Comparison of two enrichment schemes for qualitative recovery of *Salmonella* serovar Choleraesuis from rectal swabs collected from neonatal and early weaned pigs

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**Introduction**

Traditional bacteriological cultivation of samples for salmonellae typically have involved a preenrichment in buffered peptone water, particularly for samples suspected of harboring low numbers of salmonellae. Many researchers, however, have since dispensed with this initial step (4-6, 13, 17-20), opting instead for direct enrichment in tetraphionate broth, especially for samples suspected of containing nonmotile adapted serotypes. However, direct enrichment in tetraphionate may be too harsh for optimal recovery of *Salmonella* serovar Choleraesuis (3, 16). Thus, GN-Hajna broth has been recommended as a preenrichment broth for samples suspected of containing this host adapted serotype (7) and has been used in several recent studies (1, 4, 8-10). Direct comparative evidence, however, substantiating this recommendation are lacking. Presently, we report findings supporting the use of GN-Hajna as a preenrichment medium for recovery of *Salmonella* Choleraesuis.

**Materials and Methods**

Piglets (n = 12) were infected at two days of age via oral gavage as earlier described (1) with 1.1 x 10^6 colony forming units (CFU) of a novobiocin (NOV) and nalidixic acid (NAL) resistant strain of *Salmonella* Choleraesuis var. *kunzendorf* 3246pp. These piglets were farrowed in a 1.5 m x 2.1 m farrowing crate (Hog Slat Inc., Newton Grove, NC, USA) at the Food Animal Protection Research Lab facilities. At 14 days of age the piglets were weaned and were reared according to typical swine husbandry practices in a concrete floored pen (approximately 6 m^2). Pigs were phase fed rations formulated to meet or exceed NRC requirements (14).

Rectal swabs were collected in duplicate each day and each swab was initially cultured in 5 ml of either GN-Hajna broth (GN-scheme) or tetraphionate broth (TET-scheme). Following this initial step, 100 μl of each was then transferred to 5 ml Rappapor-Vassiladis (RV) broth for further enrichment. Selective differentiation was subsequently accomplished on Brilliant Green agar (BGA) containing 25 μg NOV/ml (BGA/NOV) and on BGA containing both 25 μg NOV/ml and 20 μg NAL/ml (BGA/NOV+NAL). Both plating media were used because a wildtype salmonellae, identified as a strain of *Salmonella* serovar Anatum by the National Veterinary Services Laboratory (NVSL), Ames, IA, was isolated from a fecal sample collected from the maternal dam one week prior to farrowing. The BGA/NOV was used to allow for recovery of wildtype salmonellae whereas the BGA/NOV+NAL was for optimal recovery of our NOV and NAL resistant strain of *Salmonella* Choleraesuis. All incubation steps were carried out at 37°C for 18 to 24 hr. Plates were examined for colonies exhibiting typical salmonellae morphology and suspect colonies were confirmed via serum agglutination using Salmonella Antiserum Poly A I-IV and Group C, Factors 5 and 6.

Representative colonies picked at random were also sent to National Veterinary Services Laboratory, Ames, IA, for serotyping and, except for the strain isolated from the maternal dam, all were confirmed as *Salmonella* Choleraesuis var. *kunzendorf*. Since no other salmonellae serotypes were recovered from any of the above cultured swabs or from tonsil, lung, spleen, ileocolic lymph or cecal specimens collected at necropsy at 87 days of age, we concluded that the piglets were colonized by our challenge strain only. We therefore present only those results obtained via selective differentiation on BGA/NOV+NAL. Necropsy specimens were cultured as described except 1 to 2 g of sample was preenriched in 20 ml GN-Hajna or tetraphionate broth. Logistic regression (15) was used to test for a difference between the two qualitative culture schemes.

Growth characteristics of our NOV and NAL resistant *Salmonella* Choleraesuis and the parent strain from which our mutant was propagated, were compared by inoculating these strains inoculated into porcine fecal suspensions. The fecal suspensions were prepared by mixing (1:1) freshly collected pig feces, determined by subsequent culture to contain no detectable wildtype salmonellae, with anaerobic 200 mM phosphate buffer (pH 6.8). Inoculum concentrations, determined via direct plating on BGA/NOV immediately prior to inoculation, were 2.2 x 10^8 and 2.3 x 10^7 CFU/g, respectively. Once inoculated, serial 10-fold dilutions of each fecal suspension were cultured via the TET-scheme or GN-scheme in triplicate so as to accommodate a 3-sample most probable number test (2), using as a positive result the recovery of salmonellae following selective differentiation.

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Results

Our double antibiotic resistant *Salmonella* Choleraesuis strain was recovered more effectively \((P < 0.10)\) from swabs cultured via the GN-scheme and plated to BGA/NOV+NAL than those cultured via the TET-scheme and similarly plated to BGA/NOV+NAL (Figure 1).

The most probable numbers of our double resistant *Salmonella* Choleraesuis recovered from the fecal suspensions cultured via the GN-scheme and plated to BGA/NOV or BGA/NOV-NAL were \(8.2 \times 10^4\) and \(2.4 \times 10^5\) organisms/g, respectively. For the suspensions cultured via the TET-scheme and then plated to BGA/NOV or BGA/NOV+NAL, the most probable numbers of our mutant strain were \(4.2 \times 10^4\) organisms/g in both cases. The most probable number estimate of the parent strain, plated to BGA/NOV, from the fecal suspensions cultured via the GN-scheme was \(4.2 \times 10^4\) organisms/g and was \(1.5 \times 10^4\) organisms/g when cultured via the TET-scheme.

Discussion

Our present findings support the recommendation that GN-Hajna broth be used as a preenrichment medium for the qualitative recovery of *Salmonella* Choleraesuis (7). Whereas the reliability of shedding data obtained via culture of rectal swabs from field collections has been questioned (11, 12), our challenge strain of *Salmonella* Choleraesuis was readily recovered by both culture schemes, with average daily recoveries exceeding 60% during the first 19 days post challenge. It is likely that additional selectivity was achieved with the use of our BGA medium containing both NOV and NAL since recoveries of our challenge strain were higher when the same cultured specimens were plated to BGA/NOV+NAL than to BGA/NOV (data not shown). Enhanced selectivity of BGA/NOV+NAL is also suggested by higher quantitative recovery, via most probable number estimation, of the challenge strain cultured via the GN-scheme and plated to BGA/NOV+NAL than when plated to BGA/NOV. These results indicate that the actual incidence of *Salmonella* Choleraesuis shedding in our study was underestimated by the use of BGA/NOV and tends to suggest that this likely occurs in field examinations as well.

![Figure 1. Daily incidence of *Salmonella* Choleraesuis shedding following experimental infection of 2 day old piglets.](image-url)
References


