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Prevention of *Salmonella* Infection in Chicks by Treatment with Fecal Cultures from Mature Chickens (Nurmi Cultures)

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ABSTRACT

Chicks (*Gallus domesticus*) were treated per os with 24-h-old anaerobic cultures of feces from mature chickens 1 day after hatching, challenged with *Salmonella typhimurium* in the drinking water 2 days later, and sacrificed on day 11 or 12; then the lower third of the intestinal tract was examined for salmonellae. Cultures of feces inoculated directly into the crop or added to the drinking water, even after holding at -70 C for 21 days, protected chicks against infection by *S. typhimurium*. Cultures serially subcultured daily up to four times were protective, and dilution to 1:80 in drinking water containing 4% skim milk powder did not decrease their protective effect. Treated chicks were about 1000-fold more resistant to infection by *Salmonella* than untreated chicks.

Salmonella-contaminated poultry meat is a major source of human *Salmonella* infection (*31*). Decreasing the number of *Salmonella*-infected birds would result in fewer contaminated carcasses reaching the consumer (*11*). Maintenance of *Salmonella*-free breeding flocks and use of *Salmonella*-free protein concentrates in feed have decreased *Salmonella* contamination of poultry meat in Sweden (*1*) and Denmark (*10*), but these conventional methods of control have not been applied uniformly in most other countries.

A novel concept for decreasing *Salmonella* infection in poultry has been proposed by Nurmi and his co-workers in Finland (*12,15,16,21,22*), and has been reviewed recently (*14*). The concept involves introduction of intestinal bacteria from mature chickens into newly hatched chicks. Within a few hours of treatment, the chicks are resistant to more than 1000 infectious doses per os of *Salmonella* (*20*). Others have confirmed and extended the work of Nurmi et al. using chickens (*9,17,24,25,26,32*) and turkeys (*9,24*).

Attempts to demonstrate protection using various pure cultures isolated from intestinal material of mature birds have been unsuccessful (*3,5,7*), partially successful (*18*) or successful (*28*). The reported successful treatment (*28*) using *Streptococcus faecalis* could not, however, be repeated in our laboratories (unpublished data) or elsewhere (E. Goren, personal communication). A recent report (*4*) has shown protection with a mixture of pure cultures, but the cultures are not yet available for distribution.

Administration of cultured chicken cecal content has been shown to reduce the incidence of *Salmonella*-contaminated carcasses from commercially produced broilers in one field trial in Finland (*15*), but not in another (*22*). Additional field trials are required to test the efficacy of mixed cultures of intestinal organisms.

If undefined mixed cultures of intestinal organisms can be shown to be effective in field trials using commercial broiler flocks, then the search for pure cultures which can produce the same effect would be indicated. If not, then conventional methods of control should be applied or other methods of control should be investigated. The work described in this report was initiated to develop cultures and test them for suitability for field trials.

Culture for a field trial in commercial flocks must be safe, effective and reproducible. This report describes experiments on the protective effect of fecal cultures from adult chickens in the context of (*a*) efficacy, (*b*) reproducibility, (*c*) method of administration, (*d*) effect of freezing and (*e*) effect of serial subculture. In all experiments, chicks were reared on litter to more closely approximate commercial practices.

MATERIALS AND METHODS

Poultry and housing

Six trials conducted from May to November, 1979, are described. Broiler chicks (White Rock × Cornish) were shipped from a commercial hatchery, 100 per box, on the day of hatching and usually arrived the next day. They were reared on 5 cm of new pine shaving litter in concrete-floored pens. For each trial, new pens (45-cm high) were constructed of corrugated paper. For Trials I-III, 4 quadrant-

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shaped contiguous pens arranged in a circle were constructed in each of 4 or 6 wire-walled rooms in an experimental poultry barn. In Trials IV-VI, 14 rectangular pens, contiguous in banks of seven, with the banks abutting, were constructed in each of two solid-walled isolation rooms (A and B). In each trial, 30 ± 2 randomly-selected chicks were placed in each pen to obtain a density of one chick per 0.06 m^2. Between trials, premises were cleaned and disinfected, the barn by fumigation with formaldehyde and the isolation rooms with quaternary ammonium compounds.

Temperature and lighting

Temperatures were at least 36 C under brooder lamps in each pen, and maximum distance in any pen from the brooder lamp was 2 m. Room temperature varied from 20-30 C in Trials I-III, and from 28-30 C in Trials IV-VI. Continuous overhead lighting was supplied for 3 days after placement, then about 2 h per day thereafter. Continuous light of less intensity was supplied by the brooder lamps throughout each trial.

Water and feed

Non-chlorinated well water was used for Trials I-III and chlorinated municipal water was used for Trials IV to VI. The continuous drinkers (3.1-1 inverted jugs with about 0.5 1 in the trough) were sanitized daily, usually by immersion in $\sim$ 500 ppm of chlorine for $>$ 0.5 h, followed by rinsing in tap water. Viable counts showed that this procedure destroyed $>$ 99.9% of coliform bacteria. Disinfected drinkers used for administering treatment or challenge cultures were filled with tap water, inverted, held several hours to desorb residual disinfectant, and emptied before use. Water for diluting treatment or challenge cultures was at 25-30 C. Water to which the challenge culture was added was tested to assure absence of chlorine.

