QUEENSLAND DEPARTMENT OF PRIMARY INDUSTRIES
DIVISION OF ANIMAL INDUSTRY BULLETIN No. 161

OCCURRENCE OF SALMONELLAS IN RAW AND PASTEURIZED LIQUID WHOLE EGG

by B. PEEL, B.Agr.Sc.

SUMMARY

The presence of salmonellas was determined in 8 691 samples taken from various stages in the manufacture of liquid whole egg as well as from the factory environment. Salmonellas were found in 351 samples and included 29 serotypes. Samples of bulked unpasteurized liquid egg had a much higher incidence of salmonellas (15·3%) than the individual eggs from which they were prepared (0·2%). The occurrence of these organisms in liquid whole egg did not appear to be related to the degree of bacteriological contamination of the product. Pasteurization of whole egg reduced the incidence of salmonellas from 20·6% in the samples of unpasteurized egg to 0·04% in the samples of the pasteurized product.

Of 888 samples used in a comparison of a liquid enrichment-solid medium method with a selective motility medium, 216 samples were found to be positive. Of these positive samples, 92% were positive by the selective motility medium method and only 56% by the liquid enrichment-solid medium method.

I. INTRODUCTION

Salmonella organisms are of particular concern to the poultry industry. Chickens are frequently intestinal carriers of these organisms (Buxton and Gordon 1947; Simmons and Byrnes 1972). Eggs become infected with salmonellas other than *Salmonella pullorum* from shell contamination rather than from direct ovarian transmission (Mundt and Tugwell 1958; Ross *et al.* 1964; Mellor and Banwart 1965). These organisms may remain viable on the shells for considerable periods (Buxton and Gordon 1947). Simmons *et al.* (1970) showed that, under favourable conditions, salmonella organisms can pass immediately through the external structures of the egg without prior digestion of the membranes. Because of the potential public health risk, the control of these organisms is of great importance during the manufacture of egg products.

Salmonella typhi-murium and *S. thompson* were isolated from 4.2% of liquid whole egg by Knowles (1953), and Hobbs and Smith (1955) isolated salmonellas from 34 (27%) of 128 tins of imported whole egg products. The Public Health Laboratory Service of Great Britain (Report 1958) examined 8 962 samples of

frozen whole egg being imported from a number of countries and isolated salmonellas from 43.4% of the samples from Australia. However, 77% of the strains isolated were *S. pullorum*. When this serotype was excluded, the rate of contamination of Australian frozen whole egg was 5%. Salmonella organisms were detected in 27 of 114 samples of frozen eggs by Thatcher and Montford (1962).

This paper reports on the influence of egg quality and manufacturing procedures on the incidence of salmonella contamination in liquid whole egg.

II. MATERIALS AND METHODS

Manufacturing procedure

The normal sequence of operations in the manufacture of liquid whole egg was as follows. Two methods of removing the egg contents from the shell were in use—Vinall machines and vacuum extractors.* During the Vinall machine operation, sound-shelled eggs were washed in water at approximately 64.4°C for approximately 75 s after which they were sprayed with water at approximately 82°C, dried and finally crushed in bulk. Vacuum extraction was usually used for the removal of egg contents of unwashed cracked or leaking eggs. Egg contents from both processes were collected in a tip tank while the shells were fed out of the premises into refuse bins. The liquid egg was pumped through a filter, cooled to approximately 3.3°C and discharged into a bulk storage tank to await pasteurization. Export quality liquid whole egg was manufactured from first quality shell eggs while the local quality product was manufactured largely from second-quality shell eggs.

During the pasteurization process the egg passed successively through (a) the regenerative section where the raw egg on one side of the heat exchange plates was heated by the hot pasteurized egg on the other side; (b) the heating section which heated the egg to slightly above 64.4°C; (c) the holding section which maintained the egg at 64.4°C for approximately 3.75 min; (d) the regenerative section where it was partially cooled by the raw incoming egg; and (e) the cooling section where it was cooled to approximately 3.3°C.

The pasteurized egg then flowed to a storage tank or directly to the can filling section.

Origin of samples

An egg processing factory producing whole egg was investigated during the period from March 1968 to October 1970. Samples were taken from the shell eggs, unpasteurized product, pasteurized product and the factory environment.

