RESEARCH REPORT

PORK SAFETY

Title: Rapid detection of *Yersinia enterocolitica* in pigs using the TaqMan System - **NPB #98/160**

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Date Received: 10/15/1999

ABSTRACT: The TaqMan assay, or 5' nuclease PCR assay, is a second-generation PCR detection system, which is reportedly more sensitive than conventional PCR tests. We have developed and evaluated a 5' nuclease PCR assay for the detection of *Y. enterocolitica.* The assay targets the chromosomally encoded *ail* (**a**dhesion **i**nvasion **l**ocus) gene.

Three primer/probe sets, (TM1, TM2, and TM3) amplifying different, yet overlapping, regions of *ail* were examined for specificity and sensitivity. The TM1 set displayed the highest specificity, accurately detecting each of the 26 *Y. enterocolitica* strains and none of the 21 non-*enterocolitica* strains. TM1 set detected ~0.5 pg (10^{-12} grams) of purified *Y. enterocolitica* DNA. The TM2 set was the most sensitive and detected ~ 0.25 pg of purified DNA. However, it failed to recognize 10 of the *Y. enterocolitica* strains used in this study. For TM3, sensitivities comparable to TM1 were achieved; cross-reaction with non-*enterocolitica* strains was not observed. However, TM3 did not identify all of the *Y. enterocolitica* strains tested.

The optimized TaqMan assay was compared with bacteriological culture methods and the first-generation multiplex PCR for the rapid detection of pathogenic *Y. enterocolitica* in market weight hogs (n=240) and pork products (n=650). *Y. enterocolitica* was not detected by bacteriological culture in any of the hog tissues tested (nine samples per hog) but was detected by multiplex PCR (2.0%) and TaqMan (45.6%) assays. In addition, ground pork (n=300 samples) and chitterlings (n=350) were screened for *Y. enterocolitica*. By standard culture, *Y. enterocolitica* was detected in chitterlings (8%), but not in ground pork (0%). By multiplex PCR, *Y. enterocolitica* was identified in ground pork (12%) and chitterlings (27%). In contrast, the highly specific TaqMan assay identified *Y. enterocolitica* in ground pork (52%) and chitterlings (79%).

The results of this study indicate that the TaqMan probes and primers for the *ail* gene (TM1) are more specific for pathogenic strains of *Y. enterocolitica* than either bacteriological culture or first-generation multiplex PCR assays.

INTRODUCTION: Pigs are the major animal reservoir for strains of *Yersinia enterocolitica* which are pathogenic to humans (4, 6, 8, 16). In endemic regions, pig and human isolates (serovars 0:9 and 0:5, 27) have been found to be identical by DNA fingerprinting methods. *Y. enterocolitica* is isolated from tongue, tonsils, cecum, rectum, fecal, and gut associated lymphoid tissue of pigs and retail-purchased pork. Foodborne outbreaks have involved consumption of contaminated raw or undercooked ground pork, pork tongues and chitterlings. During 1982, 172 cases of *Y. enterocolitica* serotype O:13a, 13b, were traced to pasteurized milk possibly contaminated with pig manure during transport (16).

Human yersiniosis (case rate of 1/100,000 population), is one of the 7 major foodborne diseases which is monitored by CDC FoodNet. According to CDC, the yersiniosis case rate (cases per 100,000 population) varies from 0.5 (California) to 3 (Georgia). The hospitalization rate for yersiniosis (32% of cases) is second only to listeriosis (94%) (14).

Detection methods involve enrichment in PBS (4C, 14 days) or in irgasan, ticarcillin and potassium chlorate (ITC, 28C, 3 days), followed by plating to onto cefsulodin-irgasan-novobiocin agar (CIN) agar, and the appearance of “red bulls eye” colonies (15, 18). Since colonies are not always typical, standard isolation methods may underestimate its prevalence.

The Polymerase Chain Reaction (PCR) has been used to detect *Y. enterocolitica* by amplifying genes unique to the species (2, 3,7). Virulent *Yersinia* require the expression of chromosomally encoded invasion (*inv*) and attachment invasion locus (*ail*) genes and the plasmid-encoded virulence factor (*vir f*) gene (1, 13). However, the plasmid is lost during laboratory culture, and therefore is not a stable target for molecular-based detection (3, 10). Efforts have focused on the detection of the *ail* gene, which is present in pathogenic *Y. enterocolitica* (7).

