

UC Davis

UC Davis Previously Published Works

Title

Sensitivity and specificity of on-farm scoring systems and nasal culture to detect bovine respiratory disease complex in preweaned dairy calves

Permalink

<https://escholarship.org/uc/item/29z0873h>

Journal

Journal of Veterinary Diagnostic Investigation, 28(2)

ISSN

1040-6387

Authors

Love, William J

Lehenbauer, Terry W

Van Eenennaam, Alison L

et al.

Publication Date

2016-03-01

DOI

10.1177/1040638715626204

Peer reviewed

Sensitivity and specificity of on-farm scoring systems and nasal culture to detect bovine respiratory disease complex in preweaned dairy calves

William J. Love, Terry W. Lehenbauer, Alison L. Van Eenennaam, Christiana M. Drake, Philip H. Kass, Thomas B. Farver, Sharif S. Aly¹

Abstract. The California (CA) and Wisconsin (WI) clinical scoring systems have been proposed for bovine respiratory disease complex (BRDC) detection in preweaned dairy calves. The screening sensitivity (SSe), for estimating BRDC prevalence in a cohort of calves, diagnostic sensitivity (DSe), for confirming BRDC in ill calves, and specificity (Sp) were estimated for each of the scoring systems, as well as for nasal swab cultures for aerobic bacteria and mycoplasma species. Thoracic ultrasound and auscultation were used as the reference standard tests interpreted in parallel. A total of 536 calves (221 with BRDC and 315 healthy) were sampled from 5 premises in California. The SSe of 46.8%, DSe of 72.6%, and Sp of 87.4% was determined for the CA system. The SSe of 46.0%, DSe of 71.1%, and Sp of 91.2% was determined for the WI system. For aerobic culture, the SSe was 43.4%, DSe was 52.6%, and Sp was 71.3%; for *Mycoplasma* spp. culture, the SSe was 57.5%, DSe was 68.9%, and Sp was 59.7%. The screening and diagnostic sensitivities of the scoring systems were not significantly different but the Sp of the WI system was greater by 3.8%. Scoring systems can serve as rapid on-farm tools to determine the burden of BRDC in preweaned dairy calves. However, users may expect the SSe to be less than the DSe when confirming BRDC in an ill calf.

Key words: Bovine respiratory disease; pneumonia; preweaned dairy calves; screening tests.

Introduction

Bovine respiratory disease complex (BRDC) describes the clinical entity of bronchopneumonia and other respiratory illness in calves and adult cattle associated with a variety of pathogens.³ The etiologic agents of BRDC include bacterial organisms such as *Pasteurella multocida*, *Mannheimia haemolytica*,¹⁶ and *Mycoplasma bovis*,⁹ and viruses such as *Bovine herpesvirus 1* (infectious bovine rhinotracheitis, IBR)¹⁸ and *Bovine respiratory syncytial virus* (BRSV).⁵ Control of BRDC in dairy cattle is important because respiratory disease is a major cause of economic loss in the cattle industry, accounting for 22.5% and 46.5% of dairy heifer mortality before and after weaning, respectively.³¹ The 2 primary strategies for managing BRDC are prevention and treatment. While prevention is the preferred approach, cases may be difficult to prevent because many of the etiologic agents reside in the nasal passages of healthy calves. Factors that predispose calves for BRDC are also common in modern dairy production settings, and include stress,²⁸ failure of passive transfer,³³ coinfection with both types of *Bovine viral diarrhoea virus* (BVDV),²⁶ and poor air quality.¹⁹

Accurate detection of BRDC in dairy calves remains a significant challenge in dairy production systems.³⁵ Subjective

evaluation of clinical signs is a common method to detect BRDC in calves, but the lack of pathognomonic clinical signs and variation in clinical sign severity leads to inaccurate case detection.¹⁷ Thoracic ultrasound is emerging as an antemortem reference test for BRDC; however, ultrasound requires training and expensive equipment to be used effectively.¹ The accuracy of subjective clinical assessment is also dependent on the skill and experience of the evaluator. Microbial culture and quantitative real-time polymerase chain reaction (qPCR) assays may be used to more objectively detect pathogens as putative causes of BRDC; however, such tests may be prone to false-positive results because healthy cattle can harbor etiologic organisms without disease.¹³ Hence, the diagnostic accuracy of aerobic bacterial culture of deep nasopharyngeal swabs to test for BRDC in preweaned dairy calves has not been estimated.

