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Salmonella enterica subspecies *enterica* PCR and Culture Workflow at WVDL

Salmonella diagnostic testing can be challenging to interpret and apply to clinical or pathology samples. The WVDL has a workflow for the identification of *Salmonella enterica* subspecies *enterica* utilizing molecular detection, culture, serogroup and serotyping. This workflow was created based on existing data from published literature and data collected at the WVDL. We have analyzed the data collected from 2014-2020 to provide clients with a better understanding of why the WVDL has implemented the current *Salmonella* testing scheme.

The WVDL uses real-time PCR to identify *Salmonella* nucleic acid and traditional culture methods to obtain live *Salmonella* isolates, which can be used for serotyping. Susceptibility can be performed, but there are limited interpretations and susceptibility testing is not recommended. The WVDL offers a *Salmonella* species PCR that identifies the *Salmonella enterica* subspecies *enterica*. The *Salmonella* species PCR targets the *Salmonella* genus and the assay uses conserved genes common to all *Salmonella* species. The WVDL continues to culture the buffered peptone water (BPW) that was used for *Salmonella* PCR testing when the results are ≤ 35 C_T (cycle threshold).

Since 2014, the WVDL has been analyzing data comparing our PCR and culture rates from samples that obtain a 35 or lower C_T on the *Salmonella* species PCR. Table 1 demonstrates culture rates based on C_T value for 2020. At a C_T value of ≤25, the WVDL cultures *Salmonella*, of any serotype, 98.3% of the time. That rate drops as the C_T value increases demonstrating that less *Salmonella*-specific nucleic acid is present, thus less number of live *Salmonella* organism for successful culture. As an example, a C_T value between 25-30 produces a 91.6% culture rate, but by a C_T value between 30-35 the rate drops drastically to 61.7%.

Table 1: Culture rate for *Salmonella* species post multiplex PCR for 2020.

	≤25	**25-30**	**30-35**
Total Samples	118	191	193
***Salmonella* Isolated**	117	183	156
No *Salmonella* Isolated	1	8	37
Culture Rate	98.3%	91.6%	61.7%

Looking at culture rate for samples with overall C_T of ≤ 25, ≤30 and ≤35 for 2014-2020 (Table 2), the WVDL culture rates post-BPW enrichment have remained relatively stable.

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Table 2: Culture rate for *Salmonella* species post multiplex PCR for 2014-2020.

***Salmonella* species PCR C_T Value**	**2014-2016**	**2017**	**2018**	**2020**
≤ 35	77.8%	80.7%	85.2%	81.6%
≤ 30	91.3%	93.9%	94.9%	94.2%
≤ 25	97.9%	97.6%	94.2%	98.3%

It's important to note that cultures rates do not take into account the *Salmonella* culture that may have been requested from that same animal or sample directly. This data is purely the culture rate from the inoculums of post incubation PCR enrichment broth (BPW). The BPW was inoculated with a sample, such as feces or intestine, and incubated for 18-20 hours, before an aliquot is used for PCR and a second aliquot is cultured in enrichment media and plated on selective plates a day later if the PCR obtained a C_T value of ≤35. Therefore a different sample, from the same animal or tissue, may have been directly cultured using enrichment broths and selective plates and obtained a positive *Salmonella* culture when the BPW did not. The WVDL has observed that some *Salmonella* serotypes are enriched in BPW, an example is *Samonella* ser. Cerro, and others are difficult to culture from BPW, such as *Salmonella* ser. Dublin. Additionally, culture rate does not take into account animals that were treated with antibiotics prior to submission, which can result in a negative culture, but a positive PCR result. The WVDL has been observing an increase in the isolation of *Salmonella* ser. Cerro (see J Dairy Sci. Valenzuela et al. 2017), which has also been observed in New York and Pennsylvania.

The WVDL has implemented the following workflow to assist clients in obtaining the timeliest results. Diagnostic samples, which are enriched in BPW and are run on the *Salmonella* species PCR are automatically submitted for bacterial culture when the PCR C_T value is ≤ 35 (culture rate of 81.6% in 2020). Clients who do not want this testing done should notify their case coordinator (608-262-5432).

A sample with C_T value between 37 and 40 is considered a weak positive, where little nucleic acid is found and further sampling or testing may be necessary. Any sample with C_T value >35 will not be automatically sent for culture due to the low culture success rate. Clients who would like culture performed from a BPW sample that the C_T value was >35 should notify their case coordinator of that request. When a sample results in a high C_T value (low nucleic acid found in the sample), which will not result in automatic culture (PCR >35 C_T), it is recommended that additional samples from a particular animal or farm with appropriate risk factors or history of salmonellosis should be taken and tested to prove salmonellosis. Clients can request culture on these samples by contacting their case coordinator.

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The purpose of the continued culture on a low C_T value for the *Salmonella* species PCR positives is to obtain an isolate for further serotyping and possibly susceptibility. **If culture is successful, serotyping will automatically be performed, but will have an additional charge.** Clients who do not wish to have serotyping should notify the their case coordinator. *Salmonella* serotyping can take multiple days to weeks if the isolate is typeable. Non-typeable isolates can be sent to NVSL upon requested.

