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Development of a Bovine Leukocyte Differential index for Australian feedlot cattle

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Abstract

To reduce emergence of antimicrobial resistant organisms and preserve efficacy of antimicrobials to treat bovine respiratory disease (BRD), the World Health Organization recommends testing and targeted treatment of individual animals and a net reduction in antimicrobial use. The current research explored the ability to predict BRD risk in 2,450 Australian feedlot cattle using Advanced Animal Diagnostics' QScout® Cattle Lab, a chute-side blood test which reports total leukocytes, neutrophils, mononuclear cells, eosinophils, and neutrophil:mononuclear ratio . Health data during the feeding period and lung consolidation and pleurisy at slaughter were recorded. Descriptive statistics and generalized linear mixed models were used to determine relationships between leukocyte variables and health outcomes. 3-level analysis revealed odds of BRD diagnosis were 2.11 times greater for steers with low eosinophils ($P < 0.01$; Q1, <64.72 cells/mL) than those in the reference category (Q3; 95% C.I. = [1.40, 3.16]). Similarly, in the 2-level analysis, odds of BRD diagnosis were 1.83 times greater for steers with eosinophils below the ROC-derived threshold (<238 cells/mL) than those with higher eosinophils (95% C.I. = [1.31, 2.56]). These data indicate low eosinophils at feedlot induction may be important predictors of BRD risk in Australian feedlots.

Executive summary

Respiratory disease has been one of the greatest animal health and economic challenges faced by the fed cattle industry. The resulting clinical illness, reduced performance, mortality, labor and management challenges, and antibiotic treatments all come at great cost. Antibiotic use is necessary to preserve the health and welfare of animals in the industry, but overuse of these therapies has been shown to increase the prevalence of antimicrobial resistant organisms. The World Health Organization, along with national health organizations, has recommended a net reduction in antimicrobial use coupled with individual animal testing to support the use of anti-infectives as a means to reduce the emergence of antimicrobial resistant organisms. This recommendation is of particular importance to cattle feeders as bovine respiratory disease (BRD), also known as shipping fever, is "the most significant infectious disease of feedlot cattle in eastern Australia" (Cusack and Mahony, 2016).

Causes of BRD may include viral, bacterial, and mycoplasma species, and in many cases is a complex disease resulting from co-infections of multiple organisms. Cattle deemed to be at high risk for BRD often receive metaphylactic antimicrobials to prevent disease and/or transmission, and this process has been demonstrated to reduce the number of animals pulled for BRD treatment. Still, a meta-analysis of metaphylaxis control studies reported that not all cattle in a pen benefit from metaphylaxis. Results of this study indicate that five head treated help prevent one acute case of BRD (Dedonder and Apley, 2015), suggesting that targeted use of antibiotic therapies can prevent as many health events as metaphylaxis provided those animals in need of treatment can be accurately identified. Diagnosis of BRD is most commonly performed from visual observation of clinical symptoms. This method is highly subjective, with a sensitivity of 62% and specificity of 63% (White and Renter, 2009). However, objective data from blood leukocyte differentials have been demonstrated to be associated with increased risk of BRD (Richeson et al., 2013).

Advanced Animal Diagnostics (AAD) developed the QScout® Cattle Lab technology to address the difficulties in accurately identifying animals at risk for BRD. QScout® BLD is a chute-side blood test that can provide a leukocyte count and differential in less than a minute. Test results are uploaded to a data management system that can be used to trace individual animal results or to track groups of cattle. Preliminary findings in U.S. studies on lighter feedlot cattle indicate the ability of BLD to identify a population of cattle that have higher morbidity, lower average daily gain, and lower carcass weight and value compared to other animals in their cohort.

The goal of the current work is to evaluate the applicability of QScout® BLD in the Australian cattle industry and to produce an index to allow for predictive, objective data that could be utilized for assessing individual animal and pen-level risk for BRD. Cattle populations arriving at feedyards in Australia are typically heavier and potentially have different age-associated immune status than those in the U.S. leading to different etiology of BRD across countries. As such, any index resulting from the current work may be specific to the Australian feedlot industry.