Feed was commercially-prepared, non-medicated broiler starter crumbles. It was supplied from self-filling troughs.

Treatment

Donors. Five *Salmonella*-free adult chickens were caged individually in an isolation unit. Two were obtained from specific-pathogen-free flocks housed indoors and three were obtained from separate farm flocks housed with access to soil outside of the barn. For each trial, fresh droppings from 3 to 4 donors were collected on clean waxed paper, pooled, and suspended in 4 volumes of 0.1% peptone water.

Fecal cultures (FC). VL medium (*2*) containing 0.0005% hemin, 0.00005% menadione and 0.0001% resazurin was prepared under oxygen-free CO_2 and maintained anaerobically in rubber-stoppered tubes and bottles. This medium, prewarmed to 37 C, was inoculated with 1% v/v of the above fecal suspension and incubated 24 h at the same temperature. Serial subcultures were made by transferring daily, 1%v/v of 24-h-old culture to the same prewarmed medium.

Administration of fecal culture (FC). Chicks were treated with FC when they were placed in the pens. For Trials I and II, 0.5 ml of FC was inoculated into the crop of each chick. For Trials IV - VI, 50 ml of FC was added to 450 ml of tap water or solution of 4% skim milk powder. For Trial III, both inoculation into the crop and addition to the drinking water were used. In each trial, untreated controls were given sterile VL broth undiluted or diluted 1:10 with water as appropriate. The drinkers were removed after 6 h, and the residual volumes measured to calculate the average consumption of FC per chick.

Challenge with S. typhimurium. Two days after treatment, chicks were challenged with *S. typhimurium* (*17*). The challenge was initiated by culturing 4 to 5 colonies individually in nutrient broth for 24 h at 37 C, mixing the 24-h cultures, subculturing 0.1 ml of the mixture to 9 ml of prewarmed (37 C) nutrient broth and incubating for 2 h at 37 C. Viable plate counts showed that these cultures contained $1.5\text{-}2.1\times10^7$ cells per ml. The chicks were deprived of water for 2 h before challenge. One ml of 2-h-old culture, or an appropriate dilution was added to 500 ml of water in a drinker to provide 0, 10^2, 10^3, 10^4 or 10^5 *S. typhimurium* per chick, calculated on the expected consumption of water per chick. The challenge was left in the pens for 2 h and then replaced with fresh water.

Examination of chicks for Salmonella

Chicks were sacrificed at 11-12 days of age (8-9 days post challenge) by cervical disarticulation and immersed in about 1000 ppm of benzalkonium chloride (Roccal 1:100) for 5-10 min. The portion of the intestinal tract from just above the ileo-cecal junction to the cloaca, including the ceca, was removed aseptically and cut into 3-4 pieces; these tissues are hereafter called ceca or C. Ceca were placed in 18-oz whirlpack bags and refrigerated within 2 h of removal. Ten to 15 ceca from each group of chicks were cultured individually the same day except for Trial III when ceca were frozen at -70 C for 18 days before analysis. Additionally, in Trials I, II and IV some of the ceca were frozen for subsequent study (see below).

Each cecal specimen (C) was mixed with 100 ml of nutrient broth (NB) for 30 sec in a stomacher (Colworth 400). A 1:1000 dilution of the cecal suspension (referred to as 10^{-3} C) was prepared by transferring 0.1 ml of the homogenate of C to 10 ml of NB. Both C and 10^{-3} C were preenriched by incubation at 35 C, and the next day 1 ml of each was inoculated into 10 ml of Difco tetrathionate brilliant green broth (TBG). The TBG cultures were incubated overnight at 42 ± 1C, then streaked on bismuth sulfite agar (BSA:Difco) which was incubated at 35 C for 1 and 2 days. Colonies resembling *Salmonella* on BSA were identified by their reactions in triple sugar iron and lysine iron agar slopes, and by agglutination with appropriate antisera.

Comparison of frozen and non-frozen specimens.

Non-frozen and frozen ceca from the same groups of chicks were cultured. Each sample consisted of 10 to 15 cecal specimens. The frozen ceca in Trials I, II and IV were selected for comparison if the incidence of *Salmonella* infection in non-frozen ceca from the same group of chicks was between 40 and 93% inclusive. In Trial I, frozen ceca were obtained from carcasses defrosted after 8 and 21 days at -21 C; in the other two trials, ceca were excised within 2 h of sacrifice and frozen at -17 C for 8 and 15 days (Trial II) or at -80 C for 10 days (Trial IV).

Statistical treatment

Data were assessed using Fisher's exact test (*23*) or the Chi square test, as appropriate.

Trials

Triplicate pens per challenge dose were used in Trial I, duplicate pens in the others. For Trials I and II, chicks were assigned to pens as described below; for Trials III to VI, they were assigned to pens chosen by random numbers (*29*).

Trial I was designed to establish a base line for subsequent experimentation, and to determine reproducibility in triplicate pens containing identically treated chicks. Three rooms held treated chicks inoculated into the crop with undiluted fecal culture and three rooms held control chicks inoculated with sterile medium. The challenges were identical for all six rooms: chicks in each of the four quadrant-shaped pens in each room received, in consecutive order, a calculated dose of 0, 10^2, 10^4 or 10^5 cells of *S. typhimurium* per chick. Thus unchallenged chicks were always in pens between and contiguous with pens containing chicks that received 10^2 and 10^5 cells.

