Histopathology Whisper® Proof-Of-Concept Study

Electronic stethoscope predicts the presence of histopathologic lung lesion characteristics in bovine respiratory disease complex diagnosed cattle.

Summary

Geissler Corporation has developed a non-invasive technology to be used as a point-of-care diagnostic tool for the assessment of animal lung health. This white paper reports on a pilot study using histopathology as a validation of the efficacy and feasibility of this new technology. The foundation of this technology is based on the ability to replicate through digitization algorithms, the clinical acumen of industry expert veterinarian auscultators. The technology uses a specialized proprietary electronic stethoscope and a digitized-sound analytical algorithm software product. The patented product is registered as Whisper®. The results of this study indicate that Whisper® is able to predict at a statistically significant level five characteristics of histopathologic lung lesions when tested on cattle suspected of having Bovine Respiratory Disease Complex (BRDC).

Background

Feedlot cattle with BRDC account for an estimated 57% of total feedlot mortality[(A)] and have an industry wide impact approaching $1 billion dollars[(B)] annually. Woolums *et al.* (2005) found BRDC to be the number one cause of morbidity and mortality in feedlots. Lung lesions are commonly found at harvest and are estimated to have a prevalence of 29.7% to 77%.[(C,F)] Lung lesions at harvest from cattle treated for BRDC have been associated with reduced hot carcass weight, reduced average daily gain and lower marbling scores.[(D,E)] BRDC is often missed. Research suggests that 50% or more of animals at slaughter with gross lung lesions have never been diagnosed with BRDC.[(C)] Early identification and early treatment of BRDC cattle is the mainstay of the current disease management strategies. Identification of sick cattle is a major challenge for feedlots and the success of identifying sick animals directly impacts the economics of the feedlot business as well as the welfare of the cattle or herd. This identification process would benefit from new technologies that offer an accurate diagnosis of lung health and an economically feasible disease management strategy for BRDC. This study's objective is to determine the predictive performance of Whisper® on 10 different histopathologic lung lesions associated with BRDC using computerized auscultation technology.

Lung Score

A metric for Lung Health

DeDonder *et al*. have described a lung auscultation scoring system that correlates strongly with severity of lung disease. This system was shown to have statistically significant predictability for: lung lesions at slaughter, risk of retreatment for BRDC and mortality risk from BRDC. The lung score system requires that

feedlot personnel doing the lung scoring be well-trained in clinical auscultation. The Whisper® technology was developed to meet the needs for a lung health diagnostic tool that wouldn't require the user to be an expert clinical auscultator. Whisper® was created by researching and modeling the scoring of lung sounds by industry expert veterinarian auscultators. Whisper® uses a 1 to 5 lung severity scale with '1' representing normal lung health and '5' representing a diagnosis of chronic severe lung disease.

Methods

A stratified sampling approach was used to enroll cattle for this study. Expert veterinarian clinical auscultators selected cattle (in Southwest Kansas) that were diagnosed with naturally occurring BRDC. The stratification selection criterion was based on finding animals with lung auscultation diagnosis in the following severity categories: Normal, Mild, Moderate, Severe and Chronic. Feedlot personnel identified cattle in their pens that displayed some or all of the following BRDC signs (depression, anorexia, tachypnea and nasal discharge). The cattle were placed in the hospital area, restrained in a hydraulic chute and auscultated with the Whisper® algorithm software. Following the recording of lung sounds, the cattle were released from the chute and euthanized. The feedyard's consulting veterinarian performed a necropsy on the animals and samples of lesions were taken from the periphery of the affected areas and placed in Whirlpak™ bags with 10% formalin solution. Samples were then submitted for histopathology analysis. The majority of samples went to Kansas State University Veterinary Diagnostic Laboratory in Manhattan KS. Four of the 25 samples went to University of Nebraska-Lincoln Veterinary Diagnostic Laboratory in Lincoln NE. Samples were scored from histopathology reports as either positive or negative for the ten histopathologic characteristics analyzed. (See Table 1)

Histopathologic characteristics used in study

Distribution Patterns
- Diffuse
- Lobar
- Multifocal

Pattern Type
- Interstitial
- Bronchopneumonia

Cellular Infiltrations
- Polymorphonuclear Leukocytes (PMNs)
- Monocytes
- Fibrin

Inflammation Stage
- Acute
- Chronic

Table1. List of Histopathologic Characteristics used in analysis.

