Salmonella detection in/on tomatoes

1. Introduction

EURL-*Salmonella* was requested, by EC DG SANTE, to collect information on the detection of *Salmonella* in/on tomatoes. For this, NRLs-*Salmonella* were approached if they ever had found *Salmonella* in/on tomatoes and whether they were aware of specific problems with detection of *Salmonella* in/on tomatoes. Additionally, some literature relevant for the subject was reviewed.

2. Information from NRLs-*Salmonella* on *Salmonella* in/on tomatoes

In total 22 NRLs-*Salmonella*, originating from 20 different countries replied to the request. 21/22 NRLs-*Salmonella* have never found *Salmonella* in/on tomatoes or do not test for *Salmonella* in/on tomatoes.
One NRL indicated to have in total five isolates from tomato-based food stuff in the strain collection, which belong to serovars Enteritidis, Orion, Paratyphi B and Abaetetuba (twice), found between 2008 and 2022.
In 2016, tomatoes were part of the national zoonosis monitoring in Germany, for which a protocol for preparation of tomato samples was established (see clause 4).

3. Information from literature on *Salmonella* in/on tomatoes

In 2014, an EFSA scientific opinion was published on *Salmonella* in tomatoes (EFSA, 2014), indicating that there is (very) limited data on the occurrence of *Salmonella* in/on tomatoes. In the EU only one salmonellosis outbreak associated with tomato consumption was identified in 2011. For this outbreak, microbiological analyses of the tomatoes was not possible, due to the fact that no stock was left. In this EFSA opinion, it was also indicated that in the USA, 13 salmonellosis outbreaks associated with tomato consumption have been reported in the period 2005-2012. In the majority of these outbreaks, contamination occurred at production or during minimal processing.

A later publication of Gu et al. (2018) gave information on a multi-year study (2012-2015) to investigate presumptive factors associated with the contamination of *Salmonella* within tomato fields in Virginia (USA). This was done because the Eastern Shore of Virginia was implicated in four *Salmonella* outbreaks associated with tomatoes between 2000 and 2010. Gu et al. (2018) performed several field experiments using irrigation water from a naturally contaminated pond and poultry litter from naturally contaminated local broiler farms as sources to investigate survival and transmission of *Salmonella* in tomato fields. *Salmonella* Newport was identified to be the most prevalent serovar isolated from pond water samples. *Salmonella* serovars Newport, Saintpaul, Typhimurium and Kentucky were isolated from the poultry litter used as soil amendment for fertilization in the different field trials. Additionally, one follow up field experiment was performed to quantify the likelihood and level of contamination in tomato rhizosphere, on leaves and on fruits after artificial inoculation, through drip irrigation, with *Salmonella* Newport (10^4 cfu/ml). The results of the different field experiments showed that the use of untreated pond water and raw poultry litter increased the likelihood of *Salmonella* detection in the rhizosphere of tomato plots. In several field studies (including the study with artificial inoculation of *Salmonella*), *Salmonella* was isolated from tomato leaf samples, but generally only from plots that were not staked and plastic mulch was not used; therefore, tomato plants were observed to readily contact the contaminated soil. All tomato fruits samples (n=4800) collected from the naturally contaminated plots tested *Salmonella* negative. From the artificially contaminated plot, 600 tomato fruits samples were tested and only 4 tomatoes tested *Salmonella* positive, but only from plots without staking or plastic mulch, where fruits were in contact with the (contaminated) soil.

Summary
The field studies of Gu et al. (2018) show little to no findings of *Salmonella* in/on tomatoes, even when these tomatoes are cultured on contaminated soil and/or irrigated with contaminated water. The results suggest that during production, tomatoes may become contaminated when in contact with *Salmonella* contaminated soil.
In the EFSA opinion of 2014, it is also suggested that 'testing of tomatoes for *Salmonella* could be limited to instances where other factors indicate breaches in GAP, GHP, GMP of HACCP programmes.'

4. Protocols (form literature) for preparation of tomato test portions for detection of *Salmonella*

For the detection of *Salmonella* in/on tomatoes reference is made to EN ISO 6579-1:2017(/A1:2020) or, for most of the USA studies, to the Bacteriological Analytical Manual (BAM, 2024) of the FDA.
For the preparation of the test portions of tomatoes, different protocols exist.