Trial II was designed to compare the efficacy of a 1:10 dilution of fecal culture with undiluted culture, both inoculated into the crop. The assignment of treatments and challenges to pens was the same as for Trial I, except that each treatment was in duplicate rather than in triplicate.

In Trial III, efficacy of the following were compared: (*a*) treatment by inoculation and by addition to the drinking water, (*b*) treatment in drinking water and in 4% solution of dehydrated milk and (*c*) non-frozen 48-h-old culture, and 24-h-old culture frozen at -70 C for 21 days and thawed at 37 C on the day of use. For this trial, chicks were 2 days old at time of treatment because they were in transit for 2 days rather than the usual 1 day.

In Trials IV to VI, the ability of 0, 2 and 4 serial subcultures of fecal flora to prevent infection of treated chicks by *Salmonella* was compared. Each of the three cultures used in each trial was started by inoculation of freshly gathered feces into VL medium as described above. In Trial VI, we also determined the effect of dilution of the fourth subculture on ability to protect chicks against challenge. For these trials, one member of each pair of duplicate pens was in Room A, the other in Room B.

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RESULTS

Donor birds remained free of *Salmonella* throughout these trials; we did not detect *Salmonella* in at least two pooled samples of feces for each of the six trials.

Chicks used in Trials I-IV and Trial VI were apparently free of *Salmonella* on arrival from the hatchery, as evidenced by failure to isolate *Salmonella* from feces in the shipping boxes. In Trial V, however, the feces contained *Salmonella infantis*.

Consumption of FC administered in the drinking water for 6 h was less per chick (10.2 ± 2.6 ml) than FC in 4% milk (14.3 ± 1 ml); consumption of sterile medium in water was 14.1 ± 2.2 ml.

Post-challenge enumeration of *Salmonella* in inoculated drinking water showed that the number of organisms did not change during the 2-h challenge period. The challenge doses of *Salmonella* were calculated on the average volume of *Salmonella*-inoculated water consumed per chick, 6 ml in Trials I-III and 8.3 ml in Trials IV-VI.

Spread of *Salmonella* did not occur readily between contiguous pens as salmonellae were found in the ceca of only 2 of 115 non-treated, non-challenged chicks (Tables 1, 2 and 4).

TABLE 1. (*TRIAL I*). *Protection of chicks inoculated into the crop with undiluted fecal culture and challenged with* S. typhimurium[a].

Challenge dose	Triplicate pens[b]	Number of *Salmonella*-infected ceca of 15 examined[d]			
		Treated		Not treated	
		C[c]	10^{-3}C[c]	C	10^{-3}C
0	A	0	0	1	0
	B	0	0	0	0
	C	0	0	0	0
10^2	A	0	0	8	1
	B	0	0	6	4
	C	0	0	2	0
10^4	A	6	1	15	12
	B	5	2	15	12
	C	2	0	14	13
10^5	A	14	10	15	15
	B	3 * * *	2 * * *	15	10 *
	C	3	1	15	14

[a]Treated one day after hatching (day 1), challenged on day 3 and sacrificed on day 12 (Trials I-II) or on day 11 (Trials III-VI). See text for preparation of cultures and challenge.

[b]Each replicate pen contained 30 chicks; 15 per pen were cultured in Trials I-III and 10 in Trials IV-VI.

[c]C = whole ceca; 10^{-3} C = 1:1000 dilution of ceca.

[d]No significant differences ($p > 0.05$) among proportions of *Salmonella*-infected ceca in triplicate pens (Trial I) or between duplicate pens (Trials II-VI) except as indicated: * a=0.05, * * a=0.01 and * * * a=0.001.

Trial I

The results (Table 1) indicate that inoculation with undiluted FC increased resistance to *Salmonella* infection about 100- to 1000-fold because the proportion of infected chicks in the treated groups challenged with 10^5 *S. typhimurium* (20/45) was about the same as for non-treated chicks challenged with only 10^2 *Salmonella* (16/45). Non-treated control chicks were equally infected by challenges of 10^5 and 10^4 *Salmonella* (45/45 vs 44/45) and most of them (76/90) were heavily infected, i.e., *Salmonella* was detected in 10^{-3} C. In contrast, only 20/45 treated chicks were infected by a challenge with 10^5 *Salmonella* and 13/45 with 10^4 *Salmonella*, and only a small proportion of these treated chicks (16/90) was heavily infected. Results were generally consistent among triplicate groups of chicks except for treated chicks in pen A which were challenged with 10^5 *Salmonella*. Similar exceptions were observed in Trials II-VI and will be discussed below.

Trial II

Inoculation into the crop with 1:10 diluted FC was as protective as inoculation with undiluted FC (Table 2). Protection was similar to that obtained in Trial I.

TABLE 2. (*TRIAL II*). *Protection of chicks inoculated into the crop with diluted culture*[a].