We have developed a multiplex PCR assay to identify *Y. enterocolitca*. The assay employs two primer sets which target the chromosomally-encoded *ail* gene and the plasmid-encoded *yadA* gene. PCR products are analyzed by either gel detection or by enzyme linked immunosorbent assays (ELISA; 17). Gel detection is subject to sample cross contamination and uses the carcinogen, ethidium bromide, to visualize the resultant DNA product. The results are qualitative and are scored as either positive or negative.

Second generation PCR-based assays offer qualitative as well as quantitation of specific amplicons. TaqMan is a fluorescence-based PCR detection system (Perkin Elmer Applied Biosystems), which reduces sample handling time, minimizes cross contamination, eliminates the need for gel detection, and, since reactions are done in a 96-well microplate plate, may be automated (9, 10, 12). TaqMan both detects and verifies the PCR products. As summarized in Figure 1, TaqMan utilizes specific PCR primers and a probe. The probe is labelled with both a fluorescent reporter dye (6-carboxy-fluorescein) and a fluorescent quencher dye (6-caraboxytetramethyl-rhodaine). When the probe is intact, the fluorescence from the reporter is suppressed by the quencher (Figure 1, Annealing and Extension). During amplification, the probe is displaced (Figure 1). The 5’ to 3’ nuclease activity of the *Taq* DNA polymerase digests the probe, thus liberating the reporter dye, and increasing the level of fluorescence.

Because swine are its principal reservoir, there is a need to monitor hogs for the virulent strains of *Y. enterocolitica* in order to assess the effectiveness of on-farm pathogen reduction programs. The goal of this study was to couple the detection of pathogenic *Yersinia* in pigs with the speed and elegance of the TaqMan system.

OBJECTIVES:

1. Develop and optimize the sensitivity and specificity of TaqMan system
2. Survey ground pork samples for the presence of *Y. enterocolitica*
3. Determine the prevalence of *Y. enterocolitica* in swine.

PROCEDURES

Bacterial strains and culture conditions. The strains listed in Table 1 were used to determine the specificity of the assay. Bacterial isolates were obtained from the National Animal Disease Center (NADC), the American Type Culture Collection (ATCC), and the Centers for Disease Control (CDC). Strain NADC 5571 (serotype 0:3) was isolated from a yersiniosis outbreak involving consumption of contaminated chitterlings. *Yersinia* strains were grown in either tryptic soy broth (Difco) or ITC broth (ticarcillin-irgasan-potassium chlorate) (20) overnight at 30˚C with shaking.

DNA isolation. Genomic DNA was isolated from bacterial cells using a modified guanidine/silica particle extraction protocol (5). Briefly, 1 ml of bacterial culture was pelleted (1 min at 14,000 rpm). The bacterial pellet was resuspended in 0.5 ml of diatom DNA binding solution (1% diatomaceous earth, 6 M guanidine HCl), frozen at -70°C to lyse the cells, thawed, and the diatoms pelleted (1 min, 14,000 rpm). The diatom pellet was washed with 95% ethanol and the DNA eluted by adding 50 µl dH_20 and heating at 65°C for 10 min. RNase A was added to each DNA sample at a concentration of 1 mg/ml and incubated (37˚C for 15 min). The DNA concentration of each sample was determined spectrophotometrically at 260 nm.

Sensitivity testing. *Y. enterocolitica* strains NADC 5231(ATCC 23715, serotype 0:8) and 5560 (CDC, serotype 0:8) were used as standards in determining the sensitivity of the assay. DNA samples were brought to a concentration of 0.1 µg/µl then serially diluted 10-fold to 1 fg/µl. The final range of DNA concentrations was from 500 ng to 50 fg per 50 µl reaction. The 47 *Yersinia* DNA samples used in the specificity testing were normalized to a concentration of 10 ng/µl and were present in reactions at a final concentration of 1 ng/µl.

Primers and probes. Three independent sets of primers and probes specific for the *ail* gene were designed using Primer Express™ software (PE ABI Prism). Each of the primer/probe sets amplified a different, yet overlapping, region of the *ail* gene. Each probe was labeled at the 5' end with the fluorescent reporter dye FAM (6-carboxy-fluorscein) and at the 3' end with the quencher dye TAMRA (6-carboxy-tetramethyl-rhodamine).