A total of 2,139 and 2,041 animals for health and lung data analyses, respectively, were enrolled in the study at a commercial feedlot in central VIC under the supervision of licensed veterinarians and following industry standard management practices. Cattle in this study did not receive antimicrobial metaphylaxis. Collection was done at induction processing, where a drop of blood was collected from the jugular vein of each animal using the Qdraw™ quick collection device. Blood was placed in the QScout® BLD test slide and analysed to deliver a complete white blood cell count.

Throughout the feeding period, cattle were monitored for clinical signs of disease. All cattle pulled for signs of illness were treated according to standard operating procedures at the feedlot. Morbidity

and mortality events were tracked for each individual animal. At harvest, lung consolidation and pleurisy scores were determined by trained veterinarians.

Morbidity, lung scores, and pleurisy scores were individually analysed to determine their relationship with leukocyte variables generated from the QScout® BLD. SAS (Version 9.4, SAS Institute Inc, Cary, NC) was used to carry out an ROC analysis and establish 2- and 3-level thresholds for all QScout® BLD leukocyte variables. Generalized linear models were generated utilizing the 2- and 3-level thresholds, and odds ratios, confidence intervals, and *P*-values (alpha ≤ 0.05) from these final models are reported.

Although the BRD morbidity rate was low in this study population, the 3-level analysis revealed steers with low eosinophils ($P < 0.01$; Q1, <64.72/μL) were 2.11 times greater odds of being diagnosed with BRD prior to day 50 post-enrollment than cohorts in the reference category (Q3; 95% C.I. = 1.40 to 3.16). Similarly, in the 2-level analysis steers below the ROC-derived threshold for eosinophils (<238/μL) were 1.83 times greater odds of being diagnosed with BRD prior to day 50 post-enrollment (95% C.I. = 1.31 to 2.56). Interestingly, these data are in agreement with a previous experiment that reported similar association between eosinophil concentration and BRD risk (Richeson et al., 2013). We recommend additional exploration of eosinophil concentration at induction of Australian feedlot cattle as further development of leukocyte algorithms are considered for BRD prediction.

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1 Background

Respiratory disease has been one of the greatest animal health and economic challenges faced by the fed cattle industry. The resulting clinical illness, reduced performance, mortality, labor and management challenges, and antibiotic treatments all come at great cost. Antibiotic use is necessary to preserve the health and welfare of animals in the industry, but overuse of these therapies has been shown to increase the prevalence of antimicrobial resistant organisms. The World Health Organization, along with national health organizations, has recommended a net reduction in antimicrobial use coupled with individual animal testing to support the use of anti-infectives as a means to reduce the emergence of antimicrobial resistant organisms. This recommendation is of particular importance to cattle feeders as bovine respiratory disease (BRD), also known as "shipping fever", is "the most significant infectious disease of feedlot cattle in eastern Australia" (Cusack and Mahony, 2016).

Causes of BRD may include viral, bacterial, and mycoplasma species, and in many cases is a complex disease resulting from co-infections of multiple organisms. Cattle deemed to be at high risk for BRD often receive metaphylactic antimicrobials to prevent disease and/or transmission, and this process has been demonstrated to reduce the number of animals pulled for BRD treatment (Ishmael, 2011). Still, a meta-analysis of metaphylaxis control studies has suggested that not all cattle in a pen benefit from metaphylaxis. Results of this study indicate that five head treated help prevent one acute case of BRD (Dedonder and Apley, 2015). This analysis suggests that targeted use of antibiotic therapies can prevent as many health events as metaphylaxis, provided those animals in need of treatment can be accurately identified. Diagnosis of BRD is most commonly performed from visual observation of clinical symptoms. This method is highly subjective, with a sensitivity of 62% and specificity of 63% (White and Renter, 2009). However, objective data from blood leukocyte differentials have repeatedly been demonstrated to be associated with increased risk of BRD (Richeson et al., 2012, Richeson et al., 2013).

Advanced Animal Diagnostics (AAD) developed the QScout® Cattle Lab technology to address the difficulties in accurately identifying animals at risk for BRD. QScout® BLD is a chute-side blood test that can provide a leukocyte count and differential in less than a minute. Test results are uploaded to a data management system that can be used to trace individual animal results or to track groups of cattle. Preliminary findings in U.S. studies on lighter feedlot cattle indicate the ability of BLD to identify a population of cattle that have higher morbidity, lower average daily gain, and lower carcass weight and value compared to other animals in their cohort.

