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Clarifying dairy calf mortality phenotypes through postmortem analysis

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ABSTRACT

Health problems can be thought of as phenotypic expressions of the complex relationships between genes, environments, and phenomes as a whole. Detailed evaluations of phenotypic expressions of illness are required to characterize important biological outcomes. We hypothesized that classifying dairy calf mortality phenotypes via a systematic postmortem analysis would identify different cause-of-death diagnoses than those derived from treatments alone. This cross-sectional study was carried out on a dairy calf ranch in the northwestern United States from June to September 2017 and focused on calves ≤ 90 d of age. Comparisons were made between causes of death based on 3 levels of information: on-farm treatment records alone, necropsy-based postmortem analyses in addition to treatment records, and Washington Animal Disease Diagnostic Laboratory (WADDL) results in addition to all other information. A total of 210 dairy calves were necropsied during this study, of which 122 cases were submitted to WADDL. Necropsy- and WADDL-derived mortality phenotypes were in almost perfect agreement (Cohen's $\kappa = 0.86$) when broadly categorized as diarrhea, respiratory, diarrhea and respiratory combined, or other causes. The level of agreement between on-farm treatment records and postmortem-derived results was low and varied by the level of diagnostic detail provided. There was just fair agreement ($\kappa = 0.22$) between treatment-based and necropsy-based phenotypes without WADDL input and only slight agreement ($\kappa = 0.13$) between treatment-based and corresponding necropsy-based phenotypes with WADDL input. Even for those cases in which causes of death aligned along

a comparable pathologic spectrum, the lack of detail inherent to standard treatment-based causes of death failed to identify meaningful target areas for intervention. This was especially apparent for numerous cases of necrotizing enteritis and typhlitis (cecal inflammation) that were variously categorized as diarrhea and pneumonia by treatment-based diagnoses. The specificity of these lesions stood in stark contrast to the otherwise generic cause of death diagnoses derived from treatments. The findings from this study supported the hypothesis and highlighted the value of on-farm necropsies and laboratory-based diagnostics to (1) detect antemortem disease misclassifications, (2) provide detail regarding disease processes and mortality phenotypes, and (3) direct disease mitigation strategies.

Key words: dairy calf, mortality, phenotype, postmortem, typhlitis

INTRODUCTION

Health problems can be thought of as phenotypic expressions of the complex relationships between genes, environments, and phenomes as a whole (Houle et al., 2010). Although modern dairy population medicine has focused extensively on establishing genetic associations to understand phenotypes related to productivity, disease states, and mortality (De Vries, 2017), these associations tend to explain only a small proportion of phenotypic variance (Houle, 2010). Detailed records and evaluations of data related to phenotypic expressions of illness are required to characterize important biological outcomes, including morbidity and mortality (Houle et al., 2010; Giebel et al., 2012; Gangsei et al., 2016).

Efforts to document dairy mortality have primarily focused on adult cow death (Thomsen and Houe, 2006; McConnel et al., 2015; Shahid et al., 2015; Compton et al., 2017). Research into heifer health problems tends to distill disease into preweaning or postweaning gas-

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trointestinal (GI) or respiratory disease (Gorden and Plummer, 2010; Stanton et al., 2012; Klein-Jöbstl et al., 2014; USDA, 2017). It is estimated that over 8% of nulliparous heifers die, with producer-derived surveys implicating infectious calf diarrhea as a cause of more than half (56.4%) of all preweaning calf mortality and respiratory problems as a cause of over half (58.9%) of postweaning deaths (USDA, 2017). However, diagnostic detail and accuracy is hindered by a lack of necropsies and additional laboratory work-up. Across US dairies, it is estimated that only 11% of farms necropsy heifers at all and <5% of dead heifers are necropsied as part of a postmortem examination of cause of death (USDA, 2017).

Without necropsies, it is often impossible to clarify underlying disease processes and treatment efficacy. Furthermore, inconsistencies in cause of death definitions and disease data presentation hinder descriptions, comparisons, and investigations into animal health (Kelton et al., 1998; Giebel et al., 2012). Standardizing health event nomenclature based on postmortem findings in simple and consistent terms can provide useful information not only for the analysis of deaths but for other health-related questions as well (McConnel and Garry, 2017). Without standardized methods of classification, what little information is available is often wasted (McConnel et al., 2010; Compton et al., 2017).

The correlation between suspected causes of death in heifers and causes of death verified through postmortem evaluations has been assessed to a very limited degree within Scandinavia (Gulliksen et al., 2009) and has not been evaluated at a meaningful level within the United States. Gulliksen et al. (2009) found that pneumonia was the most common cause of death among 65 necropsied calves on 35 Norwegian dairy farms but that enteritis was the most frequent postmortem diagnoses in the youngest calves. Given that <5% of dead heifers are necropsied in the United States, the reality is that many dairy calf mortalities are poorly categorized, with causes of death based solely upon previous treatment history rather than diagnostic input. The absence of diagnostic input is important because current estimations of the frequency of disease are then dependent upon standard treatment protocols serving as a proxy for unsubstantiated underlying disease. This lack of insight into heifer health problems is a major liability for efforts to accurately record disease phenotypes. Without a clearer understanding of the relationship between treatment and disease, approximations of treatment failures versus diagnostic inaccuracies are left unknown. Ultimately, understanding the timing and fates of animals that die on farms can be informative in their reflection of management conditions and production efficiencies

and provide a foundation for improved understanding of animal health and features of farm management that present risks of poor outcomes.