In 2016, NRL-*Salmonella* in Germany drafted the following protocol for preparation of tomato samples for detection of *Salmonella*:

- *Cut the unwashed tomato into quarters with a sterile knife and weigh 25 g ± 1 g.*
- *Add 225 ml 1% buffered peptone water (BPW) according to ISO 6579-1.*
- *Allow the mixture to stand for 1 h at room temperature.*
- *Adjust the mixture to pH 6.8 ± 0.2 with 1 M NaOH, if necessary.*
- *Swirl the flask several times and incubate at 37°C ± 1°C for 18 h ± 2 h.*
- *Follow ISO 6579-1.*

It was noticed, in preliminary tests, that stomaching the tomatoes in BPW had an unfavorable effect on the growth of *Salmonella* and it was advised not to stomacher the tomato sample.

In the BAM (2024), the following preparation of tomato samples is described for detection of *Salmonella* (*23.Tomatoes):*
For comminuted or cut fruit, aseptically weigh 25 g sample into a sterile blending container. Add 225 ml universal pre-enrichment broth (UPB) and blend 2 min. Aseptically transfer the homogenized mixture to sterile, wide-mouth, screw-cap jar (500 ml) or other appropriate container and let stand 60 ± 5 min at room temperature with jar securely capped. Do not adjust pH. Mix well and loosen jar cap about 1/ 4 turn. Incubate 24 ± 2 h at 35 ± 2°C. Continue as in BAM E.1-11.
Remark: EURL-*Salmonella* asked FDA for the reason of the 1 h hold time at room temperature and was informed that this is just how the method was validated. Initially, lactose broth was prescribed in the BAM, needing 1 h for the pH adjustment. Nowadays, buffered enrichment media are used, but still treated the same way as lactose broth. However, Wang et al. (2012) tested four different tomato sample preparations and this 1 h hold time at room temperature was not applied as no pH adjustment was needed for buffered broths (oral communication Mrs. Wang, FDA). The sample preparations for pre-enrichment tested by Wang et al. (2012) were 'Whole soaking' (whole tomatoes immersed in buffer), 'Quartering' (tomatoes cut into quarters and immersed in buffer), 'Stomaching' (tomatoes in buffer were stomached for 2 min before incubation), 'Blending' (tomatoes in buffer were blended for 20 sec before incubation).

In EN ISO 6887-1 or EN ISO 6887-4 no specific procedure is given for preparation of tomatoes. As tomatoes are acidic (generally having a pH of 4,1-4,9), the preparation for acidic products described in EN ISO 6887-1 and -4 needs to be followed:
It is important when preparing a suspension of acidic products that the pH is brought back to near neutrality (pH 7,0 ± 0,5). The use of buffered peptone water is sufficient for most products with pH greater than or equal to 4,5. More acidic products (greater than or equal to pH 3,5) may be brought back to the required pH using double-strength buffered peptone water, but the pH of such products should be checked when these are tested for the first time to ensure the required range is achieved.

5. Experiments at EURL-*Salmonella* for preparation of tomato samples and detection of *Salmonella*

Some steps for preparation of tomato test samples described in the three procedures above may be contradictory, e.g. do (not) adjust the pH, do (not) stomacher or blend the sample in the pre-enrichment buffer, do (not) leave the mixture of tomato sample in pre-enrichment buffer at room temperature for 1 h. Therefore a small experiment was performed by EURL-*Salmonella*.

Experimental design

- Measurement of pH of cherry tomatoes and snack tomatoes.
- Measurement of pH of cherry tomatoes and snack tomatoes (25 g sample) in 225 ml single strength Buffered Peptone Water (BPW; EN ISO 6579-1:2017):
  - Immediately after adding BPW to the tomato sample, with or without stomaching.
  - After leaving the tomato sample in BPW at room temperature for 1 h.
- Testing the recovery of *Salmonella* Strathcona from cherry tomatoes:
  - Inoculation of 8 samples of 25 g cherry tomatoes each, with 2 different levels of *Salmonella* Strathcona: approx. 5 cfu/25 g (4x) and 50 cfu/25 g (4x).
  - Addition of 225 ml single strength BPW to each 25 g sample.
  - Stomaching of half the set of tomato samples in BPW for both *Salmonella* levels and no stomaching of the other half set of samples.
  - Incubation of the initial suspensions at 34-38 °C, without 1 h hold time at room temperature.
- Following next steps of EN ISO 6579-1 for detection of *Salmonella* in food samples (selective enrichment on MSRV agar and in MKTTn broth).

Results

pH measurements

pH measurements for the cherry tomatoes and snack tomatoes are summarised in Table 1.

