Comparison of Brilliant Green Agar and Hektoen Enteric Agar Media in the Isolation of Salmonellae from Food Products

VELMA Y. L. GOO, GEORGE Q. L. CHING, AND JOHN M. GOOCH
Laboratories Branch, State Department of Health, Honolulu, Hawaii 96813

Received for publication 26 April 1973

Brilliant Green (BG) agar and Hektoen enteric (HE) agar media were compared for their efficiency in isolating salmonellae from various food products. Of the 11,226 food specimens examined, 1,662 (or 14.9%) yielded salmonellae. Of this number, 1,475 (88.7%) were recovered from BG agar and 1,315 (79.1%) were recovered from HE agar media. The results indicate that BG agar is more effective in isolating salmonellae from food products. A smaller subsidiary study showed HE agar to be more selective than BG agar. Four hundred ten specimens yielded 92 nonlactose-fermenting isolants other than salmonellae on BG agar and only 11 such isolants on HE agar.

During the past decade, Hawaii has reported the highest incidence of human salmonellosis to the Center for Disease Control in Atlanta, Ga. (4). Two surveys, conducted in 1960 to 1962 and 1967, on the epidemiological aspects of salmonellosis in Hawaii established that food products played a significant role as the source and in the transmission of salmonellae (2, 3, 5).

A Salmonella surveillance project supported by the U.S. Department of Health, Education, and Welfare from January 1970 to April 1971 investigated the sources and transmission of salmonellae in food products, animal feeds, market equipment, and abattoir environments in Hawaii. A total of 15,071 specimens was examined during this period. Of this number, 11,226 were food samples. Food and food products examined included powdered food products, eggs and egg products, carcasses and viscera of beef, poultry, and pork of intrastate, interstate, and foreign origin. In conjunction with this project, the effectiveness and selectivity of Brilliant Green (BG) and Hektoen enteric (HE) agar media for isolating salmonellae from food products were compared.

MATERIALS AND METHODS

Sampling procedures. The cotton swab method was used in obtaining carcasses and viscera samplings of beef, pork, and poultry. All the meat samplings were obtained by swabbing an area 4 by 4 inches (10.16 by 10.16 cm [2]). In instances where the surfaces of the meat products were semidy, the swabs were moistened with sterile normal saline prior to sampling. A sampling thus obtained was placed directly into a test tube containing 8 ml of Tetrathionate Brilliant Green (TBG) enrichment broth. The inoculated specimen was immediately delivered to the laboratory, assigned a number, and placed in a 37 C incubator.

Laboratory methods. Isolation procedures for foods, feeds, etc., recommended by the Center for Disease Control were followed (1). After 18 to 24 h of incubation, inoculated TBG broth was streaked onto BG and HE agar plates. The BG and HE agar plates were examined after overnight incubation for the presence of Salmonella-like colonies. Three colonies were picked from each suspicious plate and inoculated into triple sugar iron agar slants and incubated at 37 C. Cultures exhibiting Salmonella-like reactions (mainly alkaline slant, acid, and gas butt, with or without H2S formation) were checked for urease activity by the rapid urea test (a 1:20 dilution of urea and buffer solution). Urease-negative cultures were inoculated into a series of biochemical test media, including 1% sucrose, mannitol, lactose, and salicin broths, tryptone broth, 10% lactose agar slant, Simmons citrate agar slant, and motility test medium. Cultures showing biochemical reactions characteristic of the genus Salmonella were checked with Salmonella polyvalent somatic and grouping sera and submitted to the State’s Regional Salmonella Typing Center for definitive identification.

Various brands of dried milk, instant breakfasts, and powdered haupia (coconut pudding) were examined for Salmonella contamination. For each sampling, 100 g were reconstituted in a 2-liter flask with 1,000 ml of sterile distilled water containing 20 ml of a 0.1% aqueous BG solution (a 1:50,000 concentration). The
inoculated broth was incubated at 37 C. After 24 h of incubation, 10 ml of the primary broth was transferred to a 250-ml flask containing 100 ml of TBG broth and reincubated. Loopfuls of the primary and secondary broths were streaked onto BG and HE agar plates after 24 and 48 h.