Challenge dose	Duplicate pens[b]	Number of *Salmonella*-infected ceca of 15 examined[d]					
		Treated with				Not treated	
		Undiluted culture		Culture diluted 1:10		Sterile medium	
		C[c]	10^{-3}C[c]	C	10^{-3}C	C	10^{-3}C
0	A	0	-[e]	0	-	0	-
	B	0	-	0	-	0	-
10^2	A	1	1	0	0	1	1
	B	0	0	0	0	4	3
10^4	A	0	0	0	0	4	2
	B	1	0	0	0	12 * *	8
10^5	A	0	0	0	0	8	6
	B	7 * *	2	2	0	11	7

See footnotes a-d in Table 1.

[e] - = Not done.

Trial III

The following methods and treatments were equally effective: (*a*) inoculation into the crop and by addition to the drinking water, (*b*) non-frozen and frozen cultures and (*c*) administration of frozen culture in water and in milk (Table 3).

The chicks used in this trial were 2 days in transit under high ambient temperatures (mid-July, 1979) when they received neither water nor food. An unusually high proportion of chicks with poorly absorbed yolk sacs was observed at necropsy, 11 days after hatching, but there was no increase in mortality during shipping, or after

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TABLE 3. *(TRIAL III). Protection of chicks with non-frozen and frozen culture inoculated into the crop and in drinking water*[a,g].

		Number of *Salmonella*-infected ceca of 15 examined[d,f]							
		Treated with 1:10 dilution of culture by:						Not treated	
Challenge dose	Duplicate pens[b]	Inoculation		Drinking water		Drinking water with 4% dry milk		Sterile medium (1:10) in drinking water	
		C[c]	10^{-3}C[c]	C	10^{-3}C	C	10^{-3}C	C	10^{-3}C
				Non-frozen culture					
10^2	A	-[e]	-	-	-	-	-	2	2
	B	-	-	-	-	-	-	1	0
10^4	A	0	0	0	0	-	-	9	8
	B	0	0	2	0	-	-	13	11
10^5	A	0	0	4	2	-	-	-	-
	B	3	0	4	3	-	-	-	-
				Frozen culture					
10^4	A	3	0	4	0	0 }	0	-	-
	B	1	0	0	0	11 } * * *	3	-	-
10^5	A	0	0	0	0	0	0	-	-
	B	1	0	0	0	0	0	-	-

See footnotes a-d in Table 1.

[e] - = Not done.

[f] Ceca held 18 days at -70 C before examination.

[g] In Trial III only, chicks were treated on day 2 and challenged on day 4.

treatment and challenge (Table 6). The efficacy of treatment appeared equal to that obtained in chicks that were treated within 1 day of hatching (Tables 1 and 2).

Trial IV

Cultures serially subcultured two and four times were as effective in preventing infection by *Salmonella* as the culture that had been inoculated with fresh feces (Table 4).

Trial V

Some of the chicks were already infected with *S. infantis* on arrival from the hatchery, as evidenced by isolation of this serovar from a composite sample of fecally contaminated material taken from each shipping box. In our process of randomization of chicks into pens, we distributed from a box of 100 chicks, seven into each of 14 pens in Room A. This was repeated with chicks from three additional boxes. Similarly, chicks from four boxes were distributed into 14 pens in Room B. From a ninth box, we distributed two chicks to each of the 28 pens to obtain the final population of 30 chicks per pen. It appears that chicks infected with *S. infantis* were not uniformly distributed in the shipping boxes; at necropsy, *S. infantis* was isolated frequently in ceca of chicks from Room A (21 of 28 isolates), but not from Room B (one of 40 isolates). *S. typhimurium*, the challenge organism, was the only other serovar identified.

Protection by FC was not demonstrated in Room A, possibly because *S. infantis* had spread before treatment, and was minimal in Room B (Table 4). Others (*22*) have observed that infected birds do not respond to treatment.

Trial VI

Serial subculture did not reduce the efficacy of the treatment (Table 4), nor did dilution of the fourth subculture to 1:80 in 4% milk (Table 5).

Results aggregated from the six trials (Tables 1-5) are presented to summarize the protective effect obtained in pens of chicks and by individual chicks. Forty of 83 pens with treated, challenged chicks were devoid of infection and only 27 of the 83 pens (33%) contained one or more heavily infected chicks. In contrast, all 37 pens with non-treated, challenged chicks contained one or more infected chicks and 35 of the 37 (95%) contained one or more heavily infected chicks.

The aggregate data for treated, challenged chicks showed that 227 of 1035 (22%) had infected ceca, but only 97 of 1035 (9%) were heavily infected. In contrast, 340 of 465 (73%) non-treated, challenged controls had infected ceca and 293 of the 465 (63%) were heavily infected.

Thus treatment reduced the precentage of pens with infected chicks, the percentage of infected chicks and the percentage of heavily infected chicks.

Safety of treatment

There was no significant difference in numbers of deaths between treated and untreated chicks in Trials II to VI (Table 6). However, there were significantly more deaths ($p < 0.05$) among treated birds in Trial I. Most of

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TABLE 4. (*TRIALS IV, V, VI*). *Protection of chicks with serial subcultures of feces added to drinking water*[a].