The intention of the current work is to evaluate the applicability of QScout® BLD in the Australian cattle industry and to produce an index to allow for predictive, objective data that could be utilized for assessing individual animal and pen-level risk for BRD. Cattle populations arriving at feedyards in Australia are far heavier and potentially have different age-associated immune status than those in the U.S. leading to different etiology of BRD across countries. As such, any index resulting from the current work may be specific to the Australian feedlot industry.

2 Project objectives

2.1 Risk determination from QScout® BLD results

2.1.1 Specific objectives

1. Develop a risk model to predict BRD risk of Australia feedlot cattle based on Bovine Leukocyte Differential index and other information available at arrival

2. Determine relationships between the Bovine Leukocyte Differential at feedlot arrival and carcass characteristics

3 Methodology

3.1 Data Collection

A total of 2,450 feedlot steers not receiving antimicrobial metaphylaxis were enrolled at a commercial feedlot during Fall 2018 and Spring 2019 with the assistance of Dr. Tony Batterham and Apiam Animal Health. Enrollment was conducted across 2 years because of the low BRD prevalence during the initial Fall 2018 enrollment. All procedures utilising live animals were conducted under the supervision of licensed veterinarians following industry standards. Study guidelines were approved by an Animal Care and Use committee.

Initial cattle processing included scanning the NLIS tag, addition of a unique visual ear tag, treatment for parasites, vaccination for respiratory pathogens, and collection of individual body weights. A subset of the animals also received a growth promoting implant. All animals were sampled using AAD's QDraw collection device to draw blood from the jugular vein. The blood was then transferred to the BLD test slide and inserted into the QScout® Cattle Lab for leukocyte differential analysis.

Cattle were separated into feedlot pens for the duration of the feeding period (range: 45 to 195 DOF). Cattle were fed similar diets targeting clean bunk management once per day based on steam-flaked barley or wheat. Animal health was monitored daily by trained feedlot employees. Animals displaying visible signs of illness were removed from their home pen and taken to the hospital for diagnosis, where their NLIS tag was scanned, their visual ID noted, body weight and rectal temperature collected, and blood sample analysed by QScout®. Animals requiring treatment were processed through the hospital according to agreed-upon protocols. Necropsies were done on animals that died during the feeding period and, where possible, a cause of death was identified and reported.

For those animals that survived until slaughter, lung consolidation scores and pleurisy scores were assessed and recorded by licensed veterinarians. Scores were not available for any animal that died prior to slaughter and any animal that had invalid slaughter data was removed from the slaughter dataset. Scoring methodology is detailed in the Appendix.

Deads remained in the dataset used to evaluate morbidity to conform with previous methodology (Richeson et al., 2013). This included all animals that died for any reason, at any time during the study including those that died prior to day 50 post-enrollment. Details for those animals that died prior to day 50 post-enrollment (and thus had less than 49 days at risk; n=9) but were not identified as ill with BRD while they were alive are reported in the Appendix.

To create the final datasets, the following additional adjustments were made:

1) Remove steers with no BLD data (108 animals).
2) Remove steers that received an IBR vaccine, but no Rhino vaccine or growth promoting implant (203 animals).
3) Remove steers with enrollment weight <= 250 kg or >= 600 kg (2 animals).
4) For lung and pleurisy scores, animals that died before slaughter (10 animals) and those with missing scores or scores outside the grading range (88 animals) were removed.

Table 1. Demographics of the BRD study population after exclusions.

Variable	Unit	Fall 2018	Spring 2019
Head (n=)		1,252	887
Enrollment Weight (kg)	mean.sd	434.4 ± 64.3	412.6 ± 57.5
Enrollment Weight (kg)	range	270-618	282-576
ADG (kg)	mean.sd	1.73 ± 0.4	1.33 ± 0.3
ADG (kg)	range	-0.12-2.89	-0.28-2.39
BRD morbidity (%)		10.9	14.5
Overall mortality (%)		0.8	1.9

Table 2. Demographics of the Lung/Pleurisy Score study population after exclusions.

