Journal of Food Protection, Vol. 71, No. 4, 2008, Pages 835–838

Research Note

Detection of *Campylobacter jejuni* and *Campylobacter coli* from Broiler Chicken–Related Samples Using BAX PCR and Conventional International Organization for Standardization Culture

LISA K. WILLIAMS,[1]* ALISDAIR McMEECHAN,[1] TAMSIN BAALHAM,[2] LAURA WARD,[2] TOM J. HUMPHREY,[3] AND FRIEDA JØRGENSEN[1]

[1]*Foodborne Zoonoses Unit, Health Protection Agency, and* [3]*Zoonotic Infections Group, School of Clinical Veterinary Science, Division of Veterinary Pathology Infection and Immunity, University of Bristol, Langford, Bristol BS40 5DU, UK; and* [2]*Oxoid Ltd., Basingstoke, Hampshire RG24 8PW, UK*

MS 07-492: Received 14 September 2007/Accepted 12 December 2007

ABSTRACT

In this study, the conventional International Organization for Standardization (ISO) culture method was compared with the DuPont Qualicon BAX system, a high-throughput, rapid molecular assay that can be used to detect several bacterial species, including *Campylobacter jejuni* and *Campylobacter coli* in diverse sample types. Standard enrichment culture is a time-consuming process, taking up to 6 days to obtain a confirmed result. Rapid molecular assays have been developed that provide results within 24 h. Naturally contaminated samples from the poultry production chain were examined for the presence of *Campylobacter* spp. Samples from broiler chicken ceca ($n = 100$), fresh chicken carcass rinses ($n = 60$), and bootsocks (gauze sock walked through a broiler chicken house; $n = 50$) were enriched according to the ISO 10272 method in Bolton broth specifically designed to detect *Campylobacter* spp. in complex sample types. Samples were enriched without blood for use with the BAX system using the *Campylobacter* BAX kits for the detection of *C. jejuni* and *C. coli.* Samples also were directly plated onto modified charcoal cefperazone deoxycholate agar, and results were compared with those from the enriched samples for the ability to detect *Campylobacter* spp. *Campylobacter* spp. were isolated from 49% of samples with conventional enrichment cultures, from 48% with direct culture, from 68% with the BAX system and enrichment cultures, and from 62% with the BAX system used directly with samples. Overall, the BAX system detected more positive samples than did the conventional culture method and is an effective methodology for the rapid and reliable detection of *Campylobacter* spp. from diverse sample types.

Campylobacter species continue to be the leading cause of bacterial foodborne illness in both the United Kingdom and many other countries *(10).* The majority of cases of human campylobacteriosis are attributed to the consumption of poultry *(23, 25, 27, 29).* In a recent United Kingdom survey that was structured to reflect market share, approximately 50% of chickens on retail sale were contaminated with *Campylobacter* spp. *(10).* The bacteria are frequently isolated throughout poultry production, including during rearing and at slaughter *(22),* and their occurrence is well documented *(4, 5, 13, 26, 28).* However, little is known about the epidemiology of *Campylobacter* spp. in poultry flocks *(3),* making control measures more difficult to implement and monitor. Reliable detection of *Campylobacter* spp. from environmental samples is important to ensure that control measures are as effective as possible. Rapid detection and isolation methods for this important human pathogen are needed urgently. Samples are currently tested using the International Organization for Standardization (ISO) standard enrichment protocol ISO 10272 *(1),* with either Preston or Park-Sanders broth. This process can be time-consuming; a presumptive result takes 4 days, and full confirmation of *Campylobacter* spp. takes up to 6 days. In several studies *(10, 12, 14, 16, 17, 19, 24),* the effectiveness of several widely used enrichment broths has been compared; their use varies between countries and laboratories. Bolton and Preston broths are widely used for enrichment of *Campylobacter* spp., and Bolton broth was chosen for the present study because of its reported superiority over other methods *(2).* The cultivation of *Campylobacter* under laboratory conditions can be difficult because of the limitations of the culture media, which are designed primarily for the isolation of *Campylobacter jejuni (4).* The limitations of reliably isolating and identifying campylobacters by conventional culture may be addressed by the application of molecular techniques. One approach is to use PCR to detect the presence of *Campylobacter* species and to then

