

EXPERIMENT IN EGG PENETRATION BY SALMONELLA

C. H. BIGLAND¹ and GEORGIA PAPAS¹

Many chicks and poults in Alberta have been found suffering from Salmonella infections. (1) The history given with some of these cases left a possible source of infection in doubt, especially those wherein day old birds were received from the hatchery and placed in previously disinfected brooder houses only to have a Paratyphoid infection become rampant in the matter of a few days.

It is believed that organisms of the Salmonella group could be in the fertile eggs prior to hatching time; but it is also believed that organisms of the Paratyphoid group are seldom actually incorporated in the egg when laid. (2)

In an effort to ascertain if Salmonella could penetrate the egg shell and reach the interior and, if so, the length of time necessary, the following experiment was conducted.

PROCEDURE — This briefly falls into the following headings:

1. Inoculation of egg shell with Salmonella
2. Incubation at room temperature
3. Disinfection of shell
4. Culture of portions of contents
5. Identification of organisms found

INOCULATION OF EGGS

It was found, in previous unreported work at this laboratory, that little or no penetration of the eggs took place within the first seven days after inoculation. Considering this, it was decided to check egg penetration by various species of Salmonella from the seventh day to the sixteenth day after inoculation.

Approximately 140 eggs were used for each organism examined. The inoculum consisted of one pound of unsterelized poultry faeces moistened with 50 c.c. of a twenty-four hour nutrient broth culture of the Salmonella to be examined. Each egg was carefully rolled in the contaminated faeces and placed in wire baskets lined with four layers of sterile cheesecloth, which were folded over the top of the eggs to prevent outside contamination. There were ten baskets of fourteen eggs each, all contaminated on the outside by the test organism.

INCUBATION

All baskets were incubated at room temperature in the coolest room available. (See temperature and humidity chart).

¹Alberta Veterinary Laboratory, Edmonton, Alberta.

One basket was removed for checking each day, starting on the seventh day after inoculation, until all ten baskets had been examined. On Sundays or holidays the baskets were removed and placed in the refrigerator for examination the following day.

DISINFECTION OF SHELL

In order to ensure that no *Salmonella* were introduced into the egg at the time of examination, a plan of shell disinfection was necessary.

In the first experiment with *Salmonella typhimurium*, the eggs were placed in a rack; the upper portion was washed with 1:1000 solution of bi-chloride of mercury and then painted with tincture of merthiolate 1:1000.

When *Salmonella typhimurium* was recovered in ten eggs in this experiment, nine of these in one day, the possibility of introduction from the outer shell was questioned. In view of this, in the subsequent groups, the eggs were washed in 450 ppm of Roccal (a commercial quaternary ammonia compound)* for one hour, removed, dried and painted with 1:1000 tincture of merthiolate.

CULTURE OF EGG CONTENTS

After the merthiolate had dried, the upper portion of the shell was removed with sterile scissors. The contents were then mixed with sterile swabs, which were used to inoculate the mixed egg material into 10 c.c. tubes of tetrathionate broth and nutrient broth. These were incubated for twenty-four hours and then plated on MacConkeys, brilliant green and blood agar plates.

IDENTIFICATION OF ORGANISMS FOUND

All colonies suspected of being *Salmonella* were fished and seeded in Lactose, Dulcitol, Maltose, Saccrose, Dextrose, Mannite, Kligler's Iron Agar, Motility media and peptone water for indole testing.

To prove that only the test organism was recovered from the interior of the eggs, all organisms identified as *Salmonella* were submitted to the Provincial Public Health Laboratory, Edmonton, for serological typing.

RESULTS

Four *Salmonella* organisms were tested in this way: *Salmonella bareilly*, *Salmonella oranienburg*, *Salmonella kentucky* and *Salmonella typhimurium*. Two tests are included for *Salmonella typhimurium* due to variation in shell disinfection techniques. The number of *Salmonella* recoveries are given in Table 1.

Table 1 indicates a recovery of *Salmonella* from approximately 8 per cent of eggs inoculated with *Salmonella typhimurium*, approximately 3 per cent

*Alkyle-dimethylbenzyle-ammonium chlorides (technical) Winthrop-Stearns Inc.

TABLE I
EGG SHELL PENETRATION BY SALMONELLA

ORGANISM	AFTER INOCULATION												REMARKS
	7	8	9	10	11	12	13	14	15	16	17	TOTAL	
<i>S. typhimurium</i>	0	0	0	1	9	1	0	0	0	0		11 (140)	<i>S. typhimurium</i> isolated from outside of unsterilized egg at 16th day.
<i>S. typhimurium</i>	2 (13)	1	0	0	3*	2	1	0 (12)	1*	1		11 (137)	No Salmonella isolated from outside of eggs following dipping in Roccal solution.
<i>S. oranienburg</i>	1	0	0	0	2	0	0	1	0	0		4 (140)	No Salmonella isolated from outside of eggs following dipping in Roccal solution.
<i>S. bareilly</i>	0 x	0 x	0	0	0	0 (12)	0	0 (12)	0 (12)	0 (13)	x	0 (133)	<i>S. bareilly</i> recovered from outside of unsterilized egg on 16th day.
<i>S. kentucky</i> **	1 (13)		3	3	3	4 (12)	2 (13)	3 (13)	0	0	7°	26 (135)	<i>S. kentucky</i> recovered from the outside of two eggs daily before dipping in Roccal. After one hour in 450 ppm Roccal solution, Salmonella was recovered from 1 egg out of 135.