Variable	Unit	Fall 2018	Spring 2019
Head (n=)		1239	802
Enrollment Weight (kg)	mean.sd	434.1 ± 64.0	414.6 ± 57.0
Enrollment Weight (kg)	range	270-598	282-576
ADG (kg)	mean.sd	1.73 ± 0.4	1.32 ± 0.3
ADG (kg)	range	-0.12-2.89	-0.28-2.39
BRD morbidity (%)		10.6	12.3
Pleurisy > 0 (%)		23.1	17
Lung Score > 0 (%)		25.5	29.4

3.2 Data Analysis

Two datasets were created from the overall study population. One dataset was used to evaluate the relationship of BLD variables at arrival enrollment to morbidity within 50 days post-enrollment and the other dataset was used to evaluate the relationship of BLD variables at arrival enrollment to lung consolidation score and lung pleurisy score at slaughter.

Binary outcomes were created from the original datasets:

- Morbidity dataset:

    - BRD50: If an animal was identified as ill with BRD by visual detection within the first 50 days on feed post-enrollment.

- Slaughter dataset:
    - Lung Score (LS): Lung scores of 0 (normal) remained 0, any abnormal lung score (1-3) was coded as 1.
    - Pleurisy Score (PS): Pleurisy scores of 0 (normal) remained 0, any abnormal pleurisy score (1-3) was coded as 1.

Each blood variable was categorized using two methods. First, ROC analysis was performed using SAS (Version 9.4, SAS Institute Inc, Cary, NC) and a 2-level threshold (high or low) for each variable in each dataset was created by selection of the best fit probability level from the classification table for each variable. Then, each blood variable of individual animals was ranked and categorized into quartiles for each dataset (Q1, Q2, Q3) with Q1 (low; <25%), Q2 as the interquartile range (25-75%), and Q3 (high; >75%).

For each binary outcome, a generalized linear mixed model (GLMM) was fitted using the GLIMMIX procedure in SAS (Version 9.4). First, an intercept-only model was fitted using the Laplace method and empirical sandwich estimators for each individual binary outcome. Random intercepts to account for clustering within year and pen within year were evaluated. The intercept-only model was then compared with univariate models that accounted for clustering within year and pen within year for each blood variable (categorized as either low or high) and were fitted via the Laplace method and empirical sandwich estimators. Models that did not improve were noted. In some cases, the random intercept for year when the Laplace method was used interfered with convergence, optimization, or yielded invalid estimates and so it was removed. Final models for each blood variable within each outcome were fitted using the RSPL method with Kenward-Roger degrees of freedom adjustment, and random intercepts for year and pen within year were again evaluated. In most final models, the random intercept for year yielded a covariance estimate of zero. Odds ratios, confidence intervals, and *P*-values (alpha ≤ 0.05) from these final models were reported. Additional GLMMs were fitted for each individual binary outcome to evaluate the relationship of each blood variable when categorized into a three-level variable. Model building and selection effects was performed as described previously. Descriptive statistics were generated using PROC FREQ in SAS.

4 Results

Table 3. Descriptive statistics for leukocyte variables at induction for the overall study population and steers in different health outcome categories.

				Mean (SD)					
Variable	Mean	SD[A]	Range	BRD Yes	BRD No	LS Abnormal	LS Normal	PS Abnormal	PS Normal
Total leukocytes (μL)	8,499	2,404	2,046-21,147	8,313 (2,406)	8,525 (2,404)	8,265 (2,548)	8,558 (2,333)	8,485 (2,483)	8,477 (2,373)
Neutrophils (μL)	2,396	1,145	423-10,357	2,289 (1,121)	2,412 (1,148)	2,345 (1,160)	2,403 (1,135)	2,259 (1,082)	2,421 (1,154)
Mononuclear cells (μL)	5,873	1,754	1,201-18,121	5,853 (1,786)	5,876 (1,750)	5,712 (1,850)	5,913 (1,700)	6,013 (1,894)	5,818 (1,700)
Eosinophils (μL)	229	289	0-3,109	171 (243)	237 (294)	208 (251)	241 (306)	213 (259)	237 (300)
Neutrophil:mononuclear	0.4288	0.2169	0.07-1.81	0.4110 (0.2146)	0.4313 (0.2172)	0.4336 (0.2159)	0.4256 (0.2150)	0.3971 (0.2030)	0.4357 (0.2177)

[A] Standard deviation

Table 4. Demographics and health outcomes of the slaughter dataset population.