Sampling procedures

The unpasteurized liquid whole egg samples were taken from the Vinall and vacuum extraction egg breaking machines, bulk tank (export use) and bulk tank (local use) while the samples of pasteurized whole egg originated from the frozen tins, bulk tanks (local use) and cream cans (local use). Tins of farm-produced unpasteurized whole egg were sampled after their arrival at the factory. Samples of liquid whole egg were taken from the thoroughly mixed product using sterile dippers. The tins of frozen whole egg to be sampled were allowed to thaw partially and the lids removed after flame sterilization. The samples were taken with sterile stainless steel triers. All samples were placed in sterile containers.

*The use of these machines has now been discontinued.

The shells of badly soiled and cracked eggs were swabbed using cotton swabs moistened with the selective enrichment medium. The egg was then washed in warm running water and allowed to dry. After dipping the eggs in alcohol and the excess alcohol on the shells had been ignited to flame the surface, the contents were aseptically transferred to the selective enrichment medium.

Samples of shell and water were taken from the shell disposal bin and the grease trap immediately outside the factory processing area.

Detection of salmonellas

Tetrathionate broth (Oxoid) was used as the selective enrichment medium. Pasteurized and unpasteurized whole egg (50 g), water (50 ml) or egg shell material (30 g) from the shell bin or grease trap were added to 200 ml of tetrathionate broth. Each swab was added to 10 ml of tetrathionate broth. The selective enrichment medium was streaked onto plates of bismuth sulphite agar (Oxoid), Salmonella-Shigella agar (Oxoid) and desoxycholate citrate agar (Oxoid) after 24 h and onto bismuth sulphite agar after 48 h incubation at 37°C. The plates were incubated at 37°C. Three suspect salmonella colonies were transferred from each plate to tubes of triple sugar iron agar (TSI) (Oxoid). Those cultures producing salmonella like reactions in TSI agar were checked for purity and tested for the following properties: motility, fermentation of lactose, sucrose, salicin, glucose, mannital, utilization of citrate, and production of H_2S, indole, urease, lysine decarboxylase and beta-galactosidase. Finally slide agglutination tests were performed using Salmonella polyvalent 'O' and 'H' antisera (Burroughs Wellcome, England). Serological typing was done by the Salmonella Reference Laboratory, Institute of Medical and Veterinary Science, Adelaide.

During the investigation, the selective motility medium described by Harper and Shortridge (1969) was compared to the above method for the isolation of salmonellas, by inoculating 0.1 ml of the 24 h tetrathionate culture into the inner tube of the medium. Sub-cultures from the outer surface were made onto the plates of selective media. Suspicious colonies were tested biochemically and serologically to determine if they were salmonellas.

Total count

The total bacterial counts of the samples were determined by plating onto plate count agar (Oxoid) and incubating at 30°C for 72 h.

Resazurin reductase test

To determine the microbiological quality of raw liquid egg, 2 ml of fresh 0.005% resazurin (BDH Chemicals, England) solution was added to 10 ml of egg which was then incubated at 30°C. The time taken for the test to change from blue to pink was recorded.

III. RESULTS

Over a period of 3 years, 7 758 samples of whole egg were tested for the presence of salmonellas. Table I summarizes the results of the incidence of salmonellas in whole egg taken at various stages of the processing operations. While the incidence of salmonellas varied from month to month no definite seasonal effect was obvious. The total bacterial count of the samples of unpasteurized whole egg taken from the Vinall machine ranged from less than 300 to 28×10^3 g^{-1} with a mean of 750 g^{-1}. However, 21 of the 207 samples from this origin contained salmonellas. The mean total bacterial count of the whole egg taken from the vacuum extractors was 29×10^4 g^{-1}. One count of 5×10^6 g^{-1} was recorded. Salmonellas were present in 28 of the 186 samples taken from the vacuum extractors.