With that in mind, the objective of this project was to utilize necropsies, standardized death certificates listing attributable causes of death (underlying, contributing, immediate), and additional diagnostics to clarify calf mortality phenotypes for a better understanding and recording of the underlying burden of disease. Comparisons were made between standard treatment-based versus on-farm necropsy-based cause-of-death diagnoses with or without additional diagnostics at the Washington Animal Disease Diagnostic Laboratory (WADDL; College of Veterinary Medicine, Washington State University, Pullman). We hypothesized that classifying dairy calf mortality phenotypes via a systematic postmortem analysis would identify differences in cause of death diagnoses compared with those derived from treatments alone.

MATERIALS AND METHODS

Study Population

This cross-sectional study was carried out on a dairy calf ranch in the western United States between June 20 and September 14, 2017. The ranch housed approximately 25,000 heifer calves from multiple dairies through 200 d of age. Historical mortality levels approximated 2% of carrying capacity per month, with 90% of those deaths occurring at ≤ 90 d of age. This study focused on deaths of calves ≤ 90 d of age to concentrate efforts within the high-risk period for calf mortality.

Colostrum was fed to calves at the dairy of birth and approximately 40% of calves had serum total protein levels assessed at the ranch within the first week of life using a Brix refractometer. Calves were fed 2 L of a custom milk blend twice daily from 1 to 52 d of age. On d 53 to 60, they were fed 2 L once per day and weaned thereafter. The milk blend consisted of pasteurized waste milk and milk replacer targeting 13% solids, 22 to 24% fat, and 28% protein. A grain mix consisting of pellets, molasses, and whole corn was offered from d 3 of age and steadily increased to approximately 2.25 kg by d 30 with free choice thereafter. An intranasal viral respiratory vaccine (Vista Once SQ, Intervet Inc., Merck Animal Health, Omaha, NE) and enteric clostridial vaccine (Ultrabac CD, Zoetis Inc., Kalamazoo MI) were administered at arrival. A booster dose of the respiratory vaccine was administered at 21 d of age, and a 7-way clostridial vaccine (Ultrabac 7, Zoetis Inc.) was administered at 45 d of age. Calves were kept in hutches through approximately 90 d of age.

Calf Health Records

Two on-farm veterinarians oversaw calf health management and treatment protocols. Upon entry to the ranch all calves were ear notched and tested for bovine viral diarrhea virus (BVDV) using PCR. Health problems were diagnosed and treated based on input from calf health managers' clinical assessments, and health records included broad disease diagnoses and associated treatments. Morbidity and mortality records were managed on-farm using DairyComp 305 (Valley Agricultural Software, Tulare CA) with record oversight and health data compilation provided through The HEALTHSUM Syndicate LLC (Sunnyside, WA). On-farm mortality codes were based on available antemortem information and predominantly derived from salient treatment records. Plausible causes of death were recorded by 3 calf health managers without input from this study's systematic postmortem analysis, and were coded as standardized, generic disease remarks (diarrhea, pneu, resp, injury, bloat) within a "died" event.

Study Protocol

Calves that died overnight between 1700 and 0700 h and were ≤ 90 d of age were gathered and delivered by 1000 h to a dedicated, biosecure necropsy site. Time and personnel constraints dictated that necropsies were performed 2 to 3 times per week on up to 10 calves. If more than 10 calves were available for necropsy, a coin flip was used to determine the 10 calves to be necropsied. Only calves that had died the previous night were necropsied in an effort to avoid autolysis due to summer daytime temperatures. Necropsies were performed following a study protocol (Supplemental Figure S1; <https://doi.org/10.3168/jds.2018-15527>) outlining the standardized procedures for calf necropsies (Severidt et al., 2002), tissue sampling and tissue submission guidelines, and representative digital images. Once or twice per week, a coin flip was used to identify a subset of up to 6 necropsied calves to be sampled for additional diagnostics at WADDL. The minimum number of calves to be included overall was based on experiential evidence, and data related to diagnostic inaccuracies suggesting that on-farm treatment-based causes of death would agree with WADDL diagnostic results in up to 60% of cases (Gulliksen et al., 2009). Necropsy-based postmortem findings and WADDL diagnostic results were anticipated to have no less than 80% agreement. These potential differences suggested that a minimum sample size of 82 cases (compared against themselves) would allow for detection of at least a 20% difference in classification agreement with a power of 80% and a significance level of 0.05.

Necropsies were performed initially by the on-farm veterinarians and principal investigator (CSM), with help from 2 undergraduate summer interns. Within 2 wk of the study's commencement, the interns performed necropsies with or without a veterinarian present following the prescribed protocols. For all cases, a set of standard digital images was taken, demonstrating both thoracic and abdominal cavities. Additional images were taken to highlight specific pathologies and irregularities of interest. All images were uploaded into a group messenger application for smartphones (2017 WhatsApp Inc.; <https://www.whatsapp.com/>) and delivered to participating investigators for comment and discussion regarding lesions and relevant historical attributes. Images related to cases submitted to WADDL for further diagnostics were catalogued electronically at Washington State University for evaluation by the veterinary pathologists on record.

