CHAPTER 6

PROBLEMS ASSOCIATED WITH USE OF ISOLATION PENS

INTRODUCTION

The majority of experiments in this chapter were concerned with testing of caecal anaerobes for their effect on salmonella infection in young chickens. When it became apparent that the test system was not working an experiment was designed to test whether the variation that was occuring was related to time or whether there were inherent limitations in the facilities used.

EXPERIMENTS 6.1 to 6.10

MATERIALS AND METHODS

The test organisms used in experiments 6.1-6.10 had been isolated from feral chickens and are described in Chapter 3. They were AC6, AC7, AC8, AC9, AC10, AC11 and AC12. The S. faecalis culture used in Experiment 6.3 had been isolated and freeze-dried by Soerjadi (1979).

Birds were housed in the isolation pens (see Chapter 3) and cloacal swabbing was used for isolation of the salmonella.

In all experiments birds were orally dosed at day-old with sterile RCM broth (control) or different bacterial isolates and subsequently challenged with 10^6 cells of S. typhimurium on day 4. Two replicated groups of particular cultures were tested in some experiments if that culture had shown promise in a previous experiment.

RESULTS

The results of experiments 6.1 to 6.5 are given in Table 6.1 and those for experiments 6.6 to 6.10 in Table 6.2.

Experiment 6.1

Fifteen percent of the control chickens were detected as salmonella carriers. Pretreatments AC6 and AC7 significantly increased the number of salmonella carriers ($P < 0.1$ and $P < 0.05$ respectively) while AC8 significantly decreased carrier rate ($P < 0.1$). The three factor interaction, treatment by salmonella by replicate (TxSxR), was significant ($P < 0.05$ and $P < 0.01$) for both AC6 and AC7 indicating lack of consistency of the treatment by salmonella interaction over the replicates.

Experiment 6.2

Twenty percent of the control chickens were detected as carriers. Pretreatments AC9 and AC11 significantly increased carrier rate at the .1% level, AC8 at the 10% level while AC10 had no significant effect. The TxSxR interaction was significant ($P < 0.05$) for AC9 only.

Experiment 6.3

None of the control chickens were detected as carriers. Pretreatments AC9 and AC8 significantly increased carrier rate at the .1% level. AC12 had no significant effect and no carriers were detected in the S. faecalis treatment group. No significance was detected in the TxSxR interaction indicating good correlation between replicates within treatments.

Experiment 6.4

Thirty-five percent of the control chickens were detected as carriers. Pretreatments AC8 and AC12 had no significant effect on carrier rate. No significance was detected in the TxSxR interaction.

Experiment 6.5

Sixty-seven percent of the control chickens were detected as carriers. Pretreatment AC9 significantly increased the number of carriers ($P < 0.01$) while AC12 had no significant effect. The TxSxR interaction was significant for all treatments including the control indicating poor correlation between replicates within treatments.

Experiment 6.6

Seventy percent of the control birds were detected as carriers. All pretreatments significantly decreased the carrier rate, AC9 at the 1% level and the remainder at the .1% level. No significance was detected in the TxSxR interactions.

Experiment 6.7

Eighty one percent of the control birds were detected as carriers. Pretreatment AC7 had no significant effect on carrier rate while AC8, AC9 and AC11 significantly decreased it at the .1% level. The TxSXR interaction was significant for AC9 and AC11 at the 5% and 10% levels respectively.

Experiment 6.8

Fifty-eight percent of the control birds were detected as carriers. AC7 had no significant effect on carrier rate. The two applications of AC8 significantly decreased carrier rate ($P < 0.1$ and $P < 0.05$). No significance was detected in the TxSxR interactions.

Experiment 6.9

Forty percent of the control birds were detected as carriers. AC11 and one application of AC8 significantly ($P < 0.05$) decreased carrier rate while AC9 and the other application of AC8 had no significant effect. No significance was detected in the TxSxR interactions.

Experiment 6.10

Thirty-six percent of the control birds were detected as carriers. One application of both AC9 and AC8 significantly ($P < 0.05$ and $P < 0.001$ respectively) decreased carrier rate while the other applications had no significant effect. The TxSxR interaction was significant ($P < 0.1$) for one AC9 group.

Table 6.1
Effect of orally treating day-old chickens with sterile RCM broth (control) or different bacterial isolates and subsequently challenging with 10^6 cells of S. typhimurium on day 4

Treatment	Salmonella carriers[a] Rep 1	Rep 2	Overall (%)	Significance levels TxS	TxSxR[b]
Experiment 6.1					
Control	2	4	15		NS
AC6	10	3	33	-	*
AC7	13	3	40	*	**
AC8	0	11	3	-	NS
Experiment 6.2					
Control	1	7	20		*
AC8	7	8	38	-	NS
AC9	13	10[c]	59	***	*
AC10	3	10	33	NS	NS
AC11	8	16	60	***	NS
Experiment 6.3					
Control	0	0	0		NS
AC8	8[d]	4[c]	35	***	NS
AC9	8[c]	3[c]	29	***	NS
AC12	1	2	8	NS	NS
S. faecalis	0	0	0	NS	NS
Experiment 6.4					
Control	7	7	35		NS
AC8	4	9	33	NS	NS
AC12	10	10	50	NS	NS
Experiment 6.5					
Control	16[c]	10	67		*
AC9	13[d]	19	91	**	-
AC12	6	13[c]	49	NS	**

a - 20 birds per replicate
b - Treatment x Salmonella x Replicate
c - 1 bird died before challenge
d - 5 birds died before challenge

Table 6.2
Effect of orally treating day-old chickens with sterile RCM (control) or different bacterial isolates and subsequently challenging with 10^6 cells of S. typhimurium on day 4

Treatment	Salmonella carriers[a] Rep 1	Rep 2	Overall (%)	Significance Levels TxS	TxSxR
Experiment 6.6					
Control	14	14	70		NS
AC7	0	0	0	***	NS
AC8	0	1	3	***	NS
AC9	9	6	38	**	NS
AC12	8	3	28	***	NS
AC11	1	3	10	***	NS
Experiment 6.7					
Control	16[b]	14[c]	81		NS
AC7	13[c]	14[b]	73	NS	NS
AC8	5[c]	0	13	***	NS
AC9	4	11	38	***	*
AC11	1	4	13	***	-
Experiment 6.8					
Control	11	12	58		NS
AC7	14	14	70	NS	NS
AC8	10	5	38	-	NS
AC8	6	8	35	*	NS
Experiment 6.9					
Control	6	10	40		NS
AC8	8	13	53	NS	NS
AC8	4	3	18	*	NS
AC9	8	12	50	NS	NS
AC11	5	2	18	*	NS
Experiment 6.10					
Control	9[c]	5	36		NS
AC8	0	1	3	***	NS
AC8	5	7	30	NS	NS
AC9	6	9	38	NS	-
AC9	6	0	15	*	NS

a - 20 birds per treatment
b - 2 birds died before challenge
c - 1 bird died before challenge

DISCUSSION

The experiments in this series highlighted the main problem encountered in this work. That is, lack of repeatibility of experimental results. There was a vast variation in infection levels found in the control groups between experiments and no apparent reason for the discrepancy. All the experiments were run in the same facilities with birds from the same source and using the same challenge organism. Viewed individually the experiments appeared to achieve results but when viewed overall there was a confounding variation in these results. Residual pen contamination and slight temperature differences between pens due to shading and positional effects were possible factors that contributed to the variation.

