Optimising the Recovery of *Salmonella* spp. From Raw Poultry Using Indirect Impediometry.

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BACKGROUND. *Salmonella* infections in Northern Ireland were the main cause of foodborne disease until exceeded by those of *Campylobacter* in 1990. Subsequently there has been a marked increase in campylobacter infections and *Campylobacter* spp. are now the major cause of foodborne infections in the province (Anon. 1995). However *Salmonella* infections remain the second most common cause of foodborne disease and the consequences for the victims can be much more severe than those caused by campylobacters (Doyle, 1990), including death (Doyle and Cliver, 1990). Hence it is essential that methods used for the isolation of salmonellas from food stuffs are as efficient as possible. Optimising culture media and conditions by conventional methodologies is a slow and expensive process due to the considerable amount of staff-hours involved in enumerating bacteria to prepare growth curves for comparison. However Donaghy et al (1993) showed that indirect impediometry (Owens et al, 1989) can be used to rapidly estimate the growth of salmonellas in broths. This work also showed that the results obtained with pure cultures of *Salmonella* spp. were repeated with naturally contaminated meat samples. Thus the speed of impediometry, and simplicity of studies with pure cultures, could allow a rapid comparison of media and incubation temperatures. The optimum conditions determined could then be applied to meat samples.

OBJECTIVES. To isolate salmonellas from meats using selective media, such as Rappaport Vassiliadis broth, the organisms are first allowed to repair any damage and then to proliferate to increase the probability of their being detected (Anon, 1993). Buffered peptone water (BPW) is specified for this purpose and incubated for 16-20h at 35 or 37^{0}C. Using indirect impediometry the growth of pure cultures of salmonellas damaged by heating, freeze-thawing or NaCl in BPW was investigated. The aim was to determine the optimal conditions for the repair of injured salmonellas and then to compare these with the standard method using raw poultry as a meat naturally contaminated with salmonellas.

METHODS. A Rapid Automatic Bacterial Impedance Technique (RABIT) system (Don Whitley Scientific, Shipley, GB) was used for all impediometry. *Salmonella* serotypes were local isolates obtained from a range of animal feed and foodstuffs, or type cultures from NCTC London. All media were supplied by Merck Ltd, Lutterworth, GB, unless otherwise stated. Cultures were maintained on Protect beads (Technical Services Consultants Ltd, Lancaster, GB) at -80^{0}C. Prior to use cultures were grown overnight on nutrient broth (10ml) then harvested by centrifugation (1700g (av.), 10min). The pellet was resuspended in 10ml phosphate buffered saline (PBS) for subsequent use. Three stress regimes were used; i) heating in a water bath at 52^{0}C for one hour followed by cooling on ice (15min), ii) freezing at -80^{0}C for 20min followed by thawing at 25^{0}C for 50min, this cycle being repeated four times, iii) resuspending the pellet noted above in PBS supplemented with 20% (w/v) NaCl and held at 30^{0}C for 2 hours. Damage was estimated by preparing decimal dilutions of cultures and plating simultaneously on tryptone soy agar and brilliant green agar (24h 37^{0}C) to determine total counts and resistant cells respectively.
Indirect impediometry used RABIT cells to which equal volumes of molten 2% (w/v) agar (Oxoid No.1) and 0.7% (w/v) KOH solution were added. The cells were stoppered and left overnight to stabilise. Broths for study were prepared to the manufacturers specifications then dispensed (4.5ml) into sterile glass tubes (12x75mm). The inoculation volume was 0.1ml and the detection criterion for the RABIT was -20μS, with impedance values logged every 6 min for 24h.
To compare recovery systems retail packs of poultry were purchased in batches of 10 and stored overnight (4^{0}C). Next morning the skin was removed and finely chopped with scissors and mixed to homogeneity. Identical subsamples (25g) were abstracted for addition to BPW and after appropriate incubation 0.1ml was inoculated into RV broth for incubation (42^{0}C, 24h) as previously described (Donaghy and Madden, 1993). Cultures detected as positive by the RABIT were streaked onto brilliant green agar and Rambach agar for incubation (37^{0}C, 24h).

RESULTS AND DISCUSSIONS. Studies with the stressed salmonellas (4 serotypes) showed that the lethality of the regimes decreased in the order heating>freeze/thaw>salt (Fig. 1). Impedance studies were then undertaken with BPW being inoculated with decimal dilutions (0.1ml) of the stressed cells in PBS (10^{-1} to 10^{-4}). The effects of the incubation temperature on recovery was studied over the range 35-45 ^{0}C at 2^0 intervals. No injured cells grew at 45^{0}C hence no data is presented. It was also found that growth was significantly slower at 35^{0}C than at 37^{0}C hence only data derived from incubations in the range 37-43^{0}C is presented, Fig. 2-4. The time to detection (TTD) is the time taken from starting the incubation in the RABIT to the detection criteria being met. In this case about 10^7 cfu/ml salmonellae in the BPW will cause detection. Thus rapid repair and subsequent growth will give a short TTD and the growth conditions which allow rapid repair and growth are chosen by simply selecting those parameters giving the smallest TTD values. Since RABIT results are available within hours this allows a large number of parameters to be screened. In this case the effects of three physiological stresses on four *Salmonella* serotypes (in replicate) were investigated at four temperatures.
With freeze/thaw and salt stress incubation at 37-41^{0}C the TTD results are not significantly different but the results were significantly higher at 43^{0}C. Statistical analysis showed that the growth rate was highest at 39^{0}C but the lag phase was shortest at 41^{0}C. With the heat stress 39^{0}C gave the best recoveries hence it would appear that the optimum incubation temperature of BPW for recovery of *Salmonella* spp. is dependant on the stress the salmonellas have undergone. Based on the above results a recovery temperature of 41^{0}C was investigated using chilled retail packs of poultry as a naturally contaminated foodstuff.
Overall 100 packs of poultry were sampled, in duplicate. Using the conventional repair/recovery protocol of incubation at 37^{0}C 15 sub-samples were found to be positive representing a total of 12 samples. However the protocol based on the impediometric analysis

revealed 39 sub-samples to be positive, representing 25 positive packs. Thus the new recovery system was markedly more efficient for the detection of *Salmonella* spp. The serotypes isolated were *S. hadar* (12), *S. heidelberg* (5), *S. liverpool* (19), *S. newport* (3), *S. senftenberg* (1) and *S. virchow* (6). Numbers in parentheses are the number of sub-samples from which the isolates were obtained.