Dairy Calf Certificate of Death and Causes of Death

A death certificate (Supplemental Figure S2; <https://doi.org/10.3168/jds.2018-15527>) was modified from one created to document adult cows deaths (McConnel and Garry, 2017). It was completed by the interns and veterinarians for each necropsied calf on the day of the necropsy and included details related to relevant treatments, necropsy findings, significant postnatal issues, or conditions contributing to the mortality phenotype, and attributable causes of death (underlying, contributing, immediate). No information was available from the calves' dairies of origin regarding potential difficulties during the birthing process or maternal characteristics such as vaccination status. The death certificate was included as part of the WADDL submission form along with a screenshot of the DairyComp 305 CowCard, to provide a standardized accounting of treatment histories and pathological findings that helped WADDL pathologists and microbiologists determine the most logical ancillary bacteriologic and molecular diagnostics to perform.

Mortality phenotypes were diagnosed for cases with and without a WADDL workup according to the most pertinent available data from the 3 levels of diagnostic information: on-farm treatment-based records alone, necropsy-based postmortem findings in addition to treatment records, and WADDL diagnostic results in addition to all other information. On-farm necropsy-based diagnoses were formulated with input from interns, on-farm veterinarians, and the principal investigator. The WADDL-based diagnoses were determined on a case-by-case basis by the pathologist of record following completion of ancillary diagnostics including histopathology. Resultant mortality phenotypes were

then categorized broadly by the principal investigator for each of the 3 levels of information according to a modified categorization scheme for dairy calf causes of death using a calf death loss flowchart (Figure 1; Lombard et al., 2019). Although no information was available from the time of parturition, a category for calving problems was included to appropriately document trauma such as fractured ribs or pathology due to apparent hypoxic changes such as centrilobular hepatic necrosis within calves ≤ 48 h of age. Aside from those calves with lesions apparently associated with calving problems or due to a congenital defect or accident, all deaths ≤ 24 h of age were assigned to a postnatal death category as per the flowchart. Postmortem evaluations of the postnatal deaths provided insight into whether the underlying cause of death was something more specific than an ill-defined maladaptation to life. Additional categories for causes of death were as follows and depended upon the relevant treatments, timing and phenotypic expression of disease or injury: accident, diarrhea, diarrhea and respiratory, respiratory, joint or navel, lameness or injury, other digestive, other known reasons, and unknown reason.

Comparisons of Causes of Death

Although the generalities of treatment-based causes of death frequently failed to identify the same level of phenotypic detail as necropsy- or WADDL-based diagnoses, each case was assessed based on the merit of the varying levels of information to classify the cause of death within the broad categories provided within the calf death loss flowchart (Figure 1). For example, a case of bronchopneumonia had the same underlying cause of death (respiratory) listed across sources even though the extent of disease only could be exposed through postmortem analysis. Compare this to a case of necrotizing, ulcerative typhlitis (cecal inflammation) variably categorized as diarrhea, diarrhea and respiratory, and other digestive for treatment-, necropsy-, and WADDL-based mortality phenotypes, respectively. Although the underlying issue was demonstrably aligned along a comparable pathologic spectrum ranging from diarrhea to sepsis, each diagnostic level identified novel phenotypic expressions of illness and death (e.g., lung pathology, intestinal necrosis and ulcerative lesions, peritonitis) that ultimately led to reclassifications of the cause of death.

Causes of deaths based on the broad categorization scheme were compared between levels of information using descriptive statistics (Excel 2013, Microsoft Corp., Redmond, WA) and Cohen's kappa (κ) according to Landis and Koch (1977): $\kappa = (p_a - p_\epsilon)/(1 - p_\epsilon)$, where p_a represents the proportion of observations in

agreement and p_ϵ the proportion in agreement due to chance. The value of κ determined the level of agreement as follows: less than chance agreement ($\kappa < 0$), slight agreement ($\kappa = 0.01$ – 0.20), fair agreement ($\kappa = 0.21$ – 0.40), moderate agreement ($\kappa = 0.41$ – 0.60), substantial agreement ($\kappa = 0.61$ – 0.80), and almost perfect agreement ($\kappa = 0.81$ – 0.99). Because the bulk of treatment-based diagnoses fell within 3 main categories (diarrhea, diarrhea and respiratory, and respiratory), these categories were used for comparison along with all other categories collapsed together. For all cases, comparisons of diagnostic agreement were made between the standard treatment-based records with and without input from on-farm necropsy-based postmortem analyses. For cases submitted to WADDL, additional comparisons were made between treatment records and on-farm postmortem evaluations with and without input from WADDL results. For a particular pathologic outcome of interest (necrotizing, ulcerative enterocolitis and typhlitis), a χ^2 test for association was calculated based on pathogen exposure.

RESULTS

A total of 210 dairy calves were necropsied during this study, with a minimum of 1 and a maximum of 10 calves necropsied on a given day. Of those necropsied, 88% (184) were ≤ 30 d of age, 9% (19) were >30 but ≤ 60 d of age, and 3% (7) were weaned and >60 d old. Budgetary constraints dictated that 122 (58%) cases were submitted to WADDL. Of those submitted to WADDL, 89% (109) were ≤ 30 d of age, 9% (11) were >30 but ≤ 60 d of age, and 2% (2) were weaned and >60 d old but ≤ 90 d of age.

