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Detection of *Campylobacter jejuni* Added to Foods by Using a Combined Selective Enrichment and Nucleic Acid Sequence-Based Amplification (NASBA)

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An assay to detect *Campylobacter jejuni* in foods that uses a short selective enrichment culture, a simple and rapid isolation procedure, NASBA amplification of RNA, and a nonradioactive in solution hybridization was studied. The presence of high numbers of indigenous flora affected the sensitivity of the assay. However, detection of *C. jejuni* was possible up to a ratio of indigenous flora to *C. jejuni* of 10,000:1. Interference by food components was eliminated by centrifugation following the enrichment step. Fourteen food samples artificially inoculated with *C. jejuni* (1 to 1,000 CFU/10 g) were analyzed with the NASBA assay and the conventional culture method with *Campylobacter* charcoal differential agar (CCDA). A few false-negative results were obtained by both NASBA (1.42%) and CCDA (2.86%) isolation. Yet the use of enrichment culture and NASBA shortened the analysis time from 6 days to 26 h. The relative simplicity and rapidity of the NASBA assay make it an attractive alternative for detection of *C. jejuni* in food samples.

Various studies have indicated that *Campylobacter jejuni* is the most common bacterial cause of acute gastrointestinal infection in humans, exceeding rates of illness caused by both *Salmonella* and *Shigella* spp. (1, 10). In addition to *C. jejuni*, the closely related species *Campylobacter coli* and *Campylobacter lari* have been implicated as agents of gastroenteritis in humans. The size of the infective dose is extremely variable. However, as few as 500 bacteria are capable of causing infection (26). Thus, all campylobacters should be absent from foods offered for human consumption. Unpasteurized milk has been involved in a number of large outbreaks of campylobacter enteritis. However, poultry is the premier vehicle for transmitting foodborne *C. jejuni* enteritis. The numbers reported for poultry range from log 2.00 to 4.26 organisms per g (19). Outbreaks associated with poultry rarely involve a large number of individuals. A few instances of campylobacter infection have been linked to the consumption of red meat, including beef, pork, and lamb. Mushrooms may also be the cause of infection (6, 16, 19, 29, 31).

C. jejuni, *C. coli*, and *C. lari* require unusual growth conditions: reduced oxygen tension and a growth temperature of 42°C. On exposure to oxygen or in aging cultures, *C. jejuni* becomes coccal in form. This phenomenon is associated with loss of viability on culture media (2, 27). In addition, campylobacters are fastidious and slow-growing organisms, easily suppressed by other enteropathogens. Campylobacters do not ferment or oxidize carbohydrates. The identification of the *Campylobacter* species is based on biochemical tests, resistance patterns, and growth temperatures. Variation in a single test may result in misidentification of the strain, and confusion about the correct identity can easily occur. Such problems with isolation and identification often result in an inability to recover and identify *Campylobacter* spp.

DNA probes offer an attractive alternative for detection of foodborne pathogens (18, 36), including *Campylobacter* spp.. Synthetic oligonucleotide probes based on the 16S rRNA gene for the detection of all *Campylobacter* species or the thermophilic *C. jejuni*, *C. coli*, and *C. lari* have been constructed (25, 28). Korolik et al. (21) used a cloned 6.1-kb fragment of the campylobacter genome as a specific probe for the identification of *C. jejuni*. Giesendorf et al. (13) described the development of species-specific DNA probes for *C. jejuni*, *C. coli*, and *C. lari*. However, hybridization tests require, in general, the presence of 10^5 to 10^6 cells to yield a positive result. A PCR assay with a primer pair covering a region of the *flaA* gene was developed by Oyofo et al. (23), while Giesendorf et al. (12) used primers from the 16S rRNA sequence to detect *Campylobacter* spp.

Amplification procedures other than PCR have been developed, one of these being NASBA (8, 20), a technique to selectively amplify RNA. Nucleic acid amplification of RNA in NASBA is achieved through the concerted action of avian myeloblastosis virus reverse transcriptase, T7 RNA polymerase, and RNase H. Major advantages of NASBA over PCR are that NASBA is performed isothermally, which precludes the use of a special thermocycler, and that no separate reverse transcription step is required for RNA amplification. NASBA was optimized for the detection of human immunodeficiency virus type 1 sequences (20) and has already been succesfully applied to the detection of these sequences in different blood fractions (6). Van der Vliet et al. (35) used the method for identification of mycobacteria. Previously, the use of the NASBA amplification technique, based on a specific region of the 16S rRNA together with a nonradioactive hybridization procedure, to identify *C. jejuni*, *C. coli*, and *C. lari* has been described (34). A total of 6 CFU of *C. jejuni*, present in a total of 4×10^6 CFU of a mixed flora of

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gram-negative bacteria, resulted in a positive hybridization signal.