Some trends were observed with the use of the anaerobic cultures. Overall the use of AC8 was promising in reducing the level of infection in treated groups. In 9 of the 12 occassions that this organism was tested it reduced the infection level of the treated chickens. This reduction was significant on seven occassions. On two of the three occassions where it did not reduce infection level it significantly increased infection level. Conversely the use of the anaerobic culture AC9 overall increased the infection level of treated birds. This increase was significant on three occassions and non significant on two. Its use on another three occassions significantly decreased infection rate. Within the same experiments the effects of the two AC8 applications were significantly different from each other emphasising the lack of repeatibility in results.

Reviewing the experimental data it was not clear whether the variation in results was due to time or whether there was a fundamental factor in the facilities used which resulted in abberant results. Previously some of the isolation pens had been used for infectious bronchitis research in which four week old birds were subjected to challenge with IB. These birds had been reared on wire in isolation from other chickens and then placed on wire in the isolation pens at four weeks of age. Their intestinal microflora would be composed of strains of bacteria derived from their immediate environment rather than from other chickens. Bacterially therefore these birds would not be expected to interfere with the study on CE. The use of the isolation pens for IB work meant that only 3 to 6 pens were available for CE work at any one time.

To test whether the variation of results that had been obtained was due to factors inherent in the test system or in the facilities used a basic experiment was designed so that a comparison of infection levels could be obtained when birds in all pens were treated identically. For this experiment the full complement of ten pens was available.

Experiment 6.11

Four hundred day-old cockerels were randomly allocated into 20 replicates of 20 birds, two replicates being assigned to each isolation pen. All chickens were dosed with .2ml of sterile RCM broth before being placed into their cages.

Challenging, sampling (by cloacal swabbing) and identification of S. typhimurium were carried out as described in Chapter 3.

RESULTS AND DISCUSSION

The results are presented in Table 6.3 and Figure 6.1. The percentage of carrier chickens varied significantly ($P<0.001$) between 23% to 97%. The differences within replicates reached the 5% level of significance in one pen but when the combined probability over pens was calculated the differences between replicates was found to be non-significant. Hence the variation was between pens rather than within pens. This inferred that environmental conditions were variable between the different isolation pens.

Table 6.3
Effect of orally challenging 4 day old chickens with 10^6 cells of S. typhimurium

Pen	Salmonella carriers[a] Rep 1	Rep 2	Overall (%)
1	12[b]	4	42
2	4	5[c]	23
3	10	4	35
4	10	8[b]	47
5	14[d]	17[c]	86
6	15	15	75
7	16	14	75
8	20	18[c]	97
9	10	16	65
10	4	6	25

a - 20 birds per replicate
b - 2 birds died before challenge
c - 1 bird died before challenge
d - 3 birds died before challenge

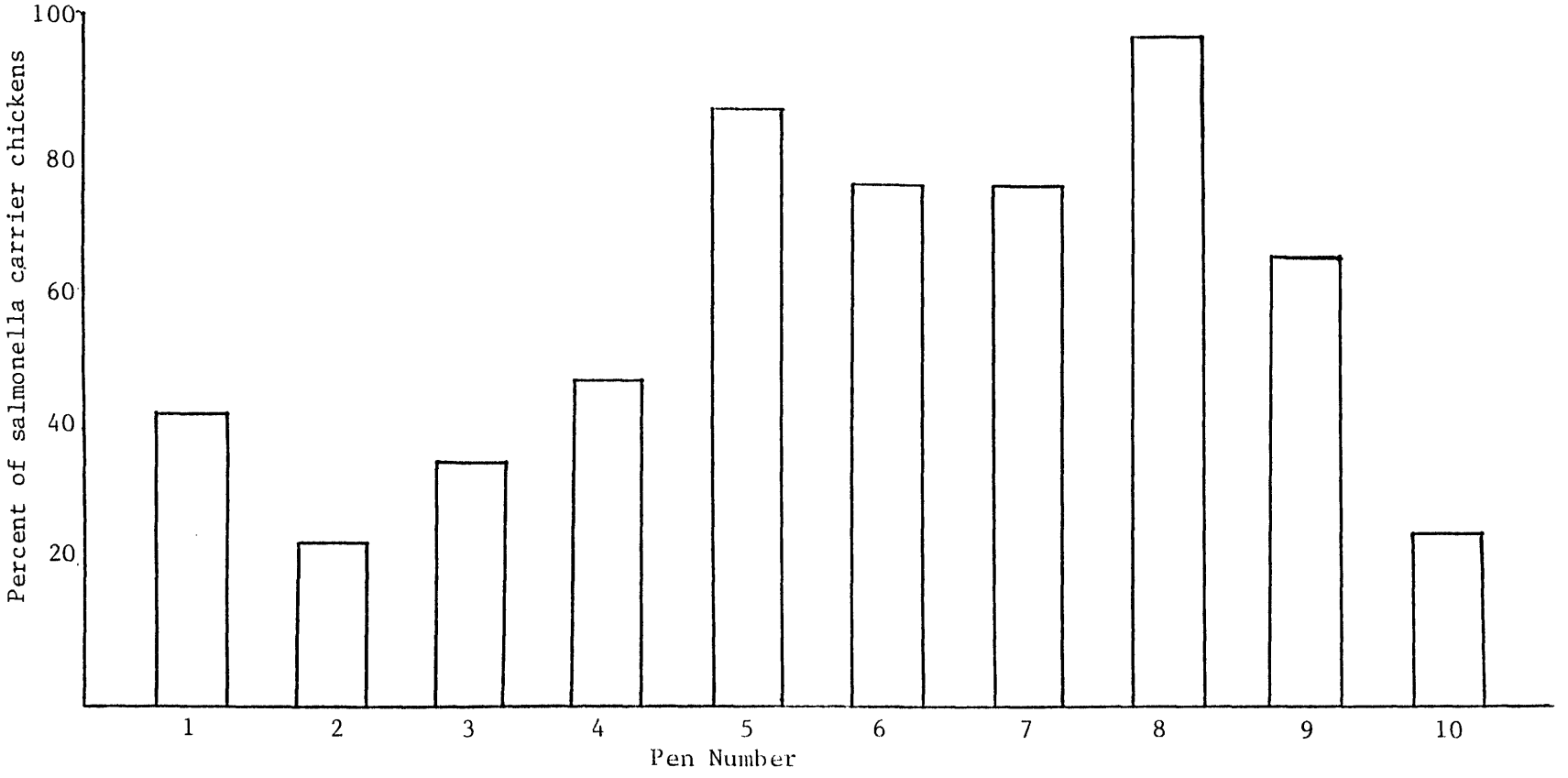

Figure 6.1

Effect of orally challenging 4 day old chickens with 10^6 cells of S. typhimurium

These results demonstrated clearly that the test system was not working. Though all birds were treated identically and housed under conditions as similar as they could be made there were large differences in the infection levels between pens. It appeared that the isolation pens were not suitable for CE work. Temperature differences between pens and residual pen contamination were possible factors contributing to the variation in infection rates.

