Salmonella Dublin diagnostics for the field veterinarian

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Abstract

Salmonella Dublin is a zoonotic, multi-drug-resistant bacteria. It is host adapted in cattle, and carrier animals shed bacteria in feces and milk, acting as a reservoir for the herd. *Salmonella* Dublin causes septicemia in young calves, who present with a high fever, increased respiratory effort and acute death. Diagnosing Salmonella Dublin in an acutely infected calf requires aseptic collection of a blood culture sample, storage at room temperature or in an incubator, and overnight delivery to a veterinary diagnostic laboratory. Diagnosing *Salmonella* Dublin post-mortem requires submission of one or more of the following organs for aerobic culture: lung, kidney, lymph node, liver or spleen. These samples should be refrigerated or frozen and delivered overnight as well. Feces and gastrointestinal samples have low sensitivity for recovering *Salmonella* Dublin and are not recommended in clinical suspects. Surveillance or monitoring for *Salmonella* Dublin antibodies can be performed with the *Salmonella* Dublin ELISA. Appropriate samples include serum, individual milk samples and bulk tank milk samples. Seroconversion time and age of animal impact the sensitivity of the *Salmonella* Dublin ELISA.

Key words: diagnostics, host-adapted, *Salmonella* Dublin

Introduction

Salmonella are a genus of flagellated, gram-negative, rod shaped bacteria. *Salmonella* enterica subspecies enterica contain over 2,5000 serovars, also called serotypes.[3] When cultured from animal specimens at the Cornell University College of Veterinary Medicine Animal Health Diagnostic Center (AHDC), the Salmonella isolate is grouped based on agglutination reactions to O and H antigens on the cell surface, then sent to the National Veterinary Services Laboratory in Ames, Iowa to be serotyped.[1] *Salmonella* serogroups infecting cattle include B (Typhimurium, Heidelberg and Agona), C (Newport, Montevideo, Kentucky), D1 (Dublin, Enteritidis), E (Anatum and Muenster) and K (Cerro) (Table 1). From 2006 to 2021, the top 5 most frequently isolated serotypes at the AHDC were Cerro (2,407), Typhimurium (1,002), Dublin (943), Montevideo (335), and Newport (227) (Figure 1). In a 2016 survey from the National Veterinary Services Laboratory, *Salmonella* enterica subspecies enterica serovar Dublin was the most frequently isolated serotype from ill cattle in the United States (U.S.), followed by Cerro, then Typhimurium.[14] Salmonella Dublin is the bovine host adapted serovar, creating a carrier state in cattle who shed the bacteria constantly or intermittently over their lifetime.[3,5,11] *Salmonella* Dublin was a recognized bovine pathogen in the western U.S. in the earlier part of the 20th century.[2,13] It marched its way east, first appearing in New York in the 1980s,[5] and is currently endemic in the northeastern dairy industry.[2] *Salmonella* Dublin is zoonotic, and human infections are typically linked to food-borne outbreaks. Cases are rare, but serious; one European study cited a 28.3% case fatality rate.[8] In 2019, the Center for Disease Control reported an outbreak of *Salmonella* Dublin linked to raw beef. There were 13 human illnesses, 9 hospitalizations and one death.[17]

Table 1: Common Salmonella serogroups and serotypes found in cattle.

Serogroup	Serotype
B	Typhimurium, Heidelberg, Agona
C	Newport, Montevideo, Kentucky
D1	Dublin, Enteritidis
E	Anatum
K	Cerro

Through passive surveillance, the AHDC has maintained a list of *Salmonella* Dublin positive dairy farms in New York (NY) state since 2006, based on either bulk tank milk *Salmonella* Dublin ELISA positive results, or isolating Salmonella Dublin from bovine samples. Currently, there are 166 farms out of approximately 4,000 dairies in the state, indicating a 4% prevalence, which is likely much lower than the actual number of infected farms.

