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Litter and aerosol sampling of chicken houses for rapid detection of *Salmonella typhimurium* contamination using gene amplification

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Rapid screening of poultry houses for contamination is critical for Salmonella control. Use of air filter sampling has great potential for efficient and reliable monitoring of Salmonella spp., as it could represent an entire poultry house and solve sample-size problems. Two sampling methods (litter and air filter) were compared for detection in four chicken pens inoculated with a S. typhimurium antibiotic resistant strain. Salmonella levels in both litter and air filter samples were determined by PCR amplification and by conventional enrichment. Although amplified DNA was not directly detected, amplified DNA could be detected using a dual probe hybridization sensor. The ratio of the positive samples to total samples determined by gene amplification was much lower than that obtained by conventional enrichments (29/128 versus 102/128 samples). However, the ratio obtained by gene amplification with air filter samples was greater than that with litter samples (26/64 versus 3/64). These results demonstrate that the air filter sampling method is an alternative method of Salmonella detection in poultry house using PCR gene amplification protocol. *Journal of Industrial Microbiology & Biotechnology* (2000) **24,** 379–382.

Keywords: *Salmonella*; aerosol sampling; PCR; poultry house

Introduction

Poultry is considered the single most important source of human exposure to *Salmonella* [7] and constitutes an important animal reservoir of asymptomatic *Salmonella* in the human food chain [16]. Because the supply of eggs or chicks from infected breeder flocks can lead to a significant increase in infection of progeny, control of infection at early stages of production is not only critical [15] but the most cost-effective strategy. There are means to minimize *Salmonella* spp. infestations in flocks, but for these programs to be effective requires the ability to screen breeder flocks, broiler flocks, layer flocks and their corresponding environments. Techniques suited for the detection of *Salmonella* and other food-borne pathogens at the production farm level and compatible with prevailing operational practices have inherent difficulties and therefore certain prerequisites. The cost of monitoring very much depends on the recovery and detection technique applied [12]. Air-borne sampling is an alternative that solves sample-size labor problems. Poultry buildings in the southeastern U.S. are commonly ventilated by wind and natural convection during warm weather, when sidewall curtains are fully opened and propeller fans blow horizontally [2]. During extensive environmental sampling, Davies and Wray [6] noted that chick dust and fluff from salmonellae-infected hatchlings contained as high as 10^4 salmonellae per gram. Likewise, studies with hatching cabinets indicate that cross-contamination of salmonellae from contaminated eggs to uncontaminated eggs may be spread by fan-driven air [1][4].

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Recently, it was reported that the PCR-amplification of *Salmonella*-specific DNA was more readily detected in air filter samples compared to litter samples when PCR was conducted with the samples that were spiked with *S. typhimurium* [8]. However, results obtained with spiked samples may not be consistent with field samples that had not been artificially spiked. In order to further investigate the potentials of the air filter sampling method, we determined the efficiency of *Salmonella* detection using a gene amplification protocol with the litter and air filter samples from a poultry house containing chicks inoculated with a *S. typhimurium* marker strain.

Materials and methods

Preparation of poultry house samples

A total of 400 chicks was randomly divided into four treatment groups placed into four chicken pens containing 100 birds/pen in a P3 facility room. As part of an ongoing experiment [5], chicks were sorted into one of four exposure groups that included either 25% or 50% challenge, and the challenged birds were inoculated by oral gavage with 10^2 CFU *S. typhimurium* on day 1. Litter samples were collected four times from each pen [5] and two air filter samples were removed from the fan filter apparatus of each pen on each sampling day. The air filter samples were 7.5×7.5 cm^2, including the pleats that are pre-made by the manufacturer (Dustgard, Precisionaire, St. Petersburg, FL). The samples were brought to the laboratory within an hour after sampling. After each litter sample was weighed, samples were diluted (w/v) 10-fold with 2% peptone and blended for 30 s in a stomacher (Lab-Blender 400, TekMar, Cincinnati, OH). To each air filter sample, 20 ml of 2% peptone was added and blended for 30 s in the

stomacher. For enumeration of the *S. typhimurium* marker strain, 0.5 ml of the original dilution was further diluted in 2% peptone for plating in duplicate onto Brilliant Green Agar (BGA, Difco Laboratories, Detroit, MI) containing 25 μg/ml novobiocin and 20 μg/ml nalidixic acid. Plates were incubated at 37°C for 2 days and the *S. typhimurium* colonies were counted.

