

ISSN 1682-8356
ansinet.org/ijps



INTERNATIONAL JOURNAL OF
POULTRY SCIENCE

ANSI*net*

308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorijps@gmail.com

Comparison of Two Types of Plating Media for Detection and Enumeration of *Campylobacter* from Poultry Samples

J.E. Line¹ and M.E. Berrang

¹USDA-ARS, Russell Research Center, Poultry Microbiological Safety Research Unit,
P.O. Box 5677, Athens, GA 30604-5677, USA
E-mail: eline@saa.ars.usda.gov

Abstract: Campy-Line agar, was compared to Campy-Cefex agar for recovery of *Campylobacter* spp. Five samples were examined from each of 18 broiler carcasses including: feathers, skin, crop, ceca and colon. An additional 16 rinse samples from fresh fully processed commercial broiler carcasses were also examined. Campy-Line agar provided *Campylobacter* spp. counts that closely mirrored those found by enumeration on Campy-Cefex agar. Campy-Cefex agar generally provided slightly higher counts ($P \leq 0.05$) for all sample types except skin. However, *Campylobacter* populations recovered with Campy-Line agar were correlated with those recovered using Campy-Cefex agar; correlation coefficient values were 0.94 for feathers, 0.95 for skin, 0.98 for crop, 0.87 for ceca and 0.88 for colon samples. Observations suggest that Campy-Line agar is easier to use due to the virtual absence of contaminating colonies.

Key words: *Campylobacter*, poultry, broiler, enumeration, media

Introduction

There are several plating media currently in use for enumeration of *Campylobacter*. One of the most popular with food safety microbiologists is Campy-Cefex agar (CCA; Stern *et al.*, 1992). This medium has been used in many published reports on the presence of *Campylobacter* on poultry and poultry related samples (Musgrove *et al.*, 2003; Siragusa *et al.*, 2004; Shih, 2000; Stern *et al.*, 2001; Stern and Robach, 2003). When working with this medium one sometimes encounters a number of non-*Campylobacter* contaminants on the agar surface. Such contamination can make detection and enumeration exceedingly difficult. Line (1999) proposed a new medium (Campy-Line Agar, [CLA]) which has been reported to allow fewer contaminants to proliferate. In studies analyzing broiler carcass rinse samples, *Campylobacter* recovery was not significantly different ($P < 0.05$) between CLA and CCA, but significantly fewer non-*Campylobacter* contaminants were observed on the CLA plates. Only carcass rinse samples were investigated in the study; other sample types were not considered. The purpose of the current study was to compare the performance of Campy-Line agar to that of Campy-Cefex agar for detection and enumeration of naturally occurring *Campylobacter* from a wider range of poultry associated sample types.

Materials and Methods

Broiler carcasses: Six broiler carcasses from the same flock were removed from the shackles at the end of the bleed tunnel in a commercial processing plant on each of three visits ($n=18$ carcasses). All birds had been without feed for approximately 12 h. Carcasses were placed in individual sterile plastic bags (Cryovac,

Duncan, SC) and kept on ice until dissection and removal of samples.

Samples from feathered carcasses: Five samples were collected from each bird. Feathers from the sternal tracts over the breast were picked by hand with new latex gloves. Breast skin from the defeathered area was collected by aseptic removal with sterile forceps and scalpel. Crop, ceca and colon were each aseptically removed with the contents intact. Tissue clamps were used to contain the contents of each organ. The colon sample included that portion of the intestine from the ileo-cecal junction to within 0.5 to 1 cm of the vent. All samples were placed into plastic bags and diluted with sterile buffered peptone water according to weight. Samples were blended for 30 s using a laboratory blender (Seward Medical, London, UK).

Processed broiler samples: In a separate experiment, 16 freshly processed broiler carcasses (post-chill, post-drip) were collected from a commercial poultry processing plant and were individually placed in large plastic bags (Cryovac, Duncan, SC). The samples were transported on ice to the Richard Russell Research Center. Sterile buffered peptone water (4°C) was added (400 ml) to each sample. The carcasses were then agitated for 2 min using a carcass shaking machine to standardize the rinse procedure (Dickens, 1985). Rinse samples were then removed (about 100 ml) and held on ice for microbiological analysis as below.