Detection may be impaired when the amplification and hybridization procedures are applied to food products (15, 17, 29, 30, 37, 38). The complex composition of food matrices and the presence of indigenous flora can hinder the amplification and lower its sensitivity. The aim of this study is to investigate the feasibility of using the NASBA method for rapid detection and identification of *C. jejuni* in food samples.

MATERIALS AND METHODS

Bacterial strains and growth conditions. The bacterial strains used in this study are *C. jejuni* LMG 6629 and *C. jejuni* LMG 6444, obtained from the culture collection of the Laboratory for Microbiology, University of Ghent. Preston medium, composed of nutrient broth (Oxoid) supplemented with Preston selective supplement (Oxoid) and *Campylobacter* growth supplement (Oxoid), was used for selective enrichment of campylobacters. The Preston medium was incubated in a jar at 42°C in an atmosphere of 5% O_2–10% CO_2–85% N_2 (OCN atmosphere) for 24 h unless otherwise mentioned. The OCN atmosphere was achieved by evacuating the normal atmosphere by a standard laboratory vacuum system and introducing the gas mixture, premade in a flask, in the jar. Isolation and enumeration of campylobacters was performed on *Campylobacter* charcoal differential agar (CCDA; Oxoid). Plates were searched for typical greyish colonies after 24 to 48 h of incubation at 42°C under OCN conditions. The strains were identified by conventional microbiological identification methods (22). Dilutions of a 24-h-old culture of *C. jejuni* in Brucella broth (BB; BBL Microbiology Systems) incubated at 37°C under OCN conditions were used for inoculation of food samples. At the time of inoculation, the number of *C. jejuni* present in the BB culture was determined by plating on CCDA. The number of indigenous bacteria in a food sample or enrichment culture was determined by using plate count agar (PCA; LabM) pour plates and aerobic incubation at room temperature (22 to 25°C) for 3 to 5 days.

Nucleic acid isolation. One milliliter of culture was used for lysis and nucleic acid isolation after guanidium thiocyanate–Triton X-100 lysis as described by Boom et al. (4). In an improved protocol for nucleic acid isolation, 10 ml of culture was transferred to a 15-ml conical tube and centrifuged for 2 min at 1,000 $\times$ *g* to remove the larger particles of food debris in the enrichment broth. If a fatty food product was used, fat accumulated in an upper layer. One milliliter of the supernatant underneath the fatty layer was added to 9 ml of lysis solution. Nucleic acid was isolated as described above.

Primers and probe. A pair of oligonucleotide primers (OT1547 and OT1118) were chosen from the 16S rRNA sequence alignment of the *Campylobacter* species and were previously shown to yield an amplification product. Hybridization with an internal probe (OT1559) occurred only between the probe and amplification product derived from *C. jejuni*, *C. coli*, and *C. lari*, not with the other *Campylobacter* spp. or some noncampylobacters tested (34).

NASBA. The NASBA reactions were performed as described by Uyttendaele et al. (34). Reaction mixtures containing no target nucleic acid but with 2 μl of RNase-free H_2O served as negative controls. The amplification products were directly processed for detection analysis by an enzyme-linked gel assay (ELGA) (see below).

ELGA. A rapid nonradioactive "in solution" hybridization assay (ELGA), using a species-specific oligonucleotide probe (ELGA probe) 5′ end labeled with horseradish peroxidase, was applied to identify the NASBA products. ELGA was performed as described by Uyttendaele et al. (34). In brief, the hybridization reaction mixtures were prepared and incubated for 10 min at 60°C. After hybridization, excess nonhybridized ELGA probes were separated from the homologous hybridized product by vertical gel electrophoresis on an acrylamide gel. Subsequently, the hybridized amplification products and the ELGA probes in the gel were visualized by staining with a substrate solution. Because of its lower mobility, the homologous hybridized product (upper blue-stained band) migrates in the gel above the free ELGA probe (lower blue-stained band).