TABLE 1

INCIDENCE OF SALMONELLAS IN WHOLE EGG AT VARIOUS STAGES OF PRODUCTION

	Unpasteurized								Pasteurized			
	Vinall Machines		Vacuum Extractors		Bulk Tank (Export Use)		Bulk Tank (Local Use)		Export		Local	
	No. of samples	Positives (%)	No. of samples	Positives (%)	No. of samples	Positives (%)	No. of samples	Positives (%)	No. of samples	Positives (%)	No. of samples	Positives (%)
Mar–Dec 1968 ..	..	..	..	..	58	10·3	202	14·4	1,327	0	..	..
Jan–Dec 1969 ..	21	28·6	21	19	277	19·9	176	17·6	1,713	0·1	..	..
Jan–Oct 1970 ..	186	8·1	165	14·5	189	28·0	244	38·5	2,048	0	560	0
Total	207	10·1	186	15·1	524	21·8	622	24·8	5,088	0·04	560	0

TABLE 2

INCIDENCE OF SALMONELLAS IN UNPASTEURIZED WHOLE EGG MANUFACTURED ON FARMS

Farm	Isolates (No. sampled)	
1	0	(76)
2	0	(4)
3	1	(41)
4	0	(14)
5	0	(42)
6	2	(22)
7	0	(8)
8	0	(108)
9	0	(34)
10	1	(85)
11	1	(28)
12	0	(50)
13	0	(7)
14	0	(36)
15	1	(2)
16	0	(14)
Total	6	(571)

Of the 524 samples of unpasteurized whole egg taken from the bulk storage tank (export use), 114 contained salmonellas. The mean total bacterial count of these samples was 62 x 10^3 g^{-1}. Resazurin tests on the unpasteurized whole egg from the bulk storage tank (local use) indicated that 66·9% of the samples contained less than 2 x 10^4 g^{-1} whole 5·5% contained greater than 3 x 10^6 g^{-1}. Of the 622 samples of unpasteurized whole egg taken from the bulk storage tank (local use), 154 contained salmonellas. Of the total of 1 539 samples taken from all sites before pasteurization, 317 (20·6%) were positive for salmonella.

Salmonellas were isolated from only two of the 5 648 samples of pasteurized whole egg. The standard plate counts of the pasteurized product showed that 97·5% contained fewer than 100 bacteria g^{-1} and only 13 contained greater than 1 x 10^3 bacteria g^{-1}.

Salmonellas were present in six of the 571 samples of unpasteurized whole egg which was produced on farms (table 2). These positive samples originated from five of the 16 farms.

Examination of 336 cracked and 511 soiled eggs resulted in the isolation of *S. anatum* and *S. singapore* from the shell of two soiled eggs. Salmonellas were not detected in the contents of the 847 eggs or in the shells of 336 cracked eggs.

The material taken from the shell disposal bin was positive in seven of the 11 samples of shell fragments. Water draining from the shell disposal bin was positive in eight of 35 samples. Salmonellas were isolated from 11 of the 40 samples of shell material taken from the grease trap.

The origin of the 29 salmonella serotypes isolated are given in table 3. *S. typhi-murium* represented 63% of the 358 strains isolated. *S. pullorum* was isolated on only one occasion. *S. cholerae-suis* v. *kunzendorf* was isolated from farm-produced liquid whole egg but was not found in whole egg in the factory.

TABLE 3

ORIGIN OF SALMONELLA SEROTYPES

Organism	Unpasteurized Whole Egg					Pasteurized Whole Egg	Factory Environment	Total
	Vinall Machines	Vacuum Extractors	Bulk Tank (Export Use)	Bulk Tank (Local Use)	Produced on Farms			
S. adelaide	..	..	2	4	1	..	1	8
S. anatum	..	4	9	8	..	..	3	24
S. birkenhead	..	..	..	1	..	..	..	1
S. bovis-morbificans	..	..	1	4	..	..	..	5
S. bredeney	..	..	1	2	..	..	..	3
S. chester	..	..	3	..	..	..	..	3
S. choleraesuis var kunzendorf	..	..	..	..	2	..	..	2
S. derby	1	..	..	3	..	..	..	4
S. give	..	..	1	1	..	..	..	2
S. havana	..	1	2	2	..	..	..	5
S. hessarek var 27	..	..	4	1	..	..	..	5
S. kottbus	..	..	1	1	2	..	..	4
S. manila	..	1	..	..	..	..	..	1
S. meleagridis	..	1	..	..	..	..	..	1
S. newbrunswick	..	..	..	1	..	..	..	1
S. newington	..	..	..	1	..	..	..	1
S. newport	..	..	..	1	..	..	..	1
S. onderstepoort	..	..	..	2	..	..	..	2
S. oranienburg	1	..	4	6	..	..	3	14
S. potsdam	1	..	..	1	..	..	..	2
S. pullorum	..	..	1	..	..	..	..	1
S. rubislaw	..	..	..	1	..	..	1	2
S. saint-paul	..	..	..	9	..	..	3	12
S. senftenberg	..	1	1	2	..	..	..	4
S. singapore	1	..	5	13	..	..	..	19
S. taxony	..	..	1	..	..	..	..	1
S. tennessee	..	..	..	3	..	..	..	3
S. typhi-murimu	17	20	79	88	3	2	17	226
S. untypable	..	..	..	1	..	..	..	1
	21	28	115	156	8	2	28	358