Culture methods: *Campylobacter* was enumerated by plating serial dilutions in duplicate onto the surface of CCA and CLA. All plates were incubated at 42°C for 36 -

Line and Berrang: Types of Plating Media

Table 1: Comparison of CCA and CLA for recovery of *Campylobacter* spp. from commercially processed (post-chill, post-drip) broiler carcass rinses (n=16)

	Campy-Cefex agar	Campy-Line agar
<i>Campylobacter</i> spp.		
Mean <i>Campylobacter</i> spp. cfu/ml	16.8	11.4
Mean log ₁₀ <i>Campylobacter</i> spp. cfu/ml	1.22	1.06
Range <i>Campylobacter</i> spp. cfu/ml	0-79	0-76
<i>Campylobacter</i> spp. standard deviation cfu/ml	28.2	21.2
Non- <i>Campylobacter</i> Contaminants		
Mean contaminant cfu/ml	14.1 ^a	0 ^b
Range contaminant cfu/ml	0-72	0

^{a,b}Results with different superscripts are significantly different (P<0.05).

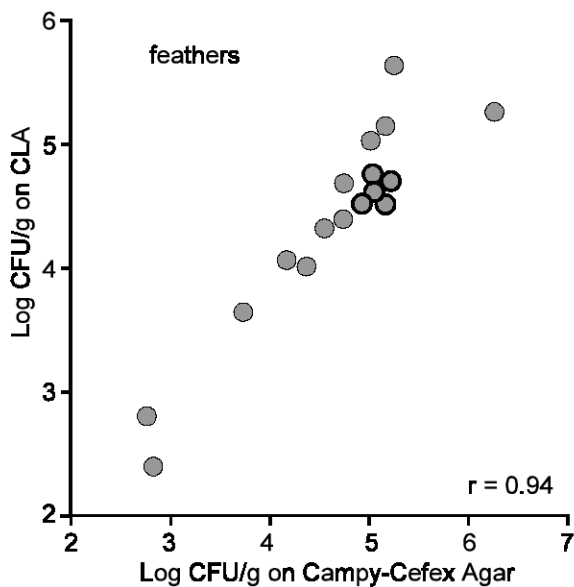


Fig. 1: *Campylobacter* recovered from feather samples using Campy-Cefex Agar and Campy Line Agar.

48 h in a microaerophilic environment (5% O₂, 10% CO₂, 85% N₂). CFUs characteristic of *Campylobacter* were enumerated. The presence or absence of non-*Campylobacter* contaminants was also noted. Every colony type counted as *Campylobacter* from each sample was confirmed as a member of the genus by examination of cellular morphology and motility on a wet mount under phase contrast microscopy. Each colony type was further characterized by a positive reaction on a latex agglutination test kit (Integrated Diagnostics Inc.; Baltimore, MD 21227).

Statistical analysis: Differences in *Campylobacter* recovery for the two media were compared using the T-test (Sigma Stat, Jandel Software, San Rafael, CA). Correlation coefficients were also determined for *Campylobacter* recovery on the two agars using the same software.

Results

Populations of *Campylobacter* recovered per gram of each non-processed sample type are shown in figures 1-5. CCA generally provided slightly higher counts (p<0.05) for all sample types except skin. Expressed as a percentage of the mean count on CCA, the differences between counts on the two plating media were 5% or less for each sample type and not likely to be microbiologically significant. The *Campylobacter* counts on CLA correlate quite well with counts from the CCA. The best correlation was found with crop samples with a correlation coefficient of 0.98. The lowest correlation was found with the ceca and colon samples with correlation coefficients of 0.87 and 0.88 respectively. CLA provided greater selectivity and allowed fewer contaminants to grow than the CCA. A virtual absence of contaminating (non-*Campylobacter*) colonies were observed on the CLA plates for the diverse sample types from feathered carcasses. A mean of 14.1 non-*Campylobacter* CFU/ml were enumerated on CCA plates from the processed poultry rinse samples; whereas, no non-*Campylobacter* contaminants were observed on the CLA plates (Table 1).