Effect of food matrices on NASBA detection of *C. jejuni*. To determine the usefulness of the NASBA and ELGA technique for detection of *C. jejuni* in food products, minced meat and chicken skin were artificially contaminated. The experimental design is shown in Fig. 1. Ten-gram amounts of food product were taken. Half of the samples were heated for 10 min at 80°C before inoculation to lower the number of indigenous bacteria; half of the samples were incubated after inoculation to increase the numbers of *C. jejuni*. Thus, four different possible mixtures of indigenous flora to *C. jejuni* in food products were created (Fig. 1). Heated but not incubated samples (situation 1) had an equal number of indigenous bacteria and *C. jejuni* but small numbers of each were present. Heated and incubated samples (situation 2) had a preponderance of *C. jejuni* over indigenous bacteria. Unheated and not incubated samples (situation 3) contained a preponderance of indigenous bacterial flora over *C. jejuni*. Unheated and incubated samples (situation 4) had equal numbers of *C. jejuni* and indigenous flora, but high numbers of each were present. It was investigated whether the combination of NASBA and ELGA was able to correctly identify the presence of *C. jejuni* in all of these situations. For each of the situations, an uninoculated sample served as a negative control. Nucleic acid isolates were used for NASBA and ELGA as described above.

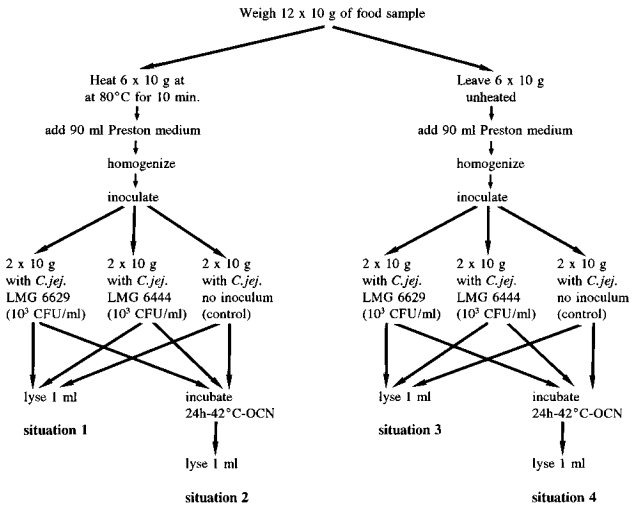

FIG. 1. Design of the experiment investigating the effect of food matrices and indigenous flora on NASBA detection of *C. jejuni* (*C.jej.*).

Effect of indigenous bacteria on sensitivity of NASBA detection. It was noted that the presence of high numbers of indigenous flora could prevent the identification of *C. jejuni* by NASBA and ELGA. Therefore, the maximum ratio of indigenous bacteria to *C. jejuni* that still allowed detection of *C. jejuni* in the sample was determined. In doing so, and taking into account the quantity of indigenous bacteria that could be attained in a Preston enriched food sample, the minimum level of *C. jejuni* which should be present for a positive hybridization signal could be determined.

For these detection limit tests, 10 g of ground beef was mixed with 90 ml of Preston medium and incubated in aerobic conditions for 24 h at 37°C. These growth conditions allowed maximum multiplication of the indigenous bacterial flora. This culture was diluted 10-, 100-, and 1,000-fold in Preston medium, and dilutions of *C. jejuni* LMG 6629 ranging from about 10^8 to 10^1 cells per ml were used for inoculating 9-ml aliquots of undiluted (10^8 CFU/ml), 10-fold-diluted (10^7 CFU/ml), 100-fold-diluted (10^6 CFU/ml), and 1,000-fold-diluted (10^5 CFU/ml) enrichment cultures of indigenous bacteria. Thus, four series of diminishing numbers of *C. jejuni* among a defined high number of non-campylobacters were obtained. After mixing, 1 ml was immediately removed for lysis, nucleic acid isolation, NASBA, and ELGA. The experiment was carried out in duplicate.