Disease transmission

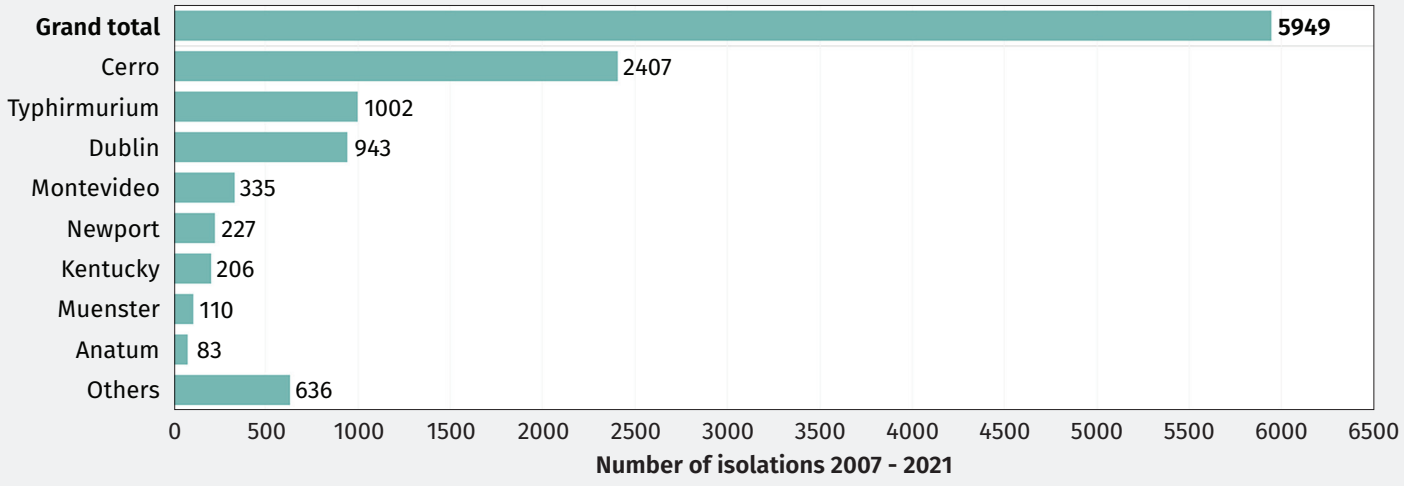
Asymptomatic carrier cattle shed *Samonella* Dublin in feces (108 – 109 organisms per day),[13,14] milk and colostrum (102- 105 organisms per mL).[6] These animals serve as an ongoing source of exposure for the rest of the herd, as 106 organisms are required for infection.[9] Clinically ill animals shed more bacteria than asymptomatic, and calves less than 1 year shed for 9 weeks and heifers and cows shed for 3 weeks after initial infection.[12] Eighteen percent of clinically ill animals become carriers and 1.5% of asymptomatic animals become carriers.[12]

Transmission occurs through ingestion of feces, raw colostrum and raw milk as well as contaminated feed, water, housing surfaces, the maternity pen and group housing.[12] From the author's experience, *Salmonella* Dublin introductions into naïve herds occur through a few common practices in dairy farms, including sending heifers to a group heifer raiser, group housed milk-fed calves, feeding raw colostrum or raw milk, open herds and attending fairs and shows.

Disease presentation

The classic presentation of salmonellosis in cattle, with a low-grade fever and diarrhea, are consistent with the non-host adapted *Salmonella* serovars, but not Dublin.[14] Salmonella Dublin causes septicemia in young calves.[2,13] In the author's experience, calves are typically less than 2 months of age and present with elevated respiratory rates and high fevers (104°-107° F). Calves can also be found dead after peracute illness, typically perceived as bovine respiratory disease by the producer. Naïve herds can experience abortion,[2] and rarely adults or calves can experience diarrhea.[5,13,14]

Figure 1:



Diagnostics for clinical suspects

The appropriate ante-mortem sample to collect on a clinical suspect is an aerobic blood culture, which must be placed in blood culture media.[4,14] To perform a blood culture, first the rubber stopper of the blood culture media is wiped with 70% alcohol and allowed to dry before inoculation. The calf is restrained and the jugular vein clipped and aseptically prepared, then allowed to dry. Sterile gloves must be worn and 2 to 3 mL of whole blood is drawn from the jugular vein. A new needle is placed on the syringe, the blood culture media is inoculated with the blood sample, then the bottle is inverted a few times. Blood culture vials can be easily contaminated, and sterility is very important when taking the sample. The blood culture media is stored at room temperature, or in an incubator, until delivery to a veterinary diagnostic laboratory. Blood culture media should not be refrigerated or frozen. The AHDC uses an automated blood culture system. Once the machine signals bacterial growth, the sample is plated and gram stained, and *Salmonella* Dublin is confirmed through the previously described process.