Discussion

Enhancing media selectivity while not adversely affecting recovery of the target organism is a common microbiological paradox. The diverse sample types examined in this study had relatively high numbers of *Campylobacter* and large populations of other bacteria. The non-*Campylobacter* colonies growing on less selective agars can mask *Campylobacter* making enumeration a challenging and tedious task. Antibiotics added to increase selectivity of a medium may harm recovery of the target organism also, especially if the target consists of cells that have undergone some form of stress. This is likely why the more selective CLA recovers slightly fewer *Campylobacter* than the less selective Campy-Cefex agar. However, the differences (5% or less) may not be microbiologically significant for these sample types. Both media are capable of maintaining culturability of *Campylobacters* from a

Line and Berrang: Types of Plating Media

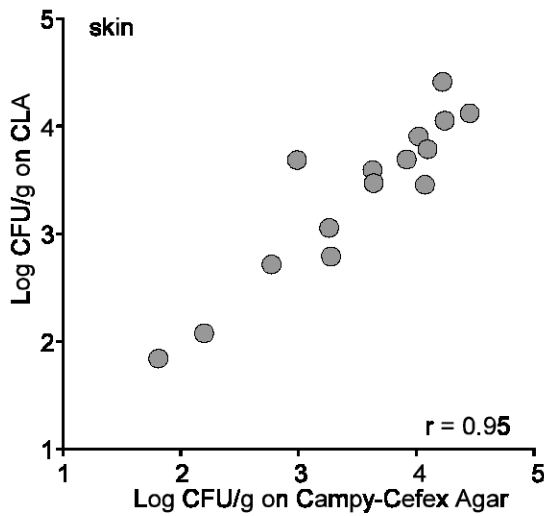


Fig. 2: *Campylobacter* recovered from skin samples using Campy Cefex Agar and Campy Line Agar

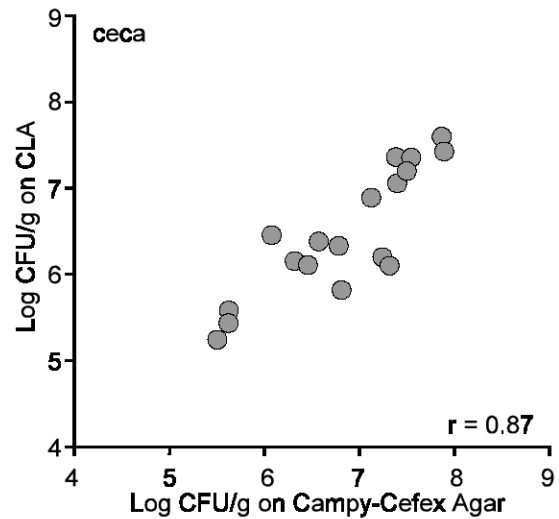


Fig. 4: *Campylobacter* recovered from ceca samples using Campy Cefex Agar and Campy Line Agar.

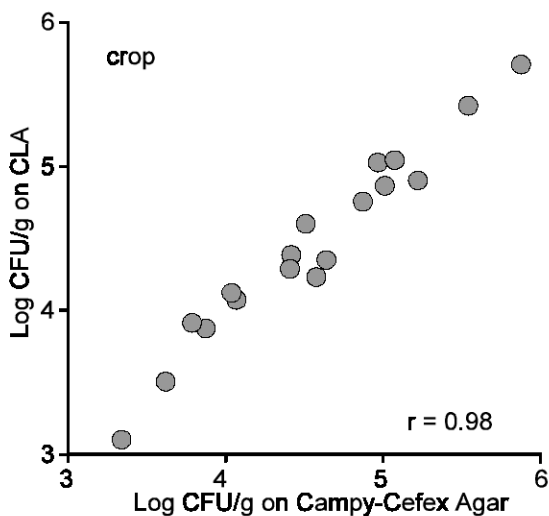


Fig. 3: *Campylobacter* recovered from crop samples using Campy Cefex Agar and Campy Line Agar.

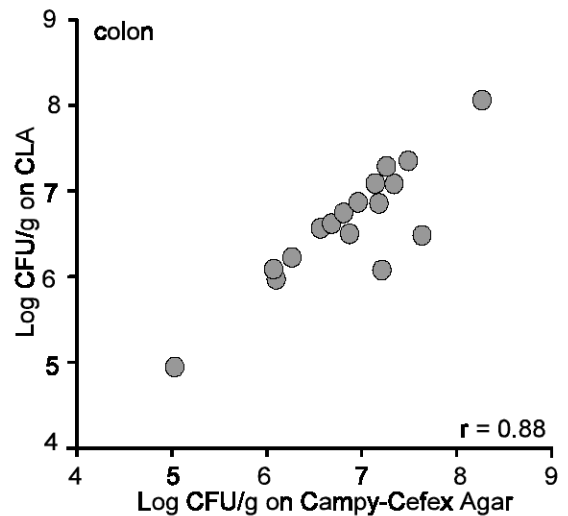


Fig. 5: *Campylobacter* recovered from colon samples using Campy Cefex Agar and Campy Line Agar.

processing environment where they are subjected to various environmental stresses including inhospitable temperatures and chlorine.