Treatments diluted 1:10 in drinking water	Challenge dose	Duplicate pens[b]	Number of *Salmonella*-infected ceca of 10 examined[d] Trial IV C[c]	Trial IV 10^{-3}C[c]	Trial V[e] C	Trial V[e] 10^{-3}C	Trial VI C	Trial VI 10^{-3}C
Sterile medium	0	A	1	0	-	-	0	0
		B	0	0	-	-	0	0
	10^2	A	-[f]	-	5	3	10	10 } *
		B	-	-	9	7	6	4 } *
	10^3	A	10	10	10	10	10	10
		B	10	10	10	10	10	10
	10^4	A	10	10	10	10	10	10
		B	10	10	10	10	10	10
	10^5	A	10	10	-	-	-	-
		B	10	10	-	-	-	-
24-h culture (not subcultured)	0	A	0	0	-	-	-	-
		B	0	0	-	-	-	-
	10^4	A	0	0	10 } ***	5 } *	9 } **	1
		B	0	0	0 } ***	0 } *	1 } **	0
	10^5	A	4	1	10	4	2	0
		B	6	0	8	0	1	0
Subcultured twice at 24- h intervals	10^4	A	10 } ***	2	10	3	0	0
		B	0 } ***	0	10	7	0	0
	10^5	A	0	0	-	-	-	-
		B	4	2	-	-	-	-
Subcultured four times at 24- h intervals	10^4	A	1	0	10	8	0	0
		B	2	1	10	7	0	0
	10^5	A	9 } ***	1	10	10	1	0
		B	0 } ***	0	10	10	0	0

See footnotes a-d in Table 1.

[e] Some chicks were already infected with *S. infantis* on arrival; most of these were apparently placed in Room A (see text for details).

[f] - = Not done.

TABLE 5. (*TRIAL VI*). *Effect of dilution of fourth serial subculture on protection of chicks*[a].

Treatment	Challenge dose	Duplicate pens[b]	Number of *Salmonella*-infected ceca of 10 examined[d] C[c]	10^{-3} C
None (sterile medium 1:10 in water)	10^4	A	10	10
		B	10	10
Culture 1:10[e]	10^4	A	0	0
		B	0	0
	10^5	A	0 } ***	0 } ***
		B	10 } ***	8 } ***
Culture 1:20	10^4	A	0	0
		B	0	0
Culture 1:40	10^4	A	0	0
		B	1	0
Culture 1:80	10^4	A	0	0
		B	1	1

See footnotes a-d in Table 1.

[e] All dilutions of culture were made with 4% aqueous solution of dry milk.

these deaths occurred within 1 day of treatment, and were apparently due to production of gas or expansion of gas introduced by inoculation of undiluted FC into the crop. In Trial II, the undiluted FC was degassed by swirling the culture flask before inoculation into the crop, and early deaths did not occur. In Trials II-VI, most deaths occurred 3-5 days after treatment.

The ceca of birds that died between challenge and sacrifice in Trials I, II and IV were cultured for *Salmonella*. Results (Table 6) suggest that the proportions of *Salmonella* infections in the treated and non-treated birds were the same.

Isolation of Salmonella *from frozen and non-frozen specimens*

Isolation of *Salmonella* from frozen ceca was compared with isolation from non-frozen ceca originating from the same groups of chicks. There was no significant difference ($p > 0.05$) in 44 of 48 comparisons while two showed a significantly higher proportion of *Salmonella*-infected specimens before freezing, and two the reverse. All regimens of temperature and time investigated appeared equally suitable for storing frozen ceca; however, for long term storage, -70 C is probably more conducive to survival of *Salmonella*.

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TABLE 6. *Deaths in treated and non-treated chicks*[a].

Trial		Total no. of chicks	Chicks that died		Number of deaths[b]	
			Number	%	Before challenge	After challenge
I	Treated	384	35	9.1	26	9(4)[c]
	Non-treated	384	14	3.6	1	13(4)
II	Treated	464	13	2.8	3	10(1)
	Non-treated	232	2	0.8	1	1(0)
III	Treated	560	1	0.2	1	0
	Non-treated	112	2	1.8	0	2
IV	Treated	540	21	3.9	1	20(18)
	Non-treated	240	7	2.9	0	7(6)
V	Treated	540	4	0.7	2	2
	Non-treated	180	1	0.6	0	1
VI	Treated	600	10	1.7	0	10
	Non-treated	240	2	0.8	0	2

[a]See Tables 1-5 for treatments and challenges.

[b]In Trial I most deaths occurred one day after treatment, in the other Trials, 3-5 days after treatment.

[c]Dead chicks were examined for *Salmonella* only in Trials I, II and IV; brackets enclose number of dead chicks that were infected with *Salmonella*.

DISCUSSION

In some broiler operations, chicks are placed on used litter and their gastrointestinal tracts are quickly exposed to adult fecal flora. This practice, although it obviates the need for Nurmi treatment, often subjects the chicks to numerous harmful infectious agents. To minimize such harmful infections, a large part of the broiler industry uses husbandry practices designed to break the infection chain. These include the all in - all out cycle, cleaning and disinfection of premises between flocks and use of new litter. These measures, unfortunately, delay colonization of the gut of the chick by some of the adult fecal flora. The delayed colonization permits *Salmonella* to grow rapidly in the gut, usually without harming the chick (*9*).

It is unlikely that, within the next several years, *Salmonella* contamination of poultry meat in North America will be decreased significantly by conventional control of breeder flocks and feed (*13*). Protective cultures, therefore, offer a promising adjunct to conventional controls, but their application on an industrial scale in North America will require registration and licensing. Before application for licensing, standardized production and testing of cultures and more extensive field trials than those reported to date in Finland (*15,22*) and in North America (*26*) will be required to prove safety and efficacy.