NASBA detection of *C. jejuni* in different food samples. In this experiment, 10-g amounts of food were mixed with 90 ml of Preston medium. After homogenization, the food samples were inoculated with dilutions of *C. jejuni* LMG 6629 resulting in very low contamination levels, about 1, 10, 100, and 1,000 CFU of *C. jejuni* per 10 g. Uninoculated food samples acted as negative controls. The samples were incubated for 18 h at 42°C under OCN conditions. Subsequently, 10 ml of Preston enrichment culture was taken and prepared for nucleic acid isolation according to the improved protocol previously described. At the same time, the numbers of indigenous bacteria and *C. jejuni* present were determined. After withdrawal of 10 ml, the Preston enrichment culture was incubated for another 6 h at 42°C under OCN conditions to fulfill the 24 h of incubation required for conventional isolation procedures. CCDA was used for isolation of *Campylobacter* spp. after 24 h of incubation. Suspected colonies were identified to the species level as mentioned before. Food samples tested in this experiment consisted of poultry products (chicken breast meat, chicken skin, turkey legs, and quail), dairy products (eggs and raw milk), red meats (ground beef, pork chops, roast beef, lamb stew, and rabbit meat), and vegetables (mushrooms, lettuce, and carrots), all purchased in a retail store with the exception of the raw milk, which was obtained from a local farm.

RESULTS

Effect of food matrices on NASBA detection of *C. jejuni*. Results are shown in Table 1 for chicken skin and ground beef.

In both cases, a preponderance of indigenous bacteria (10^7 to 10^8 CFU/ml) over *C. jejuni* (10^3 CFU/ml) (situation 3) impaired detection of *C. jejuni* by NASBA, whereas isolation of *C. jejuni* on CCDA was possible. This indicates that large numbers of indigenous bacteria interfere with the NASBA and ELGA assay for detection of small numbers of *C. jejuni*. For ground beef, equal but small numbers (10^3 to 10^4 CFU/ml) of both indigenous bacteria and *C. jejuni* (situation 1) impaired the detection of *C. jejuni* by the combined NASBA and ELGA procedure. For chicken skin, however, the small but equal numbers of both indigenous bacteria and *C. jejuni* led to a weak positive hybridization signal. Lack of hybridization for ground beef could possibly be ascribed to difficulties encountered during nucleic acid isolation from the enrichment broth due to food debris and fatty residues in the sample. This problem was resolved by modification of the nucleic acid isolation protocol. After enrichment of the food sample, the broth was prepared for nucleic acid isolation by centrifugation as described in Materials and Methods to eliminate interference from food debris and fat during the isolation procedure. For two of the four controls related to chicken skin samples, a weak hybridization signal was noticed with ELGA. As no *C. jejuni* was inoculated and no suspected colonies were found on CCDA, these results must be interpreted as false-positive results.

Effect of indigenous bacteria on sensitivity of NASBA detection. A ratio of up to 1,000:1 of indigenous bacteria to *C. jejuni* gave an unambiguous hybridization signal with ELGA (Fig. 2). When this ratio was increased up to ca. 10,000:1 or 100,000:1, only a weak hybridization signal could be noticed, indicating that the presence of high numbers of indigenous bacteria detracted from the sensitivity of the NASBA assay. It was mentioned before that a mixture of gram-negative bacteria (4×10^6 CFU/ml) had a negative influence on NASBA and ELGA detection of *C. jejuni*, although it was found that 6 CFU could be detected (34). As seen in Fig. 2, a ratio of 1,000,000:1 also resulted in a weak positive hybridization signal with 10^6 CFU of indigenous bacteria. However, the same ratio failed to produce a hybridization signal when 10^7 or 10^8 CFU of indigenous bacteria were present. Thus, when using the NASBA and ELGA technique, a minimum of 10^3 CFU of *C. jejuni* per ml are needed for detection of the pathogen among high numbers of indigenous bacteria, possibly attaining 10^8 CFU/ml (Fig. 2A).

NASBA detection of *C. jejuni* in different food samples. The time needed for *C. jejuni* to attain 10^3 CFU/ml in Preston medium when starting from a minimally contaminated food product (<10 CFU/10 g) diluted 10-fold in Preston enrichment medium (<10 CFU/100 ml) was determined. Enrichment in Preston medium for 18 h facilitates the development of small numbers of *C. jejuni*, ranging from 1 to 1,000 CFU/10 g of food product, to sufficient numbers for detection. This enables a further reduction in assay time, with 6 h needed for the NASBA and ELGA assay, compared with the 24 h of enrichment time needed for CCDA isolation.