5' nuclease PCR conditions. PCR reaction conditions were as follows: 3.5 mM $MgCl_2$, 0.2 mM dNTP's, 1X GeneAmp® PCR Gold Buffer (PE Biosystems), 1.25 U AmpliTaq Gold™ DNA polymerase, and 5 µl DNA template in a total volume of 50 µl. Each of the primers was added at a concentration of 200 nM. Probe concentrations were as follows: TM1, 25 nM; TM2, 50 nM; TM3, 100 nM. Thermal cycling conditions were 50˚C for 2 min, 95˚C for 10 min, 35 cycles of 95˚C for 15 sec and 58˚C for 1 min followed by an indefinite hold at 25˚C.

Data analysis. PCR reactions were performed in a 96-well format in the PE ABI PRISM™ 7700 Sequence Detection System (PE Applied Biosystems). Sequence Detector™ software version 1.6.3 (PE Applied Biosystems) was used in the data analysis.

Hogs. Market weight hogs (n=300) were screened for the presence of *Y. enterocolitica*. The day prior to slaughter, hogs were randomly selected at the farm and feces and tonsil scrapings were collected. At slaughter, tonsils, ileocecal, ventral thoracic, superficial inguinal lymph nodes, carcass swabs, cecal and rectal contents were collected in sterile whirl paks and transported to the lab on ice. Tonsils and lymph nodes were homogenized with 25 ml of sterile buffered peptone water (pH 7.4) in a stomacher (1 min). Tissue homogenates (1 ml) and cecal and rectal contents (1 g) were inoculated each, into 9 ml of Irgasan, ticarcillin, potassium chlorate (ITC) enrichment broth (11). Swab samples were taken from the ham and ventral surface of the carcass. Sponges (3 cm x 1cm x 0.5 cm) were placed in 9 ml of ITC and incubated (room temperature, 2 days).

Pork products. Ground pork (n=300) and chitterlings (n=350) were procured from three major packing plants, including the abattoir which processed the hogs used in this study. Each pork sample (25 g) was inoculated in to 225 ml of ITC.

Enrichment. ITC was used as a selective enrichment medium (11, 18). Previous studies conducted in our laboratory showed that ITC enrichment was superior when compared to MTSB and PBS enrichments. After enrichment (room temperature for 48 hours) 100 □l aliquot was plated to CIN agar (Oxoid, cat. # CM 653), incubated overnight at 30^0C, and examined for typical bulls eye colonies.

Genomic DNA extraction. Template DNA was extracted using guanidine extraction procedure based on the DNA purification protocol by silica particles (5), as described above, with the omission of the Rnase treatment.

PCR primers and amplification conditions. The multiplex PCR utilized primers specific for the *ail* gene (7) and the plasmid-encoded *yadA* gene. Each reaction (25 □l) contained DNA template (3 □l), reaction buffer, 200 mM dNTPs, 300 nM of each primer, and 1.25 U Taq polymerase. The reaction profile was as follows: 94^0C for 3 min (initial denaturation); 94^0C, 15 sec (denaturation of template); 56^0C, 15 sec (primer annealing); 72^0C, 15 sec (primer extension) for 35 cycles and 72^0C for 3 min (final extension). Multiplex PCR products were size separated by electrophoresis through a 1.5% agarose gel (100 V, 30 min) with Tris-Borate EDTA (1X TBE) as running buffer. The gels were stained with ethidium bromide, destained, and visualized in a Gel Doc 1000 system (Bio-Rad).

The TaqMan PCR assay utilized primers targeting the *ail* gene and amplification conditions optimized earlier.