* Author for correspondence. Present address: Zoonotic Infections Group, Division of Veterinary Pathology Infection and Immunity, School of Clinical Veterinary Science, University of Bristol, Langford, Bristol BS40 5DU, UK. Tel: +44 (0) 117 928 9245; Fax: +44 (0) 117 928 9505; E-mail: lisa.williams@bristol.ac.uk.

differentiate between species, including *C. jejuni* and *C. coli.*

The DuPont Qualicon BAX system is a rapid molecular assay that can be used to detect *C. jejuni* and *C. coli* in complex sample matrices, although at present this system does not differentiate between the two species. In previous studies, both *C. coli* and *C. jejuni* have been detected with the BAX system from environmental samples *(18)* and poultry carcass rinses *(6).* The BAX system also has been used to confirm *Campylobacter* spp. isolates in a few recent studies *(7, 8, 20).* The procedure for BAX detection involves enrichment of samples according to internationally recognized standards (ISO 10272) without the addition of blood, which can inhibit PCRs. After standard enrichment, a PCR amplification protocol is carried out before detection and analysis is undertaken. BAX detection, including the reporting of results, takes less than 4 h.

In this study, conventional enrichment culture in Bolton broth *(15)* was compared with the BAX system for detecting the presence or absence of *Campylobacter* spp., and direct culture on modified charcoal cefperazone deoxycholate agar (mCCDA) was compared with enrichment of naturally contaminated samples from poultry farms, processing plants, and retail establishments.

MATERIALS AND METHODS

Sample preparation: chicken carcass rinse ($n = 60$). Samples from 60 fresh raw retail chicken carcasses were analyzed. Of these 60 carcasses, 30 were packaged for retail sale and obtained from a processing plant and 30 were purchased from five different retail supermarkets located in South West England. Carcasses were processed and samples analyzed on the day of collection.

The neck skin (25 g) from each chicken was aseptically removed and placed into a small stomacher bag. The whole carcass was then placed in a large stomacher bag, and 300 ml of 0.85% (wt/vol) saline (Oxoid, Ltd., Basingstoke, UK) was poured through the opening into the abdominal cavity. Most of the air was removed from the bag, and the entire carcass was rinsed in the saline for 1 min. The rinse solution was then poured into the smaller bag containing the neck skin, and the contents of this bag were stomached for 1 min with a lab blender (model 400, Colworth, London, UK).

Sample preparation: bootsock ($n = 50$). A white gauze bootsock (Mike Bowden Livestock Services, Norfolk, UK) was soaked in 20 ml of saline and immediately put onto a Wellington boot; contamination of the bootsock from the boot was reduced by covering the boot with a plastic cover before the sock was added. The sock was then walked through the broiler house, ensuring that all areas of the house were covered. The bootsock was then removed from the boot, placed into a polythene bag, and transported to the laboratory at ambient temperature on the day of sampling. At the laboratory, the noncontaminated top part of each sock was removed with sterile scissors and gloved hands to limit further contamination. The remaining soiled area of the sock was cut into two pieces and placed into a filter stomacher bag. Saline (50 ml; Oxoid) was added to the bag, and the sample was homogenized for 30 s with the lab blender.

Sample preparation: ceca ($n = 100$). Ceca from broiler chickens were removed at the processing plant after slaughter, and 20 to 30 individual ceca from each flock were pooled by placing them in a polythene bag. These ceca were transported to the laboratory under chilled conditions and a pooled sample (0.3 g from each cecum) was prepared. For direct culture detection methods, a cecal suspension of 0.1 g of pooled sample vortexed in 9.9 ml of saline (Oxoid) was prepared.