Table 2 shows the results for 8 of the 14 food products tested in the experiment. The negative control samples of chicken skin, quail, and turkey legs showed a positive hybridization signal on ELGA. For chicken skin and quail, campylobacters could be isolated from the control on CCDA. These strains were identified as *C. jejuni*. Both chicken skin and quail were naturally contaminated with *C. jejuni*. No campylobacters were isolated on CCDA for the negative control or at the lowest inoculation level (2 CFU/10 g) of turkey legs, although both showed a positive hybridization signal with ELGA. But plating of 0.1 ml of the enriched Preston medium on CCDA resulted in 8 and 13 *C. jejuni* colonies for the negative control and the lowest inoculation, respectively, among an abundance of contaminating bacteria, identified as *Pseudomonas aeruginosa*. A false-negative result was obtained for NASBA and ELGA for milk holding 3 CFU/10 ml. *C. jejuni* developed only to 500 CFU/ml in the milk sample after Preston enrichment. For pork chops, the BB culture of *C. jejuni* used for inoculation of the food samples developed to 5×10^7 CFU/ml instead of the usual 10^8 CFU/ml. Therefore, the 10^{-8} dilution used for inoculation of the food sample with less than 10 CFU contained no *C. jejuni*. This resulted in an additional control.

Both the NASBA-ELGA technique and CCDA isolation detected *C. jejuni* in chicken breast meat, ground beef, and mushroom samples at the four inoculation levels (3, 30, 300, and 3,000 CFU/10 g). Similar results were also obtained with the NASBA-ELGA technique and the conventional CCDA isolation procedure for detection of small numbers of *C. jejuni* in eggs, roast beef, rabbit meat, lamb stew, lettuce, and carrots (results not shown).

No problems were encountered for localization of the horseradish peroxidase-labeled probe and interpretation of the ELGA for the different food products. Clear signals were obtained by ELGA analysis of NASBA reactions, as shown for

TABLE 1. Detection of *C. jejuni* in chicken skin and ground beef

Sample and situation	Inoculant *C. jejuni* strain	No. of organisms (CFU/ml)		Detection of *C. jejuni*	
		C. jejuni	Total	CCDA	NASBA-ELGA
Chicken skin					
1	LMG 6629	2.2×10^3	6.9×10^4	+	+
	LMG 6444	3.8×10^3	2.9×10^4	+	+
	None (control)	<10	2.2×10^4	−	+
2	LMG 6629	2.0×10^7	1.1×10^3	+	+
	LMG 6444	5.4×10^5	7.3×10^3	+	+
	None (control)	<10	6.0×10^3	−	−
3	LMG 6629	3.7×10^3	1.6×10^8	+	−
	LMG 6444	2.8×10^3	2.6×10^8	+	−
	None (control)	<10	1.1×10^8	−	+
4	LMG 6629	7.3×10^7	1.8×10^8	+	+
	LMG 6444	1.3×10^5	3.8×10^7	+	+
	None (control)	<10	8.6×10^6	−	−
Ground beef					
1	LMG 6629	1.5×10^3	4.3×10^4	+	−
	LMG 6444	4.8×10^3	5.5×10^4	+	−
	None (control)	<10	4.8×10^4	−	−
2	LMG 6629	5.8×10^7	1.2×10^3	+	+
	LMG 6444	1.7×10^7	<100	+	+
	None (control)	<10	2.4×10^3	−	−
3	LMG 6629	1.8×10^3	4.6×10^7	+	−
	LMG 6444	2.1×10^3	4.7×10^7	+	−
	None (control)	<10	3.9×10^7	−	−
4	LMG 6629	1.4×10^7	6.6×10^6	+	+
	LMG 6444	3.1×10^6	2.7×10^6	+	+
	None (control)	<10	2.0×10^6	−	−