RESULTS AND DISCUSSION

TaqMan assay optimization and sensitivity testing. The 5' nuclease PCR assay was optimized for each primers/probe set by testing a range of $MgCl_2$, primer, and probe concentrations as well as the number of amplification cycles. The optimum cycle number (35 cycles) and $MgCl_2$ concentration (3.5 mM) were identical for each primer/probe set. A positive fluorescence signal was obtained by the 35^{th} amplification cycle. Fewer cycles may lead to false negative results; additional cycles may yield false positive results. A typical amplification plot is shown in Figure 2. A positive signal is detected when the fluorescence exceeds the threshold (indicated by an arrowhead on the y axis). At the maximum DNA concentration (10 mg/ml) fluorescence is detected by the 10^{th} cycle. By cycle 20, the positive signal is first emitted from the sample with less

DNA. By cycle 34, the sample with the least amount of *Y. enterocolitica* DNA (0.1 fg or 10^{-15} grams/ml emits a positive signal. To assess the minimum amount of DNA detectable by each of the primer/probe sets, serial dilutions of known concentrations of *Y. enterocolitica* DNA were tested. The TM2 set detected 0.25 pg (0.25×10^{-12} grams of purified DNA. TM2 and TM3 sets required approximately twice as much DNA to generate a positive signal. For TM1 set the detection limit was ~ 1.5 colony forming units (CFU) per ml.

TaqMan specificity testing. Strains of *Y. enterocolitica* (n=26) and *Yersinia* species (n=9), for a total of 47 *Yersinia* strains (Table 1) were examined. Each of the primer/probe sets was tested for its ability to positively identify *Y. enterocolitica*. The TM1 set was the most specific, amplifying all the *Y. enterocolitica* and none of the non-*enterocolitica* strains. TM2 and TM3, however, were not as specific and failed to recognize all of the *Y. enterocolitica* strains (Table 1). Despite their inability to detect all strains, neither TM2 nor TM3 displayed any cross-reactivity with the non-*enterocolitica* strains under optimal PCR cycling conditions.

The 5' nuclease PCR assay is a promising tool for the rapid, sensitive, specific, and automated detection of *Y. enterocolitica*. The TM1 set of primers/probe proved to be the most specific, detecting all *Y. enterocolitica* and not cross-reacting with any non-*enterocolitica*. Although this set was not the most sensitive, it is still 100-1,000 times more sensitive than the multiplex PCR developed in this laboratory. Furthermore, the level of sensitivity achieved with the TM1 set would allow for shorter enrichments, thus providing a more rapid means of identification.

The 5' nuclease assay allows for quantification of PCR products. We have generated a standard curve for using highly purified DNA samples (Figure 3). According to these standards, very low amounts of DNA, between 5 and 50 fg (10^{-15} grams), can be accurately and reproducibly detected. The amount of *Y. enterocolitica* DNA in a sample and ultimately the bacteria present in each milliliter or gram of sample can be extrapolated from the cycle number which generates a positive signal. For example, if a sample gives a positive signal at cycle 30 it contains less *Y. enterocolitica* DNA than a sample which gives a signal at 15 cycles. The actual amount of DNA and thus CFU/ml can be determined from the standard curve. However, when this 'clean' DNA is used as a standard to quantify 'dirty' DNA prepared from field samples, accuracy is reduced. Therefore, we are currently developing a standard curve using 'dirty' DNA that will more closely reflect the condition of DNA extracted from animal tissues and food samples.

We encountered some unexpected results while developing this assay that are worth noting. First, each of the primer/probe sets amplified different strains (having the same DNA concentration per reaction) with varying efficiencies. This could be for several reasons. The genomic DNA preps could contain low levels of DNA binding proteins, possibly obstructing annealing of the primers or probes, reducing the amount of amplification and thus fluorescent signal. Alternatively, the region of *ail* targeted by the primer/probe sets vary slightly between different strains, thus decreasing primer or probe annealing and consequently the amplification signal. Second, when PCR reactions containing either TM1, TM2, or TM3 continue beyond the optimum 35 cycles some of the non-*enterocolitica* strains generate a positive signal (false positive). *Y. pseudotuberculosis* has been shown to contain a homologous *ail* locus, which could account for this result. The results obtained during development of this assay indicate that *Yersinia* species other than *enterocolitica* and *pseudotuberculosis* may harbor the *ail* gene or a degenerate version of this gene. Nevertheless, TaqMan assay using TM1

set is specific and highly sensitive for *Y. enterocolitica* if no more than 35 amplfication cycles are used.

Hog samples. In order to find the best field samples available for ultimately evaluating the TaqMan assay, we screened hog tonsils from Iowa, North Carolina, and Pennsylvania for *Y. enterocolitica*. For Iowa, hog tonsils were excised, homogenized, and cultured the same day as slaughter (n=240). For North Carolina, only tonsilar swab samples were analyzed (n=240).