Treatment-based diagnoses of causes of death based on input from calf health managers and relevant treatment records categorized almost two-thirds (65%; 137/210) of the deaths due to diarrhea or diarrhea and respiratory (Table 1). An additional 16% (34) of the total deaths were attributed to respiratory disease alone. Of the other categories for dairy calf deaths, 10% (20) of deaths fell under postnatal death, 4% (8) were unknown, and $\leq 2\%$ (≤ 4) were accounted for by each of the categories "congenital defects," "joint or navel," "other digestive," and "other known," in contrast to the findings with causes of death based on additional input from on-farm necropsy-based postmortem analysis without input from WADDL. Overall, necropsy-based postmortem diagnoses categorized 21% (44/210) of deaths due to diarrhea or diarrhea and respiratory disease. Respiratory disease alone was indicated in only 6% (13) of all cases. However, the "other digestive" category captured almost 50% (104) of cases primarily because of diagnosing necrotizing, ulcerative enteritis

and typhlitis. For those 122 cases with diagnostic input from WADDL, the percent of deaths attributed to diarrhea or diarrhea and respiratory fell to 16% (19), and respiratory disease alone accounted for only 3% of

deaths (4). On the other hand, “other digestive” deaths rose to 56% (68) due to additional histopathologic diagnoses of necrotizing, ulcerative GI lesions. Based on postmortem evaluation, each of the other categories for

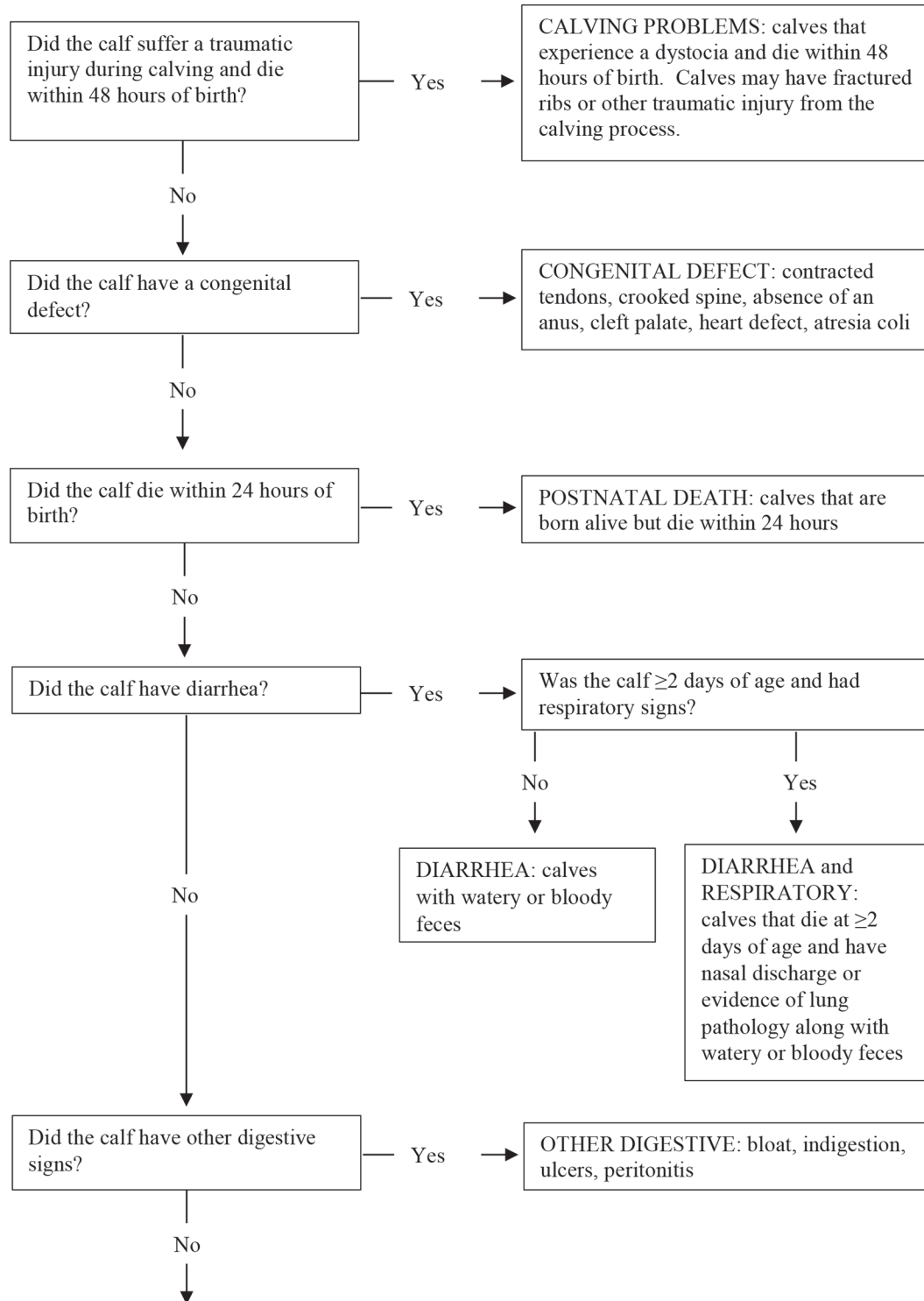


Figure 1. Calf death loss flowchart adapted from a dairy calf death categorization scheme (Lombard et al., 2019).