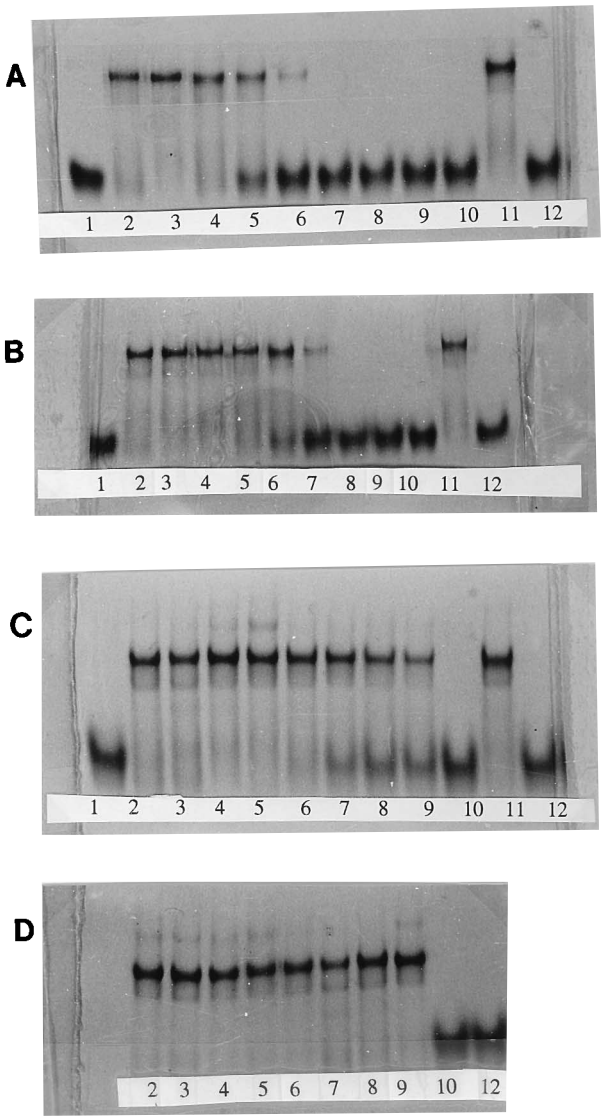

FIG. 2. ELGA analysis of NASBA reactions performed on nucleic acid isolated from a suspension of ground beef indigenous flora at 3×10^8 CFU/ml (A), 3×10^7 CFU/ml (B), 3×10^6 CFU/ml (C), and 3×10^5 CFU/ml (D), containing diminishing numbers of *C. jejuni*. The number of *C. jejuni* (CFU per milliliter) was as follows: lane 2, 4×10^7; lane 3, 4×10^6; lane 4, 4×10^5; lane 5, 4×10^4; lane 6, 4×10^3; lane 7, 4×10^2; lane 8, 4×10^1; lane 9, 4×10^0. Lane 10 is a negative control sample (indigenous flora without *C. jejuni*). Lane 11 is a positive control (4×10^7 CFU of *C. jejuni* per ml). Lanes 1 and 12 contained free ELGA probe.

three food products (mushrooms, turkey, and roast beef) tested in the experiment (Fig. 3).

DISCUSSION

NASBA amplification combined with ELGA hybridization was tested for detection of *C. jejuni* in foods. Rossen et al. (30) reported that several compounds used in culture media, DNA extraction procedures, and individual components of the food sample had inhibitory effects on the PCR. Extracts from cheese especially interfered with amplification (17, 29, 35, 38). Grant et al. (15) reported inhibition of PCR when the assay was applied directly to meat samples. Inhibition of PCR by components of foods can be overcome by dilution (24), isolation of bacteria (5, 11, 15), extensive sample pretreatments (32, 37), or extraction of DNA (12, 38). In the above-described experiments, problems associated with nucleic acid isolation from food homogenates were resolved by a supplementary centrifugation step to eliminate larger particles and fat. Subsequently, nucleic acid was isolated by a simple and rapid protocol as described by Boom et al. (4). No inhibition of NASBA amplification was encountered.

Some authors mentioned an adverse influence of contaminating bacteria on the sensitivity of the PCR assay (15, 29). The use of selective enrichment medium overcomes these difficulties by increasing the number of target organisms and diluting out contaminating bacteria. A short incubation of the enrichment culture also ensures detection in the case of nonuniform distribution of the pathogen in the food product or of a low microbial load (1 to 100 cells/10 g). For this reason, an overnight (18-h) enrichment culture of the food sample in Preston medium was chosen to enable *C. jejuni* to multiply to the detection limit of 10^3 CFU/ml required for positive ELGA detection when high numbers of indigenous flora (10^7 to 10^8 CFU/ml) are present. Rossen et al. (29) needed 2×10^5 cells of *Listeria monocytogenes* per ml for a positive signal when a combination of selective broth culture and PCR was applied.