Veterinary Medicine Teaching and Research Center, Tulare, CA (Love, Lehenbauer, Aly); and Departments of Population Health and Reproduction (Lehenbauer, Kass, Farver, Aly), Animal Science (Van Eenennaam), and Statistics (Drake), University of California, Davis, CA.

¹Corresponding Author: Sharif S. Aly, Veterinary Medicine Teaching and Research Center, School of Veterinary Medicine, University of California, Davis, 18830 Road 112, Tulare, CA 93274. saly@ucdavis.edu

Several clinical scoring systems have been developed to test for BRDC in calves. Clinical scoring systems compile clinical data into a single value to assess disease more objectively than an unstructured clinical evaluation alone. Two scoring systems for dairy calves are the Wisconsin BRDC scoring system (WI system)²³ and the California BRDC scoring system (CA system).^{2,21} The WI system assesses 5 clinical signs (<http://www.vetmed.wisc.edu/dms/fapm/fapmtools/calves.htm>): ocular discharge, nasal discharge, rectal temperature, induced or spontaneous coughing, and ear and head position. Each clinical sign is assigned 0 points for normal presentation, and an abnormal presentation is assigned 1, 2, or 3 points, with more severe presentations of clinical signs being assigned higher values. If the sum of the points for coughing, rectal temperature, nasal discharge, and the greater value of the ocular discharge or ear and head assessments for a calf exceeds 5, then the calf is BRDC test positive using the WI system. The CA system uses 6 clinical signs: spontaneous cough, nasal discharge, ocular discharge, rectal temperature ($>39.2^{\circ}\text{C}$), head and ear position (ear droop or head tilt), and respiratory quality (tachypnea or dyspnea).² Unlike the WI system, each clinical sign is dichotomized into normal and abnormal presentations, and abnormal presentations are assigned different values that range between 2 and 5. The point values for all 6 clinical signs are summed, and the calf is BRDC test positive using the CA system if the total point value is 5 or higher.

The tests used to detect BRDC may be used as screening or diagnostic tests. Screening tests are used to evaluate the prevalence of disease in a population, or to identify individuals to be investigated in more detail. In contrast, diagnostic tests are typically used to confirm disease in suspect subjects.¹² Accurate estimates of sensitivity (Se) and specificity (Sp) are needed to appropriately interpret BRDC scoring systems results, but there is little published information regarding the Se or Sp of these systems.⁷ The objective of the current study was to estimate the Sp and both the screening and diagnostic Se (SSe and DSe, respectively) of the CA and WI systems and of nasal swab culture methods in the detection of BRDC in hutch-raised dairy calves as assessed by thoracic ultrasound and auscultation.

Materials and methods

Study herds

The study was approved by the University of California, Davis Institutional Animal Care and Use Committee (protocol 17496, approval date March 21, 2013). Preweaned calves were sampled from 5 locations in the southern San Joaquin Valley of California between April and September 2013. Three locations (locations 1, 2, and 4) were dairies that raised their own calves, one location (location 3) was a calf ranch that raised calves from 3 dairy herds, and the final location (location 5) was a calf ranch raising calves from multiple

dairies from California and Arizona with unknown colostrum feeding practices. Calves from locations 1–4 were fed 4 liters of colostrum during the first 12 hr of life. Weaning age varied from 60 to 110 days. Calves at locations 1, 2, 4, and 5 were housed in elevated wooden hutches with slatted flooring and could make nose-to-nose contact with adjacent calves. Calves at location 3 were housed in larger nonelevated hutches made of wood and wire fence panels, which included an exercise area, and could not make physical contact with adjacent calves. Calves at locations 1 and 4 were housed under shade structures. Details of the participating locations are summarized in Table 1.

Sampling and evaluation

A nested case-control study was performed using incidence density sampling to select cases and healthy calves as they occurred in time. A nested case-control study is a case-control study nested within a well-enumerated cohort of subjects.²⁹ In our study, the cohort consisted of all of the calves on the farm that were free from BRDC at the beginning of the study. Only 1 location was sampled each day to reduce accidental transmission of pathogens between locations. Enrollment date ranges and number of visits to each dairy are summarized in Table 1.