The most effective safe preparations appear to be mixed cultures obtained by growing cecal or fecal material in anaerobic medium. Such cultures may contain several hundred species of bacteria in varying and unknown proportions, and the search for the most effective mixtures of pure cultures may be prolonged. Therefore standardized production and testing of fecal cultures should be developed expeditiously to proceed with field trials.

Our results showed that subculturing fecal material anaerobically four times at daily intervals in VL medium did not reduce its ability to protect chicks against infection by *Salmonella*. Similar protection has been obtained for cultures passaged four times at 4-day intervals in VL (*24*) and four times at 1-day intervals in trypticase soy broth (*32*).

Reproducibility of test results is a prerequisite to development of a safe effective product. Although efficacy of treatment was apparent in 5 of the 6 trials reported here, one or more results from triplicate or duplicate pens in each of the 5 trials were inconsistent with the general trend. The reasons for such inconsistencies have been discussed more fully (*14*), but are summarized as follows: if one or more chicks was less responsive to the treatment and more susceptible to the challenge - and became infected - it could excrete large numbers (10^7 to 10^9) of *Salmonella* per g of feces (*12*). These would contaminate litter (*17*) and drinking water (*30*). Thus the treated chicks could be subjected to continued exposure to much larger numbers of *Salmonella* than were present in the original challenge. Such exposure may be reduced by rearing chicks in wire-floored brooders with feed and water external to the wire walls. Although rearing in such brooders appears to

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provide a more reproducible test system (*12,24,28,32*), we have used chicks on litter to approximate commercial practices.

Administration of live cultures in drinking water requires assurance that disinfected drinkers are free of disinfectant, and that tap water is free of chlorine and toxic metals. Residual disinfectant and toxic metals can be inactivated with peptide-containing material such as powdered milk (*8*). We added live culture to drinking water for treatment and for challenge. During treatment, the 1.8% of peptide-containing materials of the VL medium (tryptone, yeast extract and beef extract) was diluted 1:10 with water, to 0.18%. In Trial VI, the diluted culture was further protected with a 4% solution of powdered milk (*26*). During challenge, the culture of *S. typhimurium* was not protected with organic matter. Nevertheless, the numbers of *Salmonella* remained constant during the 2-h treatment period because of precautions to eliminate chlorine from both drinkers and water. In separate experiments, 0.1 ppm of free chlorine plus 0.25 ppm of combined chlorine in water drawn from the tap destroyed over 99% of added *S. typhimurium* in less than 2 h (Blanchfield and Pivnick, unpublished data).

Although treatment with pure cultures or mixtures of pure cultures would be preferred, it appears unnecessary to delay testing of fecal cultures which may be effective in decreasing *Salmonella* contamination of poultry meat. Four serial subcultures and dilution of FC in drinking water would ensure that less than 1×10^{-11} g of donor feces was administered per chick. Parasites and viruses (*6*), and mycoplasma (*19*) would be unlikely to be present in these preparations. Numerous bacterial pathogens could, however, grow in the culture medium. These must be considered in developing a safety test although there have been no reports that fecal cultures have caused disease in Nurmi-treated chicks.

In our experiments and those of others (*15,17,24*), there was no evidence of harmful effects or decreased weight gains or feed conversion due to the fecal cultures. Even chicks stressed by 2 days in transit (Trial III) were not affected by treatment with FC. However, preparations should be subjected to more stringent tests for safety, e.g., chicks stressed by deprivation of food and water (*26*) or held at abnormal temperatures (*27*). Such stressors should approximate conditions that may occur in commercial operations. Also, the tests should be extended to about 7-8 weeks for broiler chickens.

Although eradication of infection is an unrealistic goal, abatement is not unrealistic. If, as a result of treatment, there is a decrease in the number of *Salmonella*-infected flocks, and a decrease in the number of *Salmonella* excreted by those birds that do become infected, then the treatment with fecal cultures may be of commercial advantage for reducing *Salmonella* contamination of poultry meat.

