

## Influence of Temperature and Predation on Survival of *Salmonella enterica* Serovar Typhimurium and Expression of *invA* in Soil and Manure-Amended Soil<sup>∇</sup>

R. García,<sup>1,2</sup> J. Bælum,<sup>1</sup> L. Fredslund,<sup>1</sup> P. Santorum,<sup>2</sup> and C. S. Jacobsen<sup>1,3\*</sup>

Geological Survey of Denmark and Greenland (GEUS), DK-1350 Copenhagen, Denmark<sup>1</sup>; Centro de Investigación y Formación Agrarias, Consejería de Desarrollo Rural, Ganadería, Pesca y Biodiversidad, Gobierno de Cantabria, 39600 Muriedas, Cantabria, Spain<sup>2</sup>; and University of Copenhagen, Faculty of Life Sciences, DK-1958 Frederiksberg C, Denmark<sup>3</sup>

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The effects of three temperatures (5, 15, and 25°C) on the survival of *Salmonella enterica* serovar Typhimurium in topsoil were investigated in small microcosms by three different techniques: plate counting, *invA* gene quantification, and *invA* mRNA quantification. Differences in survival were related to the effect of protozoan predation. Tetracycline-resistant *Salmonella* serovar Typhimurium was inoculated into soil and manure-amended soil at  $1.5 \times 10^8$  cells g soil<sup>-1</sup>. Population densities were determined by plate counting and by molecular methods and monitored for 42 days. Simultaneous extraction of RNA and DNA, followed by quantitative PCR, was used to investigate *invA* gene levels and expression. Analysis by these three techniques showed that *Salmonella* serovar Typhimurium survived better at 5°C. Comparing DNA and CFU levels, significantly higher values were determined by DNA-based techniques. *invA* mRNA levels showed a fast decrease in activity, with no detectable mRNA after an incubation period of less than 4 days in any of the soil scenarios. A negative correlation was found between *Salmonella* serovar Typhimurium CFU levels and protozoan most probable numbers, and we propose the role of the predator-prey interaction as a factor to explain the die-off of the introduced strain by both culture- and DNA quantification-based methods. The results indicate that temperature, manure, and protozoan predation are important factors influencing the survival of *Salmonella* serovar Typhimurium in soil.

*Salmonella* bacteria excreted in the feces of asymptomatic animals may constitute an important source of freshwater and food contamination when manure is spread directly on land (43). The bacteria can be shed from manure and are reported to survive in soil for 160 to 200 days (23, 25).

Numerous methods have been developed for the detection and quantification of *Salmonella* in different matrices (11, 14, 35). The introduction of molecular techniques has become an especially important advance in reducing the time required for detection of *Salmonella* and in detecting active bacteria in environmental samples through their DNA and RNA (10, 18, 51).

Due to a lack of sensitivity and problems with PCR inhibitors in environmental samples, quantitative PCR (qPCR) of *Salmonella* using DNA isolated directly from soil or manure without enrichment has not yet been widely applied (34). Another problem with DNA-based detection assays is also the possible detection of DNA from inactive or nonviable bacteria, causing false-positive results (31). Quantification of mRNA, however, allows analysis of which genes are being expressed and thereby quantitative measurement of the activity levels of specific functional traits of interest. As mRNA, in general, is considered an extremely labile molecule, it is commonly ac-

cepted that analysis of mRNA is a better measure of microbial activity than is DNA analysis (46).

A sequence of the *invA* gene (10) has been utilized as a target for the detection of *Salmonella* nucleic acids in soil samples. This gene is highly conserved in almost all *Salmonella* serotypes (7, 17, 44), and detection of *Salmonella* based on the presence of this gene has previously been reported (11, 12, 24). *invA* mRNA has been used as a biomarker for active cells (13, 18, 26); it has been discussed, however, whether transcription of the *invA* gene may differ with different physiological states of the cell, which may affect assay specificity (13). Jacobsen and Holben (26) showed a detection limit of  $5 \times 10^4$  seeded *Salmonella* serovar Typhimurium cells per g of soil. Based on these data, *invA* mRNA seems to be a feasible candidate for reverse transcriptase PCR assays to specifically identify living *Salmonella* cells.

