a less efficient colonizer of the intestinal tract than *S. kedougou*.

DISCUSSION

The artificial contamination of feed requires the dispersal of small volumes of fluid containing the organisms in relatively large volumes of dry feed. Cox, Bailey and Thomson (1982) and Schleifer *et al.* (1984) achieved this by using a 'V'-shaped twin-shelled mixer, while for the experiments summarized in this paper a two-stage mixing process, involving desiccated coconut as a premix, was used (Hinton, 1986).

It is possible to initiate infection in a proportion of young chicks when they are given feed containing small numbers (< 1/g) of salmonellas (Schleifer *et al.* 1984 and these studies) while a dose response was demonstrated when feed containing between 10^0 and 10^2 *S. kedougou*/g was fed during the first 3 weeks after hatching. As the dose increased so did the proportion of (1) groups with infected birds (Table 2) and (2) birds with *S. kedougou* in their cloacal faeces (Table 3) and caecal contents (Table 4). The median number of salmonellas in the caecal contents also increased with increasing numbers of salmonellas in the feed although the maximum count never exceeded 10^8/g contents at any dose (Table 4).

The intestinal tract of the chicken is sterile at hatching and the complex microflora of the caeca, where the 'food-poisoning' salmonellas tend to localize, takes several weeks to develop (Barnes *et al.* 1972). The young bird is clearly very susceptible to colonization with salmonellas. However, it is not clear from the available data whether the dose response recorded was principally a reflection of

the numbers of salmonellas in the feed, or evidence of the fact that, within limits, even the 'rudimentary' intestinal flora of the young chick has some inhibitory influence on colonization, or a combination of both factors.

Culture of the cloacal faeces for salmonellas is a simple and easily repeated method of detecting carriers although, as this study confirms, it under-estimates the proportion of infected birds. For instance, of the birds given feed containing between 10^0 and 10^2 *S. kedougou*/g for 3 weeks 203 were classed as negative following culture of the cloacal faeces using enrichment (Table 5). However, 46 (23%) of these birds had detectable salmonellas in their caecal contents with the numbers exceeding 10^3/g in 11 (5·4%).

The artificial contamination of feed did not eliminate variability in infection rates in groups of birds, particularly those given feed containing between 1 and 30 organisms/g (Tables 2 and 4). This suggests that factors other than the dose determine whether salmonellas colonize the intestinal tract of young chicks. These factors probably reflect differences between chicks, or groups of chicks, since salmonellas readily survive for 6 months or more in feed stored at ambient temperatures (Hinton, 1986). Certainly the intestinal flora of newly hatched chicks varies in terms of both the number of organisms and the complexity of the flora (Mead & Impey, 1986; Hinton, Lim & Linton, 1987). Secondly the age of the bird at the time of challenge may be relevant since newly hatched chicks may be up to 2 days of age when they leave the hatchery and it is recognized that they become less susceptible to infection during the first few days of life (Milner & Shaffer, 1952; Sadler, Brownell & Fanelli, 1969; Impey, Mead & Hinton, 1987).

The method of exposure employed in these experiments simulates that likely to occur under commercial husbandry conditions and the technique can be used easily to study critically a number of factors which may influence feed borne infections. These include (1) feed disinfection procedures and other techniques available for reducing the number of organisms in the feed, (2) the influence of growth promoting antibiotics, probiotics and other feed additives on salmonella carriage, (3) the phenotypic characteristics (e.g. virulence factors) which influence the colonization of the intestinal tract and the development of immunity, and (4) competitive exclusion as a means of salmonella control (Impey *et al.* 1987). However, in view of the fact that there are (1) differences in the infectivity of different serotypes and (2) variations in the infection rate in different groups exposed to comparable numbers of the same serotype it is essential to repeat experiments involving exposure via the feed several times so that sufficient data are accumulated to allow adequate statistical evaluation of the results obtained.

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