

gle wild boar has tested positive for *Trichinella* since official active surveillance was implemented in 2013. This outbreak should raise attention on the preventive key role of epidemiological veterinary surveillance, and the need to optimize sampling procedures and targeted health education

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#### PS15.01 (407)

##### Characterization of the Role of FruR in *Listeria monocytogenes* Virulence and Identification of Its Regulons

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**Purpose:** *Listeria monocytogenes* is a Gram-positive intracellular pathogen that cause listeriosis, a fatal foodborne disease for human and animals. Pathogenesis of *L. monocytogenes* is tightly controlled by transcriptional regulators. Among these regulators, we found the genome of *L. monocytogenes* F2365 contains seven members of the DeoR-family of regulators. Although some members of the DeoR-family have been studied in several bacteria, its function in *L. monocytogenes* is unexplored, even though sugar metabolism is crucial for virulence. The goal of the present study was to assess the importance of *fruR* (LMOF2365\_2307), which encodes a DeoR-family transcriptional regulator, in the virulence of *L. monocytogenes*.

**Methods & Materials:** Overlap extension PCR and allelic exchange were used to construct an *L. monocytogenes* strain lacking *fruR* ( $\Delta fruR$ ). To determine the role in *in vivo* and intracellular virulence, mice model challenges and plaque assay in murine L2 fibroblast cell lines were conducted, respectively. Furthermore, RNA-seq analysis were performed to identify upregulation and downregulation of genes due to deletion of *fruR*.

**Results:** In the murine model, deletion of *fruR* severely decreased virulence compared to parent F2365 strain, and constitutive activation of *PrfA*, a key virulence regulator of *L. monocytogenes*, did not restore virulence to  $\Delta fruR$  strain. Moreover,  $\Delta fruR$  strain was defective for cell-to-cell spread in L2 fibroblast cells. The  $\Delta fruR$  strain had an increased lag phase compared to F2365 during growth in media with H<sub>2</sub>O<sub>2</sub>, suggesting that FruR contributes to survival of *L. monocytogenes* during oxidative stress. Furthermore, RNA-seq analysis revealed that glycolysis and pentose phosphate pathway (PPP) genes were significantly affected by *fruR* deletion. In particular, FruR induces expression of genes encoding PPP enzymes and suppresses glycolysis genes. Taken together, these results suggest a novel virulence mechanism whereby FruR upregulates the PPP, and this upregulation is necessary for full protection from oxidative stress.

**Conclusion:** This study clarifies the role of FruR in controlling *L. monocytogenes* carbon metabolism and provides a new mechanism allowing metabolic adaptation of *L. monocytogenes* to oxidative stress and may assist in the development of intervention strategies to control *Listeria* infection.

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#### PS15.02 (377)

##### Updated Algorithm for the Enhanced Detection, Isolation, and Identification of Shiga Toxin-Producing *Escherichia coli*, NYSDOH, 2011-2020

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**Purpose:** Shiga toxin-producing *Escherichia coli* (STEC) are an important cause of enteric infections and isolation is critical for outbreak investigations. A shift from enzyme immunoassays to more sensitive, culture independent molecular methods at submitting laboratories has resulted in an increase in specimen submissions to public health laboratories. The Wadsworth Center (WC) saw a 172% increase in the number of STEC specimens received in 2019-2020 compared to 2011-2012. During the last decade, WC has updated testing algorithms to improve efficiency and optimize recovery of STEC isolates for surveillance and outbreak detection.

**Methods & Materials:** WC utilizes an initial molecular screen to detect the presence of Shiga toxin genes (*stx*) and O157 DNA by real-time PCR. PCR positive samples are cultured to isolate the STEC organism. Real-time PCR is used to identify serogroups O26, O103, O111, O45, O121, and O145. Individual colonies and/or pools of colonies are screened for *stx* and immunomagnetic bead separation is performed as needed for serogroups identified by PCR. PCR positive isolates are confirmed as *E. coli* biochemically and sent for whole genome sequencing analysis.

**Results:** From 2011-2020, 3,637 primary specimens were received at WC and 2,815 were positive by PCR. STEC was isolated from 63% (2,282/3,637) of these. Furthermore, 23% (822/3,637) of specimens were determined to be *stx* DNA negative. The most frequently isolated serogroup was O103, representing 20% (447/2,282) of total STEC isolated from primary specimens. Serogroup O157 was identified most frequently when isolates and primary specimens were assessed together. Our results indicate real-time PCR cycle threshold values (CT) are inversely related to isolate recovery from primary specimens. Isolate yield decreases with CT values >25 and is further reduced with CT's >30. CT values >30 may indicate that the organism is not viable or is not present in sufficient quantities for culture recovery.

**Conclusion:** Isolation and characterization of STEC is essential for public health surveillance to monitor foodborne infections and outbreaks. The WC has determined that establishing a CT cutoff helps predict isolation of organism. The implementation of this updated algorithm that utilizes a CT cutoff allows for the most streamlined and efficient recovery of STEC.

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#### PS15.03 (941)

##### Insufficient heat treatment of eggs due to following cooking instructions in the labels

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**Purpose:** The demand for ready-to-eat, prepared meals has risen over the years. Following a recipe is a reason pointed by some consumers for not monitoring doneness during cooking. Our aim was to investigate the inactivation of *Salmonella* in prod-

ucts with eggs during cooking according to instructions in labels/recipes.