For culture enrichment, 1-ml aliquots from the original litter or filter sample suspensions were added to a duplicate sterile screw-cap tube containing 20 ml of Universal Preenrichment broth (Difco Laboratories, Detroit, MI) per sample and the remainder of each sample was stored at −4°C for polymerase chain reaction (PCR) analysis at a later time. To determine culture enrichment response, Universal Preenrichment broths were incubated overnight at 37°C and then streaked onto BGA plates containing novobiocin and nalidixic acid and incubated for 2 and 3 days for growth.

Gene amplification–hybridization sensor protocol

The primers were designed to amplify a 152-bp region of the DNA binding protein gene *hns*. The sequence for the *hns* primers were as follows: upstream (located between 531 and 552 bp): 5′-TAC CAA AGC TAA ACG CGC AGC T-3′ and downstream (located between 662 and 684 bp): 5′-TGA TCA GGA AAT CTT CCA GTT GC-3′ [EMBL accession no. X14375, [10]]. Gene amplification was performed using a Perkin Elmer DNA thermal cycler and a Gene-Amp kit with AmpliTaq DNA polymerase (Applied Biosystems, Foster City, CA). Ten microliters of each sample (2% peptone) was overlayed with 15 μl of mineral oil to prevent evaporation and incubated at 98°C for 10 min to lyse cells. A reaction mix containing 2.5 μl of the 10×PCR buffer, 2.5 μl (1 μM) of each primer, 2.5 μl of 2.5 mM $MgCl_2$, 0.5 μl of each dNTP stock solution (10 mM), 0.125 μl of the AmpliTaq DNA polymerase (0.625 total units) and 2.8 μl H_2O was added during a "hot-start" incubation of 80°C for 30 min to prevent false priming. The target DNA sequences were amplified through 35 cycles of 94°C for 1 min, 60°C for 1 min and 72°C for 1 min, followed by a final cycle of 72°C for 7 min. The *hns* amplicons were then confirmed in solution using the Threshold™ analysis system (Molecular Devices, Menlo Park, CA). The approach using the Threshold™ analysis system has been described in detail previously [9][13]. Briefly, the assay relies on a biotinylated oligomer and fluoresceinated oligomer simultaneously hybridizing to the amplified product in solution, capture by streptavidin to a biotinylated membrane, and detection via pH changes of an antifluorescein–urease polyclonal antibody complex bound to the fluoresceinated probe after exposure to urea (assayed with a pH-sensitive silicon sensor) [9][13].

Results and discussion

It was previously reported that amplification of the *hns* gene sequences of *S. typhimurium* in air filter samples spiked with *S. typhimurium* marker strain exhibited less interference than in litter samples [8]. One of the major problems in using spiked samples is that the association between the organism and the sample matrix is unlike that in "natural" unspiked samples [14]. Previously it was observed that the detection sensitivity dropped from 100% to 15% when unspiked samples rather than artificially contaminated food samples were used for validating *Listeria monocytogenes* detection in foods [3][11]. Some of this has been attributed to bacteria naturally contaminating samples having reduced viability compared to bacteria regularly grown in the laboratory [3]. The use of naturally contaminated samples permits a more realistic evaluation of the test protocols in terms of sensitivity, specificity and reproducibility.

Therefore, the poultry house samples originated from infected birds and all of the air samples were analyzed for *S. typhimurium* using both growth enrichment and the PCR gene amplification protocol. Previously, the efficiency of gene amplification was evaluated by the intensity of specific PCR products as visualized on agarose gel after staining the gel with ethidium bromide [8]. However, the amount of PCR products in this study was too low to be visualized by gel electrophoresis (results not shown). Therefore, a dual probe hybridization sensor approach was used to amplify the signal of specific PCR products as described previously [9][13]. The results are as shown in Table 1 for litter samples and Table 2 for air filter samples.

The number of positive samples for air filter and litter samples determined by the gene amplification protocol (29 of a total of 128=0.23) was much lower than that obtained by enrichment cultures (102/128=0.79). Moreover, the ratio obtained by PCR gene amplification with air filter samples(26/64=0.41) was much greater than that with litter samples (3/64=0.05). It may be that air filter samples contained less PCR-inhibiting compound than litter samples. This result is consistent with the results obtained with previous spiked samples [8], when filter and litter samples obtained from a poultry house spiked with the

Table 1 Detection of *S. typhimurium* in litter samples from a poultry house using selective enrichment and threshold detection of PCR products

Pen[a]	Detection method	Days post inoculation			
		2	5	7	9
		Number of positive samples			
A	Enrichment	0	4	4	4
	PCR	0	0	0	0
B	Enrichment	2	3	4	4
	PCR	1	0	0	0
C	Enrichment	3	4	4	4
	PCR	1	0	0	0
D	Enrichment	2	4	4	4
	PCR	0	0	1	0

[a]Four pens (A–D) and four samples per pen.