All hutch-housed calves on the participating locations were eligible for enrollment into the study, unless they had been vaccinated in the previous 14 days or treated with antibiotics in the previous 10 days. Eligible calves ($N = 1083$) were visually evaluated during a morning walk-by at the start of each visit. Signs indicative of BRDC included depression, sunken eyes, coughing, and abnormal respiration; calves with any of these signs were considered clinically suspect calves and marked for more detailed examination before being enrolled in the study to contribute to the Se estimation.

An additional set of eligible calves was randomly selected at each visit by generating a random list of numbers that ranged from 1 to the size of the calf herd in hutches specific to each dairy. The exact number of calves selected randomly varied due to the number of visually suspect calves; however, ~12 calves were sampled each day in total due to time constraints. The randomly selected calves were marked for further examination on the same day before being examined, confirmed free of BRDC, and enrolled in the study to contribute to the Sp estimation.

All calves marked for further examination were evaluated using a standardized procedure composed of the following steps: clinical sign assessment and scoring, thoracic auscultation, thoracic ultrasound, decision to enroll, and collection of nasal swabs for culture from enrolled calves. Prior to handling each calf, the following clinical signs were visually assessed and recorded: respiratory rate, nasal discharge, ocular discharge, coughing, ear flick or drooped ears, head shake or head tilt, dyspnea, and diarrhea. After the clinical signs were recorded, a study team member

Table 1. Summary of 5 locations in the California southern San Joaquin Valley that participated in the study to estimate the sensitivity and specificity of 2 clinical scoring systems and 2 microbial cultures to detect bovine respiratory disease complex in preweaned calves performed from April through September 2013. The numbers of calves present and eligible to be sampled each day were estimated from records. Vaccination protocols were reported by the day of age the vaccines were typically administered with the specific vaccine product administered being denoted by symbols (see footnotes).

Location	Milking cows	Primary breeds	Estimated calves/day		Enrollment date range (mm/dd/yyyy)	No. of visits	Colostrum Source	Milk source	Weaning age (days)	Vaccination (days of age)	Sampling ages (days)
			Total	Eligible							
1	1,800	Holstein	200	90	05/30/2013, 06/26/2013	9	Cows	Pasteurized waste milk and replacer; 2–3 L every 12 hr	80–85	1*, 60†, 81‡	15–60
2	3,000	Jersey	350	267	06/27/2013, 09/10/2013	18	Cows, quality tested	Pasteurized waste milk; 1.5 L every 12 hr	110–130	70*, 110†	1–70, 84–110
3	Calves only	Holstein	600	216	04/29/2013, 07/23/2013	33	Cows	Milk replacer + neomycin; 2 L every 8–12 hr	60–65	2*, 15†, 30‡, 75‡	42–75
4	3,200	Holstein & Jersey	1,050	310	07/22/2013, 08/15/2013	9	Cows and heifers, quality tested	Pasteurized waste milk and replacer; 2 L every 12 hr	60–65	3*, 14†, 28‡	42–weaning
5	Calves only	Holstein	6,000	200	09/03/2013, 09/11/2013	6	Cows and heifers, pasteurized	Pasteurized waste milk and nonfat dry milk; 2 L every 12 hr	60–65	10§, 24§, 38‡	52–weaning

* Inforce3 (modified-live infectious bovine rhinotracheitis, *Bovine parainfluenza virus 3*, *Bovine respiratory syncytial virus* intranasal vaccine; Zoetis, Florham Park, New Jersey).

† Bovi-shield 4 (modified-live *Bovine viral diarrhea virus 1*, infectious bovine rhinotracheitis, *Bovine parainfluenza virus 3*, *Bovine respiratory syncytial virus*; Zoetis, Florham Park, New Jersey).

‡ Bovi-shield Gold 5 (modified-live *Bovine viral diarrhea virus 1* and 2, infectious bovine rhinotracheitis, *Bovine parainfluenza virus 3*, *Bovine respiratory syncytial virus*; Zoetis, Florham Park, New Jersey).