Table 1. Cause of death diagnoses for 210 dairy calves based on 3 levels of information: on-farm treatment records alone, necropsy-based postmortem analyses plus treatment records, and Washington Animal Disease Diagnostic Laboratory (WADDL) results in addition to all other information¹

Source(s) of information determining cause of death:			No. of cases by age		
Treatment record	Necropsy input	Diagnostic laboratory input	≤30 d	31–60 d	>60 d
Congenital defect	Congenital defect	Congenital defect	1		
		NA ²	2		
Diarrhea	Accident	Accident	2		
		Diarrhea	4		
	Diarrhea and respiratory	Diarrhea and respiratory	1		
		Other digestive	1		
		NA	5		
		Diarrhea and respiratory	4		
		NA	5		
		Joint or navel	1		
		Other digestive	32	1	
		NA	25		
Diarrhea and respiratory	Accident	NA	1		
		Diarrhea	1	1	
	Diarrhea and respiratory	NA	1		
		Diarrhea and respiratory	4	2	1
		Other digestive	2		
		NA	7	1	1
		Joint or navel		1	
		NA	1		
		Other digestive	20	1	
		NA	9		1
Joint or navel	Other known	Other known	1		
		Joint or navel	2		
Other digestive	Other known	NA	1		
		Other digestive	1		
Other known reasons	Other known	Other known	1		
		NA	1	2	
Postnatal death	Accident	Accident	1		
		Postnatal death	1		
		Calving problems	1		
		Postnatal death	11		
Respiratory	Accident	NA	7		
		NA		1	
		Calving problems	1		
		Congenital defect	1		
		Diarrhea	1		
		Diarrhea and respiratory	1		
		Joint or navel	1		
		NA	1		
		Other digestive	5	2	
		NA	2	1	1
		Other known	1		
		Respiratory	1		
		Calving problems	1		
		Other known		1	
		Respiratory	2	1	1
		NA	3	2	2
Unknown reason	Unknown	Other known		1	
		Unknown	1		
		Accident	1		
		Congenital defect	1		
		Diarrhea	1		
Unknown reason	Unknown	Other digestive	3		
		Unknown	1		
		NA	1		

¹Cause of death comparisons were based on modified categories for dairy calf deaths (Figure 1).

²NA refers to cases without a WADDL submission.

dairy calf deaths contained ≤4% of cases, both with (≤5) and without (≤8) input from WADDL, aside from the 9% of cases assigned to postnatal death both with (11) and without (19) input from WADDL.

Comparative results of diagnostic agreement between the standard treatment-based records with and without input from on-farm necropsy-based postmortem analyses are presented in Table 2 (part a). The proportion

Table 2. Agreement between dairy calf cause of death diagnoses from 3 levels of information: on-farm treatment records alone, necropsy-based postmortem analyses plus treatment records, and Washington Animal Disease Diagnostic Laboratory (WADDL) results in addition to all other information¹

Item	Cause of death	Diarrhea	Diarrhea and respiratory	Respiratory	Other	Total
Part a: Cause of death based on treatment records and necropsy-based postmortem evaluations ²						
Cause of death based on treatment records only	Diarrhea	11	9	0	61	81
	Diarrhea and respiratory	3	18	0	35	56
	Respiratory	1	1	13	19	34
	Other	1	0	0	38	39
	Total observations	16	28	13	153	210
Proportion of observations in agreement (p_a)		11	18	13	38	80
Proportion in agreement due to chance (p_ε)		6.2	7.5	2.1	28.4	44.2
Cohen's κ			0.22 (95% CI: 0.15–0.28)			
Part b: Cause of death based on treatment records, necropsy-based postmortem evaluations and WADDL results ³						
Cause of death based on treatment records only	Diarrhea	4	5	0	37	46
	Diarrhea and respiratory	2	7	0	25	34
	Respiratory	1	0	4	13	18
	Other	0	0	0	24	24
	Total observations	7	12	4	99	122
Proportion of observations in agreement (p_a)		4	7	4	24	39
Proportion in agreement due to chance (p_ε)		2.6	3.3	0.6	19.5	26.0
Cohen's κ			0.13 (95% CI: 0.06–0.21)			
Part c: Cause of death based on treatment records, necropsy-based postmortem evaluations, and WADDL results ³						
Cause of death based on treatment records and necropsy-based postmortem evaluations	Diarrhea	7	1	0	1	9
	Diarrhea and respiratory	0	11	0	2	13
	Respiratory	0	0	4	2	6
	Other	0	0	0	94	94
	Total	7	12	4	99	122
Proportion of observations in agreement (p_a)		7	11	4	94	116
Proportion in agreement due to chance (p_ε)		0.5	1.3	0.2	76.3	78.3
Cohen's κ			0.86 (95% CI: 0.76–0.97)			

¹Cause of death comparisons were based on modified categories for dairy calf deaths (Figure 1) and focused on diarrhea, diarrhea and respiratory, respiratory, and other categories collapsed together. Agreement between on-farm treatment records with or without input from necropsy-based postmortem evaluations but no input from WADDL is shown in part (a); agreement between on-farm treatment records with or without input from postmortem evaluations including WADDL results is shown in part (b); agreement between on-farm treatment records with input from postmortem evaluations but with or without input from WADDL results is shown in part (c).

²Includes all cases submitted (122) and not submitted (88) to WADDL.