The survival of *Salmonella* in environmental habitats can be influenced by different factors. In several studies, survival of *Salmonella* in soils has been examined by culture-dependent methods testing the influence of different factors like manure addition, temperature, and interaction with other microorganisms (23, 28, 39, 48). *Salmonella* spp. spread with manure have been reported to survive for up to 300 days in soils (4, 29), but the duration of their survival depends on several factors such as, for example, the incubation temperature (20, 50). Another important factor influencing *Salmonella* survival in soil is predation by protozoa. The role of protozoa in food-borne pathogens' survival in the environment is often a neglected factor in microbial ecology, and therefore this has only been investi-

\* Corresponding author. Mailing address: Department of Geochemistry, Geological Survey of Denmark and Greenland (GEUS), Øster Voldgade 10, DK-1350 Copenhagen, Denmark. Phone: 45 38142313. Fax: 45 38142050. E-mail: csj@geus.dk.

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gated in a few studies (5). Brandl et al. (8) investigated the viable form of *Salmonella enterica* in vesicles of the protozoan *Tetrahymena*. They showed that this protozoan releases vesicles containing a high density of *S. enterica*, leading to an underestimation of actual population sizes of the pathogen during predation studies.

The primary objective of the present study was to evaluate the survival of tetracycline-resistant *Salmonella* serovar Typhimurium in soil and manure-amended soil at three different temperatures (5, 15, and 25°C) by using three different techniques: plate counting, *invA* DNA qPCR, and direct quantification of *invA* mRNA. Furthermore, the role of predation was evaluated to relate the survival of *Salmonella* as measured by different methods with the estimated most probable number (MPN) of protozoa present in soil and manure-amended soil.

#### MATERIALS AND METHODS

**Soils.** Soil samples were collected from Sjælland Odde, Denmark. Topsoil was obtained from the 0- to 30-cm layer of an agricultural field containing 19% clay, 18% silt, 62% sand, and 1.2% carbon and with a pH of 7.2. Approximately 100 kg of soil was obtained in total by using a manual composite sampling technique in which subsamples were taken from scattered locations within a 10-m<sup>2</sup> area, mixed thoroughly, and stored frozen at -20°C in aliquots of ~2 kg. Prior to experiments, aliquots were thawed and acclimatized in the dark at 10°C for 10 days as described by Mortensen and Jacobsen (38). Three subsamples of soil were negative for *Salmonella* when tested by qPCR, and no CFU of tetracycline-resistant bacteria were detected according to the procedure described below (detection limit, 100 CFU g<sup>-1</sup>).

**Manure.** Fresh manure was obtained from dairy cows. It was stored at 5°C for 1 week before the beginning of the assay. Physical/chemical analysis of subsamples indicated a moisture content of 90.4%, an ammonia N concentration of 1.94 g kg<sup>-1</sup>, and total N, phosphorus, potassium, copper, and magnesium concentrations of 3.94, 0.77, 3.63, 0.00995, and 1.284 g kg<sup>-1</sup>, respectively (wet basis). qPCRs were negative for *Salmonella*, and no CFU of tetracycline-resistant bacteria were detected in three manure subsamples.

**Bacteria.** *Salmonella* serovar Typhimurium tetracycline-resistant DSM554 was obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany, and grown in Müller broth (Oxoid) containing 25 µg/ml tetracycline (Sigma-Aldrich) at 37°C in the dark for 24 h at 100 rpm. Ten milliliters of the culture was placed into 100 ml Müller broth with tetracycline and incubated again at 37°C for at least 4 h until an optical density at 600 nm of ~0.6 was attained. Subsequently, the culture was centrifuged (6,000 × g, 10 min) and resuspended in 100 mM sterile phosphate buffer (PB), pH 7, resulting in an inoculum of 2 × 10<sup>9</sup> CFU ml<sup>-1</sup> based on direct plate counts (Müller agar plates with 25 µg/ml tetracycline, 24 h, 37°C).

**Microcosm setup and *Salmonella* serovar Typhimurium inoculation.** Triplicate microcosms of topsoil and manure-amended topsoil were set up in 100-ml glass flasks with airtight glass stoppers (Schott Glaswerke, Mainz, Germany).

Prior to inoculation, 30 g (wet weight) of soil was placed in each flask and sterilized PB or liquid manure was added to bring the soil to 85% of the field water-holding capacity. The amount of manure in the manure-amended topsoil corresponded to a manure application rate of 3 kg m<sup>-2</sup>, which is commonly practiced on farms in the region.

At the onset of the experiment, 1 ml *Salmonella* serovar Typhimurium (Tet<sup>+</sup>) culture (described above) was inoculated into the microcosm soil surface without mixing, producing 1.5 × 10<sup>8</sup> CFU g soil<sup>-1</sup> in each microcosm. In manure-amended topsoil microcosms, manure amended with the bacteria was applied to the soil surface, producing the same level of CFU g soil<sup>-1</sup>. The two different soil scenarios (with/without manure) were incubated in triplicate at three different temperatures, 5, 15, and 25°C.