The pens were originally built for infectious bronchitis research in which 4 weeks old birds were used. Some difficulty was encountered in adapting the pens for use with day-old birds. The pens were on different parts of the hill and in addition some pens were not facing the exact direction of the majority. These two factors could result in some pens being subject to draughts. Trees shaded some pens and the combination of shading and the presence of draughts could alter the temperature within pens.

Soerjadi (1979) reported a 78% salmonella carrier rate in chickens kept in a cold environment (18°-22°C) and challenged with 10^3 cells of S. typhimurium as compared with 0% infection rate in chickens kept in a warm environment (32°-36°C). In addition he found that cold stressing 12 days old chickens that had been challenged with 10^5 cells of S. typhimurium at 4 days of age caused a significant ($P < 0.01$) increase in the shedding of salmonella. If there were slight temperature differences between the different isolation pens higher infection rates could result in the pens that had the lower temperatures.

To minimise positional effects treatments were randomized over pens for each experiment. The citrobacter work showed that despite rigorous cleaning some bacterial pen contamination remained. It was possible therefore that in some pens birds were exposed to protective bacteria

from a previous trial which increased their resistance to salmonella challenge. Thus the low infection rates found in control birds in pens 2, 3 and 10 may have been the result of birds coming into contact with protective bacteria. Conversely the high infection rates found in other pens may have been influenced by lower temperatures.

Due to the difficulty in achieving a consistent infection rate in the isolation pens subsequent experiments were conducted in one or two temperature controlled rooms in the University of New England's Animal House. The use of these rooms permitted control over the temperature of the chicks' environment and gave much greater efficiency in cleaning. The chicks were housed in experimental brooders within the rooms and these brooders could be dismantled completely for cleaning.

CHAPTER 7

NALIDIXIC ACID AND SULPHAPYRIDINE SENSITIVITY

The experiments in this chapter were conducted in one or two temperature controlled rooms and were aimed at establishing a working test system.

Experiment 7.1

Two hundred and fifty day-old cockerels were randomly allocated into 10 replicates of 25 birds, two replicates being assigned per treatment. The replicates were placed in separate brooders in an temperature controlled room as described in Chapter 3. Four groups were dosed with either anaerobic culture AC12, AC13, AC14 or AC15 (see Chapter 3) while the fifth group were dosed with sterile RCM broth (control).

Challenging, sampling (by cloacal swabbing) and identification of S. typhimurium were carried out as described in Chapter 3.

RESULTS AND DISCUSSION

The results are presented in Table 7.1. Eighteen percent of the control birds were detected as being carriers of S. typhimurium. All treatments significantly ($P < 0.01$) reduced the number of salmonella carrier chickens. No significant differences were found in the TxSxR interactions.

Table 7.1
Effect of orally treating day-old chickens with sterile RCM broth (control) or different bacterial isolates and subsequently challenging with 10^6 cells of S. typhimurium on day 4

Treatment	Salmonella carriers[a] Rep 1	Rep 2	Overall (%)	Significance Levels TxS	TxSxR
Control	4	5	18		NS
AC12	0	0	0	**	NS
AC13	0	0	0	**	NS
AC14	1	1	4	**	NS
AC15	0	0	0	**	NS

a - 25 birds per treatment

The low numbers of chickens detected as being carriers of S. typhimurium could have been due to either one or a combination of the following factors:

1. Residual protective bacteria in the rooms. Two days prior to the experiment commencing the rooms were occupied by chickens involved in an infectious bronchitis trial

2. Failure to infect the birds with salmonella

3. Failure to isolate the salmonella

4. Presence of high levels of antibiotics in the feed

To determine whether feed type or level of S. typhimurium challenge affected the number of birds that became carriers of S. typhimurium the following experiment was designed.

Experiment 7.2

Three hundred day-old cockerels were randomly allocated into 20 replicates of 15 birds, two replicates being assigned per treatment. Five groups were placed on a ration of commercial starter crumbles and the remaining 5 groups were fed a sorghum based diet that was mixed in the laboratory (See Chapter 3). On day 4 the groups were orally dosed with either 10^5, 10^6, 10^6, 10^7 or 10^8 cells of S. typhimurium (two groups of 10^6 were included as this was the normal challenge dose).

On day 7 the birds were swabbed for salmonella following the procedures described in Chapter 3.

RESULTS AND DISCUSSION

The results of this swabbing indicated that only 3 out of the 300 birds were carriers of S. typhimurium. From this result it was suspected that the low number of S. typhimurium carriers was due to a failure to detect the salmonella rather than failure to infect the birds. Review of the experimental procedure revealed no difference to that of Soerjadi (1979) except that nalidixic acid was included in the bacteriological media. The S. typhimurium when streaked onto brilliant

green agar that contained 1 g/l sulphapyridine and 100 mcg/l of sodium nalidixate grew well. To test whether it was still resistant after being passaged through chickens the following experiment was designed.

Experiment 7.3

Two identical groups of sixty birds were made up from the 10^6, 10^7 and 10^8 treatments from experiment 7.2. These birds were swabbed and the swabs from one group placed in selenite broth that contained 1 g/l of sulphapyridine and 100 mcg/ml of sodium nalidixate and those from the other group were placed in selenite broth that contained sulphapyridine but not the sodium nalidixate. After the standard 24 hour incubation at 37°C a loopful of broth from each of the swabs was streaked onto:-

1. brilliant green agar containing 1 g/l sulphapyridine and 100 mcg/ml of sodium nalidixate

2. brilliant green agar containing 1 g/l sulphapyridine and no sodium nalidixate

These plates were then incubated for 24 hours at 37°C after which they were examined for growth of salmonella.

RESULTS AND DISCUSSION

The results are presented in Table 7.2. Use of sodium nalidixate in the selenite broth prevented growth of salmonella. When it was included in the brilliant green agar alone it severely reduced the numbers of salmonella positive chickens detected. High numbers of salmonella carriers were detected when the media did not contain sodium

nalidixate. When the S. typhimurium was passaged through chickens it apparently lost its nalidixic acid resistance. George et al (1983) found that in the absence of selection pressure resistance to nalidixic acid in E. coli fell to a low level within 100 generations of growth. The nalidixic acid resistant induced strain of S. typhimurium which was being used was not being subjected to selection presure for at least 5 days in which time the resistance could fall to a very low level. As there were no problems of contamination by other bacteria nalidixic acid was excluded from the bacteriological media in subsequent experiments.