³Includes only cases submitted (122) to WADDL.

of observations in agreement ($p_a = 80$) and calculated to be in agreement due to chance ($p_\varepsilon = 44.2$) equated to a value of Cohen's κ ($\kappa = 0.22$; 95% CI: 0.15–0.28) indicating fair agreement between the levels of information. The addition of diagnostic results from WADDL to the postmortem evaluations (Table 2, part b), led to only slight agreement with treatment-based records ($\kappa = 0.13$; 95% CI: 0.06–0.21). On the other hand, there was almost perfect agreement ($\kappa = 0.86$; 95% CI: 0.76–0.97) between dairy calf deaths categorized by necropsy-based postmortem findings with and without input from WADDL (Table 2, part c).

The primary driver of discrepancies between treatment-based diagnoses and those established following postmortem evaluations had to do with the reassig-

ment of cases from diarrhea or diarrhea and respiratory to the category “other digestive” (Table 1). Only occasional treatment-based diagnoses entirely failed to account for the pathophysiologic system underlying the mortality phenotype identified through postmortem evaluations. For those few cases, treatment-based diagnoses such as diarrhea or unknown overlooked issues such as esophageal tubing injuries (accidents) or omphalitis (joint or navel).

Many of the cases that presented clinically with and were treated for diarrhea and potentially respiratory distress ultimately proved to suffer from specific lesions and infections that could only be diagnosed by necropsy or histopathology (Table 3). This was especially apparent for 83 cases of necrotizing, ulcerative enterocolitis

and typhlitis that fell within the “other digestive” category because they typically demonstrated severe peritonitis with obvious macroscopic, perforating ulcerative lesions. When submitted to WADDL, those cases had consistent histologic evidence of severe intestinal necrosis with histologic diagnoses such as fibrinonecrotic, transmural, ulcerative enterocolitis or typhlitis. Moreover, WADDL further elucidated the problem by demonstrating that of those necrotizing, ulcerative cases submitted for molecular diagnostics, 98% (44/45) had *Rotavirus* detected by PCR, whereas only 31% (14/45) had *Coronavirus* and 2% (1/45) had *Cryptosporidium* spp. detected by PCR (Table 4). When compared against nonulcerative cases submitted to WADDL for molecular diagnostics, the evidence suggested that necrotizing, ulcerative cases were 3.0 times more likely to be diagnosed with *Rotavirus* alone than with no *Rotavirus* or *Rotavirus* in combination with either or both *Coronavirus* or *Cryptosporidium* spp. (odds ratio = 3.0; 95% CI: 1.2–7.8; Pearson χ^2 P -value = 0.02). Although those necrotizing, ulcerative cases arguably had clinical signs and lesions aligned along a pathophysiologic continuum from GI injury to sepsis, the lack of detail inherent to standard treatment-based causes of death led to different categorizations along the calf death loss flowchart and failed to identify meaningful characteristics necessary for understanding the progression of disease and pathways for intervention.

Informative phenotypic detail across the spectrum of dairy calf death categories was provided by postmortem evaluations founded on necropsies with input from WADDL when available (Table 3). Aside from the specific lesions and infectious agents detailed within the “other digestive” category, most cases assigned to the other broad categories also benefited from insight into aspects of disease progression, severity, duration, and infection. For example, enterocolitis could be found across categories (diarrhea, diarrhea and respiratory, other digestive), but associated pathology (e.g., bronchopneumonia, peritonitis, septicemia) and level of severity (e.g., mild, suppurative, ulcerative) ultimately dictated how a case aligned within the calf death loss categorization scheme. The specificity of the mortality phenotypes stood in stark contrast to the otherwise generic cause of death diagnoses derived from treatments alone and provided meaningful insight into pathophysiological processes capable of informing therapeutic and preventive practices. This held true even for those cases that were assigned to the postnatal death category as a straightforward function of the timing of death (≤ 24 h of age). Specific pathologic findings and infections still spoke to consequential problems beyond an ill-defined maladaptation to life. In fact, postmortem evaluations were informative even for the 1% (3/210)

of cases for which a cause of death remained unknown, to the extent that the findings documented the absence of specific issues of concern.

DISCUSSION

An accurate description of dairy mortality is needed to reduce economic and animal welfare costs, as well as the reputational risk posed to the industry by preventable deaths (Compton et al., 2017). Necropsies are warranted when morbidity or mortality exceeds historic or comfortable levels, when there is a perceived treatment failure, for acquiring information necessary for confirmation of a tentative clinical diagnosis, when presenting signs are dramatic or unusual, or to characterize a disease process when no antemortem observation has been made (Mason and Madden, 2007; Thomsen et al., 2012). Information derived from a necropsy and associated diagnostics should be viewed in conjunction with background information related to management factors such as the nutritional regimen, and clinical history, including treatments, to form a systematic postmortem evaluation. The findings from this study support the hypothesis that classifying dairy calf mortality phenotypes via a systematic postmortem analysis can identify differences in cause-of-death diagnoses compared with those derived from treatments alone. Differences in the characterization of underlying pathologies highlighted the value of on-farm necropsies and laboratory-based diagnostics to (1) detect antemortem disease misclassifications, (2) provide detail regarding disease processes and mortality phenotypes, and (3) direct disease mitigation strategies related to prevention and treatment.