Direct plate counts of *Salmonella* serovar Typhimurium were done on days 0, 1, 2, 4, 7, 11, 15, 19, and 24 after inoculation and once a week thereafter until the pathogen concentration dropped below the detection limit (100 CFU g soil<sup>-1</sup>). Samples of 0.5 g (wt/wt) as a composite of about five plugs were removed from the flasks and transferred to 50-ml screw-cap glass tubes, 9.5 ml sterile PB (100 mM, pH 7) was added, and the mixture was agitated on a horizontal shaker (150 rpm, 30 min). Serial 10-fold dilutions were prepared in PB, and 0.1-ml aliquots were surface plated on Müller agar containing tetracycline. Colonies were counted after incubation for 24 h at 37°C.

At the same time points as for direct plate counting, another 0.5 g of soil was taken for nucleic acid extraction. In order to ensure a snapshot freeze event, these samples were frozen immediately in liquid nitrogen and stored at -80°C.

**DNA/RNA extraction and cDNA synthesis.** DNA and RNA were coextracted as described by Nicolaisen et al. (40). In short, the method involves bead beating in the presence of cetyltrimethylammonium bromide (CTAB) buffer, phenol, and chloroform, followed by phenol extraction and precipitation of nucleic acids from the aqueous phase by 30% polyethylene glycol 6000 (PEG). Phenol and CTAB buffer were added to the frozen samples, and cell lysis was performed for 2 × 15 s at a speed setting of 5.0 m s<sup>-1</sup> with intermittent cooling to prevent overheating of the sample. The following modification, described by Bælum et al. (3), was applied to the protocol. One microliter of glycogen (Roche, Basel, Switzerland) was added to PEG to aid in nucleic acid precipitation. After the extraction procedure, 7-µl aliquots of each sample were used for RNase-free DNase I treatment (Promega, Madison, WI) according to the manufacturer's protocol as modified by Jacobsen and Holben (26). Reverse transcription (RT) was performed using an Omniscript RT kit from Qiagen (Crawley, United Kingdom) with 2 µl of DNase-treated extract as the template, 40 pmol of *invA* reverse primer (described below), and a reaction volume of 10 µl. RT reaction temperatures and time of incubation were as described previously by Bælum et al. (3).

**qPCR.** qPCR was carried out in an iCycler (Bio-Rad, Hercules, CA). To include impacts of the soil matrix on extraction efficiency and coextracted PCR enzyme inhibitors, standards for quantification were prepared by individual inoculations of 10-fold dilutions (10<sup>1</sup> to 10<sup>7</sup> g soil<sup>-1</sup>) of *Salmonella* serovar Typhimurium to aliquots of 0.5 g soil, followed by nucleic acid extraction as described above. The primers employed were based in those described by Chiu and Ou (10) with the modifications described by Jacobsen and Holben (26). The primer sequences were as follows: *invA* forward, 5'-ACAGTGCTCGTTTACGACC-3'; *invA* reverse, 5'-ACTGGTACTGATCGATAAT-3'. All qPCRs were performed in a final volume of 20 µl containing 10 µl of premixed mastermix (Dynamo HS SYBR green qPCR kit; Finnzymes, Helsinki, Finland), 0.4 µM forward primer, 0.4 µM reverse primer, 20 µg bovine serum albumin (New England Biolabs Inc., Ipswich, MA), 7.2 µl of deionized PCR grade water, and 1 µl of template DNA (~10 to 50 ng). DNA extractions were diluted 10-fold prior to PCR quantification to avoid interactions from coextracted enzyme inhibitors. All DNA and cDNA samples were quantified in triplicate, including negative controls (containing all of the reagents except the template DNA). Another control using DNase-treated RNA samples was performed to discard possible DNA contamination which would interfere with cDNA-based quantification of *invA* gene expression levels. Each qPCR consisted of the following steps (6): 15 min initial denaturation and enzyme activation at 95°C, followed by 40 cycles of 30 s denaturation at 95°C, 30 s annealing at 55°C, 30 s elongation at 72°C, and 15 s at 77°C for quantification of the *invA* product. The procedure ended with one cycle of 6 min at 72°C for elongation and a melting curve analysis generated by analyzing the amount of double-stranded DNA after each 0.5°C increase in temperature up to 95°C.