The specificity and reliability of the protocol developed for NASBA and ELGA detection were evaluated for a number of food products with a low microbial load of the target organism (1 to 1,000 CFU of *C. jejuni* per 10 g) alongside the conventional method, CCDA isolation. The NASBA protocol and CCDA isolation were shown in this study to detect *C. jejuni* in foods with equal efficiency. False-negative results for both NASBA (1.42%) and isolation on CCDA (2.86%) were obtained because of low levels of *C. jejuni* among high levels of indigenous bacteria. A milk sample holding 500 CFU of *C. jejuni* with a background of 5.8×10^6 CFU of indigenous bacteria (Table 2) failed to produce a positive hybridization signal. That ratio of indigenous flora to *C. jejuni* (11,600:1) was the highest encountered during the experiment investigating the application of the NASBA technique for detection of *C. jejuni* inoculated into foods. Turkey leg samples holding 80 and 130 CFU of *C. jejuni* among 1.0×10^5 and 1.2×10^5 CFU of indigenous bacteria, respectively, gave positive hybridization signals, while false-negative results were obtained by CCDA isolation. Approximately 500 to 1,000 CFU/ml is necessary for isolation of the pathogen from an enrichment culture by surface inoculation with an inoculation loop (10 μl) on a selective agar medium. No false-positive results occurred for either CCDA isolation or NASBA and ELGA. The absence of a hybridization signal associated with indigenous flora in the NASBA and ELGA method confirms the specificity of the ELGA probe for *C. jejuni.*

The NASBA and ELGA procedure allowed detection of less than 10 CFU of *C. jejuni* per 10 g for different foods except raw milk. This corresponds to the detection limits obtained by Bohnert et al. (3) and Wang et al. (37), who identified 10 and 4 to 10 cells of *L. monocytogenes*, respectively, in 25 g after 48 h and overnight incubation, respectively, by PCR. Other authors reported detection limits of about 10^2 CFU/10 g by PCR after 18 to 24 h of enrichment culture for identification of *L. monocytogenes* (9, 14) and *C. jejuni* (12).

Raw milk, along with poultry products, is one of the most important sources of *C. jejuni* infection. In general, these products are heavily contaminated with *C. jejuni* (10^2 to 10^4 CFU/

TABLE 2. Detection of *C. jejuni* in different food products by CCDA isolation and the NASBA-ELGA technique

Food sample and inoculum (CFU of *C. jejuni*/10 g)	Indigenous bacteria (CFU/ml) after 18 h in Preston medium	*C. jejuni* (CFU/ml) after 18 h in Preston medium	CCDA isolation of *C. jejuni*	NASBA-ELGA detection of *C. jejuni*
Chicken breast meat				
0 (control)	5.0×10^7	<10	−	−
3	7.5×10^6	1.1×10^3	+	+
30	1.1×10^6	2.9×10^4	+	+
300	3.0×10^6	2.9×10^5	+	+
3,000	3.9×10^6	3.5×10^6	+	+
Chicken skin				
0 (control)	1.3×10^5	3.1×10^6	+	+
3	2.8×10^4	1.8×10^6	+	+
30	1.2×10^4	5.3×10^6	+	+
300	4.8×10^4	7.5×10^5	+	+
3,000	1.4×10^4	2.2×10^6	+	+
Turkey legs				
0 (control)	1.0×10^5	8.0×10^1	−	+
2	1.2×10^5	1.3×10^2	−	+
20	3.3×10^7	3.2×10^3	+	+
200	5.3×10^7	6.4×10^3	+	+
2,000	3.9×10^7	1.1×10^5	+	+
Quail				
0 (control)	1.0×10^7	5.4×10^4	+	+
0 (control)	4.2×10^7	5.0×10^5	+	+
4	2.3×10^7	1.6×10^6	+	+
40	1.3×10^6	2.0×10^6	+	+
400	3.4×10^6	9.0×10^5	+	+
Raw milk				
0 (control)	5.5×10^6	<10	−	−
3	5.8×10^6	5.0×10^2	+	−
30	3.2×10^6	3.4×10^4	+	+
300	1.8×10^6	1.5×10^5	+	+
3,000	2.1×10^6	1.0×10^7	+	+
Ground beef				
0 (control)	2.3×10^6	<10	−	−
3	8.1×10^6	1.5×10^3	+	+
30	7.6×10^6	1.5×10^5	+	+
300	6.0×10^6	2.8×10^5	+	+
3,000	5.0×10^7	5.9×10^6	+	+
Pork chops				
0 (control)	2.2×10^6	<10	−	−
0 (control)	2.8×10^6	<10	−	−
5	2.5×10^6	1.9×10^3	+	+
50	3.1×10^6	1.6×10^5	+	+
500	1.1×10^6	6.1×10^5	+	+
Mushrooms				
0 (control)	1.5×10^3	<10	−	−
3	2.1×10^4	3.6×10^4	+	+
30	6.8×10^3	6.8×10^4	+	+
300	1.0×10^3	1.3×10^6	+	+
3,000	2.5×10^4	4.5×10^6	+	+