Table 7.2
Effect of altering the sodium nalidixate content in the selenite broth and BGA on the detection of S. typhimurium carriers

Challenge dose	Salmonella carriers[a]			
	Selenite broth		Selenite broth(nal$^+$)	
	BGA	BGA(nal$^+$)	BGA	BGA(nal$^+$)
10^8	14	6	0	0
10^7	11	0	0	0
10^6	8	1	0	0
10^6	6	1	0	0

a - 15 birds per treatment

Experiment 7.4

Since high numbers of salmonella were detected once sodium nalidixate was excluded from the isolation media work commenced on testing anaerobic cultures for their effect on salmonella infection.

Two hundred and fifty day-old cockerels were randomly allocated into ten replicates of 25 birds, two replicates being assigned per treatment. Four groups were dosed with anaerobic cultures AC12, AC13, AC14 and AC15 while the fifth group was dosed with sterile RCM broth

(control). Challenging, sampling (by cloacal swabbing) and identification of S. typhimurium was carried out as described in Chapter 3.

Table 7.3
Effect of orally treating day-old chickens with sterile RCM broth (control) or different bacterial isolates and subsequently challenging with 10^6 cells of S. typhimurium on day 4

Pen	Salmonella carriers[a] Rep 1	Rep 2	Overall (%)	Significance Levels TxS	TxSxR
Control	4[b]	10	30		-
AC12	1[c]	3[c]	8	***	NS
AC13	2	5	14	-	NS
AC14	9[c]	1[c]	21	NS	**
AC15	1[c]	3[c]	8	**	NS

a - 25 bird per treatment
b - 3 birds died before challenge
c - 1 bird died before challenge

RESULTS

The results are presented in Table 7.3. Thirty percent of the control birds were detected to be carriers of salmonella. Treament AC14 had no significant effect on the carrier rate. Treatments AC12, AC13 and AC15 significantly decreased the number of salmonella carrier chickens detected ($P < 0.001$, $P < 0.1$ and $P < 0.01$ respectively). The TxSxR interaction was significant at the 10% and 1% levels for the control and AC14 treatments respectively and non-significant for the remaining treatments.

DISCUSSION

Though the anaerobic treatments were able to reduce the carrier rate it was considered that 30% infection in the controls was too low. An experiment was designed to investigate whether different challenge levels would increase the infection level in control birds.

Experiment 7.5

Three hundred and fifty day-old cockerels were randomly divided into 16 replicates of 25 birds, two replicates being allocated per treatment. Four groups were dosed with anaerobic cultures AC12, AC13, AC14 and AC15 while the remaining groups were dosed with sterile RCM broth. On day 4 the groups dosed with the anaerobic cultures and one of the RCM groups were dosed with 10^6 cells of S. typhimurium. The remaining groups were dosed either (a). 10^6 cells of S. typhimurium that had been passaged through a chicken in a previous trial; (b) 10^7 cells of S. typhimurium; (c) 2×10^7 cells of S. typhimurium; (d) 10^8 cells of S. typhimurium;

On day 7 one hundred and fifty birds were swabbed following the procedures in Chapter 3.

RESULTS AND DISCUSSION

Only three birds of the one hundred and fifty birds swabbed were positive for salmonella. From this result it was suspected that the strain of S. typhimurium being used had lost its virulence in prolonged storage in freeze dried cultures. The following experiment was designed to test other isolates of Salmonella typhimurium for their virulence.

Experiment 7.6

Three S. typhimurium isolates (82/13, 80/1205/ and AN 80/449), were obtained from Dr A R B Jackson of the Regional Veterinary Laboratory, Armidale. These isolates had been obtained from field outbreaks of salmonellosis in poultry.

Three hundred and twenty day-old cockerels were randomly allocated into 16 replicates of 20 birds, two replicates were assigned per treatment. On day 4 the treatment groups were dosed with either 10^7 or 10^8 cells of the following treatments:

1. S. typhimurium 82/13;
2. S. typhimurium 80/1205;
3. S. typhimurium 80/449; or
4. the original Armidale strain of S. typhimurium

On day 7 the chickens were assayed for the presence of S. typhimurium using the cloacal swab technique as described in Chapter 3.

RESULTS

Only 2 of the three hundred and twenty chickens were detected as being carriers of S. typhimurium. Under the conditions which the birds were kept even a low challenge dose would be expected to result in a moderate infection level. With a challenge dose of 10^7 organisms quite high infection rates would be expected. It therefore appeared that the apparent lack of infection might be due to failure to detect infection. Careful review of experimental procedure revealed no difference from that used by Soerjadi (1979). As there appeared to be no obvious reason why salmonella was failing to be detected birds were sent for bacteriological examination to two other laboratories which were routinely assaying for salmonella.

Experiment 7.7

Sixty birds were selected from the previous experiment so that 3 identical groups of 20 birds were obtained. One group of birds was sent to the Regional Veterinary Laboratory, Armidale, another was sent to the Department of Microbiology of this university and the remaining group was dealt with in this laboratory. All groups were post-mortemed at the respective laboratories and the number of salmonella infected birds determined.

RESULTS AND DISCUSSION

The Regional Veterinary Laboratory detected 12 of the 20 birds as being carriers of salmonella, the Department of Microbiology detected 18 and this laboratory detected only one bird as being a carrier of salmonella. Careful checking of procedures revealed that they were identical in the three laboratories except that sulphapyridine was included in the bacteriological media only in this laboratory. Bacteriological examination in this laboratory of a further 20 birds

from Experiment 7.6 using bacteriological media not containing sulphapyridine detected 16 of the 20 birds as being salmonella carriers. It was then evident that the sulphapyridine had been inhibiting the salmonella in the bacteriological media.

Soerjadi (1979) used sulphapyridine to prevent the growth of Citrobacter cloacae and its use has also been recommended by several other workers (see Fagerberg and Avens 1979). Routine laboratory checks had shown that the S. typhimurium used had grown on the BGA containing sulphapyridine before being passaged through the test birds. Once passaged it had apparently lost its resistance to sulphapyridine.

Watanable and Jukasawa (1961) showed that resistance by bacteria to sulfonamides was carried on a plasmid. Hardy (1981) stated that plasmids may be lost unless a selection pressure is applied. In this work no selection pressure was applied to the salmonella until after it was passaged through the chicken by which time many generations may have passed.

Fagerberg and Avens (1979) cite several authors who found problems in controlling the selectivity of brilliant green sulphapyridine agar. Some lots of commercial brilliant green agar to which sulfadiazine had been added would not support satisfactory growth of salmonella.

It appeared therefore that though sulphapyridine could be added to the bacteriological media to control the growth of contaminants, on some occassions it could be inhibitory for salmonellae. As it had been included as a precaution only it was excluded from the media in subsequent experiments.

CHAPTER 8

PROBLEMS ASSOCIATED WITH USE OF BROODERS AND SINGLE ROOMS

INTRODUCTION

Having resolved the problems encountered in Chapters 5,6 and 7 work appeared to be at a stage to test bacterial isolates. In addition to testing individual isolates a mixture of equal volumes of the isolates used in a particular experiment were tested. This was to determine whether combinations of organisms were more effective than individual isolates.