**Counting of protozoa.** Samples for counting of protozoa were taken four times during the experiment. A 3-fold dilution series of soil and manure-amended soil samples was prepared in 96-well microtiter plates (Nunc-Thermo Scientific, Roskilde, Denmark). Dilutions were made in modified Neff's amoeba saline buffer with 0.10 g liter of tryptic soy broth<sup>-1</sup> as described by Page (41). Well dishes were incubated at 15°C in the dark, and protozoan enumeration was done by visual inspection of single wells with an inverted microscope after 7 and 21 days of incubation. MPN calculations were performed by the computer-assisted method developed by Briones and Reichardt (9). The method uses Microsoft Excel and its associated Solver tool to generate MPNs, error estimates, and 95% confidence limits.

**Statistical analysis.** All amplification and plate counting experiments were performed in triplicate, and mean values and standard deviations are given. Statistical analyses were carried out using SPSS statistics 17.0. Results were compared for statistically significant differences by using a one-way analysis of variance test. Correlation between protozoan MPN and *S. Typhimurium* survival levels was evaluated using Pearson's coefficient. For all tests, a *P* value of ≤0.05 was considered significant.

## RESULTS

**Quantification of *Salmonella* serovar Typhimurium by plate counting and DNA-based methods.** Figure 1 shows levels of *Salmonella* serovar Typhimurium in soil and manure-amended soil samples incubated at three temperatures: 5, 15, and 25°C.