When presented with a post-mortem *Salmonella* Dublin suspect, on gross necropsy the lungs may have a variety of appearances, but are typically described as "wet and heavy". Other descriptions from necropsies performed the AHDC include interstitial pneumonia, affecting all lung tissue, to fibrinous bronchopneumonia with pleuritis and pleural adhesions, to hematogenous spread appearing as multifocal randomly distributed dark red areas of lung parenchyma. Hepatomegaly may be present, giving the liver a swollen appearance with rib impressions. Other gross findings include meningitis and petechia on the serosal surface of the intestines. Collection of 1-inch pieces of the organs of septicemia for aerobic culture are indicated to diagnose Salmonella Dublin. This includes lung, kidney, spleen and lymph node.[13] The liver, although typically a poor tissue choice for diagnosing septicemia due to bacterial contamination from the nearby gastrointestinal tract, does reliably culture *Salmonella* Dublin (Figure 2). All fresh tissues should be stored in individual sterile containers, and sent with ice packs for overnight delivery to a veterinary diagnostic laboratory for culture. Fresh tissue samples can be frozen until shipment if next-day delivery is not possible. Swabs of organs can also be collected and stored in bacterial transport media. Formalin-fixed sections of all organs, specifically affected organs, at the junction between normal and abnormal tissue, should be submitted for histopathology. Histologic descriptions of lung parenchyma may vary depending on stage of disease, but include neutrophilia, fibrin and necrosis,[13] and lymphohistiocytic alveolitis with fibrin deposits, hemorrhage, and fibrin thrombi.a Of all *Salmonella* Dublin isolates cultured at the AHDC from 2006 to August 2021, lung was the most frequently culture positive organ, followed by the gastrointestinal tract (including feces), then kidney, lymph node, liver and whole blood. It must be noted that feces and gastrointestinal samples are poor samples for diagnosing *Salmonella* Dublin, and Nielson et al demonstrated very poor sensitivity (6-14%) for recovery of Salmonella Dublin in feces from asymptomatic carrier animals.[8,12] Recovery of Salmonella Dublin from feces of clinical animals may be closer to 25-50%, which is still relatively poor.[10] Our data may be skewed due to high numbers of fecal samples submitted in comparison to other sample types, but submitting feces or intestines for culture to diagnose *Salmonella* Dublin is not recommended due to poor sensitivity.

Salmonella Dublin has been resistant to ampicillin, chloramphenicol, neomycin and tetracycline (the last two likely due to feed additives) since the 1970s.[5] In 2018, Cummings et al published resistance to amoxicillin/clavulanic acid, ampicillin, cefoxitin, ceftiofur, ceftriaxone, chloramphenicol, streptomycin, sulfisoxazole and tetracycline.[2] Recent mean inhibitory concentration (MIC) panels on isolates from the AHDC show a consistent pattern of resistance to ampicillin, ceftiofur, clindamycin, florfenicol, gentamycin, neomycin, penicillin, tetracycline and tilmicosin. The MIC panel is derived from the Clinical Laboratory Standards Institute (CLSI) Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated from Animals, 5th edition.[18] Enrofloxacin is not labeled for *Salmonella* Dublin, and extra-label use of this antimicrobial is illegal in food-producing animals.[7]

At this time, polymerase chain reaction (PCR) is not an adequate single diagnostic test for *Salmonella* Dublin, because bacterial culture isolates are required to perform antimicrobial susceptibility testing as well as *Salmonella* grouping and serotyping. At the AHDC, an overnight enrichment step is required before *Salmonella* culture and PCR can be performed, resulting in minimal decrease in turn-around time for the PCR over bacterial culture.