Item	N=	%
BRD50[A]	232/2041	11.4
Abnormal LS[B]	552/2041	27.1
Abnormal PS[C]	422/2041	20.7
BRD50_LS[D]	84/232	36.2
BRD50_PS[E]	72/232	31.0

[A] Number and percentage of the slaughter dataset population that was treated for bovine respiratory disease before day 50 post-enrollment

[B] Number and percentage of the population the slaughter dataset population that had an abnormal lung score at slaughter

[C] Number and percentage of the slaughter dataset population that had an abnormal pleurisy score at slaughter

[D] Number and percentage of BRD cases in the slaughter dataset population that had an abnormal lung score at slaughter

[E] Number and percentage of BRD cases in the slaughter dataset population that had an abnormal pleurisy score at slaughter

Table 5. Thresholds for the leukocyte variables and bovine respiratory disease (BRD) incidence before day 50 post-enrollment determined by receiver operating characteristic (ROC) analysis.

Variable	AUC[A]	Threshold	*P*-value[B]
Total leukocytes (μL)	0.53	9,015	0.18
Neutrophils (μL)	0.54	2,801	0.10
Mononuclear cells (μL)	0.51	-	0.85
Eosinophils (μL)	0.60	238	<0.01
Neutrophil:mononuclear	0.54	0.5184	0.17

[A] Area under the curve

[B] Contrast between parameter AUC and chance AUC

Table 6. Thresholds for the leukocyte variables and lung score (LS) incidence at slaughter determined by receiver operating characteristic (ROC) analysis.

Variable	AUC[A]	Threshold	*P*-value[B]
Total leukocytes (μL)	0.55	7,837	0.01
Neutrophils (μL)	0.52	3,249	0.30
Mononuclear cells (μL)	0.55	6,426	0.02
Eosinophils (μL)	0.53	586	0.02
Neutrophil:mononuclear	0.51	0.7098	0.45

[A] Area under the curve

[B] Contrast between parameter AUC and chance AUC

Table 7. Thresholds for the leukocyte variables and pleurisy score (PS) incidence at slaughter determined by receiver operating characteristic (ROC) curves analyses.

Variable	AUC[A]	Threshold	*P*-value[B]
Total leukocytes (μL)	0.50	-	0.95
Neutrophils (μL)	0.54	3,725	0.01
Mononuclear cells (μL)	0.53	7,229	0.04
Eosinophils (μL)	0.53	1,696	0.12
Neutrophil:mononuclear	0.56	0.6000	<0.01

[A] Area under the curve

[B] Contrast between parameter AUC and chance AUC

Table 8. Significance levels (*P*-values) for the association between leukocyte variables and the diagnosis of clinical bovine respiratory disease before day 50 post-enrollment and the incidence of lung score (LS) and pleurisy score (PS) at slaughter resulting from the univariate generalized linear mixed models accounting for year and pen within year.

	Categorization (2L)[A]			Categorization (3L)[B]		
Variable	BRD50[C]	LS[D]	PS[E]	BRD50	LS	PS
Total leukocytes (μL)	0.95	0.13	-	0.28	0.16	0.49
Neutrophils (μL)	0.67	0.34	0.56	0.55	0.17	0.76
Mononuclear cells (μL)	-	0.60	0.11	0.57	0.55	0.38
Eosinophils (μL)	<0.01	0.57	0.85	<0.01	0.96	0.87
Neutrophil:mononuclear	0.70	0.61	0.62	0.43	0.81	0.04

[A] Two level (2L) categorization originated from the ROC analysis; missing *P*-values indicate poor ROC values existed for that variable and further statistical analysis was not conducted

[B] Three level (3L) categorization based on variable distribution: low = lower quartile; medium = values within the interquartile range bounds; and high = higher quartile

[C] Bovine respiratory disease incidence before day 50 post-enrollment (yes = 1; no = 0)

[D] Lung score incidence (abnormal = 1; normal = 0)

[E] Pleurisy score incidence (abnormal = 1; normal = 0)

Table 9. Results of the generalized linear mixed model analysis for risk of clinical bovine respiratory disease diagnosis before day 50 post-enrollment (BRD50) and pleurisy score at slaughter (PS). Only leukocyte variables with significant fixed effects for BRD50 or PS ($P \leq 0.05$) are included.