Table 2 Detection of *S. typhimurium* in air filter samples from a poultry house using selective enrichment and threshold detection of PCR products

Pen[a]	Detection method	Days post inoculation			
		2	5	7	9
		Number of positive samples			
A	Enrichment	0	2	2	2
	PCR	0	1	1	0
B	Enrichment	1	2	2	2
	PCR	0	0	0	2
C	Enrichment	0	1	2	2
	PCR	1	0	2	0
D	Enrichment	1	1	2	2
	PCR	1	2	1	2

[a]Four pens (A–D) and two samples per pen.

same *Salmonella* culture were used as sources of template in PCR amplification using *hns* gene specific primers. The *hns* gene was more effectively amplified with air filter samples as compared to litter samples when the spiked samples were used to prepare for the source of template DNA. However, when DNA extraction was used to prepare a template for the PCR reaction, no differences were detected in the intensity of PCR product visualized on agarose gel.

As shown in Figure 1, the number of CFU of the *S. typhimurium* marker strain was lower in air filter samples compared to that of litter samples. The rapid detection of microbial pathogens in complex environmental matrices by gene amplification protocols is presently far from optimal due to interference from organic matrices. However, the results of this study show that the gene amplification protocol in conjunction with dual probe hybridization probe sensor has great potential to

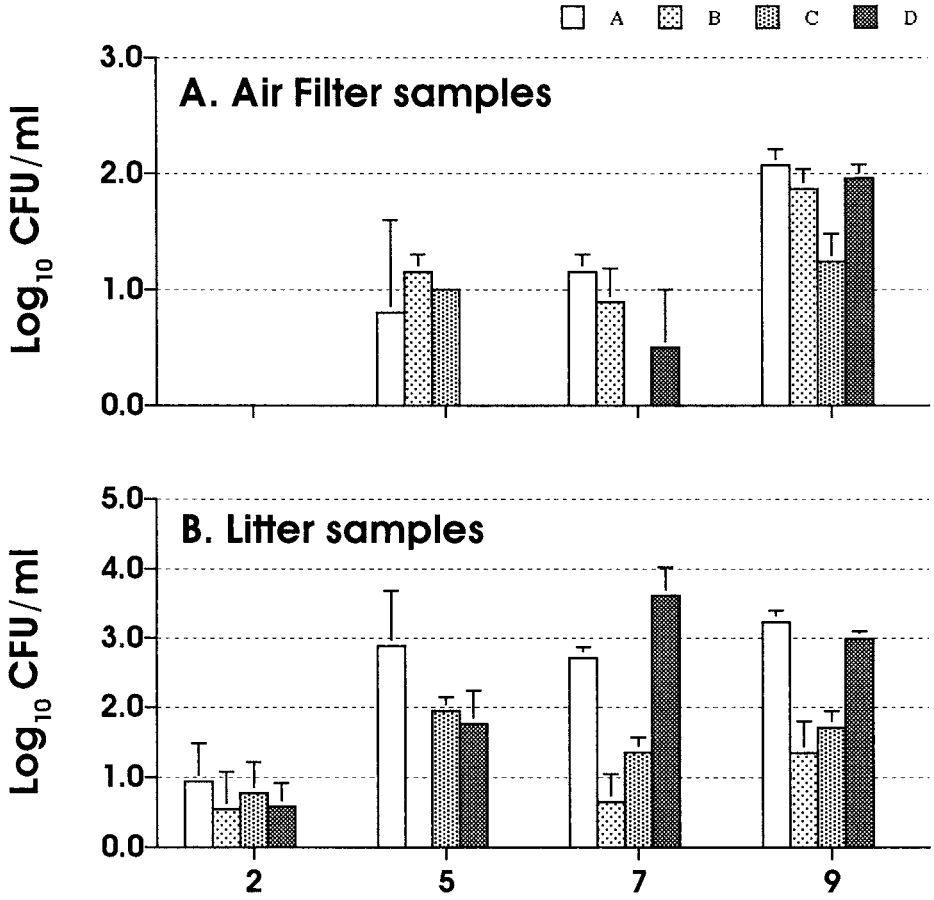

Figure 1 The CFU per milliliter of the initial dilution of the *S. typhimurium* marker strain recovered from the litter and air filter samples. Air filter samples were taken from four pens (A–D) and standard error bars are derived from the average of two samples per pen. Litter samples were taken from four pens (A–D) and standard error bars are derived from the average of four samples per pen.

be used to screen air filter samples from poultry houses rapidly, reliably and inexpensively for the presence of *Salmonella* spp.

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