Detection methods: BAX protocol. The BAX PCR was performed according to the manufacturer's instructions (DuPont Qualicon, Wilmington, Del.). Cells contained in 5 μl of the sample (either enriched or nonenriched sample) were lysed by adding them to 200 μl of protease-containing lysis reagent. Samples were heated at 37°C for 20 min and then at 95°C for 10 min and allowed to cool, and 50 μl of this lysed solution was added to a 0.2-ml PCR tube with a tablet containing the reagents needed to carry out the PCR: deoxynucleoside triphosphates, two forward and reverse primer pairs (one for *C. jejuni* and one for *C. coli*), and an internal control primer (DuPont Qualicon). The PCR was performed in the BAX cycler-detector, and results were reported via the software at the end of the cycle. The BAX kit contains internal controls, but we also ran a *C. jejuni* NCTC 11168 sample as a positive control and sterile deionized water as a negative control. On several occasions, we also used *Salmonella* Typhimurium and *Salmonella* Enteritidis as negative controls. The BAX system can detect as few as 10^4 CFU/ml of sample (enriched or nonenriched).

Detection methods: direct culture. Chicken carcass, bootsock, and cecal suspension samples were streaked onto mCCDA (Oxoid) with a 10-μl loop. Plates were incubated at 41.5°C in a microaerobic atmosphere (5% O_2, 10% CO_2, and 2% H_2) for 48 h. The theoretical detection limit for direct plating of chicken carcass rinse and bootsock samples is 100 cells per g, whereas the limit for cecal samples is 1,000 cells per g.

Detection methods: direct BAX. The BAX PCR protocol was applied to chicken carcass, bootsock, and cecal suspension samples without an enrichment step. The initial sample preparations in saline were used for this assay, and the protocol described above was followed.

Detection methods: standard enrichment in Bolton broth. Twenty five milliliters each of chicken carcass rinse and bootsock samples were transferred to 250-ml sterile plastic containers. Bolton broth (225 ml) with 5% lysed defibrinated horse blood (Oxoid) was added to each of the containers. Each cecal sample (1 g) was placed in a 100-ml sterile container, and 90 ml of Bolton broth with 5% lysed defibrinated horse blood was added. Regardless of container size, <1 cm of headspace remained in the containers, and lids were tightly capped. All containers were incubated at 37°C for 4 h and then transferred to 41.5°C for 42 to 44 h in aerobic conditions. The theoretical detection limit for enrichment culture is 1 cell per g of sample.

After incubation, 10 μl of each enrichment sample was streaked onto mCCDA, and the plates were incubated at 41.5°C under a microaerobic atmosphere for 48 h. Presumptive *Campylobacter* colonies were confirmed by growth on duplicate plates of Columbia blood agar with 5% (vol/vol) defibrinated horse blood (Oxoid). The two plates were incubated under different conditions at 37°C for 48 h; one aerobically and one under microaerobic conditions. When growth appeared on the aerobic plate, then the isolate was determined not to be *Campylobacter* spp., which cannot grow aerobically. After 48 h of incubation, isolates were confirmed by microscopy and by positive oxidase reaction. Suspect colonies also were confirmed with the BAX system by preparing a suspension of the colony in saline (Oxoid) to a McFarland standard of 0.5 and following the BAX protocol.

Detection methods: modified enrichment. Samples were enriched in Bolton broth without 5% lysed defibrinated horse blood (Oxoid). After 48 h of incubation, the BAX system protocol was followed.

Statistical analysis. The results obtained from the two methods were compared based on Cohen's kappa tests *(9, 11).*

RESULTS AND DISCUSSION

Conventional enrichment culture is widely used to identify *Campylobacter* spp. from complex sample types according to standard recommendations (ISO 10272 *(1)* and AOAC *(15)*). Bolton enrichment broth usually is favored because it has been effective for isolating campylobacters from poultry-related samples *(2).* Although these methods are considered adequate for the isolation of *Campylobacter* spp., a presumptive result takes 4 days, and an additional 1 to 2 days is required for absolute confirmation. Reducing the time needed to confirm the presence or absence of *Campylobacter* spp. would have many advantages for diagnostic, commercial, and research applications. Conventional enrichment is expensive and time-consuming, and when contamination levels are high, definitive species identification may be inhibited by the enrichment broth. The lengthy identification process can be considerably reduced with the application of rapid methods, which can give a confirmed result usually within 24 h. The BAX system is a commercially available PCR method for the rapid and sensitive detection and/or quantification of a range of pathogens and food quality organisms. The assay used in this study has recently been superseded by a real-time PCR assay for use with the new BAX Q7 machines. This new assay provides detection and quantification of *C. jejuni, C. coli,* and/or *Campylobacter lari.*