	BRD50			PS		
Variable	OR[A]	95% CI[B]	*P*-value[C]	OR	95% CI	*P*-value
Categorization (2L)						
Eosinophils						
<238	1.83	1.31 – 2.56	<0.01	-	-	-
≥238	-	-		-	-	
Categorization (3L)						
Eosinophils						
<64.72	2.11	1.40 – 3.16	<0.01	-	-	-
64.72 – 136.91	1.60	1.10 – 2.33		-	-	
>136.91	-	-		-	-	
NMR[D]						
<0.2813	-	-	-	1.16	0.77 – 1.62	0.04
0.2813 – 0.3780	-	-		0.77	0.55 – 1.08	
>0.3780	-	-		-	-	

[A] Odds ratio

[B] 95% confidence interval for the odds ratio; 95% confidence intervals that do not contain 1.0 are statistically different ($P \leq 0.05$) from the reference category

[C] *P*-values represent the overall fixed effect of the variable

[D] Neutrophil:mononuclear cell ratio

5 Discussion

5.1 Project Objective 1: Develop a risk model to predict BRD risk of Australia feedlot cattle based on Bovine Leukocyte Differential index and other information available at arrival

Results indicate that Australian feedlot cattle with low eosinophil concentration at induction have increased odds of bovine respiratory disease before day 50 post-enrollment (both 2- and 3-level categorizations). In a similar retrospective analysis of leukocyte concentration and health outcome, Richeson et al. (2013) also observed significant association between low eosinophils and BRD risk in stocker calves arriving at a research facility in the United States. Biological hypotheses for reduced eosinophils and increased clinical BRD may be explained 3-fold: 1) stimulation of the hypothalamic-pituitary-adrenal axis from stress may result in a reduction in the concentration of eosinophils

because of the effect of cortisol on haematopoiesis, 2) the peripheral blood concentration of eosinophils may be less in animals at greater risk for BRD in favour of other leukocytes (i.e., neutrophils) that specialize in viral and bacterial infection, rather than parasitic infection, and 3) eosinophils in animals at greater BRD risk may be more greatly redistributed from the blood to the lymphatic system or sites of infection to aid in resolving inflammation. While the overall effect of the variable NMR was significant in the model, neither the contrast between Q1 and Q3 or Q2 and Q3 were significant.

5.2 Project Objective 2: Determine relationships between the Bovine Leukocyte Differential at feedlot arrival and carcass characteristics

There were no observed trends in prediction of the lung and pleurisy scores from the QScout BLD variables. In this study population, the overall morbidity rate was low (11.4%). However, the percentage of steers that presented an abnormal lung and pleurisy score upon slaughter was 27.1 and 20.7 %, respectively. Furthermore, only 36.2 and 31.0 % of the BRD cases in this study that survived to slaughter had abnormal lung and pleurisy scores, respectively. These data further illustrate the challenges associated with BRD prediction and detection in the commercial feedlot.

Inclusion of data on carcass characteristics such as meat quality grade would enable exploration of additional performance metrics. Additionally, enrollment of a study with more uniform population metrics with respect to enrollment weight and days on feed until slaughter would facilitate examination of the relationship between blood leukocyte differential at processing and average daily gain.

6 Conclusions/recommendations

Although the BRD morbidity rate was low in this study population, the 3-level analysis revealed steers with low eosinophils ($P < 0.01$; Q1, <64.72/μL) were 2.11 times greater odds of being diagnosed with BRD prior to day 50 post-enrollment than cohorts in the reference category (Q3; 95% C.I. = 1.40 to 3.16). Similarly, in the 2-level analysis steers below the ROC-derived threshold for eosinophils (<238/μL) were 1.83 times greater odds of being diagnosed with BRD prior to day 50 post-enrollment (95% C.I. = 1.31 to 2.56). Interestingly, these data are in agreement with a previous experiment that reported similar association between eosinophil concentration and BRD risk (Richeson et al., 2013) in high risk US feeder calves.

These data suggest that QScout BLD is a viable option for guiding the targeted treatment of BRD in Australian feedlot cattle. We recommend additional exploration of eosinophil concentration at induction of Australian feedlot cattle as further development of leukocyte algorithms are considered for BRD prediction.