**Methods & Materials:** We have chosen a very popular and traditional Portuguese dish made with salted codfish, fried potatoes and eggs – “Bacalhau À Brás”. We bought in a supermarket three packages of a frozen, precooked dish of “Bacalhau à Brás”, all belonging to the same production lot. The package contained a 300 g meal, with the codfish and fried potatoes already precooked, being the consumer responsible to add one raw egg in the end of the recipe. In the package, the cooking instructions are provided: “Conditions of use/preparation: Without defrosting, place the stew in a non-stick frying pan, then place the potatoes over the top and let them cook on a low heat so that it defrosts completely. Add a beaten egg, slowly wrapped for about 2 minutes so that the egg is sufficiently cooked. Garnish with whole olives or slices and chopped fresh parsley.”

These instructions were followed in the laboratory where a domestic food preparation was mimicked. Eggs contaminated with different levels of *Salmonella* were used.

**Results:** The 2 min at low temperature after the addition of the raw egg, as indicated in the package of the pre-cooked dish tested, was not enough to complete inactivate *Salmonella*. The risk for *Salmonella* survival and recovery of injured cells increases with the initial level of contamination.

**Conclusion:** Recipes where raw eggs are added by the consumers at home might constitute a safety issue due to failure in proper inactivation of *Salmonella* as the manufacturer’s instructions might not be specific enough to guarantee sufficient time and temperature, which may lead to the survival of *Salmonella*. This study highlights the need to provide scientifically-based information on food labels to increase food safety.

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**PS15.04 (687)**

**Unravelling the Microbiological Hazards in Small Scale Dairy Goat Farming in Chile**

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**Purpose:** Sheep and goats production in Small Family Farming systems is one of the main sources of income and meat production and plays a vital role in food security worldwide. In Chile, there are many Small Family Farming farms; however, there is a lack of sanitary formalization and the implementation of product safety assurance systems is scarce. These issues result in a higher risk to consumers’ health and fewer commercial opportunities in markets with greater stability and economic prospects that contribute to the improvement of the production processes. The goal of the study was to characterize small family farming goat production in Chacabuco Province, Chile, based on the microbiological hazards associated with the dairy products.

**Methods & Materials:** Raw milk and fresh cheese samples were taken from 21 family farming producers from Chacabuco Province, Metropolitan Region, Chile, during the months of November and December 2019. Microbiological analysis was performed according to the requirements established in the Chilean Food Sanitary Regulations. For milk, the Mesophilic Aerobic Count was performed, and for fresh cheese the following microorganisms were detected:

*Escherichia coli*, *Staphylococcus aureus*, *Salmonella* spp., Enterobacteriaceae and presence/absence of *Listeria monocytogenes*.

**Results:** The results showed the presence of microbiological hazards in raw milk and fresh cheese. In milk, 28.6% of the farmers exceeded the accepted limit for Mesophilic Aerobic Count, while, in fresh cheese, 71.4% of farmers did not comply with the maximum limit allowed for *E. coli* and all farmers (100%) had an Enterobacteriaceae count that does not comply with the Food Sanitary Regulation. However, when assessing foodborne pathogenic bacteria, neither *Salmonella* spp. nor *Listeria monocytogenes* were present in any sample, complying with the Food Sanitary Regulation. Finally, only 4.8% of the farmers did not comply with the acceptance for *Staphylococcus aureus*.

**Conclusion:** These results demonstrate deficient manufacturing practices and, consequently, a potential risk to consumer’s health. All these results emphasize the need to apply and maintain good hygienic practices, and to study risk factors to prevent contamination and bacterial growth.

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**PS15.05 (332)**

**Trends of Amoebic Dysentery in Thailand in 2016-2020: Successful Control Program or Decreased Surveillance?**

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**Purpose:** Amoebic dysentery ranks third as the most prevalent food and waterborne diseases in Thailand and is responsible for 5-15% of all diarrheal deaths annually. Children and infants are more vulnerable for this fecal-oral transmitted disease. In prior years, this disease received less public health attention. Fortunately, improvements in water supply infrastructures, waste management, and community awareness on hygiene indirectly affect the prevalence of this disease. This study evaluated the trends of amoebic dysentery and related factors in the past five years in Thailand.

**Methods & Materials:** We collected and analysed data of amoebic dysentery annual prevalence, age groups, population density, water sources and household utilities from all provinces in Thailand during 2016-2020. Data were retrieved from National Statistical Office and Bureau of Epidemiology, Ministry of Public Health, Thailand.

**Results:** There was a constant fall in amoebic dysentery morbidity rates from 2016 (4.65/100.000) to 2020 (0.95/100.000), with an average annual reduction of 0.8 points, and the highest decline from 2019 to 2020. The most vulnerable age group was children below 15 years, followed by geriatrics of over 55 years, with geometric means of 35.2% and 26.4%, respectively. The decrease in cases is correlated with increased number of clean water supplies with over 600,000 artesian and shallow wells countrywide (accounting for roughly 1 well for 11 people), and 89% of households having access to tap water. Interestingly, the highest case contributions persistently stemmed from the provinces at the country borders with Myanmar (Chiang Mai, Mae Hong Son, Tak), Cambodia (Sisaket, Ubon Ratchatani), and Malaysia (Songkhla, Yala), which accounts for an average of 49.8% of total cases. Further exploration