Statistical Methodology

The association of the presence or absence of histopathologic characteristics and Whisper® lung score was analyzed using logistic regression modeling with a binary logit dependent variable and Fisher's scoring optimization technique. SAS [9.2] software procedure *Logistic* (SAS Inc., Cary NC) was used to perform the analysis. Univariate analysis was done with lung score as the independent variable and the presence/absence of a specific histopathologic lesion as the dependent variable. Positive histopathology

findings were assigned a score of '1' and negative or absence of findings, a score of '0.' Wald chi-square statistics were calculated and evaluated for each model. Odds ratio estimates were obtained and evaluated using 95% Wald confidence limits. Maximum likelihood estimates for each model's intercept and Whisper® lung score coefficient were obtained with Wald 95% confidence intervals. There were 25 samples from 15 animals. Ten animals were auscultated and necropsied on both the right and left lungs, and 5 animals were evaluated on the right lung only. This model did not control for within-animal effects.

Results

The logistic regression analysis indicates a statistically significant association between Whisper® lung score and the following histopathologic lesions: Interstitial lung pattern, PMNs, Monocytes, Diffuse distribution and Chronic inflammation. (See Table 2, Graphs 1–5)

Interstitial lung pattern
Wald Chi-Square = 5.5794, p=0.0182
Odds Ratio = 4.927
Lower 95%CI for odds ratio = 1.312

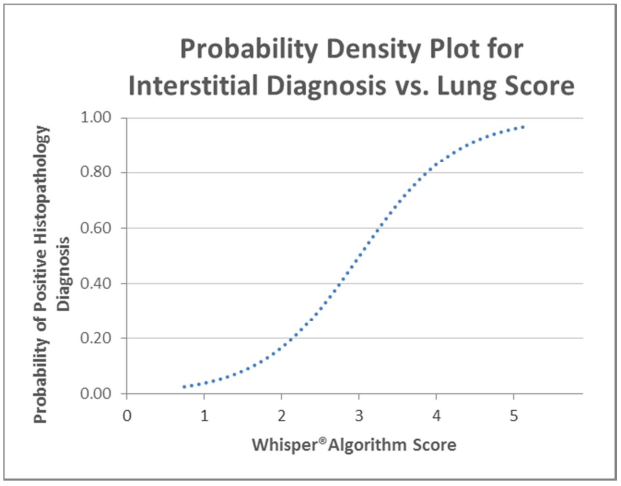
PMN infiltration
Wald Chi-Square = 5.2285, p=0.0222
Odds Ratio = 4.640
Lower 95%CI for odds ratio = 1.245

Monocyte infiltration
Wald Chi-Square = 5.1173, p=0.0237
Odds Ratio = 5.323
Lower 95%CI for odds ratio = 1.250

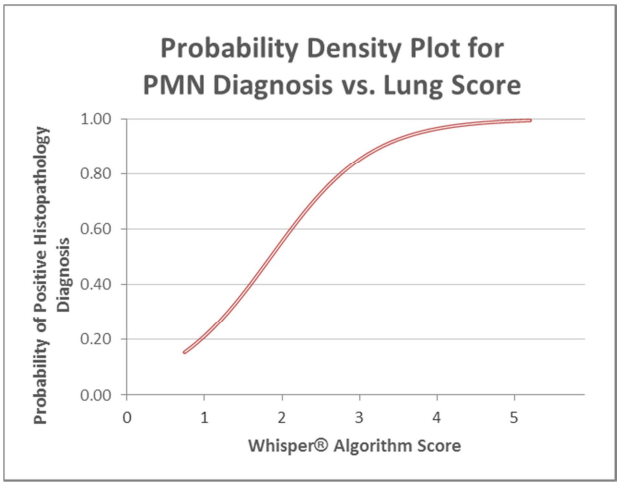
Diffuse distribution
Wald Chi-Square = 4.8405, p=0.0278
Odds Ratio = 3.788
Lower 95%CI for odds ratio = 1.156

Chronic Inflammation
Wald Chi-Square = 4.4938, p=0.0340
Odds Ratio = 3.964
Lower 95%CI for odds ratio = 1.109

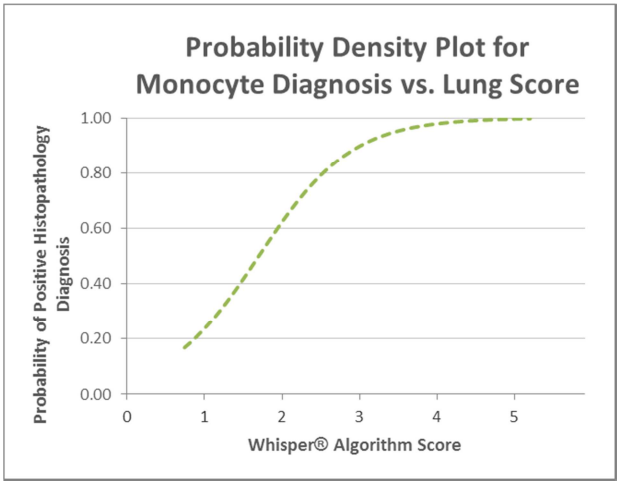
Table 2. Statistically significant univariate results.