A dozen eggs was considered as a single specimen. Each dozen eggs was cracked manually (by using a sterile glove for each specimen) into a 2-liter flask containing 1,000 ml of nutrient broth. The inoculated broth was incubated at 37 C. After 24 h, 10 ml of the primary broth was transferred to a 250-ml flask containing 100 ml of TBG broth and reincubated. Loopfuls of the primary and secondary broths were streaked onto BG and HE agar plates after 24 and 48 h.

Each 30 g of egg noodle specimen was inoculated into a 250-ml flask containing 100 ml of nutrient broth. After 24 h of incubation, 1 ml of the primary broth was inoculated into a test tube containing 9 ml of TBG broth and reincubated. The primary and secondary broths were streaked onto BG and HE agar plates after 24 and 48 h.

RESULTS

Effectiveness of BG agar and HE agar media. A comparative study of BG and HE agar media was undertaken from January 1970 to April 1971 to determine their effectiveness for isolating salmonellae from food products. Of 11,226 food samplings, 1,662 (or 14.9%) were positive for salmonellae on one or both isolation media (Table 1). Although BG agar detected significantly more specimens positive for salmonellae (1,475 of 11,226) than did HE agar (1,315 of 11,226), and the difference is significant ($X^2 = 10.47, p < 0.005$), there were 187 positives that would not have been detected if HE agar were not used as the second plating media and 347 if BG had not been used.

Table 2 summarizes the incidence of Salmonella in the food products examined during the study.

Eggs and egg products. Of the 200 dozen (from 7 farms) local cracked eggs, 4 (or 2.0%) were positive. Salmonella infantis was isolated from three of the four dozen eggs which were positive. From 236 dozen local whole eggs (from 9 brands), 3 (or 1.3%) were positive. S. cerro was isolated from the two dozens of eggs which were positive.

From the 103 mainland whole eggs (from 2 brands) examined for Salmonella, no positives were isolated.

No positives were isolated from the 14 local egg noodle samplings.

Powdered food products. A total of 101 powdered products was examined for Salmonella, with negative results. Samples included 74 (from 7 brands) dried milk, 25 (from 3 brands) instant breakfasts, and 2 (from 1 brand) haupias (coconut puddings).

| Selective medium | Food specimens examined (11,226) | Positive specimen | Negative specimen |
|------------------|---------------------------------|-------------------|------------------|
| BG agar          | 1,475*                          | 9,751             |
| HE agar          | 1,315*                          | 9,911             |

*Three hundred forty-seven of 1,475 positives were isolated on BG agar only.

* One hundred eighty-seven of 1,315 positives were isolated on HE agar only.

Table 2. Incidence of Salmonella in food products of intrastate, interstate, and foreign origin

| Food products                        | Total examined (11,226) | No. positive | Incidence (%) |
|--------------------------------------|------------------------|--------------|---------------|
| Eggs and egg products                |                        |              |               |
| Local cracked egg                    | 200                    | 4            | 2.0           |
| Local whole egg                     | 236                    | 3            | 1.3           |
| Mainland whole egg                  | 103                    | 0            | 0             |
| Local egg noodle                    | 14                     | 0            | 0             |
| Powdered food products               |                        |              |               |
| Dried milk                           | 74                     | 0            | 0             |
| Instant breakfast drink              | 25                     | 0            | 0             |
| Haupia (coconut pudding)            | 2                      | 0            | 0             |
| Bovine carcasses and viscera         |                        |              |               |
| Local beef carcass                   | 354                    | 0            | 0             |
| Mainland beef carcass                | 253                    | 0            | 0             |
| Foreign beef                         | 211                    | 1            | 0.5           |
| Local beef viscera                   | 16                     | 0            | 0             |
| Mainland beef viscera                | 26                     | 0            | 0             |
| Pisces carcasses                     | 77                     | 0            | 0             |
| Poultry carcasses and viscera        |                        |              |               |
| Local chicken carcass                | 2,927                  | 65           | 2.2           |
| Mainland chicken carcass             | 62                     | 0            | 0             |
| Local chicken viscera                | 599                    | 8            | 1.3           |
| Mainland chicken viscera             | 125                    | 2            | 1.6           |
| Porcine carcasses and viscera        |                        |              |               |
| Local pork carcass                   | 2,543                  | 234          | 9.2           |
| Mainland pork carcass                | 891                    | 38           | 4.3           |
| Local pork viscera:                  |                        |              |               |
| Not pasteurized                      | 2,126                  | 1,298        | 61.1          |
| Pasteurized                          | 170                    | 9            | 5.3           |
| Mainland pork viscera                | 192                    | 0            | 0             |