This study adapted a calf death loss flowchart and dairy calf death categorization scheme (Lombard et al., 2019) to compare 3 levels of diagnostic information: on-farm treatment-based records alone, necropsy-based postmortem findings in addition to treatment records, and WADDL diagnostic results in addition to all other information. This categorization scheme is particularly useful in that it discriminates between uncomplicated diarrhea and other specific digestive ailments such as GI ulceration and peritonitis, and acknowledges the fact that many calf deaths present with ante- and postmortem evidence suggestive of both GI and lung pathology. In total, treatment-based records attributed 65% (137) of the 210 deaths evaluated in this study to diarrhea with or without respiratory problems, and 16% (34) were attributed to respiratory disease alone. Only 4% (8) had an unknown reason for the cause of death based on treatment records. Given that this study was 97% (203) populated with preweaning calves ≤ 60 d of age, these findings are similar to results from the USDA National Animal Health Monitoring System (NAHMS)

Table 3. Specific on-farm necropsy-based and Washington Animal Disease Diagnostic Laboratory (WADDL) informed postmortem findings associated with modified categories for dairy calf deaths (Figure 1)

Categories for dairy calf deaths (no. of cases ¹)	Specific postmortem findings
Accident (6)	Aspiration bronchopneumonia Cellulitis: cervical, peri-tracheal
Calving problems (3)	Esophagitis: necrotizing, ulcerative Fractured ribs Hypoxia: hepatic necrosis
Congenital defect (5)	Multi-organ hemorrhage
Diarrhea (14)	Atresia coli, enteritis, septicemia <i>Clostridium perfringens</i> Colitis: crypt abscesses, fibroplasia, neutrophilia <i>Coronavirus</i> , <i>Rotavirus</i> , <i>Cryptosporidium</i> spp. Enterocolitis: mild multifocal <i>Escherichia coli</i> attachment pili F5 (K99), heat-stable enterotoxin (STa)
Diarrhea and respiratory (24)	Bronchopneumonia: interstitial, suppurative <i>Coronavirus</i> , <i>Rotavirus</i> , <i>Cryptosporidium</i> spp. Enterocolitis: mixed inflammation, suppurative, thrombosis Ileitis: polymicrobial Pleuropneumonia: fibrinosuppurative, <i>Mannheimia haemolytica</i> , <i>Trueperella pyogenes</i> Salmonellosis: <i>Salmonella</i> Dublin
Joint or navel (8)	Omphalitis Peritonitis Septicemia
Other digestive (110)	Abomasitis: fibrinonecrotizing, <i>Clostridium perfringens</i> genotype A Duodenitis: ulcerative Enterocolitis: erosive, necrosuppurative, ulcerative Ileo-cecal rent Mesenteric torsion Obstruction Peritonitis: fibrinosuppurative Rumenitis: necrotizing Rumen putrefaction: parakeratotic hyperkeratosis Salmonellosis: choledochal cyst, <i>Salmonella</i> Dublin, Mbandaka, Newport, ulcerative Septicemia Typhlitis: fibrinonecrotic, transmural necrosis, ulcerative
Other known (8)	Bovine viral diarrhea virus-persistent infection Hydronephrosis Disseminated intravascular hemolysis Thoracic abscess: extrapulmonary
Postnatal death (18)	Bronchopneumonia <i>Clostridium perfringens</i> genotype A Enteritis: <i>Salmonella enterica</i> Mbandaka, <i>Salmonella</i> Muenster <i>Escherichia coli</i> attachment pili F5 (K99), heat-stable enterotoxin (STa) Septicemia
Respiratory (11)	Bronchopneumonia: bronchiolitis obliterans, fibrinosuppurative, pleuritis, necrosuppurative Pneumonia: interstitial, fibrinosuppurative, <i>Mycoplasma</i> spp., <i>Trueperella pyogenes</i> Salmonellosis: <i>Salmonella enterica</i> Dublin
Unknown (3)	Lymphadenitis Renal hemorrhage Systemic inflammation

¹Number of cases based on field necropsy-based postmortem findings inclusive of WADDL input when available.

Dairy 2014 study, which indicated that preweaning heifer deaths were predominantly attributed by producers to a generic category encompassing diarrhea or other digestive problems (56%). Respiratory problems accounted for approximately one-fourth (24%) of producer-attributed preweaning heifer deaths, and producers reported that only 6% of preweaning heifer deaths were due to unknown causes (USDA, 2017). The similarity between the current study's treatment-based causes of death and those recorded in the NAHMS study was not unexpected given that only 11% of US

operations performed necropsies on heifers and only 5% of dead heifers were necropsied (USDA, 2017).