7 Key messages

Blood concentration of eosinophils at induction into the feedlot as measured by the QScout Cattle Lab has promise in evaluating an animal's risk for BRD during the first 50 days on feed. Further exploration of this concept is warranted. Adoption of this technology could lead to decreased need for antibiotic use in feedlots, lessening costs for producers and helping to address concerns related to antibiotic resistance.

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9 Appendix

9.1 Lung and pleurisy scoring methodology

The basic concept behind this lung scoring method is to calculate the percentage of lung that is consolidated. This is done by examining the lung, lobe by lobe and summing the percentage of consolidation. The schematic shows the contribution that each lung lobe makes toward the overall percentage of the lung.

At the abattoir, on the offal table, the lung is examined by observation and palpation and the percentage of consolidation is estimated lobe by lobe and summed to give a total percentage of consolidation for that lung. This is then recorded alongside the body number.

Pleurisy and pleuritic:

These lesions are score from 0 to 3 using the following criteria.

Pleurisy score 0:	No Pleurisy or pleuritic evident on lungs or thoracic wall.
Pleurisy score 1:	Pleuritic tags between lung lobes with or without small pleuritic tags on the lung surface.
Pleurisy score 2:	Significant pleuritic tags on the lung surface, small pieces of lung maybe adhered to the thoracic wall requiring trimming with or without significant amounts of pleuritic tags on the lung surface or lung margins (fringing).
Pleurisy score 3:	Lung is adhered to the thoracic wall or the rumen. The lung will not be present on the offal table. The pleura will require stripping.

Lung Abscess:

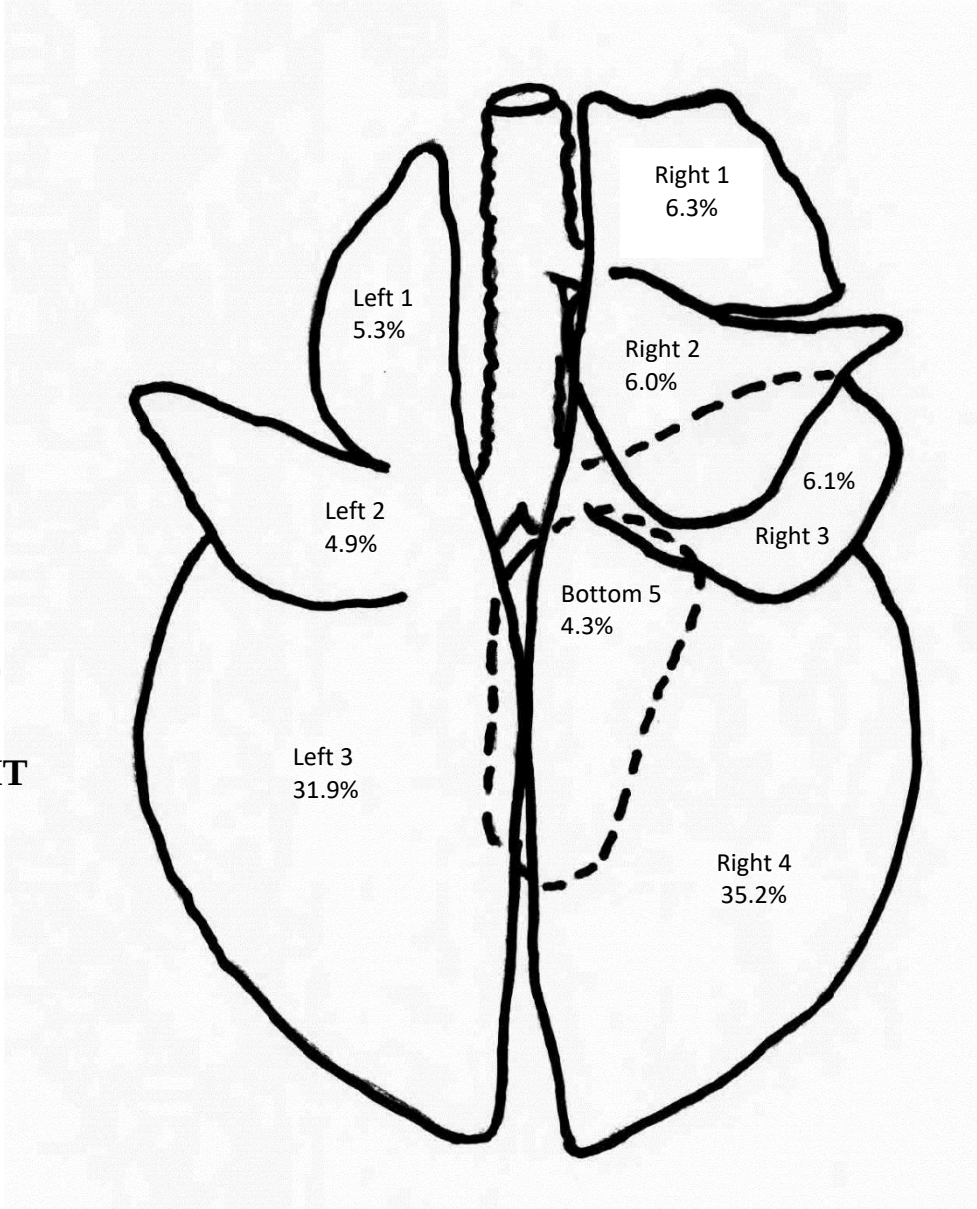
Lung abscesses may sometimes occur. These lesions create a very high risk of carcass contamination.