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REFERENCES

1. Almlof, J. 1977. National approach to *Salmonella* control. pp. 239-252. In D. A. Barnum (ed.) Proc. intern. symposium on *Salmonella* and prospects for control. Univ. Guelph, Canada.
2. Barnes, E. M., and C. S. Impey. 1971. The isolation of the anaerobic bacteria from chicken caeca with particular reference to members of the family *Bacteroidaceae*. pp. 115-123. In D. A. Shapton and R. G. Beard (eds.) Isolation of anaerobes. S.A.B. Technical Series No. 5. Academic Press, London.
3. Barnes, E. M., C. S. Impey, and D. M. Cooper. 1980. Competitive exclusion of salmonellas from the newly hatched chick. Vet. Rec. 106(3):61.
4. Barnes, E. M., C. S. Impey, and D. M. Cooper. 1980. Manipulation of the crop and intestinal flora of the newly hatched chick. Amer. J. Clin. Nut. 33:2426-2433.
5. Barnes, E. M., C. S. Impey, and B. J. H. Stevens. 1979. Factors affecting the incidence and anti-salmonella activity of the anaerobic caecal flora of the young chick. J. Hyg., Camb. 82:263-283.
6. Hitchner, S. B., C. H. Domermuth, H. G. Purchase, and J. E. Williams. (eds.) 1975. Isolation and identification of avian pathogens. Amer. Assoc. Avian Pathologists, College Station, Texas 77843.
7. Idziak, E. S., and M. Caldwell. 1977. The influence of normal intestinal flora of chicks on *S. infantis* and *S. typhimurium*. pp. 267-268. In D. A. Barnum (ed.) Proc. intern. symposium on *Salmonella* and prospects for control. Univ. Guelph, Canada.
8. Jordan, F. T. W., and T. J. Nassar. 1973. The survival of infectious bronchitis (IB) virus in water. Avian Pathol. 2:91-101.
9. Lloyd, A. B., R. B. Cumming, and R. D. Kent. 1977. Prevention of *Salmonella typhimurium* infection in poultry by pretreatment of chickens and poults with intestinal extracts. Australian Vet. J. 53:82-87.
10. Marthedal, H. E. 1977. The occurrence of salmonellosis in poultry in Denmark 1935-75, and the controlling programme established. pp. 78-94. In D. A. Barnum (ed.) Proc. intern. symposium on *Salmonella* and prospects for control. Univ. Guelph, Canada.
11. McBride, G. B., B. J. Skura, R. Y. Yada, and E. J. Bowmer. 1980. Relationship between incidence of *Salmonella* contamination among pre-scalded, eviscerated and post-chilled chickens in a poultry processing plant. J. Food Prot. 43:538-542.
12. Nurmi, E., and M. Rantala. 1973. New aspects of *Salmonella* infection in broiler production. Nature 241:210-211.
13. Pivnick, H. 1978. Canadian perspective on *Salmonella* control in poultry. Proc. 82nd annual meeting, U. S. Animal Health Assoc., Buffalo, N.Y., U.S.A.
14. Pivnick, H., and E. Nurmi. The Nurmi concept and its role in the control of salmonellae in poultry. In R. Davies (ed.) Developments in food microbiology - 1. Applied Science Publishers Ltd., London. (In press)
15. Raevuori, M., E. Seuna, and E. Nurmi. 1978. An epidemic of *Salmonella infantis* in Finnish broiler chickens in 1975-76. Acta Vet. Scand. 19:317-330.
16. Rantala, M. 1974. Cultivation of a bacterial flora able to prevent the colonization of *Salmonella infantis* in the intestines of broiler chickens, and its use. Acta Path. Microbiol. Scand. Section B. 82:75-80.
17. Rigby, C., and J. R. Pettit. 1980. Observations on competitive

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exclusion for preventing *Salmonella typhimurium* infection of broiler chickens. Avian Dis. 24:604-615.
18. Rigby, C., J. Pettit, and A. Robertson. 1977. The effect of normal intestinal flora on the *Salmonella* carrier state in poultry with special reference to *S. thompson* and *S. typhimurium*. p. 263. In D. A. Barnum (ed.) Proc. intern. symposium on *Salmonella* and prospects for control. Univ. Guelph, Canada.
19. Rodwell, A. W., and A. Mitchell. 1979. Nutrition, growth and reproduction. pp. 103-139. In M. F. Barile and S. Razin (eds.) The mycoplasmas I. Cell biology. Academic Press, New York.
20. Seuna, E. 1979. Sensitivity of young chickens to *Salmonella typhimurium* var. *copenhagen* and *S. infantis* infection and the preventive effect of cultured intestinal microflora. Avian Dis. 23:392-400.
21. Seuna, E., and E. Nurmi. 1979. Therapeutical trials with antimicrobial agents and cultured cecal microflora in *Salmonella infantis* infections in chickens. Poultry Sci. 58:1171-1174.
22. Seuna, E., M. Raevuori, and E. Nurmi. 1978. An epizootic of *Salmonella typhimurium* var. *copenhagen* in broilers and the use of cultured chicken intestinal flora for its control. Br. Poultry Sci. 19:309-314.
23. Siegel, S. 1956. Nonparametric statistics for the behavioral sciences. McGraw Hill, New York. pp. 92-104.
24. Snoeyenbos, G. H., O. M. Weinack, and C. F. Smyser. 1978. Protecting chicks and poults from salmonellae by oral administration of "normal" gut microflora. Avian Dis. 22:273-287.
25. Snoeyenbos, G. H., O. M. Weinack, and C. F. Smyser. 1979a. Further studies on competitive exclusion for controlling salmonellae in chickens. Avian Dis. 23:904-914.
26. Snoeyenbos, G. H., O. M. Weinack, and C. F. Smyser. 1979b. Competitive exclusion as a method of controlling *Salmonella* in poultry. Proc. 21st World Veter. Congr., Moscow, U.S.S.R., July 1-7.
27. Soerjadi, A. S., J. H. Druitt, A. B. Lloyd, and R. B. Cumming. 1979. Effect of environmental temperature on susceptibility of young chickens to *Salmonella typhimurium*. Australian Vet. J. 55:413-417.
28. Soerjadi, A. S., A. B. Lloyd, and R. B. Cumming. 1978. *Streptococcus faecalis*, a bacterial isolate which protects young chickens from enteric invasion by salmonellae. Australian Vet. J. 54:549-550.
29. Steel, R. G. D., and J. H. Torrie. 1960. Principles and procedures of statistics. McGraw Hill, New York. 481 p.
30. Stersky, A., B. Blanchfield, C. Thacker, and H. Pivnick. 1981. Reduction of *Salmonella* excretion into drinking water following treatment of chicks with Nurmi culture. J. Food Prot. 44:917-920.
31. Todd, E. 1980. Poultry-associated foodborne disease - its occurrence, cost, sources and prevention. J. Food Prot. 43:129-139.
32. Weinack, O. M., G. H. Snoeyenbos, and C. F. Smyser. 1979. A supplemental test system to measure competitive exclusion of salmonellae by native microflora in the chicken gut. Avian Dis. 23:1019-1030.