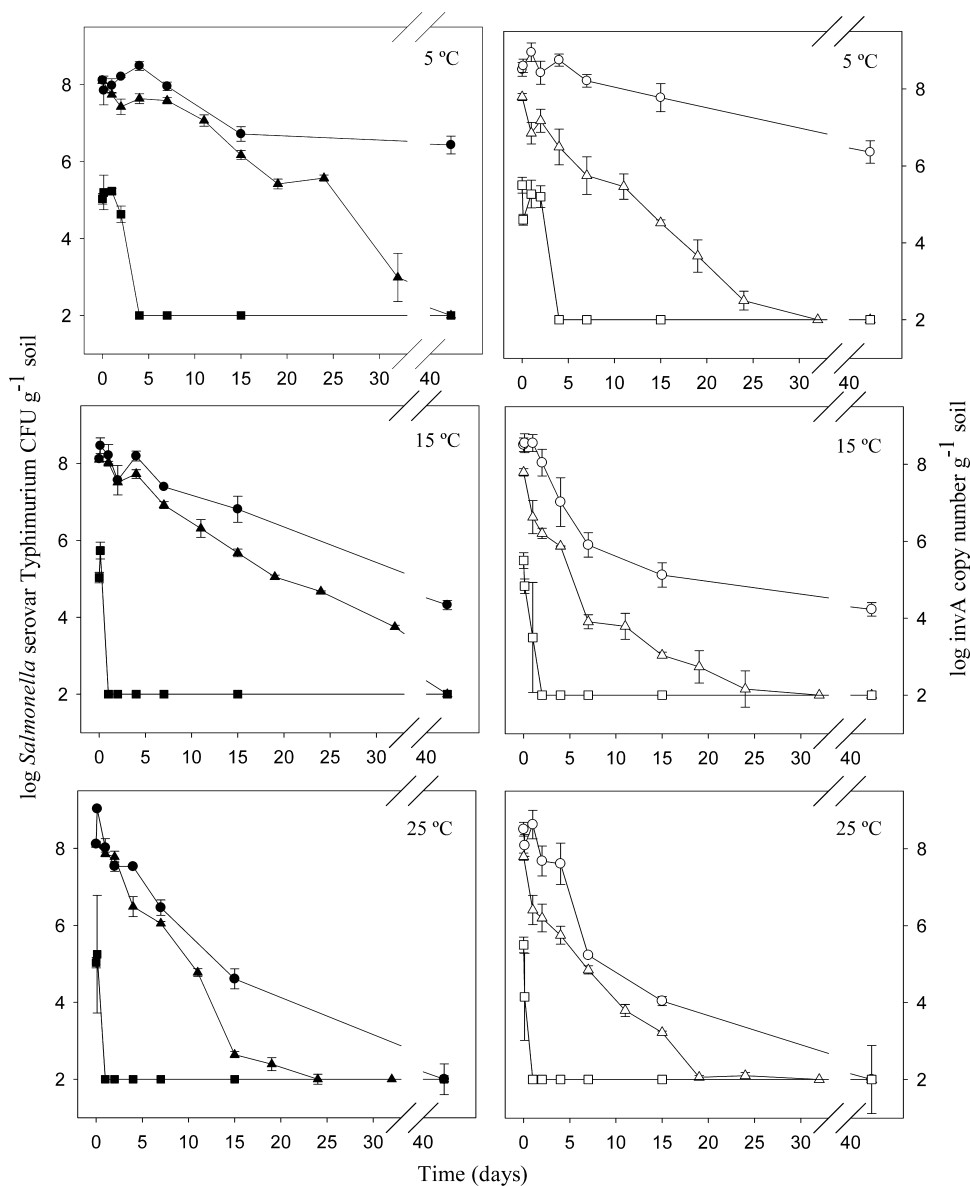


FIG. 1. Evolution of *Salmonella* serovar Typhimurium ( $Tet^+$ ) measured by qPCR and plate counting in soil samples (closed symbols) and manure-amended soil samples (open symbols) at three different temperatures. Symbols: ● and ○, log *invA* DNA copy number gram soil $^{-1}$ ; ▲ and △, log CFU count gram soil $^{-1}$ ; ■ and □, log *invA* mRNA copy number gram soil $^{-1}$ . Each point represents the mean of triplicate quantifications from one sample. Detection limit =  $10^2$ . Error bars represent standard errors of the mean. Please note the broken x axes.

A decrease in *Salmonella* serovar Typhimurium levels and activity was observed with all of the three methods used throughout the experiment.

Using plate counting, we observed a significantly ( $P < 0.001$ ) lower level of survival of *Salmonella* serovar Typhimurium in soil samples incubated at 25°C compared to 5°C as early as 4 days. This tendency was maintained throughout the experiment. At 5°C and 15°C, the addition of manure reduced the survival of *Salmonella* serovar Typhimurium, while at 25°C no significant difference was observed. In manure-amended soil samples, a significant difference between temperatures was found no earlier than after 11 days, with the highest survival at 5°C.

Using qPCR based on *invA* DNA copy numbers, we ob-

served a 1.5- to 2-log decrease at 5°C, a 4-log decrease at 15°C, and a >6-log decrease at 25°C over a period of 42 days (Fig. 1). The generally lower *invA* gene levels detected in soil samples incubated at 25°C than in those incubated at 5°C corresponded well to the results obtained by the plate counting technique. Contrary to the plate counting results, however, the evolution of *invA* genes did not present significant differences between the soil and manure-amended soil scenarios, and only from 4 to 15 days at 15°C did we observe slightly lower levels of *invA* genes in soil samples with manure than in those without manure.

**Quantification of *Salmonella* serovar Typhimurium activity levels based on *invA* mRNA.** At 5°C,  $\sim 10^5$  *invA* mRNA copies g soil $^{-1}$  were detected until 48 h after inoculation, while in the

15 and 25°C experiments we only detected *invA* mRNA until 3 h after inoculation (Fig. 1). In the scenarios without manure addition, the *invA* mRNA levels increased until 3 h at the three temperatures assayed, while in manure-amended soil samples the amount of mRNA decreased immediately after inoculation. After 4 days of incubation, the level of *invA* mRNA was lower than the detection limit in all of the soil scenarios. *invA* mRNA could only be detected when more than  $10^6$  *Salmonella* serovar Typhimurium bacteria per g of soil were detected by plate counting, and the relative amount of mRNA in the samples was, in general, less than 1% of the *invA* DNA detected.

**Comparison of plate counting and DNA quantification methods.** A regression of numbers of *invA* DNA copies compared to numbers of CFU of *Salmonella* serovar Typhimurium per gram of soil resulted in good correlations for the soil scenarios performed at both 15°C and at 25°C ( $R^2$ , >0.9). For the soils incubated at 5°C, however, a correlation coefficient ( $R^2$ ) of <0.7 was observed (Fig. 2). In the samples at this low temperature, DNA quantification values were very high even when *Salmonella* serovar Typhimurium was not detected by plate counting. At 5°C, significant differences ( $P < 0.001$ ) between DNA and CFU levels were found as early as 4 days in manure-amended soil and from 21 days in soil samples.

Despite the good correlation between CFU values and DNA levels at 25°C, a comparison of regression slopes using the Fisher test indicated a significant difference between soils with and without manure. Also at 5°C, at high levels of CFU and *invA*, significant differences were found between slopes for the soils with and without manure. Addition of manure caused decreases in CFU levels especially in the beginning of the assay. This trend was not observed when quantifications were based on *invA* genes.