WVDL does not perform antimicrobial susceptibility testing automatically for *Salmonella* isolates obtained from fecal samples. The WVDL will continue to perform *Salmonella* serotyping automatically and will do antimicrobial susceptibility testing if it is requested by the submitting veterinarian. Some of the reasons for this change in laboratory policy are listed below:

1. There are no veterinary approved Clinical and Laboratory Standards Institute (CLSI) break-points for *Salmonella*. The only break-points available are for human use[1].
2. Oral antibiotics cause gut microbiome dysbiosis (less diversity) leading to an increased probability of *Salmonella* colonization and increased risk of invasive *Salmonella* complications[2].
3. Oral antibiotics increases the risk of antimicrobial resistance (AMR) for many different types of gut bacteria including *E. coli* and *Salmonella*[2].
4. Enteric *Salmonella* are often resistant to the most common classes of oral antibiotics that are approved for use in livestock.
5. Oral antibiotics increase the number of *Salmonella* super-spreaders and their use also increases the duration of *Salmonella* shedding[3]. It has been estimated that super-spreaders account for roughly 80% of *Salmonella* transmission[3]. Super-spreaders are especially problematic since they cause an increase in environmental load of *Salmonella* which serves as a reservoir of new infections.
6. For dairy beef and veal operations, oral antibiotics particularly those that use the aminoglycoside class of antimicrobial drugs increase the risk of drug residues being detected at the time of slaughter.
7. *Salmonella* are intrinsically resistant to the aminoglycoside class of antibiotics which includes Neomycin[4].
8. Some commercial non-medicated probiotics have good activity against enteric *Salmonella*, do not cause drug residues and have been shown to improve gut microbiome diversity[5,6]. This means there are viable alternatives to oral antibiotics in livestock that are relatively inexpensive.

Please be aware that when the WVDL cultures *Salmonella* serotypes from environmental samples, these isolates will not undergo antimicrobial sensitivity testing. Interpretation of PCR results should be done with contamination control in mind. Proper

sample acquisition requires practitioners to be cognizant of potential contamination from vaccines as well as other environmental sources such as clothing and gloves. *Salmonella* nucleic acid is typically prevalent in the environment on the modern dairy and poultry production facility. Use caution to not over interpret environmental samples submitted for PCR. Environmental samples should be cultured to confirm presence of viable pathogens.

Salmonella ser. Dublin does have a lower culture rate (~60%) and therefore it may be necessary to use the PCR results along with clinical history of the herd or animal tested to dictate changes to management practices or antimicrobial therapy. The antimicrobial sensitivity for *Salmonella* ser. Dublin has not changed in the recent past (Appendix 1). Data from WVDL shows that only enrofloxacin, gentamicin, and trimethoprim sulfa (TMS) have consistent susceptibility, but we remind the practitioner about the FDA restricted uses of fluoroquinolones and aminoglycosides in food producing animals. Fifteen of 27 isolates were susceptible to TMS.

In the United States, TMS only available for food animals in oral formulation, appropriate for use in calves <2-3 weeks of age in the pre-ruminant stage of life. Enrofloxacin is labeled for bovine respiratory disease complex pathogens and in dairy animals less than 20 months of age only – off label use is strictly prohibited. Aminoglycosides are still part of a voluntary ban and carry 18-24 month slaughter withhold.

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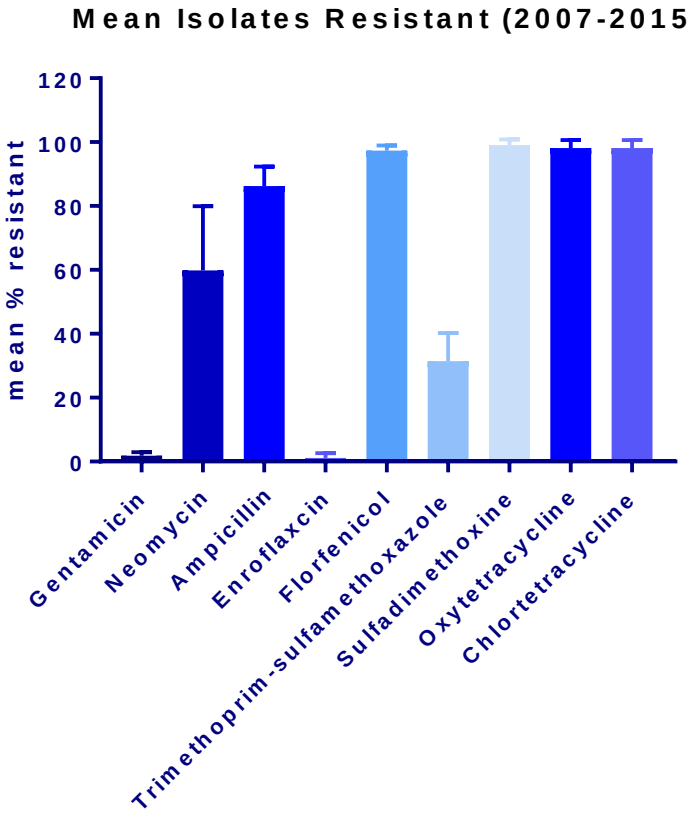

Appendix 1

These data indicate that enrofloxacin, trimethoprim sulfamethoxazole (TMS), and gentamicin should be considered potential therapeutic antimicrobials if necessary. Enrofloxacin is labeled for respiratory disease and in dairy animals less than 20 months of age only- off label use is illegal. TMS is only available in oral formulations, appropriate for use in calves < 2-3 weeks of age. Aminoglycosides are still part of a voluntary ban and carry a 18-24 month slaughter withhold.

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