As shown in Table 2, preliminary data using the multiplex PCR indicated that there may be a regional distribution of *Yersinia*, perhaps concentrated in the Eastern US where 32% of the tonsils examined (n=97) yielded pathogenic *Y. enterocolitica*. In contrast, *Y. enterocolitica* was not detected in tonsils of Iowa hogs and infrequently in samples obtained from North Carolina (2.1%). Whether other strains of *Yersinia* were present in these market weight hogs was not determined. Unfortunately, these samples were inadvertently discarded prior to screening by the TaqMan assay.

A second attempt was made to compare the sensitivity of bacteriological culture, multiplex PCR and TaqMan assays. Tissues from Iowa hogs (n=240 hogs) and pork products, including ground pork (n=300) and chitterlings (n=350) were tested. For hog tissues (Table 3), the TaqMan assay (overall, 45.6% samples positive) was at least 20-times more sensitive than the multiplex PCR (overall, 2% of samples positive). Screening large numbers of hogs for *Y. enterocolitica* typically relies on either tonsil scrapings or fecal samples. In this study, *Y. enterocolitica* was not detected by culture in any of the nine types of hog samples tested, including tonsil scraping or feces. By multiplex PCR *Y. enterocolitica* was detected in 3% of fecal samples (n=240) and 2% of tonsil scrapings (n=240) collected within 24 hrs prior to slaughter. By TaqMan, these samples yielded values of 46% and 42%, respectively. This indicates that the TaqMan assay may be sufficiently sensitive to screen the large numbers of fecal samples for *Y. enterocolitica* anticipated during the NAHMS 2000 survey. Of the carcass swabs tested (n=240), 44% were positive for *Y. enterocolitica* by TaqMan. This may reflect fecal contamination of the carcass during slaughter. The thoracic (39% positive by TaqMan) and superficial inguinal (41% positive by TaqMan) lymph nodes were included in this study since they may be components of meat products. The TaqMan assay indicated high levels of *Y. enterocolitica* in these sites. The highest prevalence of *Y. enterocolitica* was in ileocecal lymph nodes (n=240) both by multiplex PCR (7%) and TaqMan (55%) assays. However, these tissues are of limited public health concern since they are not used in the products for human consumption.

Pork products. Human yersiniosis is associated with preparation of pork chitterlings rather than consumption of ground pork. In order to determine the prevalence of *Y. enterocolitica* in hog intestines, a pilot sample (n=10) of chitterlings was collected from Pennsylvania and cultured for *Yersinia*. Samples were collected from Pennsylvania since preliminary data indicated a high prevalence of *Y. enterocolitica* in hog tonsils from that state. All chitterling samples yielded pathogenic strains of *Y. enterocolitica*, as indicated by the presence of both the chromosomal *ail* and the virulent plasmid *yadA* genes.

In an expanded survey, as summarized in Table 3, *Y. enterocolitica* was detected in chitterling samples (n=150) by bacteriological culture (10%), multiplex PCR (51%), and TaqMan assay (85%). Ground pork samples (n=300) were also screened. *Y. enterocolitica* was not isolated from ground pork. Yet it was detected by both multiplex PCR (40%) and TaqMan (80%) assays.

Conclusion. The results suggest that the TaqMan 5'nuclease PCR assay is more sensitive when compared to the multiplex PCR and bacteriological culture. Also, by the TaqMan assay, *Y. enterocolitica* was more frequently detected in ground pork (52%) and chitterlings (79%) than on carcass swabs of freshly slaughtered hogs (44%). This may suggest post-slaughter contamination of pork.