Campylobacter spp. were detected in 49% of samples with conventional enrichment culture, in 48% with conventional direct culture, in 68% with the BAX system and enriched samples, and in 6% with the BAX system and samples that had not been enriched. Overall, when considering all sample types with either direct or modified enrichment culture, the BAX system identified significantly more *Campylobacter*-positive samples ($P < 0.05$) compared with the conventional method. Detection of *Campylobacter* spp. from cecal samples was improved when direct culture or direct BAX was used compared with enrichment culture and BAX following enrichment (Fig. 1). However, for cecal samples, there was no significant difference ($P > 0.05$) when comparing direct culture to direct BAX (Fig. 1) and comparing enrichment culture to BAX following enrichment (Fig. 1). For bootsock samples, BAX on either direct or enriched samples resulted in the detection of significantly more *Campylobacter*-positive samples ($P < 0.01$) than with direct or enrichment culture, respectively (Fig. 1). For retail chicken carcass rinses, BAX analysis on either direct or enrichment cultures (Fig. 1) resulted in detection of significantly more *Campylobacter*-positive samples ($P < 0.02$) than were detected with conventional culture.

Results obtained in this study indicate that the BAX system can be used for the rapid and reliable detection of *C. jejuni* and *C. coli* in samples obtained from various poultry production environments. The BAX system was comparable or better than conventional culture, depending on sample type (Fig. 1). In previous studies *(6–8, 18, 20),* the BAX system successfully identified *C. jejuni* and *C. coli* from naturally contaminated samples and was comparable to other molecular methods.

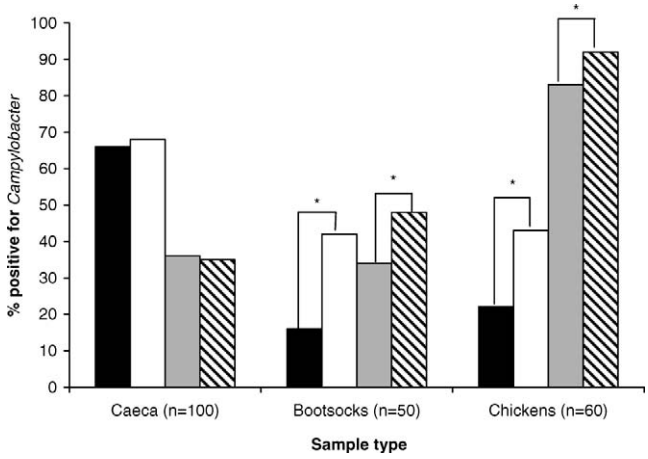

FIGURE 1. *Detection (% positive by each method) of* Campylobacter *spp. from different types of samples. Solid bars represent direct culture, open bars represent direct BAX, shaded bars represent ISO enrichment culture, and hatched bars represent BAX with modified enrichment culture. An asterisk above the bar indicates a significant difference.*

The BAX system usually is used for analyzing enriched samples. In the present study, the BAX was successfully extended for analyzing samples for the presence of campylobacters immediately after sampling, without enrichment. By omitting the enrichment step of the protocol, a result can be obtained on the day of sample collection, whereas with conventional culture a presumptive result requires at least 24 h of incubation. Same-day results are particularly useful for those sample types that do not perform well under enrichment culture conditions, such as ceca, or when degradation of the sample is likely to occur. The direct BAX method detected *C. jejuni* and/or *C. coli* from cecal samples as well as the direct culture method detected these *Campylobacter* species (Fig. 1), but fewer cecal samples were positive when examined after enrichment (Fig. 1). This result is consistent with previous work performed in our laboratory (unpublished data), where recovery of *Campylobacter* spp. from cecal material after standard enrichment, regardless of sample size in the enrichment broth, was less effective than isolation by direct plating. A similar result was obtained by Musgrove et al. *(21)* when they compared direct plating on Campy-cefex agar to enrichment in Bolton broth. They found *Campylobacter* spp. in 100% of directly plated samples but only 6% of enriched samples. This finding suggests that the enrichment procedure can be severely compromised by the many competing nontarget bacteria present.