Certain aspects of operational management such as the restricted milk feeding in this study may not correspond across calf rearing systems and undoubtedly influence calf health differentially; however, within a given contextual framework, a necropsy can discriminate clinical signs, such as respiratory distress due to concurrent GI disease and debility, from a distinct pathology, such as bacterial bronchopneumonia. Disease processes operate along a continuum with the potential

Table 4. Washington Animal Disease Diagnostic Laboratory molecular diagnostic (PCR) results for *Rotavirus* (*Ro*), *Coronavirus* (*Co*), and *Cryptosporidium* (*Cr*) spp. from cases with (45) and without (30) necrotizing, ulcerative enterocolitis or typhlitis, organized by the treatment-based dairy calf death category

Treatment-based diagnosis	Necrotizing, ulcerative enterocolitis or typhlitis	<i>Rotavirus</i> , <i>Coronavirus</i> , or <i>Cryptosporidium</i> spp.				
		<i>Ro</i> only	<i>Ro</i> and <i>Co</i>	<i>Ro</i> and <i>Cr</i>	<i>Ro</i> , <i>Co</i> , and <i>Cr</i>	No <i>Ro</i> , <i>Co</i> , or <i>Cr</i>
Diarrhea	No	2	4	0	2	1
	Yes	21	6	0	0	1
Diarrhea and respiratory	No	6	2	0	2	1
	Yes	8	6	0	1	0
Joint or navel	No	0	1	0	0	1
	Yes	0	0	0	0	0
Other digestive	No	0	0	0	0	0
	Yes	1	0	0	0	0
Postnatal death	No	1	0	0	0	1
	Yes	0	0	0	0	0
Respiratory	No	2	2	1	0	0
	Yes	0	1	0	0	0
Unknown	No	1	0	0	0	0
	Yes	0	0	0	0	0
Total combined	No	12	9	1	4	4
	Yes	30	13	0	1	1

to affect multiple organ systems and manifest across the clinical spectrum. Without the benefit of information provided by necropsies as part of systematic post-mortem evaluations, it is difficult to ascribe meaningful detail to causes of death. As demonstrated within the current study, the additional detail allowed for scrutiny of antemortem diagnostic accuracy and clarification of phenotypic expressions of illness and death. This was especially apparent concerning the surprising number of cases of necrotizing, ulcerative enterocolitis and typhlitis that particularly reduced agreement between levels of information (Table 2). The clinical presentations and treatment regimens documented for those cases belied the specificity and severity of the pathophysiologic causal pathway. Without the detail provided by extensive postmortem evaluations that integrate gross, histological, and microbiological findings, those cases would have been relegated to categories that failed to acknowledge the particular pathology. As with human cases of necrotizing enterocolitis, the exact role of microbes in bovine cases remains incompletely understood and the apparent dysbiosis associated with the lesions certainly does not imply cause and effect (Coggins et al., 2015; Adaska et al., 2017). However, the notion that a specific pathogen such as *Rotavirus* might have played a role in a rarely identified pattern of lesions potentially linked to stress-induced metabolic, immunologic, and microbial GI disturbances (Mitchell et al., 1981; Aoki-Yoshida et al., 2016) provides support for additional nutritional, therapeutic, and diagnostic investigations.

Certainly, not all deaths in this study were clearly aligned with diagnostic pathology. In a small subset of

cases (3), the cause of death remained unknown (Table 1). There were also cases (7%; 8/122) for which the necropsy- and WADDL-based mortality phenotypes differed even though there was almost perfect overall agreement between the 2 levels of information (Table 2, part c). It should be noted as well that the limited diagnostic agreement between treatment-based and necropsy-based postmortem findings was undoubtedly influenced by necropsy practices, including tissue selection and death certificate content. Nonetheless, the postmortem evaluation as a whole proved informative for the majority of cases and provided insight into many deaths that would otherwise have remained poorly or inaccurately classified. In the end, postmortem evaluations provided the detail required to describe specific lesions and pathogens and corroborated the accuracy of antemortem diagnoses and efficacy of therapeutic interventions.

For ongoing reference and education, the truncated categorizations of disease and death in on-farm record systems can be complemented with additional documentation of postmortem findings and relevant perspective through the use of death certificates, necropsy photos, and other diagnostic results. The inclusion of salient postmortem information can describe the causal pathway in such a way as to educate management moving forward. Describing the process leading to a death and mortality phenotype helps provide a narrative with the ability to convey complex and multi-layered ideas in a simple and memorable form to culturally diverse audiences. The power of the causal narrative is its ability to stimulate interest in a problem, facilitate learning, influence communication and cross-cultural under-

standing, and drive change within a farm (Snowden, 1999, 2000a,b).

Formulating a narrative based on systematic post-mortem evaluations therefore provides an avenue for exploring common sense solutions to otherwise complex problems, and affords an opportunity for real-time intervention in the form of employee education. Rather than viewing an individual calf death as a demoralizing end-point, the narrative provides an understanding from which to elicit change across the population. Rearing dairy replacement heifers should focus on limiting environmental impact, protecting animal welfare, and minimizing required inputs while returning the most profitable outputs (Hoffman and Funk, 1992; Heinrichs et al., 2017). As such, reducing economic costs and impairment to animal welfare through improved understanding of dairy calf mortality provides a critical control point for accelerating whole-farm efficiency and sustainability.

“The promises of a diagnosis, even if speculative, are always more welcome than the absolute certainties of an autopsy” (Nichols, 2017). This sentiment often holds true in dairy population medicine, with farmers and veterinarians certainly preferring to anticipate problems and avoid them, rather than explaining them in retrospect. The NAHMS Dairy 2014 study indicated that very few (<5%) dead heifers are necropsied as part of a postmortem examination. Yet without information provided from postmortem examinations, a dairy calf health management team is limited in their assessment of outcomes related to antemortem clinical diagnoses and treatments. Rather than wading through the complexities of treatment records and making decisions founded on speculation, evidence-based veterinary medicine would suggest that necropsies and additional laboratory-based diagnostics should be used as part of a systematic postmortem examination of dairy heifer causes of death.

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