The chain will be stopped to allow the carcass to be handled in an appropriate manner. These cases Will be recorded as lung abscess against the body number.

Grading will occur on all carcasses at approximately 24 time after slaughter at (0-2 oC). Data collected on carcasses will include: kill date, body number, sex, dentition, butt shape, fat depth (P8), fat colour, meat colour, MSA boning group, hump height, tropical breed content, MSA marbling, Ausmeat Marbling, Ossification, EMA pH, Rib Fat, EMA, total hot dressed weight, left side bruising, left side weight, right side bruising, right side weight, and MSA Index. All the following data will be collected for a MSA Un-grade except the MSA Index.

BOVINE LUNG DORSAL (TOP) VIEW

PERCENTAGE OF TOTAL LUNG FOR EACH LOBE



LEFT

RIGHT

LEFT 1 – Left Cranial Apical Lobe	RIGHT 1 – Right Cranial Apical Lobe
LEFT 2 – Left Caudal Apical Lobe	RIGHT 2 – Right Caudal Apical Lobe
LEFT 3 – Left Diaphragmatic Lobe	RIGHT 3 – Middle Lobe
BOTTOM 5 – Accessory Lobe	RIGHT 4 – Right Diaphragmatic Lobe

9.2 Descriptive data for animals (n=9) that died prior to the day 50 post-enrollment risk period for any reason.

These animals were not identified as BRD cases prior to their deaths but were included in the morbidity analysis to conform with prior methodology.

ID	Pen	BRD Case	Dead Diagnosis	Dead DOF	Year	3-level classification					2-level classification				
						Eos	Mono	Neut	WBC	NMR	Eos	Mono	Neut	WBC	NMR
F40646	1916F1	No	BRD	7	2019	Q1	Q2	Q3	Q2	Q3	LOW	LOW	HIGH	LOW	HIGH
42313	1918F1	No	Other	2	2019	Q1	Q2	Q1	Q1	Q1	LOW	LOW	LOW	LOW	LOW
F13020	1822F1	No	BRD	29	2018	Q2	Q1	Q2	Q1	Q2	LOW	LOW	LOW	LOW	LOW
65701	1820C1	No	BRD	34	2018	Q2	Q3	Q2	Q3	Q1	LOW	LOW	LOW	HIGH	LOW
41950	1918F1	No	Unknown	8	2019	Q3	Q3	Q2	Q3	Q1	HIGH	LOW	LOW	HIGH	LOW
65886	1820C1	No	Abomasal Ulcer	29	2018	Q3	Q3	Q2	Q2	Q2	HIGH	LOW	LOW	HIGH	LOW
65899	1820C1	No	Abomasal Ulcer	11	2018	Q3	Q2	Q3	Q2	Q3	HIGH	LOW	HIGH	LOW	HIGH
65868	1820C1	No	Septic Arthritis	16	2018	Q3	Q3	Q3	Q3	Q2	HIGH	LOW	HIGH	HIGH	LOW
42301	1918F1	No	Sent to slaughter	45	2019	Q1	Q2	Q2	Q2	Q2	LOW	LOW	LOW	LOW	LOW