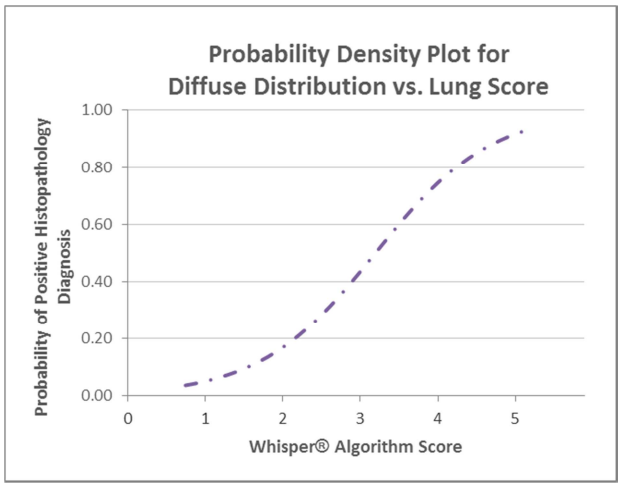
Graph1. Interstitial Diagnosis Probability by Lung Score.



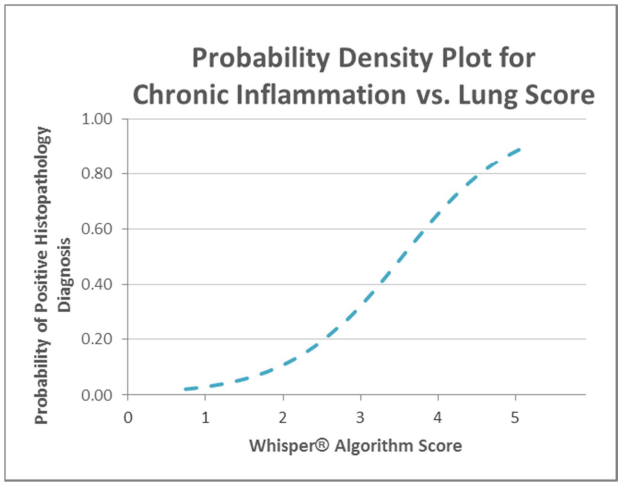
Graph2. PMNs Diagnosis Probability by Lung Score.



Graph3. Monocyte Diagnosis Probability by Lung Score.



Graph 4. Diffuse Distribution Diagnosis Probability by Lung Score.



Graph 5. Chronic Inflammation Diagnosis Probability by Lung Score.

The logistic regression analysis did not find statistically significant association between Lung Score and the following histopathologic lesions: Fibrin infiltration, Bronchopneumonia, Lobar distribution, Multifocal distribution and Acute inflammation. (See Table 3)

Fibrin Infiltration
Wald Chi-Square = 2.5271, p=0.1119
Odds Ratio = 2.049
Lower 95%CI for odds ratio = 0.846

Bronchopneumonia
Wald Chi-Square = 2.4983, p=0.1140
Odds Ratio = 2.271
Lower 95%CI for odds ratio = 0.821

Lobar distribution
Wald Chi-Square = 1.1452, p=0.2846
Odds Ratio = 1.713
Lower 95%CI for odds ratio = 0.639

Multifocal distribution
Wald Chi-Square = 0.3143, p=0.5751
Odds Ratio = 0.805
Lower 95%CI for odds ratio = 0.376

Acute Inflammation
Wald Chi-Square = 0.2770, p=0.5987
Odds Ratio = 1.221
Lower 95%CI for odds ratio = 0.581

Table 3. Non-significant univariate results.

Discussion

These results clearly show that Whisper® technology can detect the presence of lung disease as validated by gross pathology and histopathology. This study was designed to explore the efficacy and feasibility of using Whisper® technology to assess animal lung health. Histopathology was employed as a validation of proof-of-concept. The predictability of these disease characteristics is quite impressive given the difficult environment the lung sounds are obtained in. The recording of lung sounds must overcome animal to animal variations in body type, animal disposition at time of auscultation and feedyard ambient noise levels. Having access to only the animal's lung sound, as the scope is blinded to all other signs of disease state, the predictability was quite remarkable. These results are very promising and support a

proof-of-concept claim for this technology and its applications.