MATERIALS AND METHODS

The experiments were conducted in one or two temperature controlled rooms as described in Chapter 3. The bacterial isolates (AC16, AC17, AC18, AC19, AC20, AC22 and AC23) used in the following experiments are described in Chapter 3. Caecal culture was used as the method of salmonella sampling for experiments 8.1 to 8.5 after which cloacal swabbing was used so that infection levels could be assessed earlier.

Experiment 8.1

Five hundred day-old cockerels were randomly allocated into 20 replicates of 25 birds, four replicates being assigned per treatment. Two replicates of each treatment were kept in one of 2 rooms. Four

groups were dosed with either treatment AC14, AC16, AC17 or a mixture of all these isolates while the fifth group was dosed with sterile RCM broth.

Challenging, sampling and identification of salmonella were carried out as described in Chapter 3.

Experiment 8.2

Four hundred day-old cockerels were randomly allocated into 20 replicates of 20 birds, two replicates being assigned per treatment. Eight different isolates (AC13, AC16, AC18, AC19, AC20, AC21, AC22 and AC23) were tested. In addition one treatment group was dosed with caecal contents from a feral bird (see Chapter 3) and one group was dosed with sterile RCM broth as a control.

Challenging, sampling and identification of salmonella were carried out as described in Chapter 3.

Experiment 8.3

Four hundred day-old cockerels were randomly allocated into 20 replicates of 20 birds, two replicates being assigned per treatment. The ten groups were orally dosed with either AC13, AC14, AC16, AC18, AC19, AC20, AC21, AC22, a mixture of all of these isolates or sterile RCM broth.

Challenging, sampling and identification of salmonella were carried out as described in Chapter 3.

This experiment was repeated three times.

Experiment 8.4

Four hundred day-old cockerels were randomly allocated into 20 replicates of 20 birds. Four replicates were allocated to treatments AC13, AC18 and AC19, while treatments AC14 and AC20, a mixture of all the bacterial isolates and sterile RCM broth were each assigned two replicates.

Challenging, sampling and identification of salmonella were carried out as described in Chapter 3.

Experiment 8.5

Four hundred day-old cockerels were randomly allocated into 20 replicates of 20 birds, two replicates being assigned per treatment. Five treatment groups were dosed with either AC13, AC14, AC18, AC19 or AC20. Two groups were dosed with a mixture of AC14 and S. faecalis or AC20 and S. faecalis while the ninth and tenth groups were dosed with either a mixture of all the isolates or sterile RCM broth (control).

Challenging, sampling and identification of salmonella were carried out as described in Chapter 3.

Experiments 8.6 and 8.7

These experiments were of the same format as previous ones. However results from the first swab indicated extremely low levels of infection in the controls and so the experiments were terminated.

RESULTS

Experiment 8.1

These are presented in Table 8.1. Fifty-four percent of the control chickens were detected as being salmonellae carriers. No significant differences in carrier rate was detected between the control

and treatment groups.

Table 8.1
Effect of orally treating day-old chickens with sterile RCM broth (control) or different bacterial isolates and subsequently challenging with 10^6 cells of S. typhimurium on day 4

Treatments	Salmonella carriers[a]				
	Rep 1	Rep 2	Rep 3	Rep 4	Overall (%)
Control	7(4)[b]	7(6)	7(3)	15	54
AC14	11(6)	8(3)	13(2)	9(1)	60
AC16	6(7)	8(8)	7(2)	6(1)	44
AC17	4(8)	4(5)	12(3)	10(3)	49
Mixture[c]	8(7)	12(6)	2(2)	8(3)	48

a - 25 birds per replicate
b - the number of birds that died before challenge are given in parentheses
c - mixture comprises of equal portions of cultures AC14, AC16 and AC17

Experiment 8.2

These are presented in Table 8.2. Sixty-six percent of the control chickens were detected as being salmonellae carriers. Isolates AC16, AC21 and AC22 had no significant effect on the carrier rate while isolates AC13, AC19, AC20 and AC23 reduced the carrier rate significantly at the 5% level. Isolate AC18 and the feral caecal group both significantly reduced the carrier rate at the .01% level. The TxSxR interaction was significant ($P < 0.1$) for AC19 and AC23.

Table 8.2
Effect of orally treating day-old chickens with sterile RCM broth (control), feral caecal material (FCM) or different bacterial isolates and subsequently challenging with 10^6 cells of S. typhimurium on day 4

Treatment	Salmonella carriers[a] Rep 1	Rep 2	Overall (%)	Significance Levels TxS	TxSxR
Control	12(1)[b]	13(1)	66		NS
FCM	3(3)	0(4)	9	***	NS
AC13	10(2)	4(4)	41	*	NS
AC16	10(1)	8(1)	47	NS	NS
AC18	2(4)	5(1)	20	***	NS
AC19	4(2)	13(1)	46	*	-
AC20	7(9)	3(4)	36	*	NS
AC21	5(2)	7(5)	36	NS	NS
AC22	13	13(1)	67	NS	NS
AC23	12	5	40	*	-

a - 20 birds per replicate
b - numbers in parentheses represent the number of birds that died before challenge

Experiment 8.3

These are presented in Table 8.3. In experiment 8.3a fifteen percent of the control chickens were detected as salmonellae carriers. Carrier rate was significantly decreased by treatments AC13, AC22 and the mixture at the 10% level and by AC16 and AC20 at the 5% level. No other treatments significantly affected the carrier rate. No significance was noted in the TxSxR interaction.

In experiment 8.3b seventy-six percent of the control chickens were detected as salmonellae carriers. All treatments significantly decreased carrier rate. AC18, AC21 and AC22 at the 1% level and the remainder at the .1% level. The TxSxR interaction was significant for the mixture ($P < 0.1$) and for the control, AC18 and AC19 ($P < 0.05$) and for AC20 ($P < 0.01$).

In experiment 8.3c fifty-four percent of the control chickens were detected as salmonellae carriers. The carrier rate was significantly decreased by treatments AC19 and the mixture ($P < 0.1$) and by AC14 ($P < 0.01$). No other treatments significantly effected carrier rate. The TxSxR interaction was significant only for AC13 ($P < 0.05$).