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Archiv Lebensmittel-hyg. 23:4-9.
12. Emard, L. O., and R. H. Vaughn. 1951. Selectivity of sorbic acid media for catalase-negative lactic acid bacteria and clostridia. J. Bacteriol. 63:487-494.
13. Enfors, S. -O., and G. Molin. 1978. Mechanism of the inhibition of spore germination by inert gases and carbon dioxide. pp. 80-84. In G. Chambliss and J. V. Vary (eds.) Spores VII. American Society for Microbiology, Washington, D. C.
14. Enfors, S. -O., G. Molin, and A. Ternström. 1979. Effect of packaging under carbon dioxide, nitrogen or air on the microbial flora of pork stored at 4°C. J. Appl. Bacteriol. 47:197-208.
15. Gray, R. J. H., and T. Sórhaug. 1981. Response regulation and utilization of microbial activities at low temperature. Economic Microbiology Series, Vol. 7. Food Microbiology, A. H. Rose (ed.) Academic Press, London. (In press).
16. Haines, R. B. 1933. The influence of carbon dioxide on the rate of multiplication of certain bacteria, as judged by visible counts. J. Society Chem. Ind. 52:13-17.
17. Hanna, M. O., D. L. Zink, Z. L. Carpenter, and C. Vanderzant. 1976. *Yersinia enterocolitica*-like organisms from vacuum-packaged beef and lamb. J. Food Sci. 41:1254-1256.
18. Jarvis, B., V. H. Lach, and J. M. Wood. 1977. Evaluation of the spiral plate maker for the enumeration of microorganisms in food. J. Appl. Bacteriol. 43:149-157.
19. King, D. A., Jr., and C. W. Nagel. 1967. Growth inhibition of a *Pseudomonas* by carbon dioxide. J. Food Sci. 32:575-579.
20. King, D. A., Jr., and C. W. Nagel. 1975. Influence of carbon dioxide upon the metabolism of *Pseudomonas aeruginosa*. J. Food Sci. 40:362-366.
21. Lawrie, R. A. 1974. Meat science. Pergamon Press, New York. p. 178-182.
22. Ledward, D. A., D. J. Nichol, and M. K. Shaw. 1971. Microbiological and colour changes during ageing of beef. Food Technol. Aust. 23:30-32.
23. Mitsuda, H., K. Nakajima, H. Mizuno, and F. Kawai. 1980. Use of sodium chloride and carbon dioxide for extending shelf-life of fish fillets. J. Food Sci. 45:661-666.
24. Ogilvy, W. S., and J. S. Ayres. 1951. Post-mortem changes in stored meats. II: The effect of atmospheres containing carbon dioxide in prolonging the storage life of cut-up chicken. Food Technol. 5:97-102.
25. Park, H. S., and E. H. Marth. 1972. Inactivation of *Salmonella typhimurium* by sorbic acid. J. Milk Food Technol. 35:532-539.
26. Robach, M. C. 1978. Effect of potassium sorbate on the growth of *Pseudomonas fluorescens*. J. Food Sci. 43:1886-1887.
27. Robach, M. C., E. C. To, S. Meydav, and C. F. Cook. 1980. Effect of sorbates on microbiological growth in cooked turkey products. J. Food Sci. 45:638-640.
28. Sander, E. H., and H. M. Soo. 1978. Increasing shelf-life by carbon dioxide treatment and low temperature storage of bulk pack fresh chickens packaged in Nylon/Surlyn film. J. Food Sci. 43:1519-1527.
29. Silliker, J. H., and S. K. Wolfe. 1980. Microbiological safety considerations in controlled atmosphere storage of meats. Food Technol. 34:59-63.
30. Smith, H. W. 1963. The use of carbon dioxide in transportation and storage of fruits and vegetables. Adv. Food Res. 12:96-138.
31. To, E. C., and M. Robach. 1980. Potassium sorbate dip as a method of extending shelf-life and inhibiting the growth of *Salmonella* and *Staphylococcus aureus* on fresh whole broilers. Poultry Sci. 59:726-730.
32. Valley, G. 1928. The effect of carbon dioxide on bacteria. Quart. Rev. Biol. 3:209-224.
33. Windsor, M. L., and T. Thoma. 1974. Chemical preservation of industrial fish: new preservative mixtures. J. Sci. Food Agric. 25:993-1005.
34. Wolfe, S. K. 1980. Use of CO and CO_2 -enriched atmosphere for meats, fish and produce. Food Technol. 34:55-58.

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