More *Campylobacter*-positive bootsock and chicken rinse samples were detected with the direct BAX method than by isolation using the conventional direct culture method (Fig. 1). This difference may reflect the increased

ability of the direct BAX method to detect dead or injured cells, which are otherwise undetectable by direct culture. The significant increase in detection of *Campylobacter* spp. in chicken carcass rinse samples using BAX and enrichment compared with using conventional ISO enrichment and isolation may be related to the fact that detection is not hampered by competing microflora when using the BAX system.

In this study, the BAX system provided a reliable, rapid, and cost-effective method for detection of *Campylobacter* spp. in chicken-related samples and could represent considerable time savings for various high-throughput applications.

ACKNOWLEDGMENTS

We are grateful to the farmers and processing plant staff who provided us with the samples used in this project. We also thank Mr. Roger Lovell for his excellent technical assistance. This work was funded by Oxoid, Ltd.

REFERENCES

1. Anonymous. 1998. Microbiology of food and animal feeding stuffs—horizontal method for detection of thermotolerant *Campylobacter* BS EN 5763 (ISO 10272). British Standards Institution, London.
2. Baylis, C. L., S. MacPhee, K. W. Martin, T. J. Humphrey, and R. P. Betts. 2000. Comparison of three enrichment media for the isolation of *Campylobacter* spp. from foods. *J. Appl. Microbiol.* 89:884–891.
3. Bull, S. A., V. M. Allen, G. Domingue, F. Jørgensen, J. A. Frost, R. Ure, R. Whyte, D. Tinker, J. E. L. Corry, J. Gillard-King, and T. J. Humphrey. 2006. Sources of *Campylobacter* spp. colonizing housed broiler flocks during rearing. *Appl. Environ. Microbiol.* 72:645–652.
4. Corry, J. E. L., D. E. Post, P. Colin, and M. J. Laisney. 1995. Culture media for the isolation of campylobacters. *Int. J. Food Microbiol.* 26:43–76.
5. Denis, M., J. Refregier-Petton, M. J. Laisney, G. Ermel, and G. Salvat. 2001. *Campylobacter* contamination in French chicken production from farm to consumers. Use of a PCR assay for detection and identification of *C. jejuni* and *C. coli. J. Appl. Microbiol.* 91:255–267.
6. Englen, M. D., and P. J. Fedorka-Cray. 2002. Evaluation of a commercial diagnostic PCR for the identification of *Campylobacter jejuni* and *Campylobacter coli. Lett. Appl. Microbiol.* 35:353–356.
7. Englen, M. D., P. J. Fedorka-Cray, S. R. Ladely, and D. A. Dargatz. 2005. Antimicrobial resistance patterns of *Campylobacter* from feedlot cattle. *J. Appl. Microbiol.* 99:285–291.
8. Englen, M. D., A. E. Hill, D. A. Dargatz, S. R Ladely, and P. J. Fedorka-Cray. 2007. Prevalence and antimicrobial resistance of *Campylobacter* in US dairy cattle. *J. Appl. Microbiol.* 102:1570–1577.
9. Fleiss, J. L. 1975. Measuring agreement between two judges on the presence or absence of a trait. *Biometrics* 31:651–659.
10. Food Standards Agency. 2003. UK wide survey of *Salmonella* and *Campylobacter* contamination of fresh and frozen chicken on retail sale. Available at: http://www.foodstandards.gov.uk/multimedia/pdfs/campsalmsurvey.pdf. Accessed 20 April 2007.
11. Hale, C. A., and J. L. Fleiss. 1993. Interval estimation under two study designs for kappa with binary classifications. *Biometrics* 49: 523–534.
12. Humphrey, T., M. Mason, and K. Martin. 1995. The isolation of *Campylobacter jejuni* from contaminated surfaces and its survival in diluents. *Int. J. Food Microbiol.* 26:295–303
13. Humphrey, T. J. 2006. Are happy chickens safer chickens? Poultry welfare and disease susceptibility. *Br. Poult. Sci.* 47:379–391.