**Growth of protozoa and its relation to *Salmonella* serovar Typhimurium survival.** The initial number of protozoa was 1.5 log higher in the manure-amended soil compared to the soil samples without manure. After 24 days, a bloom in protozoan numbers was observed in manure-amended soil at all three temperatures, with the highest levels ( $10^6$ ) observed at 5 and 15°C (Fig. 3). In soils without manure, a slower increase in protozoan numbers was observed at 5 and 15°C, with the highest levels observed at the end of the experiment (42 days). The differences in protozoan evolution between the two soil scenarios, we believe, was a determining factor in the significant differences in *Salmonella* serovar Typhimurium survival. The decrease in CFU levels was faster in manure-amended soil samples at 5 and 15°C, where a faster increase in protozoan numbers was also observed. In all microcosms, prior to the bloom of protozoa, significant negative correlations were found between the abundance of protozoa and *Salmonella* serovar Typhimurium plate counting results (Pearson coefficient, <-0.5).

## DISCUSSION

Temperature has been shown to be an important factor in the survival of pathogenic bacteria in environmental samples (1, 22, 36). In our study, significant differences in *Salmonella* serovar Typhimurium survival at different temperatures were found by using three different detection methods. By the plate counting technique, the largest decline in bacterial levels was

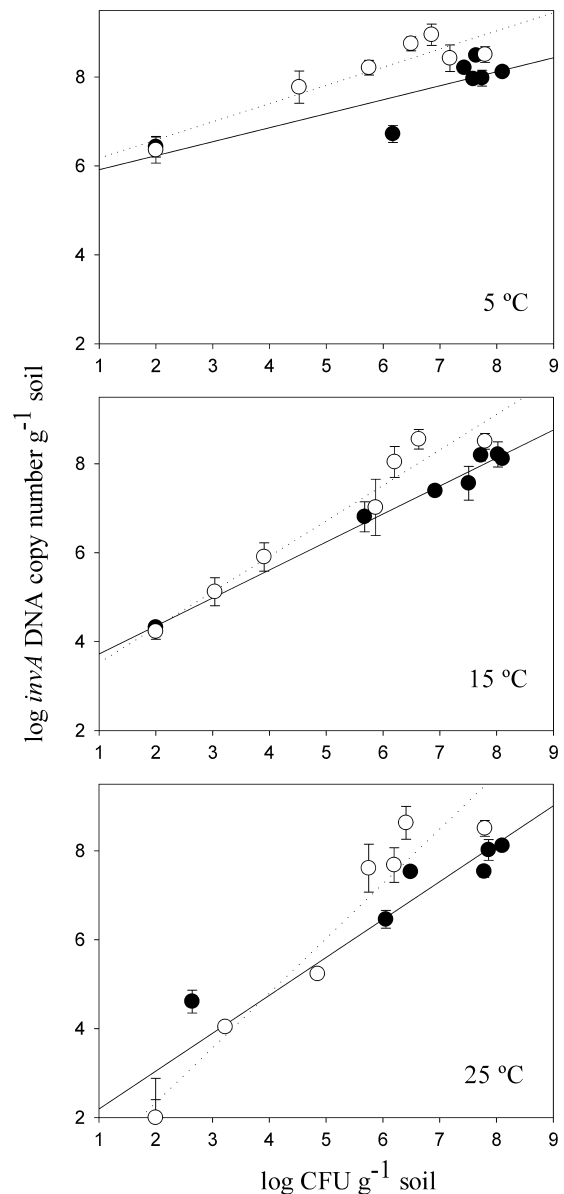


FIG. 2. Linear regression of the levels of *Salmonella* serovar Typhimurium ( $Tet^+$ ) quantified by plate counting and qPCR. The logarithm of the *invA* DNA copy number is plotted versus the logarithm of the *Salmonella* serovar Typhimurium CFU count per gram of soil at three different temperatures: 5°C, 15°C, and 25°C. Symbols: ●, soil; ○, manure-amended soil. Error bars represent the standard error of the mean.

observed at the highest temperature (25°C), which corresponds to what was published earlier (54). Semenov et al. (47) showed that survival of *Salmonella* serovar Typhimurium cells in cow manure decreased faster at 23°C than at 7°C, and similar results were reported by Holley et al. (23). In several studies, enhanced survival of allochthonous bacteria in manure-amended soils has been proposed to be due to an increase in nutrient availability (16, 23, 27). In our study, however, manure addition induced a level of *Salmonella* serovar Typhimurium survival significantly lower than that in nonamended soil. This was proven both at 5°C and at 15°C. In our case, the high