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TABLE 1. Specificity of fluorogenic 5' nuclease assay for detecting *Y. enterocolitica.*

				Amplification		
Strain	**Number**	**Serotype**	**Origin** [a]	**TM1**	**TM2**	**TM3**
Yersinia bercovieri	5230		ATCC	-	-	-
Yersinia enterocolitica	5231		ATCC	+	+	+
Yersinia enterocolitica	5232		ATCC	+	-	+
Yersinia enterocolitica	5233		ATCC	+	+	+
Yersinia enterocolitica	5234		ATCC	+	+	+
Yersinia enterocolitica	5235		ATCC	+	-	+
Yersinia kristensensii	5236		ATCC	-	-	-
Yersinia enterocolitica	5237		ATCC	+	+	-
Yersinia enterocolitica	5559	O:4,32	CDC	+	+	+
Yersinia enterocolitica	5560	O:8	CDC	+	+	+
Yersinia enterocolitica	5561	O:9	CDC	+	+	+
Yersinia enterocolitica	5562	O:18	CDC	+	-	+
Yersinia enterocolitica	5563	O:20	CDC	+	+	+
Yersinia enterocolitica	5564	O:21	CDC	+	+	+
Yersinia enterocolitica	5565	O:13	CDC	+	+	+
Yersinia enterocolitica	5566	O:5,27	CDC	+	+	+
Yersinia enterocolitica	5567	O:1,2,3	CDC	+	-	+
Yersinia enterocolitica	5568	O:2,3	CDC	+	-	+
Yersinia enterocolitica	5569	O:3	CDC	+	+	+
Yersinia enterocolitica	5570	O:3 H	CDC	+	+	+
Yersinia enterocolitica	5571		CDC	+	-	+
Yersinia enterocolitica	5610		ATCC	+	+	+
Yersinia enterocolitica	5611		ATCC	+	+	+
Yersinia aldovae	5612		ATCC	-	-	-
Yersinia aldovae	5613		ATCC	-	-	-
Yersinia aldovae	5614		ATCC	-	-	-
Yersinia bercovieri	5615		ATCC	-	-	-
Yersinia frederiksenii	5616		ATCC	-	-	-
Yersinia frederiksenii	5617		ATCC	-	-	-
Yersinia frederiksenii	5618		ATCC	-	-	-
Yersinia intermedia	5619		ATCC	-	-	-
Yersinia intermedia	5620		ATCC	-	-	-
Yersinia intermedia	5621		ATCC	-	-	-
Yersinia kristensensii	5622		ATCC	-	-	-
Yersinia kristensensii	5623		ATCC	-	-	-
Yersinia kristensensii	5624		ATCC	-	-	-
Yersinia mollaretii	5625		ATCC	-	-	-
Yersinia pseudotuberculosis	8119		ATCC	-	-	-
Yersinia pseudotuberculosis	8120		ATCC	-	-	-
Yersinia pseudotuberculosis	8121		ATCC	-	-	-
Yersinia ruckeri	8122		ATCC	-	-	-
Yersinia ruckeri	8123		ATCC	-	-	-
Yersinia enterocolitica	8177		Eckstrom	+	+	+
Yersinia enterocolitica	8178		Eckstrom	+	-	+
Yersinia enterocolitica	8179		Eckstrom	+	-	+
Yersinia enterocolitica	8180		Eckstrom	+	-	+
Yersinia enterocolitica	8181		Eckstrom	+	-	+

TABLE 2. Recovery of *Y. enterocolitica* from pigs.

State	Number of Pigs	% Positive
Iowa	240	0%
North Carolina	240	2.1%
Pennsylvania	97	32%

TABLE 3. Summary of detection of *Yersinia enterocolitica* by culture, multiplex PCR and the TaqMan assays.

	Number of positive isolates (% of isolation)		
HOGS (n*~240)	**Culture Method**	**Multiplex PCR**	**TaqMan PCR**
Pre slaughter			
Fecal sample	0 (0%)	5 (2.0%)	100 (42%)
Tonsil scrapings	0 (0%)	3 (1.0%)	111 (46%)
Post slaughter			
Ileocecal LN	0 (0%)	17 (7.0%)	131 (55%)
Tonsils	0 (0%)	9 (3.7%)	125 (52%)
Rectal contents	0 (0%)	3 (1.0%)	114 (47%)
Cecal contents	0 (0%)	0 (0.0%)	102 (42%)
Carcass Swab	0 (0%)	4 (1.6%)	106 (44%)
Thoracic LN	0 (0%)	2 (0.8%)	94 (39%)
Superficial inguinal LN	0 (0%)	3 (1.3%)	98 (41%)
AVERAGE	**0%**	**2.0%**	**45.6%**
PORK PRODUCTS			
Chitterlings (n*=350)	27 (8.0%)	95 (27%)	278 (79%)
Ground pork (n*=300)	0 (0%)	35 (12%)	157 (52%)
AVERAGE	**4%**	**18.5%**	**66.%**