Campylobacter recovery from processed carcass rinse samples plated on CCA and CLA was similar in this study to that observed in previous reports. In the current study, CCA recovered an average of about 0.16 Log CFU more *Campylobacter* than CLA, with a mean of 14.1 contaminants per ml on the CCA and none on the CLA ($P < 0.05$). The previous study reported an average difference in recovery of about 0.11 Log between the agars and about 8.7 contaminant CFU/ml on CCA and none on CLA (Line 2001). These data demonstrate the enhanced selective ability of the CLA in eliminating

non-*Campylobacter* competitors on the plates. Other researchers have demonstrated efficacy of CLA in isolating *Campylobacter* from poultry associated samples. Cole *et al.* (2004) used CLA to successfully isolate *Campylobacter* from a variety of turkey reproductive system samples including semen and segments of the reproductive tracts of both male and female turkeys. Researchers have also demonstrated the efficacy of CLA in isolating *Campylobacter coli*, *jejuni* and other *Campylobacter* spp. from swine production. Samples included sponge sampling of skin surfaces, fecal swabs, colon samples and environmental samples from slaughter and processing equipment (Pearce *et al.*, 2003). It is not surprising then that CLA

Line and Berrang: Types of Plating Media

was successful in isolating *Campylobacter* from poultry feather, skin, crop, ceca or colon samples in the current study.

The slight depression observed in recovery of stressed cells on CLA as compared to Campy-Cefex agar must be balanced against the ease of use of the media. Subjective observations as well as plate count data suggest that CLA is easier to use with most sample types due to the virtual absence of contaminating colonies. Given the high level of correlation measured between counts from the two types of media, it is recommended that ease of use be taken into account when choosing between Campy-Cefex agar and CLA for a selective plating media to enumerate *Campylobacter* from poultry feather, skin, crop, ceca or colon samples.

References

- Cole, K., A.M. Donoghue, P.J. Blore and D.J. Donoghue, 2004. Isolation and prevalence of *Campylobacter* in the reproductive tracts and semen of commercial turkeys. *Avian Dis.*, 48: 625-630.
- Line, J. E., 1999. Development of a selective differential agar for isolation and enumeration of *Campylobacter* spp. 10th International Workshop on *Campylobacter*, *Helicobacter* and Related Organisms. Baltimore MD.
- Musgrove, M.T., N.A. Cox, M.E. Berrang and M.A. Harrison, 2003. Comparison of weep and carcass rinses for recovery of *Campylobacter* from broiler carcasses. *J. Food Prot.*, 66: 1720-1723.
- Pearce, R.A., F.M. Wallace, J.E. Call, R.L. Dudley, A. Oser, L. Yoder, J.J. Sheridan and J.B. Luchanski, 2003. Prevalence of *Campylobacter* within a swine slaughter and processing facility. *J. Food Prot.* 66: 1550-1556.
- Shih, D.Y., 2000. Isolation and identification of enteropathogenic *Campylobacter* spp. from chicken samples in Taipei. *J. Food Prot.*, 63: 304-308.
- Siragusa, G.R., J.E. Line, L.L. Brooks, T. Hutchinson, J.D. Laster and R.O. Apple, 2004. Serological methods and selective agars to enumerate *Campylobacter* from broiler carcasses: data from inter- and intra laboratory analyses. *J. Food Prot.*, 67: 901-907.
- Stern, N.J., J.E. Line and H. Chen, 2001. *Campylobacter*. In *Compendium of Methods for the Microbiological Examination of Foods, Fourth Edition*. ed. Downes, F. P. and Ito, K. Washington, D. C. American Public Health Association.
- Stern, N.J. and M.C., Robach, 2003. Enumeration of *Campylobacter* spp. in broiler feces and in corresponding processed carcasses. *J. Food Prot.*, 66: 1557-1563.
- Stern, N.J., B. Wojton and K. Kwiatek, 1992. A differential selective medium and dry ice generated atmosphere for recovery of *Campylobacter jejuni*. *J. Food Prot.*, 55: 514-517.