Figure 2:

Herd testing procedure	HSe
Bulk tank milk LPS ELISA at cut-off OD=0.4	38%
Culture of dung-pits	45%
Drinking water cultures	5%
Bulk tank milk filter cultures	7%
Faecal culture of animals with current or earlier signs of salmonellosis	38%
Serology of all young stock	100%
Serology of all young stock between 4 to 6 months	91%
Serology of animals with current or previous signs of salmonellosis	80%
Combination of bulk tank milk ELISA and serology of animals with current or previous signs of salmonellosis	91%
Combination of bulk tank milk ELISA and serology of all young stock between 4 to 6 months	99%
Combination of bulk tank milk ELISA in four samples collected over 5 to 12 months	95%

Salmonella dublin ELISA

The *Salmonella* Dublin antibody test at the AHDC is the commercially available PrioCHECK™ *Salmonella* Dublin ELISA.b It can be performed on bovine serum, individual milk or bulk tank milk samples, and can be used for surveillance or monitoring of herds or individual animals. The manufacturer recommendation for a positive result is an optical density (OD) of ≥35%.19 At OD ≥35%, the ELISA has a sensitivity of 65% (95% CI 55-75%) and a specificity of 97% (95% CI 96-98%),8 but there are a few important considerations that will impact the interpretation of this test. *Salmonella* Dublin primarily affects young calves and has a long seroconversion time of up to 7 weeks.[10] The ELISA will detect maternal antibodies in calves, as well as the commercially available Salmonella Dublin vaccine.[15] The PrioCHECK™ Salmonella Dublin ELISA detects O antigen factors 1, 9 and 12, and can cross react with other Salmonella serotypes containing those factors, for example Salmonella Typhimurium.[19] The ELISA has the most sensitivity when performed on calves from 4-6 months of age,[16] likely due to the disease affecting neonatal calves, the prolonged seroconversion time, and a lack of maternal antibodies in this age group. Screening all heifers age 4-6 months at once results in 91% sensitivity for detecting Salmonella Dublin in the herd.[16] There is currently no recommendation for maximum or minimum number of cows in a bulk tank milk Salmonella Dublin ELISA sample, due to inherent fluctuation in antibody titers within a single animal and a herd on a day-to-day basis. The sensitivity of a single bulk tank milk sample is only 38% for detecting Salmonella Dublin antibodies, but screening all heifers age 4-6 months and performing a single bulk tank milk ELISA results in a 99% sensitivity.[11] Four bulk tank milk samples collected over a 5-12-month period results in 95% sensitivity for detecting Dublin (Figure 3),[16] and may be more cost effective on large dairies.

Since 2017, 20,284 ELISA serology samples have been tested at the AHDC and 13,693 were from NY state alone. Overall, 1,391 (6.9%) were positive. More than half of the samples were submitted for export purposes, and may not have been clinical suspects. In the same timeframe, 657 bulk tank milk ELISAs were submitted, and 553 were from NY state. One hundred and eighteen (18%) were positive. The increase in prevalence in bulk tank milk versus serum is likely impacted by reason for testing (export versus herd surveillance), as well as the ability of bulk tank milk to be positive due to a fraction of positive animals in the herd.

Performing ongoing surveillance to ensure a herd is negative is recommended. For herds that are positive, monitoring prevalence in the 4-6-month-old animals at set intervals (for example quarterly or twice yearly), can be used to assess the impact of management changes on controlling Salmonella Dublin in a herd. Carrier animals maintain a positive titer for 3 consecutive tests over an 8-month period.[9,11,14] The Salmonella Dublin ELISA can be used serially to determine carrier status, but if the positive animal is left in the group, she may shed *Salmonella* Dublin and infect cohorts in between testing dates. Due to delayed seroconversion, this can lead to perpetuation of Salmonella Dublin within herds. Monitoring certain animals to detect carriers can be costly and ineffective in controlling *Salmonella* Dublin in endemic herds, and once herds become infected, it is nearly impossible to eradicate. Preventing the disease from entering a dairy farm is the best management tool for *Salmonella* Dublin; once it becomes endemic, exquisite management of young stock is required to interrupt transmission and control *Salmonella* Dublin outbreaks in calves.

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Endnotes

a. Author's experience reviewing AHDC pathology reports.
b. PrioCHECK® *Salmonella* Dublin ELISA, ThermoFisher Scientific, Waltham, MA.

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