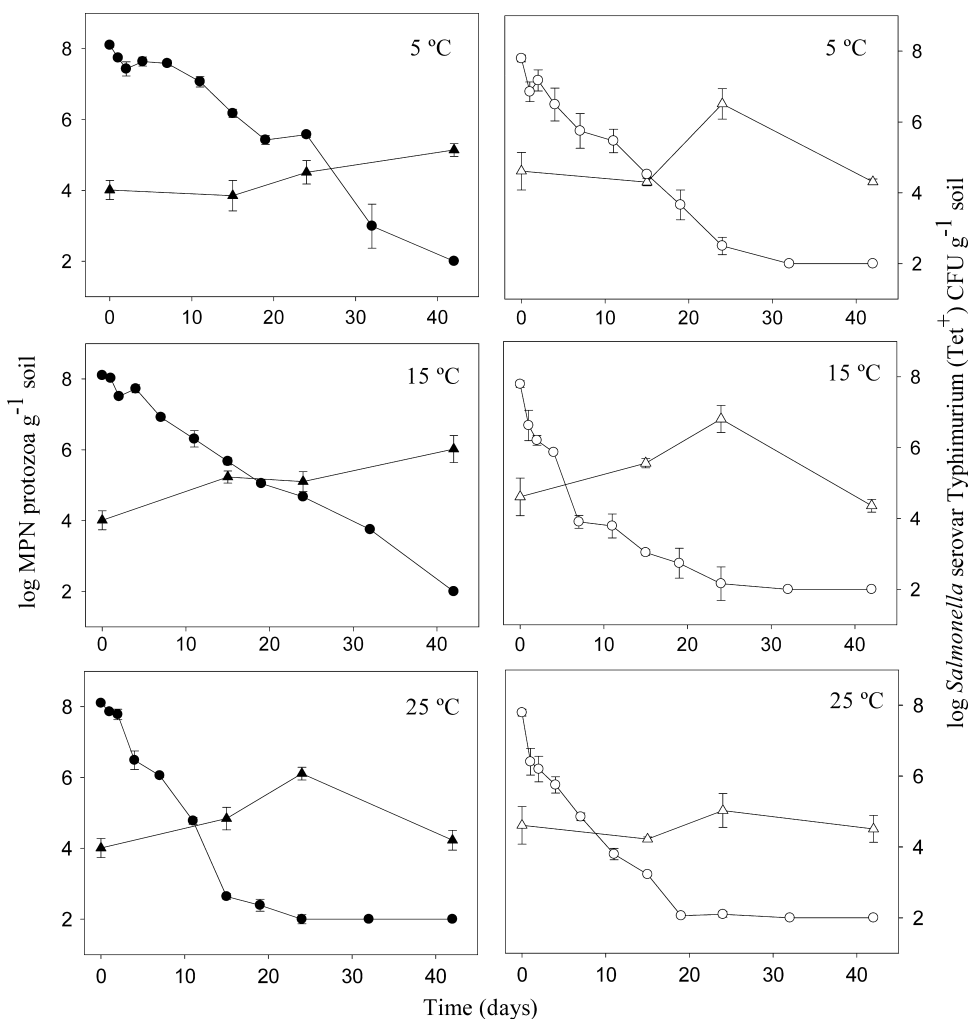


FIG. 3. Evolution of *Salmonella* serovar Typhimurium CFU counts (●, ○) and protozoan levels (△, ▲) in soil samples (closed symbols) and manure-amended soil samples (open symbols) incubated at three different temperatures. Protozoan counting was done at 15, 24, and 42 days after the beginning of the assay. Protozoan levels at time zero were determined before the addition of bacteria. Error bars represent the standard error of the mean.

nutrient availability due to manure addition might have increased the activity of the native soil microbial community. Increasing the general competition between bacteria could lead to decreased survival of the introduced pathogen (15). The faster decrease in CFU levels in manure-amended soil can also be related to evolution in protozoan levels, since predation by protozoa is another factor affecting bacterial survival in soil. After inoculation of *Salmonella* serovar Typhimurium at 5 and 15°C, blooms in the numbers of protozoa occurred within 24 days, while in soils without manure the highest level of protozoa was not observed until the end of the assay. In all microcosms, before the maximum level of protozoa was reached, a significant negative correlation was found between the abundance of protozoa and *Salmonella* serovar Typhimurium plate counting results, corroborating other studies of the role of predation in pathogen survival (2, 45).

In all of the previous works, the influence of factors like temperature, manure addition, or protozoan predation on the survival of *Salmonella* in environmental samples was deter-

mined by plate counting techniques. Herein, however, we report that in addition to survival of *Salmonella* serovar Typhimurium based on CFU levels, these factors may influence the survival of *Salmonella* serovar Typhimurium based on quantification of *invA* DNA or mRNA as well.