In the comparison of the liquid enrichment-solid media (L-S) method with the liquid enrichment-selective motility tube-solid media (L-T-S) method, salmonellas were isolated from 216 of the 888 samples examined. There were 105 samples from which salmonellas were isolated by both methods and 672 samples from which salmonellas were not isolated by either method. Salmonellas were isolated from 16 samples by the L-S method only and from 95 samples by the L-T-S method only. Salmonellas were isolated from 92% of the positive samples by means of the L-T-S method while the L-S method isolated the organism from only 56% of the positive samples. More than 30% of these samples would have been reported as containing no salmonellas if the selective motility medium had not been incorporated in the testing procedure. On four occasions, the L-T-S method isolated two different salmonella serotypes from the one sample. On two occasions, a different serotype was isolated by the L-T-S method to that by the L-S method.

IV. DISCUSSION

It has been shown that unpasteurized liquid whole egg is frequently contaminated with salmonella organisms. Of the unpasteurized liquid whole egg samples taken at the factory, 20·6% were positive for salmonellas. Although this figure is higher than those reported by Knowles (1953) and Salowey *et al.* (1946), it is in general agreement with those of Hobbs and Smith (1955) and Thatcher and Montford (1962) and, in fact, lower than that reported by Garibaldi *et al.* (1969). The marked seasonal effects on the incidence of salmonellas as reported by Garibaldi *et al.* (1969) were not obvious in the present investigation.

Bierer and Barnett (1965) showed that the washing of eggs in water at 150°F (65·5°C) for 3 min was sufficient to kill *S. pullorum, S. gallinarum* and *S. typhi-murium* on the shells. They claimed that washing of eggs at 150°F (65·5°C) for either 1 or 3 min would have an application in the control of salmonellas during the manufacture of egg products. The incidence of salmonellas in the samples of liquid whole egg taken from the Vinall machines was therefore surprising. It was expected that the shell treatment in the water bath would have produced a lower incidence. It is possible that the incidence of salmonellas could be reduced if the time of washing in the Vinall machine was increased to 2 to 2·5 min instead of the 1·25 min used.

Salowey *et al.* (1946) and Kraft *et al.* (1967) reported a higher incidence of salmonellas in liquid whole egg produced from dirty eggs than in that from clean eggs. In the present survey, although the total bacterial contamination of the liquid whole egg (local use) was much higher than that of the liquid whole egg (export use), there was little difference in the incidence of salmonellas in the samples from either source. The total bacterial counts of the samples of liquid whole egg taken from the Vinall machine were very low, and yet 10% of these samples contained salmonellas. Therefore, a low bacterial count does not necessarily indicate the absence of pathogenic bacteria.

In an examination of English egg products (Report 1959), it was shown that egg products from small producers appeared less likely to be contaminated with salmonellas than those from large producers. This was found also to be the case in the present study. The incidence of salmonellas in liquid whole egg produced on farms was only 1·1%. Only two shells of the 847 eggs examined harboured salmonella organisms. While the chances of finding salmonellas in or on an individual hen's egg is quite small, examination of a mixture of

thousands of eggs, such as used for processing, very often reveals the presence of these organisms. The results of this investigation suggest that the probability of isolating salmonellas increases as the volume of liquid whole egg, from which the sample is taken, increases. A solution to this problem appears to be the elimination of salmonella contamination of eggs at the farm level.

Through the receipt and handling of salmonella contaminated eggs there is a possibility of contaminating the factory environment. Bearing in mind the capacity of salmonellas to remain viable for long periods, the importance of careful cleaning, sanitation and vermin control is obvious.