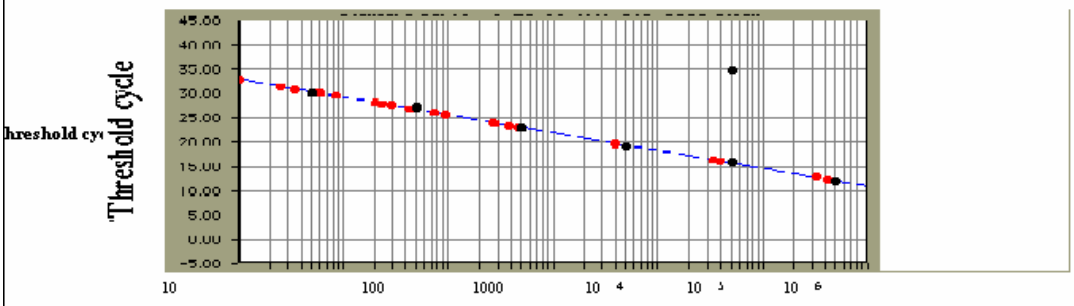
n* is the number of samples tested.

FIGURE 1. The 5' nuclease assay depends on the 5'-3' nuclease activity of *Taq* DNA polymerase. The *Taq* polymerase cleaves an internal fluorogenic probe, which consists of an oligonucleotide with a reporter and quencher dye. (1) During PCR, the fluorogenic probe binds to the target DNA located between the two primers. (2) The probe is displaced as the PCR product is formed. (3) During amplification cleavage by the 5' nuclease activity of *Taq* plymerase liberates the fluorescent reporter dye from the probe. This increases the fluorescent emission of the reaction. An increase in fluorescence indicates amplification of target *ail* DNA of *Yersinia enterocolitica.*

FIGURE 2. A positive signal is detected when the fluorescence exceeds the threshold (indicated by an arrowhead on the y axis). At the maximum DNA concentration (0.10 mg/ml) fluorescence is detected by the 10^{th} cycle (1^{st} arrow on the x axis). By cycle 20, the positive signal is first emitted from the sample with less DNA. By cycle 30, the sample with even less *Y. enterocolitica* DNA (1 fg or 10^{-15} grams/ml, 3^{rd} arrow on the x axis) emits a positive signal.

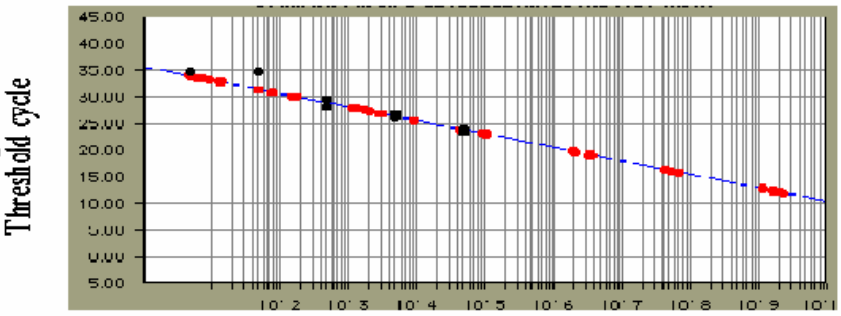
FIGURE 3. Standard curve for the quantitation of *Yersinia enterocolitica*. The amount of *Y. enterocolitica* DNA in a sample and ultimately the bacteria present in each milliliter or gram of sample can be extrapolated from the cycle number which first generates a positive signal. For example, a sample which first gives a positive signal at cycle 30 it contains less *Y. enterocolitica* DNA (~10^2 pg)than a sample which first gives a signal at 15 cycles (10^8 pg). The actual amount of DNA and thus CFU/ml can be determined from the standard curve.

Standard Curve for the Quantitation of *Yersinia enterocolitica*



Picograms of Clean DNA per 50 µl Reaction

Standard Curve for the Quantitation of *Yersinia enterocolitica*



Picograms of Dirty DNA per 50 µl Reaction

Typical amplification plot of highly purified *Yersinia enterocolitica* DNA