CONCLUSIONS. Indirect impediometry proved a rapid and effective means of studying the effects of temperature on the growth of injured salmonellas in buffered peptone water. For heat injured cells 39^{0}C was optimal but for salt and freeze/thaw stress 41^{0}C was best. This allowed the design of an improved recovery stage in salmonella isolation, which was tested with 100 samples of retail packs of raw chilled chicken, in duplicate. The results clearly showed that allowing salmonellas to repair at 41^{0}C rather than 37^{0}C resulted in recoveries of salmonellas more than doubling. Hence repair at 41^{0}C is proposed for samples in which salmonellas have not suffered heat stress, such as raw meats.

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REFERENCES.

1. Anonymous (1993) Methods for microbial examination of food and animal feeding stuffs. Part 4. Detection of *Salmonella*. BS5763. British Standards Institute.
2. Anonymous (1995) The health of the public in Northern Ireland. DHSS. HMSO, Belfast, Northern Ireland.
3. Donaghy, J.A. and Madden, R.H. (1993). Detection of salmonella in animal protein by Rappaport Vassiliadis broth using indirect impediometry. International Journal of Food Microbiology. 17(4):281-288.
4. Doyle, M.P. (1990) *Campylobacter jejuni* In *Foodborne Diseases* ed. Cliver, D.O. pp. 217-22. San Diego : Academic Press.
5. Doyle, M.P., and Cliver, D.O. (1990) *Salmonella* In *Foodborne Diseases* ed. Cliver, D.O. pp. 186-204. San Diego : Academic Press.
6. Owens, J.D., Thomas, D.S., Thompson, P.S., & Timmerman, J.W. (1989) Indirect conductimetry: a novel approach to the conductimetric enumeration of microbial populations. Letters in Applied Microbiology. 9:245-249.

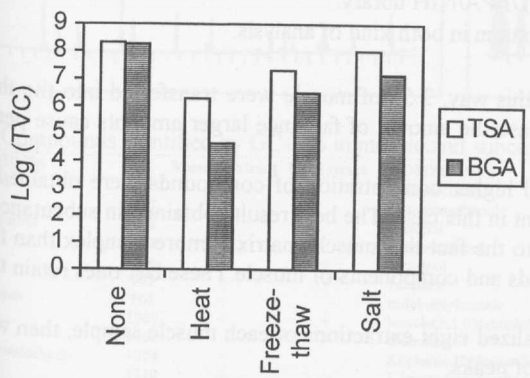

Fig 1. Effect of three stress regimes on 8 *Salmonella* serotypes, assessed by growth on 2 media.

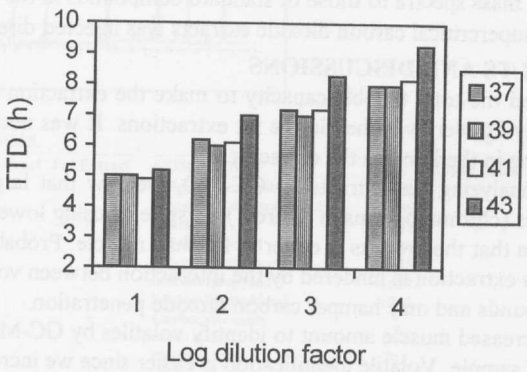

Fig 2. Effect of freez/thaw stress regime on subsequent growth 4 *Salmonella* serotypes in BPW at four temperatures.

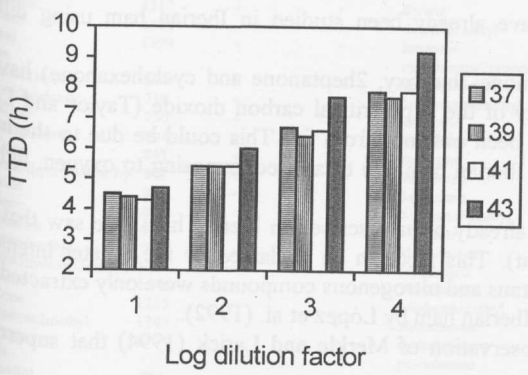

Fig 3. Effect of salt stress regime on subsequent growth of 4 *Salmonella* serotypes in BPW at four temperatures.

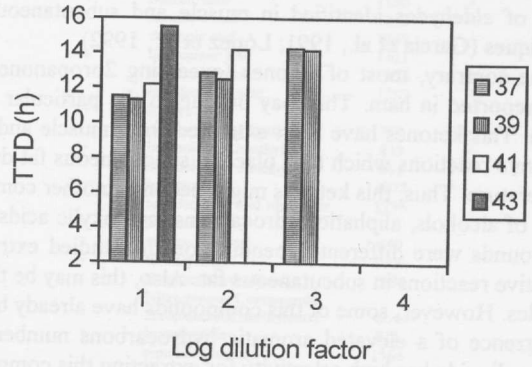

Fig 4. Effect of heat stress regime on subsequent growth of 4 *Salmonella* serotypes in BPW at four temperatures.