ACM/587

ADVISORY COMMITTEE ON THE MICROBIOLOGICAL SAFETY OF FOOD

SURVEILLANCE WORKING GROUP

1. In connection with the planned Food Standards Agency survey of *Salmonella* contamination of UK-produced shell eggs, the ACMSF's Surveillance Group commented on an ADAS validation for a protocol for the isolation of *Salmonella* from external shell surfaces of eggs. This was regarded as important if the planned survey was to be able to differentiate shell from contents contamination. A copy of the Surveillance Working Group's comments is attached.

2. Having consulted widely, the FSA concluded that no technique was currently available which would effectively differentiate between *Salmonella* contamination on the shell and that in the contents. The Agency therefore proposes to adopt the same method as was used in the 1995/96 Department of Health-funded survey carried out by the Public Health Laboratory Service. Although it will not be possible to be certain that *Salmonella* on shell surfaces will not contaminate egg contents at breaking, this is likely to be a rare event, and the proposed protocol will enable an indication to be obtained of the relative proportion of contamination on the shell or in the contents. It will also enable a direct comparison to be made with results from the 1995/96 survey.

Secretariat
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Advisory Committee on the Microbiological Safety of Food

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Dear Sonia

FSA SURVEY OF *SALMONELLA* CONTAMINATION OF UK-PRODUCED SHELL EGGS

1. Thank you for sending me a copy of the ADAS validation for a protocol to determine salmonellae on external shell surfaces of eggs. As requested, I have consulted the ACMSF Surveillance Working Group. The feeling of the Group is that the ADAS work does not advance matters and that the results do not accord with those from previous trials. The Group's detailed comments appear below.

Shell penetration

2. In general, when eggs are contaminated naturally, the source is either faecal contamination or, rarely, contamination in the shell gland. Chicken faeces are a quite dry material and *Salmonella* is unlikely to get into the pores of eggs with any regularity. The protocol chosen by ADAS appears unrealistic, given that immersion in broth would have allowed *Salmonella* to invade deeply into the pores. Moreover, the use of such high numbers of organisms would also have facilitated the crossing of the shell membrane. If salmonellae were present in the pores, they may have been protected from the alcohol. Table 2 data show that 45 of 50 eggs were contaminated internally after alcohol immersion. Even with "low" levels of inoculum, 36 of 50 eggs were positive for *Salmonella* (Table 4). It should also be borne in mind that shell contamination will occur as a discrete event on one area of the shell, rather than all over the shell. **Nascimento** *et al* (1992)[1] showed that egg shells have defined areas which will differ in shell quality and membrane cover. The literature quoted in Annex A suggests that the

ADAS technique would have allowed the contamination of egg contents during egg immersion, thus explaining why so many eggs were found to be contaminated in their contents after disinfection. In addition to the problems caused by the method of contamination, the numbers used were much too high at c. 100,000 and c. 10,000 cells of *Salmonella* per egg. These levels of contamination would not be seen on the shells of naturally-contaminated eggs because the environment is quite hostile.

Shell disinfection

3. This is a tricky area. The issue is not whether all of the *Salmonella* from the shell can be recovered but whether enough can be removed to allow subsequent detection. Thus, the numbers given in the ADAS report are misleading. Surprisingly, there are relatively few published papers on this. Professor Humphrey's initial work at Exeter used sterile faeces contaminated with *S. enteritidis*. The faeces were allowed to dry, as much faecal material as possible was removed, and the egg was swabbed and then immersed in 70% ethanol for 5 minutes. *S. enteritidis* was not obtained from either egg contents or disinfected shells.

4. **Himathongkham** *et al* (1999)[2] compared a variety of methods for shell disinfection and used essentially the same method as ADAS. Perhaps not surprisingly, they also found that ethanol was not wholly efficient and that the only method that disinfected all shells was immersion in boiling water for 3 seconds. However, this sometimes caused eggs to crack.

5. **Gast RK** (1993)[3] also explored this and compared immersion in iodine with immersion in boiling water for 5 seconds. As with the Himathongkham study, boiling eliminated all *Salmonella* on egg shells. In Gast's study, eggs were also contaminated by dipping in broth containing *Salmonella*.

6. If ADAS are to sample eggs by immersion, they will need to disinfect by immersion in boiling water as this will reach bacterial cells in the pores. A better technique would be to swab the eggs, as the aim is to establish presence/absence, not to recover large numbers.

7. There remains the problem of what to do with the shells post-swabbing and disinfection. Disregarding them could result in *Salmonella* being missed, although safeguarding against that eventuality would mean that the study would be out of step with other similar studies undertaken in the past. A possible compromise would be to add disinfected shells to the shell swabs.

8. Another option would be to swab the surface and test this separately from the contents (and discard the shell). Considering the problem pragmatically, one risk comes from the outside of the egg when handled (this will be assessed by testing the swabbed surface). A

second risk comes from what might be consumed when the egg contents are eaten (which will be assessed by testing the contents). What is left in the pores and membrane at the time of eating the egg is not likely to impact on its safety, and it is unlikely that this will occur without the contamination also being found on the external shell or in the contents. That brings into question the justification for testing the shell after swabbing.

9. To summarise, the Surveillance Working Group's preference would be to go for swabbing of the exterior shell using a sterile sponge or ball of cotton wool. Under no circumstances should a rinse technique be used as this risks introducing *Salmonella* into the egg contents. The Group is confident that using a big swab for each batch of 6 eggs will recover *Salmonella* from the exterior shell. Immersion in 70% ethanol will successfully eliminate *Salmonella* naturally present on egg shells. As an alternative, the Group would be content with the use of immersion in boiling water for 5 seconds. Shells should be discarded rather than cultured.

10. I hope you will find the above comments helpful.

Yours sincerely

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Administrative Secretary

cc : Professor Humphrey
Professor Gasson
Mrs Jefford
Mr Kyriakides
Dr O'Brien
Ms Hoad

ANNEX A

Shell penetration

Nascimento *et al* (1992)[1] found that the cuticular layer on egg shells was rarely present as an even covering over the shell surface and that shell membranes are invariably pitted with holes larger than bacterial dimensions.

Sauter *et al* (1979)[4] showed that *Salmonella* could be isolated with regularity from inside the shell membrane when eggs at 22°C were immersed in broth at 4°C. Thus, 42% of samples were positive for *Salmonella typhimurium*. This technique clearly exaggerates the ingress of contaminated liquid because chilled broth was used. However, if the broth is at any temperature below that of the egg, the egg contents will contract, forming a partial vacuum which will draw liquid into the egg.

Peel and Simmons (1976)[5] found essentially the same as the above workers.

Javed *et al* (1994)[6] did a rather more sophisticated study. They applied 2 dyes which identified areas of possible penetration on egg shells. These areas were then challenged with a variety of *Salmonella* strains. With the most vulnerable areas, 30% of eggs showed penetration through the cuticle, shell and inner and outer shell membranes.

Miyamoto *et al* (1998)[7] also demonstrated shell penetration using the egg immersion method.

Padron (1995)[8] examined water uptake during immersion and found that it occurred with many eggs.

ANNEX B

REFERENCES

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7. Miyamoto T, Horie T, Baba E, Sasai K, Fukata T, Arakawa A. *Salmonella* penetration through egg shell associated with freshness of laid eggs and refrigeration. J Food Prot 1998; **61(3) :** 350-353.

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