* January 1970 to April 1971.

* Dozen.
The beef carcasses isolated from the local and mainland beef mixtures, consisting of 370 (354 carcasses and 16 tripe) and 279 (253 carcasses and 26 tripe) specimens, respectively. The beef samplings were obtained from two meat companies, one sausage factory, and four supermarkets.

Fish carcasses. Approximately 77 island "reef" fishes were examined for salmonella contamination. BG and HE agar media failed to detect the presence of salmonella on fish carcasses. The fish samplings were obtained from one open market and a supermarket.

Poultry carcasses and viscera. Of 3,713 chicken carcasses and viscera, 3,526 were of local and 187 were of mainland origin. Out of 2,927 local chicken carcasses, 65 (or 2.2%) were positive. BG agar isolated 59 (90.7%) positives from the carcasses and HE agar yielded 16 (24.6%) positives. From 599 local chicken viscera, 8 (or 1.3%) were positive. BG agar isolated five (62.5%) positives from the viscera and HE agar isolated four (50%) positives. The predominant Salmonella serotypes isolated were S. heidelberg, S. typhi, and S. saint paul. The chicken specimens were obtained from four local abattoirs and two supermarkets.

No positives were found in the 62 mainland chicken carcass samplings. Of 125 mainland chicken viscera, 2 (or 1.6%) were positive. The two serotypes isolated were S. typhi and S. derby. They were isolated from BG agar medium. The mainland samples were obtained from one sausage factory, three supermarkets, and one drive-in restaurant.

Porcine carcasses and viscera. A total of 5,922 pork carcasses and viscera was examined for salmonellae. Salmonellae were isolated from 234 (or 9.2%) of the 2,543 local pork carcasses and 1,397 (or 56.9%) of the 2,296 local hog viscera. A 3-month period viscera pasteurization study was carried out in the hog slaughterhouse from September to December, 1970, by utilizing methods described by Paul Yoder (personal communication). Prior to the study, the contamination percentage ranged from 70 to over 90. Due to the pasteurization treatment of all hog viscera before marketing, the degree of contamination fell considerably. A total of 170 hog viscera was subjected to pasteurization, of which 9 (5.3%) yielded salmonella. S. derby and S. anatum were the two serotypes that survived a few pasteurization treatments. The predominant serotypes isolated were S. derby, S. anatum, and S. typhi.

From 891 mainland pork carcasses, 38 (or 4.3%) were positive. S. typhi and S. derby were the predominant serotypes isolated. No salmonellae were recovered from 192 mainland viscera samplings. The mainland pork samplings were obtained from three supermarkets and one restaurant.

Table 3 summarizes the distribution of Salmonella serotypes isolated from the various food products. A total of 28 serotypes was isolated. Of the 15 serotypes frequently isolated, only 1 (S. worthington) was isolated more frequently on HE agar medium, but the difference was not statistically significant.

Selectiveness of BG agar and HE agar. To compare the selectiveness of BG and HE agar media, a 2-week study was conducted from 1–15 April 1971. A total of 410 food specimens was examined. Of this number, three specimens were positive for Salmonella on BG and four on HE agar media. There was a remarkable difference in the number of nonlactose-fermenting (Proteus, Pseudomonas, Paracolon) subcultures made from BG agar medium (92 subcultures) and HE agar medium (11 subcultures) (Table 4). This study demonstrated that HE agar medium is far more selective than BG agar medium.