Since DNA can persist in dead cells and thereby may bias the number of viable bacteria, quantification based on the extraction of DNA from environmental samples should be carefully evaluated (30, 42). Even though it has been argued that the half-life of DNA in environmental samples may be very short because of the presence of nucleases (32, 53), also very old DNA has been shown to persist in soil (33). Levels of *Salmonella* serovar Typhimurium based on DNA quantifications were significantly higher than those obtained by plate counting, especially in manure-amended samples at low temperatures. Such a trend has also been confirmed by others (11, 51). As previously reported by Turpin et al. (52), detection of *Salmonella* using a culture-based technique was not possible in any of the temperature scenarios after 42 days. Based on *invA*

DNA levels, however, high levels of *Salmonella* serovar Typhimurium were still present at this time point, especially at 5°C, where a difference of 4 orders of magnitude between DNA and CFU levels was observed at the end of the assay, showing a nonculturable response higher at 5°C than at 25°C, as demonstrated by Gupte et al. (21). Due to a faster decrease in CFU levels, this difference was even more pronounced in the soil-with-manure scenario at 5°C.

The elevated *invA* gene levels found might be related to the presence of viable but nonculturable *Salmonella* serovar Typhimurium cells, as also reported by Marsh et al. (35). Especially at 5°C and 15°C, the ratio of *invA* gene to CFU levels was higher in soil with manure than in soil without manure. The addition of manure to soil seems to decrease the culturability of *Salmonella* serovar Typhimurium and support the presence of high levels of *invA* DNA.

The initial numbers of protozoa in our manure-amended soils were 1.5 logs higher than those in soil samples without manure, indicating that a significant number of protozoa was added along with the manure. These favorable conditions for predatory activity can be related directly to the fast decrease in *Salmonella* serovar Typhimurium CFU levels in the manure-amended soil. Brandl et al. (8) observed that the protozoan ciliate *Tetrahymena* contained intracellular feeding vesicles with high densities of ingested *S. enterica*. The subsequent release of these vesicles seemed to prolong bacterial survival in natural environments such as sites contaminated with manure, where this pathogen is present. The presence of *Salmonella* within intracellular protozoan vesicles likely underestimates the actual population of the pathogen because they cannot be detected by plate counting. Hence, if *Salmonella* is able to survive in protozoan vesicles, as other authors have reported (8, 19), it is in agreement with our results, where we found high numbers of *invA* gene levels versus low levels of CFU under conditions where predatory activity was higher. Our results also indicate that predation can be proposed to explain *Salmonella* dynamics in nonamended soils; the faster decline of cultivable *Salmonella* serovar Typhimurium at 25°C is consistent with the greater predatory levels observed under warm than under cooler soil conditions (5).

With the above-mentioned dilemma in mind, quantification of mRNA transcribed from a genus-specific gene could be the ideal biomarker for metabolically active *Salmonella* cells in environmental samples and it would help to determine their viability (49). In our study, we chose expression of the *invA* gene as such a biomarker. We observed a dramatic decrease in *invA* mRNA levels after only 48 h of inoculation at 5°C. At 15 and 25°C, this decrease was even faster and *invA* mRNA could only be detected in the 25°C microcosms until 3 h after the beginning of the experiment. Fey et al. (14) have done a study on the detection of *invA* mRNA in water samples, and they found a significant decrease in *invA* mRNA levels 3 h after inoculation, coinciding with the late logarithmic phase. In our study, however, this significant decrease in *invA* mRNA levels was detected 48 h after the inoculation of *Salmonella* serovar Typhimurium into samples incubated at 5°C.

In the present study, we were only able to detect *invA* mRNA in samples with more than 10<sup>6</sup> cells per g of soil (detected by plate counting), and this detection limit is higher than what has been observed in previous studies on mRNA

quantification in soil samples (26, 37, 40). The fact that the number of *invA* transcripts in general was lower than the *invA* gene copy numbers may indicate that *invA* expression is down-regulated as a response to stress due to suboptimal conditions in the environmental sample.

This study compared, for the first time, the fate of *Salmonella* serovar Typhimurium in soil and manure-amended soil at different temperatures by culturing and molecular methods. The *Salmonella* serovar Typhimurium levels detected by the three methods indicate that this pathogen survives better at 5°C. However, at this temperature, greater differences between *invA* DNA and CFU levels was observed. We propose the role of the predator-prey interaction as a factor to explain the differences between the culture and DNA quantification methods. The quantification of *invA* mRNA with our method confirms that *Salmonella* serovar Typhimurium shows invasive activity 48 h after inoculation into soil samples, although a high detection limit is observed. More work has to be done to improve molecular detection methods to evaluate the infectiousness of this strain in soils samples and how this ability may be affected by environmental conditions.

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