*One egg shell found cracked during dipping in Roccal. Salmonella recovered in each case.
x Including one turkey egg in each instance. **Small pullet eggs used. °This basket refrigerated on the 17th day but not cultured until 24th day.

inoculated with *Salmonella oranienburg*, and approximately 19 per cent inoculated with *Salmonella kentucky*, no recoveries being made from eggs inoculated with *Salmonella bareilly*. In each case serological typing of the organism recovered revealed it to be the same as the organism inoculated on the shell.

TEMPERATURE AND HUMIDITY READINGS

It was hoped that daily temperature and humidity reading might yield some information. Nothing significant was apparent from the readings except that the temperature at which the eggs were kept may have been higher than egg storage on the farm. Average temperature and relative humidity readings are given in Table 2.

DISCUSSION

The results of this experiment indicate that three of the four Salmonella used can penetrate the unbroken egg shell. This penetration taking place at least from the seventh to sixteenth day after inoculation. It seems possible that

TABLE 2
AVERAGE TEMPERATURE AND HUMIDITY READINGS

	AVERAGE TEMPERATURES	AVERAGE RELATIVE HUMIDITY	EGG PENETRATION
<i>S. kentucky</i>	73(72-76)*	42(31-57)	26/135
<i>S. bareilly</i>	69(66-72)	61(48-73)	0/133
<i>S. oranienburg</i>	71(70-72)	20(19-21)	4/140
<i>S. typhimurium</i>	73(70-78)	24(21-25)	11/137
<i>S. typhimurium</i>	69(66-72)	19(14-28)	11/140

*Maximum and Minimum Temperatures.

penetration may continue as long as the organism remains viable on the shell. The inoculum was heavy in this case to give every opportunity for penetration but there is every possibility of eggs being contaminated by infected birds naturally and so introducing Salmonella organisms into hatching eggs. The degree of contamination should have a bearing on the percentage of eggs penetrated. The methods employed should give the maximum penetration under conditions used.

It would appear that a method of disinfecting the shells of hatching eggs shortly after laying should preclude Salmonella penetration during storage and incubation. This could reduce the number of Paratyphoid outbreaks in chicks and poults as the only source of infection would be the Salmonella actually within the egg when laid. The disinfectant used would have to be efficient and yet sufficiently mild to ensure it did no harm to the hatchability of the egg itself.

No explanation can be given for the lack of egg penetration in the case of *Salmonella bareilly*, unless this organism is not as actively motile as the others or a "weak" strain was used in this experiment. Death of the organism is ruled out by the isolation of *Salmonella bareilly* from the outside of an unsterilized egg on the 16th day after inoculation. The other organisms, too, were viable on untreated eggs on the 16th day of the experiment.

Washing and dipping eggs for one hour in 450 ppm Roccal solution appeared quite effective in ridding the shells of the organisms even before painting with merthiolate solution. For example, in the test of *Samonella kentucky*, the

shell of each of the 135 eggs were cultured by swabbing after the Roccal treatment and this organism was recovered from only one egg shell.

The condition and quality of the shell may be a factor favouring, or assisting to inhibit, *Salmonella* penetrations. The eggs used in these experiments were all fresh market quality eggs.

The possibility of human *Salmonella* infection from the consumption of raw eggs, or raw egg products contaminated by *Salmonella* through egg penetration, is also present.

SUMMARY

An experiment in the penetration of the shells of commercial grade eggs by *Salmonella* between the 6th and 16th day after inoculation, and stored at room temperature is given. Results indicating a shell penetration of approximately 8 per cent for *Salmonella typhimurium*, 3 per cent for *Salmonella oranienburg*, and 19 per cent for *Salmonella kentucky* was found in this test. No egg penetration was found when *Salmonella bareilly* was used.

The possibility of shell disinfection of hatching eggs to preclude shell penetration of *Salmonella* is discussed. The possible transmission to humans through the shell penetration of commercial eggs is also mentioned.

ACKNOWLEDGEMENTS

The authors would like to express their thanks to Mr. F. J. Higginson, Poultry Commissioner, and Mr. T. Hughs, Poultry Plant Manager, Alberta Poultry Branch, for supplying the eggs used in this experiment; also to Drs. Ballantyne, Wilton, Weir and O'Donoghue for suggestions and encouragement, and to Dr. R. D. Stuart and Miss M. Findlayson of Provincial Public Health Laboratory for serological checking on *Salmonella* types isolated.

REFERENCES

- 1Annual Reports of Veterinary Services Branch for the Province of Alberta, 1948, 1949, 1950, 1951.
 - 2Beister, H. E. and Schwarte, H. L., *Diseases of Poultry*—second edition 1948.
-