g). Few problems in the sensitivity of NASBA detection with 18 h of enrichment time are expected. But as no campylobacters can be tolerated in foods for human consumption, NASBA should also be able to identify very small numbers of *C. jejuni*. Both poultry and raw milk possess an initial total flora of 10^4 to 10^5 CFU/g. Sometimes the indigenous flora of these food products is able to proliferate during enrichment in Preston medium, up to 10^6 to 10^7 CFU/ml. The outgrowth of low numbers of *C. jejuni* ($<10^2$ CFU/10 g) can be restrained in these circumstances, preventing them from attaining the required detection limit. In addition, *C. jejuni* is often stressed by chilling and exposure to air, which prolongs the lag phase of the organism and retards multiplication. As the level of competing organisms for NASBA amplification is unknown, it is

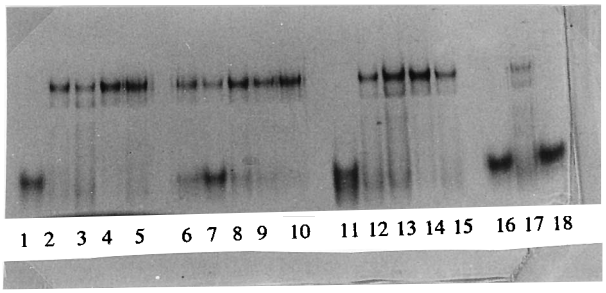

FIG. 3. ELGA analysis of NASBA reactions performed on nucleic acid isolated from mushrooms (lanes 1 to 5), turkey legs (lanes 6 to 10), and roast beef (lanes 11 to 15) inoculated with the indicated amounts of *C. jejuni* per 10 g: Lane 1, 0 (control); lane 2, 2 CFU; lane 3, 20 CFU; lane 4, 200 CFU; lane 5, 2,000 CFU; lane 6, 0 (control); lane 7, 10 CFU; lane 8, 100 CFU; lane 9, 1,000 CFU; lane 10, 10,000 CFU; lane 11, 0 (control); lane 12, 10 CFU; lane 13, 100 CFU; lane 14, 1,000 CFU; lane 15, 10,000 CFU. Lane 16, negative control; lane 17, positive control; lane 18, free ELGA probe.

important to have a high level of target cells. To ensure the presence of at least 10^3 CFU/ml, the enrichment time in Preston medium can be extended from 18 to 20 to 24 h. The same considerations apply to red meats. Processed foods especially have a high initial flora (up to 10^6 CFU/g). Vegetables initially carry a high indigenous flora (10^6 to 10^7 CFU/g), but a significant reduction (10^3 to 10^4/ml) occurs in Preston medium, while campylobacters develop unrestrictedly. Even 18 h of enrichment is sufficient to acquire a positive detection signal.

In summary, it was demonstrated that the NASBA assay, performed with nucleic acid obtained from an 18-h selective enrichment culture, combined with a rapid nonradioactive hybridization procedure (ELGA) provides a sensitive and specific method for detection of *C. jejuni* in foods. The complete procedure takes 26 h to complete and can detect as few as 3 to 5 CFU in 10 g of the original food sample.

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