Table 8.3
Effect of orally treating day-old chickens with sterile RCM broth (control) or different bacterial isolates and subsequently challenging with 10^6 cells of S. typhimurium on day 4

Treatment	Salmonella carriers[a] Rep 1	Rep 2	Overall (%)	Significance levels TxS	TxSxR
Experiment 8.3a					
Control	3	3	15		NS
AC13	0	1	3	-	NS
AC14	1	1	5	NS	NS
AC16	0	0	0	*	NS
AC18	2	2	10	NS	NS
AC19	3	0	8	NS	NS
AC20	4	9[e]	35	*	NS
AC21	1	1	5	NS	NS
AC22	1	2[f]	3	-	NS
Mixture	1	0	3	-	NS
Experiment 8.3b					
Control	11[g]	18	76		*
AC13	3	7	26	***	NS
AC14	3[e]	6[b]	22	***	NS
AC16	3[f]	9[g]	32	***	NS
AC18	10	6	40	**	*
AC19	5[f]	3	21	***	*
AC20	6[g]	2[c]	21	***	**
AC21	7[g]	9[f]	43	**	NS
AC22	5[g]	8	34	**	NS
Mixture	4[f]	3[f]	18	***	-
Experiment 8.3c					
Control	12[f]	9	54		NS
AC13	5	12[f]	44	NS	*
AC14	4	7[b]	25	**	NS
AC16	8[d]	9[g]	43	NS	NS
AC18	12[d]	8	49	NS	NS
AC19	8[f]	5[c]	33	-	NS
AC20	12	5[c]	42	NS	NS
AC21	9	11[f]	51	NS	NS
AC22	9[d]	10[f]	48	NS	NS
Mixture	9	4[f]	28	-	NS

a - 20 birds per replicate unless noted
b - 24 birds per replicate
c - 21 birds per replicate
d - 22 birds per replicate
e - 3 birds died before challenge
f - 1 bird died before challenge
g - 2 birds died before challenge

Experiment 8.4

These are presented in Table 8.4. Eighty-three percent of the control chickens were detected as salmonella carriers. AC13 had no significant effect on carrier rate in either application. AC18 in one application significantly decreased carrier rate ($P < 0.01$) whilst in the other had no significant effect. AC19 was similar to AC18 in that one application significantly ($P < 0.001$) reduced carrier rate and the other had no significant effect. AC14 and AC20 significantly reduced carrier rate ($P < 0.01$ and $P < 0.05$ respectively). The mixture of isolates significantly reduced the carrier rate ($P < 0.05$). The TxSxR interaction was significant for one application of AC19 and for AC20 at the 10% and 1% levels respectively.

Experiment 8.5

These are presented in Table 8.5. No statistical analysis was done on these results because of the lack of consistency in the control replicates. This discrepancy between replicates is also present in treatments AC19, AC14 and S. faecalis and AC20 and S. faecalis. The AC13, S. faecalis and mixture treatment groups appeared to decrease the carrier rate while the others had either no effect or increased it.

DISCUSSION

From the results of the first two experiments in this series it appeared that the test system was working. Though there were not many treatments that gave significant protection this was perhaps to be expected in a biological system.

Table 8.4
Effect of orally treating day-old chickens with sterile RCM broth (control) or different bacterial isolates and subsequently challenging with 10^6 cells of S. typhimurium on day 4

Treatment	Salmonella carriers[a] Rep 1	Rep 2	Overall (%)	Significance Levels TxS	TxSxR
Control	16	17	83		NS
AC13	16[b]	19[b]	92	NS	NS
AC13	13	17	75	NS	NS
AC14	9[b]	8[b]	45	***	NS
AC18	14	19	83	NS	NS
AC18	10	9	48	**	NS
AC19	10	7	43	***	NS
AC19	12[c]	19[b]	86	NS	-
AC20	5	17[d]	58	*	**
Mixture	10	13[b]	59	*	NS

a - 20 birds per replicate
b - 1 bird died before challenge
c - 3 birds died before challenge
d - 2 birds died before challenge

Table 8.5
Effect of orally treating day-old chickens with sterile RCM broth (control) or different bacterial isolates and subsequently challenging with 10^6 cells of S. typhiurium on day 4

Treatment	Salmonella Carriers[a] Rep 1	Rep 2	Overall (%)
Control	1(5)	10	31
S. faecalis	2[c]	1	7
AC13	3(1)	1(1)	11
AC14	6(7)	2	24
S. faecalis + AC14	4	12(3)	43
AC18	14	10	60
AC19	10(6)	2(1)	36
AC20	7(5)	14	60
S. faecalis + AC20	3	12	38
Mixture	3	0	8

a - 20 birds per replicate unless noted
b - the number of birds that died before challenge are given in parentheses
c - 24 birds per replicate

The replicated experiment showed that there was some factor/s that prevented duplication of results. The carrier rate in the control chickens varied considerably as did that of the treatments. The next two experiments showed the carrier rate in the control birds to vary widely from 83% to 31%. In two further experiments such low carrier rates were recorded that the experiments were terminated.

The experimental work had reached a stage where time was limited and it was apparent that the test system was not working sufficiently well for the results obtained to be viewed with any degree of confidence. Two factors which may have been influencing the carrier rate were feed and the supersaturation of the facilities with bacteria. Seuna and Nurmi (1979) and Williams and Whittenmore (1980) suggested that the level of therapeutic agent in the cloaca of the chicken was high enough to inhibit in vitro recovery of salmonella. They found that most of the antimicrobial agents were excreted with the urine into the cloaca. Though the feed used for the experiments (see Chapter 3) was meant to be free of antibiotics it was possible that an error at the feed mill may have resulted in antibiotics in the feed which could therefore affect in vitro recovery of salmonella.

Rantala and Nurmi (1973) reared treatment groups separately in isolation. Soerjadi (1979) did not continually repeat experiments involving different bacteria. Impey et al (1982) used flexible wall isolators under negative pressure. In this present work the facilities available did not allow the treatments to be kept separately. In addition though the rooms and brooders could be cleaned satisfactorily for general pruposes it was impossible to eliminate all bacteria and thus ensure that bacteria did not carry over from trial to trial. The aberrant results obtained could have been connected with either feed or

supersaturation of the environment with bacteria. The final two experiments were concerned with these aspects.

Experiment 8.8

This experiment was designed to test whether a suitable infection level could be obtained by varying feed type or day of challenge. Feed type was varied to check that the commercial feed did not contain any additives that reduced the detection of salmonellae in the live bird. A sorghum based ration that did not contain any antibiotics or drugs (see Chapter 3), was tested against the commercial ration. If the facilities were supersaturated with 'protective' bacteria then challenging with salmonellae earlier would result in higher carrier rates as birds had less time to obtain protective bacteria from their environment.

Three hundred and twenty day-old cockerels were randomly allocated into 16 replicates of 20 birds. Two replicates were assigned per treatment. Half the replicates were fed commercial chicken starter whilst the remainder were fed a sorghum based ration.

Treatments were:

1. Challenge with salmonella on day 1
2. Challenge with salmonella on day 2
3. Challenge with salmonella on day 3
4. Challenge with salmonella on day 4

Challenging, sampling (by cloacal swabbing) and identification of salmonella were carried out as described in Chapter 3.

Experiment 8.9

This experiment was designed to test whether keeping control birds in a separate room or brooder from birds dosed with anaerobic bacteria had any affect on infection level. Treated birds and control groups were challenged with salmonellae on day 3 as the results from the previous experiment had shown this to result in a suitable carrier rate in the control birds. Groups that were challenged on days 2, 3 and 4 were included as a comparison.