DISCUSSION

An ideal selective medium for the isolation of Salmonella from various kinds of specimens should be inhibitory against the rapid lactose-fermenting coliforms and other nonlactose-fermenting bacteria such as Proteus, Pseudomonas, Citrobacter, etc., and expedite the identification of Salmonella by eliminating the bacteriological examination of these extraneous colonies.

On HE agar medium, Salmonella colonies appear as transparent blue-green colonies, with or without black centers (H2S production). The size of the colonies ranged from 0.5 to 2 mm. HE agar medium inhibited most of the coliforms and other nonlactose-fermenting bacteria, thereby facilitating the identification of Salmonella from food products.

BG agar medium supported the growth of most of the nonlactose as well as the rapid lactose-fermenting bacteria. On BG agar medium the Salmonella colonies ranged from 0.5 to 1 mm in size. The other nonlactose fermenters...
| Salmonella serotype | Total | Poultry viscera | Eggs whole | Eggs cracked | Porcine carcasses | Porcine carcasses | Porcine carcasses | Eggs whole | Eggs cracked | Porcine carcasses | Porcine carcasses | Porcine carcasses | Porcine carcasses |
|---------------------|-------|----------------|------------|------------|-----------------|-----------------|-----------------|------------|-------------|-----------------|-----------------|-----------------|-----------------|
| Total               | 343   | 361            | 68         | 3          | 286             | 4               |                 | 30         | 1           | 0               | 0               | 0               | 0               |
| bovidae             | 49    | 43             | 1          | 0          | 0               | 0               |                 | 0          | 0           | 0               | 0               | 0               | 0               |
| carysil            | 6     | 2              | 2          | 0          | 0               | 0               |                 | 0          | 0           | 0               | 0               | 0               | 0               |
| cerro               | 4     | 0              | 0          | 0          | 0               | 0               |                 | 0          | 0           | 0               | 0               | 0               | 0               |
| colorado            | 1     | 0              | 0          | 0          | 0               | 0               |                 | 0          | 0           | 0               | 0               | 0               | 0               |
| derby               | 780   | 775            | 82         | 7          | 682             | 7               | 720             | 2          | 2           | 0               | 0               | 0               | 0               |
| enteritis           | 1     | 0              | 0          | 0          | 0               | 0               |                 | 0          | 0           | 0               | 0               | 0               | 0               |
| giv                 | 5     | 0              | 0          | 0          | 0               | 0               |                 | 0          | 0           | 0               | 0               | 0               | 0               |
| heidelberg         | 56    | 54             | 32         | 0          | 4               | 0               | 29              | 0          | 0           | 0               | 0               | 0               | 0               |
| infantis           | 24    | 22             | 3          | 0          | 0               | 0               | 2               | 0          | 0           | 0               | 0               | 0               | 0               |
| kentucky           | 5     | 2              | 0          | 0          | 0               | 0               | 0               | 0          | 0           | 0               | 0               | 0               | 0               |
| lanka              | 1     | 0              | 0          | 0          | 0               | 0               | 0               | 0          | 0           | 0               | 0               | 0               | 0               |
| livingston          | 8     | 6              | 0          | 0          | 0               | 0               | 0               | 0          | 0           | 0               | 0               | 0               | 0               |
| manhattan          | 16    | 15             | 2          | 0          | 0               | 0               | 0               | 0          | 0           | 0               | 0               | 0               | 0               |
| montevideo          | 4     | 2              | 1          | 0          | 0               | 0               | 0               | 0          | 0           | 0               | 0               | 0               | 0               |
| muenchen           | 1     | 1              | 0          | 0          | 0               | 0               | 0               | 0          | 0           | 0               | 0               | 0               | 0               |
| newington           | 53    | 49             | 14         | 0          | 39              | 0               | 36              | 0          | 0           | 0               | 0               | 0               | 0               |
| newport            | 16    | 13             | 3          | 0          | 0               | 0               | 1               | 0          | 0           | 0               | 0               | 0               | 0               |
| panama              | 32    | 29             | 3          | 0          | 20              | 2               | 20              | 0          | 0           | 0               | 0               | 0               | 0               |
| saint paul         | 20    | 18             | 2          | 0          | 7               | 3               | 7               | 0          | 0           | 0               | 0               | 0               | 0               |
| san diego          | 1     | 0              | 0          | 0          | 0               | 0               | 0               | 0          | 0           | 0               | 0               | 0               | 0               |
| senftenberg        | 0     | 0              | 0          | 0          | 0               | 0               | 0               | 0          | 0           | 0               | 0               | 0               | 0               |
| simbury            | 0     | 0              | 0          | 0          | 0               | 0               | 0               | 0          | 0           | 0               | 0               | 0               | 0               |
| tennessee          | 1     | 1              | 0          | 0          | 0               | 0               | 0               | 0          | 0           | 0               | 0               | 0               | 0               |
| thompson           | 1     | 1              | 0          | 0          | 0               | 0               | 0               | 0          | 0           | 0               | 0               | 0               | 0               |
| typhimurium        | 230   | 223            | 19         | 0          | 187             | 14              | 180             | 0          | 0           | 0               | 0               | 0               | 0               |
| willemsden         | 23    | 20             | 3          | 0          | 20              | 0               | 15              | 0          | 0           | 0               | 0               | 0               | 0               |
| worthington         | 59    | 52             | 5          | 0          | 52              | 0               | 62              | 0          | 0           | 0               | 0               | 0               | 0               |