The efficiency of pasteurization is illustrated by the fact that only two of more than 5 500 samples of pasteurized liquid whole egg examined contained salmonella organisms. Through pasteurization, the likelihood of egg products being responsible for the spread of salmonella organisms is almost eliminated.

The incorporation of the selective motility medium into the procedure for the examination of liquid egg for the presence of *Salmonella* resulted in a significantly higher isolation rate. The performance of the selective motility medium during this study on whole egg was comparable to that described by Harper and Shortridge (1969) when testing faecal specimens.

V. ADDENDUM

Subsequent to the study described in this paper, the factory has reduced the holding time of the pasteurizer from 3 min 45 s to 3 mins 5 s.

VI. ACKNOWLEDGEMENTS

Acknowledgement is made to the South Queensland Egg Marketing Board for facilities given to carry out investigational work at their factory. The author is indebted to the staff of the laboratory at the South Queensland Egg Marketing Board for technical assistance and to the Salmonella Reference Laboratory, Adelaide, for identifying the salmonella cultures.

REFERENCES

BIERER, B. W., and BARNETT, B. D. (1965).—Killing Salmonella on eggshells by increasing wash water temperature. *J. Am. vet. med. Ass.* 146:735-6.

BUXTON, A., and GORDON, R. F. (1947).—The epidemiology and control of *Salmonella thompson* infection in fowls. *J. Hyg., Camb.* 45:265-81.

GARIBALDI, J. A., LINEWEAVER, H., and IJICHI, K. (1969).—Number of *Salmonellae* in commercially broken eggs before pasteurisation. *Poult. Sci.* 48:1096-101.

HARPER, J., and SHORTRIDGE, K. F. (1969).—A selective motility medium for routine isolation of Salmonella. *J. Hyg., Camb.* 67:181-6.

HOBBS, B. C., and SMITH, M. E. (1955).—Outbreaks of paratyphoid B fever associated with imported frozen egg. II Bacteriology. *Proc. Soc. appl. Bact.* 18:471-7.

KNOWLES, N. R. (1953).—Observations on the microbiology of raw and heat-treated liquid egg. *Proc. Soc. appl. Bact.* 16:107-18.

KRAFT, A. A., TERREY, G. S., AYRES, J. C., and FORSYTHE, R. H. (1967).—Factors influencing bacterial contamination of commercially produced liquid egg. *Poult. Sci.* 46:1204-10.

MELLER, D. B., and BANWART, G. J. (1965).—*Salmonella derby* contamination of eggs from inoculated hens. *J. Food Sci.* 30:330-6.

MUNDT, J. O., and TUGWELL, R. L. (1958).—The relationship of the chicken egg to selected paratyphoids. *Poult. Sci.* 37:415-20.

REPORT (1958).—The contamination of egg products with *Salmonellae* with particular reference to *Salmonella paratyphi* B. *Mon. Bull. Minist. Hlth* 17:36-51.

REPORT (1959).—The examination of English egg products. A report of the Public Health Laboratory Service. *Mon. Bull. Minist. Hlth* 18:158-61.

ROSS, W. J., FORSYTHE, R. H., AYRES, J. C., HAGLUND, J. R., and KRAFT, A. A. (1964).—Salmonella recovery from fresh eggs laid by artificially infected hens. *Poult. Sci.* 43:1357.

SALOWEY, M., SPAULDING, E. H., and GOERESLINE, H. E. (1946).—An investigation of a source and mode of entry of Salmonella organisms in spray-dried whole egg powder. *Fd Res.* 11:380-90.

SIMMONS, E. R., AYRES, J. C., and KRAFT, A. A. (1970).—Effect of moisture and temperature on ability of Salmonellae to infect shell eggs. *Poult. Sci.* 49:761-8.

SIMMONS, G. C., and BYRNES, R. V. (1972).—The origin of salmonellas in chickens and chicken carcases. *Aust. vet. J.* 48:186-9.

THATCHER, F. S., and MONTFORD, J. (1962).—Egg products as a source of *Salmonellae* in processed foods. *Can. J. publ. Hlth* 53:61-9.

(Received for publication 12 March 1975)

The author is a bacteriologist in the Pathology Branch at the Animal Research Institute, Queensland Department of Primary Industries, Yeerongpilly, Q. 4105.