Six hundred and forty day-old cockerels were randomly allocated into 32 replicates of 20 birds. Two replicates were assigned per treatment. Seven groups were dosed with either S. faecalis, AC13, AC14, AC18, AC19, AC20 or sterile RCM broth and challenged with salmonella on day 3. Two groups in the same room but kept in a different brooder were dosed with sterile RCM broth and challenged with salmonella either on day 3 or day 4. In the adjoining temperature controlled room 3 groups were dosed with sterile RCM broth and then challenged with salmonella either on day 2, day 3 or day 4. In a seperate room in a separate building (room 15) three groups were dosed with RCM broth on day 1 then challenged with salmonella either on day 2, 3 or 4. This room had been occupied two days earlier by adult birds in an infectious bronchitis experiment and had been thoroughly hosed out and a clean brooder placed in it. Challenging, sampling (by cloacal swabbing) and identification of salmonella were carried out as described in Chapter 3.

RESULTS

Experiment 8.8

These are presented in Table 8.6. Challenging on days 1, 2 or 3 gave significantly ($P < 0.001$) higher infection rates than challenging on day 4. When fed on the commercial ration, one hundred percent infection was obtained when challenging on days 1 or 2, seventy-four percent on day 3 and only fifteen percent when challenging on day 4 . When fed on university mixed feed one hundred percent infection was obtained on day 1 challenge, 65% on day 2 and 30% on days 3 and 4.

Experiment 8.9

These are presented in Table 8.7. Seventy-one percent of the day 3 challenge chickens (control) kept in a separate brooder were detected as salmonellae carriers. Significant decreases in carrier rate was affected by AC18, AC19 ($P < 0.05$), AC20 ($P < 0.01$) and the mixture of organisms ($P < 0.001$). Treatments AC13, AC14 and S. faecalis though decreasing infection levels had no significant effect on carrier rate. Only 46% of the day 3 challenged control chickens that were kept in the same brooder as the treatments were detected as salmonella carriers. Seventy-eight percent of the day 4 challenge chickens kept in the separate brooder were detected as being salmonellae carriers. The carrier rates of the day 2, 3 and 4 challenge treatment groups kept in the next room were not significantly different from that of the control.

In room 15 the carrier rates of the day 2, 3 and 4 challenge groups were 72%, 50% and 25% respectively. The day 3 and 4 challenge groups had significantly less carrier birds ($P < 0.1$ and $P < 0.05$ respectively) than did the control.

Table 8.6
Effect of varying feed type and day of salmonella challenge on the salmonella infection rate in young chickens

Day of Challenge	Feed type	Salmonella carriers[a] Number	Percentage
Day 1	Commercial(C)	20	100
	University(U)	18[b]	100
Day 2	C	20	100
	U	13	65
Day 3	C	14[c]	74
	U	6	30
Day 4	C	3	15
	U	6	30

Significance (Chisquare)

Factors	Significance
Commercial Feed	
Day 2 v Day 3	*
Day 2 v Day 4	***
Day 3 v Day 4	***
Commercial v University	
Day 2(C) v Day 2(U)	**
Day 3(C) v Day 3(U)	**
Day 4(C) v Day 4(U)	NS

a - 20 birds per treatment
b - 2 birds died before challenge
c - 1 bird died before challenge

Table 8.7
Effect of isolating experimental groups of day-old chickens and orally treating them with either sterile RCM broth (control) or different bacterial isolates and subsequently challenging with 10^8 cells of S. typhimurium on day 4

Treatment	Salmonella carriers[a] Rep 1	Rep 2	Overall (%)	Significance Level TxS	TxSxR
Room 2 - Brooder 1					
Control	16[b]	11	71		*
Day 4	12[b]	17[b]	NS	**	**
Room 2 - Brooder 2					
AC13	11[c]	12	58	NS	NS
AC14	12[c]	11	60	NS	NS
AC18	9[c]	9[b]	48	*	-
AC19	12	7[c]	52	*	NS
AC20	6	9	38	**	*
S. faecalis	18[c]	8	67	NS	NS
Mixture	8	5	32	***	NS
Day 3	14[c]	4	46	*	NS
Room 3					
Day 2	12[d]	10[b]	63	NS	NS
Day 3	10[d]	17[b]	77	NS	***
Day 4	8[b]	17[d]	71	NS	***
Room 15					
Day 2	10[c]	18	72	NS	***
Day 3	11[c]	8[c]	50	-	NS
Day 4	3[d]	6[c]	25	*	NS

a - 20 birds per treatment
b - 2 birds died before challenge
c - 1 bird died before challenge
d - 3 birds died before challenge

DISCUSSION

The results of the final two experiments demonstrated clearly that some of the difficulties encountered in this work were due to the supersaturation of the facilities with bacteria. Though there were some differences in carrier rates in the two feed groups it was evident that feed was not the cause of the low carrier rates. Housing the control birds in the same brooder as the treated chickens gave them access to protective bacteria. The longer the period between treatment and challenge the longer the control chickens had to acquire protective bacteria. This is shown clearly in the last experiment where control chickens housed in a separate brooder had a much higher infection rate than did the control chickens housed in the same brooder as the treated birds. The same was also demonstrated in room 15 which had had adult chickens in two days prior to the experimental chickens. Though the room had been cleaned between experiments there was obviously some residual contamination with protective bacteria. This was demonstrated by the rapidly decreasing numbers of infected birds when the challenge day was delayed. This effect would have been exacerbated by the slightly higher room temperature as the birds would be less stressed than those in the rooms kept at 19° C.

CHAPTER 9

GENERAL DISCUSSION

The experimental techniques used in this work were developed by Soerjadi (1979). Soerjadi isolated 2 bacteria (S.faecalis and B.fragilis) from the caecal contents of a backyard bird, that on their own, were able to significantly increase the resistance of day-old chicks to salmonella infection. Although statistically not different the level of protection given by either S. faecalis or B.fragilis was never quite as good as that given by caecal material. The approach used by the majority of researchers in competitive exclusion is to test caecal contents for its protective ability and to attempt to enumerate and classify the bacteria in the mixture (Impey et al 1982, Pivnick and Nurmi 1982). In this work a search was undertaken to isolate bacteria that would complement the two isolated by Soerjadi (1979) and bring the level of protection given by the resultant mixture of organisms up to that of caecal material. If isolated the organisms would have been identified and their growth requirements determined. The resultant mixture, of for example 5 or 6 organisms, could then have been registered as a specific group to be used for salmonella control. This would alleviate the problems of attempting to register a 'black box' mixture of organisms.

The donor birds used in the majority of the experiments presented here were White Leghorn-Black Australorp crossbred cockerels that had been dosed at day-old with faecal material from feral birds. The feral birds, captured on an unihabited island off the Queensland coast, had not been exposed to antibiotics or drugs and therefore would have a very stable and 'normal' intestinal flora. This contrasts with the intestinal flora of the modern broiler which, as it had been acquired not from adult birds but from its environment and human attendants, could be considered 'abnormal' or artificial. When comparing the two sources of birds (feral versus modern broiler) the ferals would be expected to have greater resistance to salmonella challenge due to to their very stable intestinal flora. Bowman et al (1976), Soerjadi (1979) and Snoeyenbos et al (1979) all reported differences between chicken populations in their ability to serve as sources of protective bacteria. These differences would be due to differences in the species and strains of intestinal bacteria.