It is noteworthy to point out that the predictive curves for histopathologic characteristics display a disease stage progression across lung score levels. This is consistent with pathophysiology of lung disease. For example, we see that the cellular infiltrates Monocytes and PMNs, are more likely at lower lung scores (first responders to early onset of disease) than Chronic inflammation which rises in likelihood at the higher lung score levels (late in the process after long standing disease). The likelihood of a positive diagnosis for Chronic inflammation is 29.9 times higher for a Whisper® lung score of 5 compared to a score of 1 where the likelihood is very low. (See Table 4 and Graph 6)

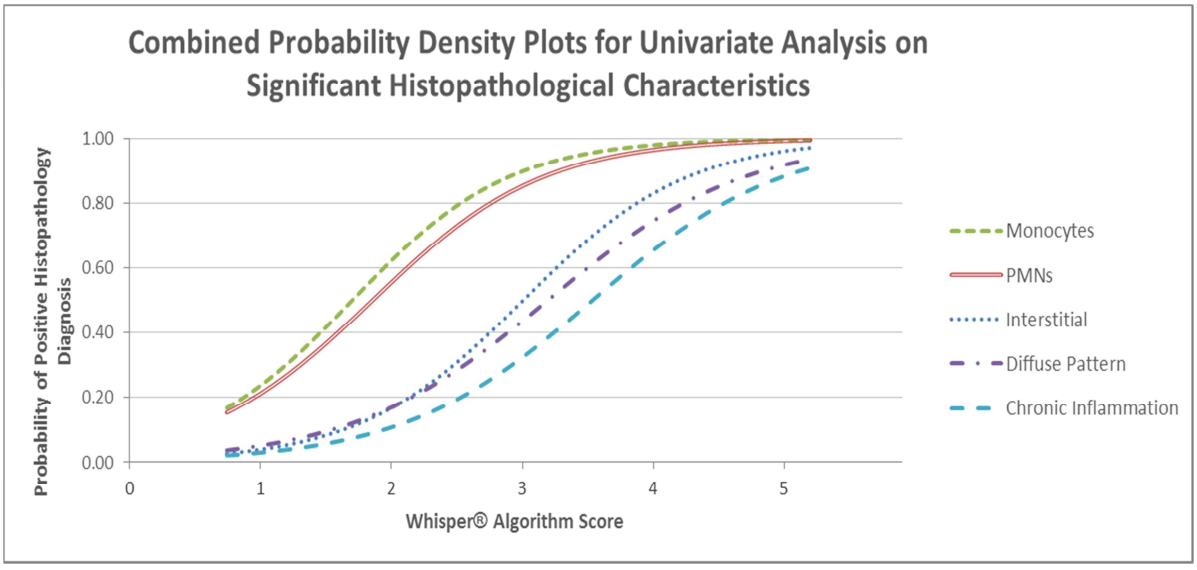
In general these results support proof-of-concept and ongoing research in this type of technology approach to animal health.

Kurt Brattain M.D.

Likelihood Ratios of Positive Histopathologic Diagnosis for Normal State Whisper® agorithm score (1) vs.scores (2,3,4 and 5)

Lung Score Comparison	Monocyte infiltration	PMN infiltration	Diffuse distribution	Interstitial pattern	Chronic Inflammation
1 vs 2	2.6	2.6	3.3	4.3	3.6
1 vs 3	3.8	4.0	8.5	12.7	11.0
1 vs 4	4.1	4.6	14.6	21.2	22.2
1 vs 5	4.2	4.7	18.0	24.5	29.9

Table 4. Likelihood ratios for histopathologic characteristics by Lung Score level (1) vs. levels (2, 3, 4 and 5)



Graph6. Displaying disease stages progressing across lung score levels: Cellular Infiltrates/early stage/lower lung score → Chronic Inflammation/late stage/higher lung score.

References

(A) G. H. Loneragan, D.A. Dargatz, P.S. Morley, Marty A. Smith, *JAVMA*, Vol. 219, No. 8 1122-1127

(B) D. Griffin. Veterinary Clinics of North America: Food Animal Practice' 13:3 pp. 367-377

(C) P. N. Thompson, A. Stone, and W. A. Schultheiss, J Anim. Sci, 84:488-498

(D) M. J. Schneider, R. G. Tait Jr., W. D. Busby, and J. M. Reecy, J Anim Sci 87:1821-1827.

(E) LK Bryant, LJ Perino, D Griffin. *Bov Pract.* 33(2):163-173

(F) Gardner BA, Dolezal HG, Bryant LK, *et al.*: *J Anim Sci* 77:3168-3175, 1999.

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