*January, 1970, to April, 1971.*
Table 4. Comparison of the efficiency of BG agar medium and HE agar medium*

| Food product | No. examined | Sal. monella | BG agar medium | HE agar medium |
|--------------|--------------|--------------|----------------|----------------|
| Intrastate   |              |              |                |                |
| Whole Egg    | 20           | 0            | 16             | 1              |
| Poultry      | 75           | 0            | 11             | 3              |
| Chicken Carcass | 175       | 2            | 47             | 6              |
| Porcine Pork Carcass | 140      | 2            | 18             | 1              |

*1–15 April 1971.

* Includes Enterobacter, Escherichia, and Paracolons (Citrobacter and Arizona).

* Includes predominantly Proteus and Pseudomonas.

as well as the Salmonella colonies appear as transparent red colonies, with or without H$_2$S formation. Therefore, more suspicious BG agar plates than HE agar plates were selected out for picking, and this may account for the higher number of Salmonella isolates from BG agar.

In conclusion, BG agar merits consideration as an isolation medium for food surveys because of its effectiveness in isolating salmonellae. HE agar, although not as effective, was far more selective, and its use would require less effort and supplies to eliminate Salmonella-like isolants.

The epidemiological implications of survey results are presented and discussed in a companion paper.

ACKNOWLEDGMENTS

This study was supported by Public Health Service research grant CC-00424 from the National Communicable Disease Center, Atlanta, Ga.

Appreciation is expressed for the sampling and laboratory technical assistance of A. Simao, R. Ohye, and M. Butler.

LITERATURE CITED

1. Galton, M. M., G. K. Morris, and W. T. Martin. 1968. Salmonella in foods and feeds (review of isolation methods and recommended procedures). National Communicable Disease Center, Atlanta, Ga.

2. Gooch, J. M., F. T. Lynd, R. B. Hall, and V. Goo. 1968. Prevalence of Salmonella in meat and meat products of intrastate, interstate, and foreign origin marketed in Hawaii. Salmonella Surveillance Report no. 76, p. 2-3. National Communicable Disease Center, Atlanta, Ga.

3. Gould, K. L., G. Q. L. Ching, and J. M. Gooch. 1972. Epidemiologic aspects of salmonellosis in Hawaii. Amer. J. Pub. Health 62:1216-1221.

4. National Communicable Disease Center. 1963-1971. Salmonella surveillance annual summaries. U. S. Department of Health, Education, and Welfare, Atlanta, Ga.

5. Wilcox, K., and G. Q. L. Ching. 1968. A baseline study of Salmonella infections and carriers on Oahu, Hawaii. Salmonella Surveillance Report no. 76, p. 2-3. National Communicable Disease Center, Atlanta, Ga.