Initial experiments demonstrated that pretreatment of day-old chickens with faecal material from healthy adult feral or backyard chickens increased the chick's resistance to salmonella challenge. The level of resistance attained was very considerable (an average infection rate in the feral and backyard treatements of 3% compared with an average of 79% in the controls) and contrasts to that found by Lloyd et al (1977) and Soerjadi (1979), both of whom considered that, as in most biological control systems, resistance could be overcome by a substantial challenge or when there is a concurrent stress.

Thereafter considerable trouble was encountered in attaining a reliable infection rate in control groups. The first problem experienced was the sudden appearance of Citrobacter freundii. This

organism, a member of the family Enterobacteriacea, grew profusely in the isolation media making it extremely difficult to isolate the salmonella. After determining that the source of the organism was the hatchery where the chickens were obtained, means of inhibiting it in the isolation media were investigated. These investigations were non-productive and the extent of the problem realised. One of the mechanisms of CE is thought to be simply competition for attachment to the mucosal lining in the chickens gut. If the attachment sites are already occupied by an organism then neither the salmonella nor the 'protective' organisms may attach. Other sources for experimental birds were investigated and a Sydney hatchery was identified as being suitable to supply birds for future experiments.

A small trial with the Sydney chickens showed that there were still some residual pen contamination by C. freundii but that this was slight. Continual disinfecting between experiments was expected to clean this up. To facilitate the ease of isolating salmonella and as a safeguard against further contaminants the existing strain of S. typhimurium was induced to be resistant to nalidixic acid. In subsequent experiments nalidixic acid was included in both the enrichment broth and the brilliant green agar.

The next problem encountered was the lack of duplication of experimental results. Though the experimental procedure was rigorously standardized a consistent level of infection in the control treatments could not be attained and the caecal isolates tested also produced varying infection levels in the different experiments. An experiment was run processing all birds as controls and it was found that the between pen variation was highly significant indicating that environmental conditions varied between pens. There was some variation

within pens but this variation was not significant. Two factors, temperature and residual contamination by protective bacteria, were considered to be major factors in contributing to between pen variation. As the pens could not be further modified without incurring great expense the venue for experimentation was changed to two temperature controlled rooms that had become available.

The third problem encountered was that of very low infection levels. Feed type and level of salmonella challenge were investigated to determine if infection rates could be increased by varying these factors. When there was no apparent response to these changes the experimental procedure was closely examined to check whether there were any changes that could have resulted in the apparently low infection rates. Nalidixic acid supplementation of the bacteriological media was an innovation from the procedure developed by Soerjadi (1979). Subsequent experimentation showed that the S. typhimurium had lost its resistance to nalidixic acid and was being inhibited by its inclusion in the media. Removal of nalidixic acid resulted in the detection of high infection levels.

The fourth problem encountered was that even with the removal of the nalidixic acid from the media subsequent experiments were not able to establish a satisfactory infection level. High challenge doses and different strains of S. typhimurium did not increase the number of infected birds detected. Soerjadi (1979) encountered a similar problem in his work and had resolved it by cold stressing his experimental birds. The birds used in these experiments were under a moderate cold stress and still apparently not becoming infected. Revision of experimental methods did not reveal any diversion from that used for previous experiments or from that of Soerjadi (1979). Birds then were

sent to other laboratories that were routinely isolating salmonellae from chickens. Results from their examinations revealed that the birds were salmonellae carriers. Checking of bacteriological techniques showed that sulphapyridine was included in the media in this laboratory only. A subsequent examination showed that by excluding sulphapyridine this laboratory detected high infection levels.

Even with these problems resolved considerable difficulty was still encountered in achieving a consistent infection level in the controls. After several experiments in which the infection rate in the control birds varied widely the remaining two experiments were designed to gain more understanding on factors effecting infection level in the control treatments. These experiments demonstrated that the control birds were acquiring protective bacteria from the environment. The longer the period before challenge the greater the resistance to salmonella infection. Control birds kept in the same brooder as treated birds were relatively resistant to salmonella challenge by day 3.

At the start of this work none of the above problems were envisaged as both Soerjadi (1979) and Frazer (pers.comm.) had used similar facilities and had not encountered any such problems. Examination of their work showed that though it was on competitive exclusion the format varied quite markedly from this present study. Neither of them were continually testing caecal isolates and subsequently challenging with salmonella. Their work was either testing the caecal isolates in the laboratory or in isolation without challenging with salmonella or simply challenging with salmonella. Thus they were not continually attempting to challenge birds with salmonella in an area which apparently became saturated with protective bacteria. The other problems of citrobacter contamination, different environmental conditions existing between pens

and susceptibility to both nalidixic acid and sulphapyridine made the results difficult to interpret and it was only towards the end of the study that the saturation of the facilities with protective bacteria became apparent.

When viewed overall it is apparent that some of the problems encountered arose from the solution of other problems. For example, changing the venue to the temperature controlled rooms led to the close contact between control and treated groups and this contact gave control birds some resistance to infection. Using nalidixic acid and sulphapyridine as means of facilitating isolation of salmonella led to its actual inhibition. The basic lack of success in acheiving the aims of the work, i.e. that of isolating caecal bacteria to complement those isolated by Soerjadi (1979) may be attributed to simply lack of adequate facilities. The continual use of caecal isolates saturated the experimental facilites and led to aberrant infection levels of control birds. What was required was access to isolators which could be sterilised between experiments and kept as discrete units during experiments. This would have prevented the build up of organisms and ensured that birds were not exposed to organisms other than those which had been allocated as a treatment to them. Any differences in resistance would then be directly attributable to the isolate given to the chick.

The results obtained on the apparent build up of organisms in the environment are extremely favourable for the use of protective organisms in a commercial situation. If the broiler units became saturated with protective organisms this would alleviate the necessity to treat each batch of chickens, instead with the judicious use of old litter and reliance on the build up of protective organisms, treatment of day-old

chicks with a protective culture may only need to be done occasionally. In addition the rapid lateral spread of protective organisms shown to occur here and also reported by Frazer (pers.comm.) alleviates the need to inoculate every day-old chick.

Some trends were apparent in the experiments conducted in the isolation pens. The isolate, AC8, was able to increase the resistance of chicks to salmonella challenge on 9 of the 12 occassions where it was tested. Though some caution should be exercised in interpreting any data on the protection afforded by the caecal isolates tested it would appear that AC8 would merit further testing. Additionally the use of faecal material in all experiments where it was tested was able to very significantly reduce salmonella infection. The results of the initial experiments where the experimental birds were kept on litter under brooders can be viewed with considerable confidence. These showed that use of single isolates from the caecae were effective in reducing salmonella infection rates.

Future research is indicated on examining caecal isolates for their effect on salmonella infection rates in young chickens. This present work needs to be repeated with the use of isolators to ensure reproducible conditions from trial to trial. The method of isolating and storing caecal organisms appears satisfactory. Once a collection of isolates that are able to reduce salmonella infections levels is acquired they should be tested together with the streptococcus and bacteroides bacteria and the experimental period extended to assess their long term effect on the carrier rate of chickens.

CHAPTER 10

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