Table 1. pH measurements of cherry and snack tomatoes with and without BPW and after different treatments

Tomato type (25 g)	pH, no BPW added	225 ml BPW added, **no** stomaching		225 ml BPW added, **with** stomaching	
		pH immediate after adding BPW	pH after storage at room temp for 60 min	pH immediate after adding BPW	pH after storage at room temp for 60 min
Cherry tomatoes	4,3	7,0	6,9	6,8	6,8
Snack tomatoes	4,1	7,0	6,9	6,8	6,8

The NRL-*Salmonella* in Italy performed similar tests for pH measurement in BPW for 11 different types of tomatoes: with and without stomaching and with and without the one hour hold time at room temperature. Comparable results were found, with a pH varying between 6,8 and 7,0. The pH of the tomatoes without BPW were all approx. 4.

Recovery of Salmonella *Strathcona from cherry tomatoes*

4 samples of 25 g cherry tomatoes each were artificially contaminated with 2-3 cfu/ 25 g *Salmonella* Strathcona.
4 samples of 25 g cherry tomatoes each were artificially contaminated with 30-40 cfu /25 g *Salmonella* Strathcona.

225 ml BPW was added to each sample and 2 samples of each contamination level were stomached before incubation at 37 °C and the other samples were placed into the 37 °C incubator without stomaching.
After following all culture and confirmation steps of EN ISO 6579-1, all samples tested positive for *Salmonella*.

In this small experiment, stomaching did not affect the recovery of *Salmonella* Strathcona from cherry tomato samples.

6. Conclusions

- In the EFSA scientific opinion of 2014 it was already indicated that there is limited data on the occurrence of *Salmonella* in/on tomatoes.
- The number of salmonellosis outbreaks associated with tomato consumption in the EU is very limited.
- Several salmonellosis outbreaks associated with tomato consumption were reported in the USA. In the majority of these outbreaks, contamination occurred at production or during minimal processing.
- This latter was confirmed from field studies performed in the USA (Gu et al., 2018) where tomato fields were contaminated with *Salmonella*. *Salmonella* was detected in the rhizosphere of the tomato plots and in some of the leaf samples, but the latter mainly from tomato plants which had been in contact with the (contaminated) soil. *Salmonella* was hardly ever found from the tomato fruits. Only 4/5400 tomato fruits tested *Salmonella* positive and only from plots where fruits were in contact with the (artificially contaminated) soil.
- It could be questioned if the low findings of *Salmonella* in/on tomatoes can be a result of different sample preparation procedures. A small experiment performed by EURL-*Salmonella* showed:
  - ‘Normal’ (single strength) BPW (EN ISO 6579-1) has sufficient buffering capacity to bring the pH of naturally acidic tomatoes towards pH 7. Hence, pH adjustment of the initial suspension in BPW is not necessary. Neither is there a need to leave the initial suspension for 1 h at room temperature before starting the incubation.
  - In the small experiment, stomaching of the initial suspension did not negatively influence the recovery of *Salmonella* (Strathcona) from tomatoes. This confirms the findings of Wang et al. (2012) who found good recovery results after stomaching (or blending) the initial suspension of tomato samples.
- It can be concluded from literature information, as well as from the small EURL-*Salmonella* experiment, that the ‘normal’ procedure for the sample preparation of ‘one type of fruit or vegetable’ (clause 9.7.2 of EN ISO 6887-4:2017) can be applied for the preparation of tomato samples as well. Information is given in clause 7 (below).

7. Sample preparation for the detection of *Salmonella* in/on tomatoes

For preparation of tomato samples for detection of *Salmonella*, use the information in EN ISO 6887-1 and EN ISO 6887-4 for preparation of acidic samples and fresh fruit and vegetables:
Tomatoes are acidic products (pH generally 4,1-4,9), but the use of (single strength) buffered peptone water (BPW; EN ISO 6579-1) is mostly sufficient to bring the pH back to near neutrality (pH 7,0 ± 0,5; EN ISO 6887-1:2017, clause 8.6).

- Using aseptic techniques, cut the unwashed tomatoes in pieces, e.g. on a sterile half of a Petri dish. Include especially the stem scar and the skin of the tomatoes.
- Weigh 25 g ± 1 g tomato pieces into a stomacher bag.
- Add 225 ml (single strength) BPW (EN ISO 6579-1).
- Homogenize by using a peristaltic homogenizer (generally for 1 min).
- Follow EN ISO 6579-1:2017(/A1:2020) for the next steps for detection of *Salmonella* in a food product.

References

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