14. Humphrey, T. J., K. W. Martin, and M. J. Mason. 1996. Isolation of *Campylobacter* species from non-clinical samples. *PHLS Microbiol. Digest* 13:86–88.
15. Hunt, J. M., C. Abeyta, and T. Tran. 1998. Isolation of *Campylobacter* species from food and water, p. 7.01–7.24. *In* Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, Md.
16. Jørgensen, F., R. Bailey, S. Williams, P. Henderson, D. Wareing, F. J. Bolton, J. A. Frost, L. Ward, and T. J. Humphrey. 2002. Prevalence and numbers of *Salmonella* and *Campylobacter* on raw whole chickens at retail sale in England. *Int. J. Food Microbiol.* 76:151–164.
17. Josefsen, M. H., P. S. Lubeck, B. Aalbaek, and J. Hoorfar. 2003. Preston and Park-Sanders protocols adapted for semi-quantitative isolation of thermotolerant *Campylobacter* from chicken rinse. *Int. J. Food Microbiol.* 80:177–183.
18. Manfreda, G., A. De Cesare, V. Bondioli, and A. Franchini. 2003. Comparison of the BAX® system with a multiplex PCR method for simultaneous detection and identification of *Campylobacter jejuni* and *Campylobacter coli* in environmental samples. *Int. J. Food Microbiol.* 87:271–278.
19. Mason, M. J., T. J. Humphrey, and K. W. Martin. 1999. Isolation of sublethally injured campylobacters from poultry and water sources. *Br. J. Biomed. Sci.* 56:2–5.
20. Miller, W. G., M. D. Englen, S. Kathariou, I. V. Wesley, G. Wang, L. Pittenger-Alley, R. M. Siletz, W. Muraoka, P. J. Fedorka-Cray, and R. E. Mandrell. 2006. Identification of host-associated alleles by multilocus sequence typing of *Campylobacter coli* strains from food animals. *Microbiology* 152:245–255.
21. Musgrove, M. T., M. E. Berrang, J. A. Byrd, N. J. Stern, and N. A. Cox. 2001. Detection of *Campylobacter* spp. in ceca and crops with and without enrichment. *Poult. Sci.* 80:825–828.
22. Nebola, M., and I. Steinhauserova. 2006. PFGE and PCR/RFLP typing of *Campylobacter jejuni* strains from poultry. *Br. Poult. Sci.* 47: 456–461.
23. Neimann, J., J. Engberg, K. Molbak, and H. C. Wegener. 2003. A case control study of risk factors for sporadic *Campylobacter* infections in Denmark. *Epidemiol. Infect.* 130:353–366.
24. Paulsen, P., P. Kanzler, F. Hilbert, S. Mayrhofer, S. Baumgartner, and F. J. M. Smulders. 2005. Comparison of three methods for detecting *Campylobacter* spp. in chilled or frozen meat. *Int. J. Food Microbiol.* 103:229–233.
25. Rodrigues, L. C., J. M. Cowden, J. G. Wheeler, D. Sethi, P. G. Wall, P. Cumberland, D. S. Tompkins, M. J. Hudson, J. A. Roberts, and P. J. Roderick. 2001. The study of infectious intestinal disease in England: risk factors for cases of infectious intestinal disease with *Campylobacter jejuni* infection. *Epidemiol. Infect.* 127:185–193.
26. Stern, N. J., P. Fedorka-Cray, J. S. Bailey, N. A. Cox, S. E. Craven, K. L. Hiett, M. T. Musgrove, S. Ladely, D. Cosby, and G. C. Mead. 2001. Distribution of *Campylobacter* spp. in selected US poultry production and processing operations. *J. Food Prot.* 64:1705–1710.
27. Studahl, A., and Y. Andersson. 2000. Risk factors for indigenous *Campylobacter* infection: a Swedish case control study. *Epidemiol. Infect.* 125:269–275.
28. Wagenaar, J. A., D. J. Mevius, and A. H. Havelaar. 2006. *Campylobacter* in primary animal production and control strategies to reduce the burden of human campylobacteriosis. *Rev. Sci. Technol.* 25:581–594.
29. Wingstrand, A., J. Neimann, J. Engberg, E. M. Nielsen, P. Gerner-Smidt, H. C. Wegener, and K. Molbak. 2006. Fresh chicken as main risk factor for campylobacteriosis, Denmark. *Emerg. Infect. Dis.* 12: 280–285.