§ Titanium 5 + PH-M (modified-live *Bovine viral diarrhea virus*, infectious bovine rhinotracheitis, *Bovine parainfluenza virus 3*, *Bovine respiratory syncytial virus*, and killed *Mannheimia haemolytica*, *Pasteurella multocida*, and leukotoxin parenteral vaccine; Elanco, Greenfield, Indiana).

entered the hutch and immediately measured the calf's rectal temperature and heart rate. The clinical signs were scored according to the CA and WI systems. Next, the calf's lung fields were auscultated. Auscultation was considered to be abnormal if any of the following lung sounds were detected in any lung fields: rales, crackles, wheezing, moist lung sounds, pleural friction, alveolar snapping, or muffled lung sounds. Finally, the calf's lungs were examined by ultrasound. The calf's lateral thorax was prepared by clipping hair from both sides of the thorax in a triangle bounded by the olecranon, the caudal angle of the scapula, and the transverse process of the 10th thoracic vertebra, and the skin moistened with 70% isopropyl alcohol. A portable

ultrasound unit^a with an 8–5-MHz 66-mm multifrequency linear transducer was used to observe the lung parenchyma via intercostal spaces (Ollivett T. Thoracic ultrasonography and bronchoalveolar lavage fluid analysis in Holstein calves affected with subclinical lung lesions [dissertation]. Guelph, Ontario: University of Guelph, 2014). Abnormal ultrasound findings were classified into 4 categories of lesions or artifacts indicative of BRDC: small comet tails, large comet tails, consolidation, and abscesses. Small comet tails described reverberation artifacts originating in the pleura that were <5 mm wide. Large comet tails described reverberation artifacts originating from the pleura or parenchyma that were ≥5 mm wide. Consolidation

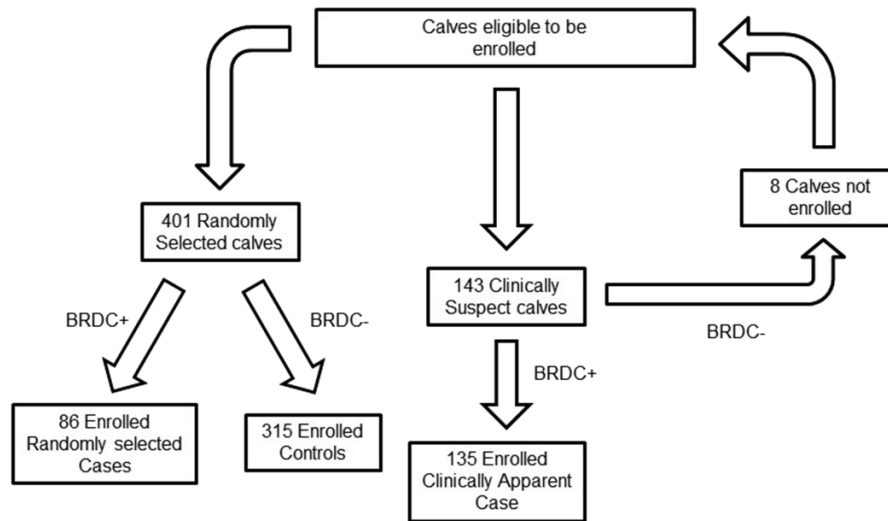


Figure 1. Flowchart for selection of preweaned dairy calves on 3 dairies and 2 calf ranches in the California San Joaquin Valley to validate 2 scoring systems for detection of bovine respiratory disease complex (BRDC). In addition to randomly selected calves, clinically suspect calves were enrolled if presented with dehydration, depression, cough, or abnormal respiration. Calves with abnormal lung ultrasound or auscultation findings were considered cases, and those with normal findings were considered controls.

described hypoechoic regions of lung parenchyma, typically containing 1 or more hyperechoic air bronchograms. Abscesses appeared as well demarcated areas with hyperechoic borders containing hypoechoic or heterogeneous material. Thoracic ultrasound findings were classified as abnormal if any of the following were detected: many small comet tails in multiple fields, multiple large comet tails, focal or extensive consolidation, or abscesses.

Clinically suspect calves with abnormal thoracic ultrasound or auscultation findings were labeled “clinically apparent cases.” Specifically, ultrasound and auscultation were interpreted in parallel, meaning calves with abnormal thoracic ultrasound and/or auscultation results were classified as cases.¹² Clinically suspect calves with normal ultrasound and auscultation were not enrolled, as these calves may have been subclinical or not exhibiting enough lung pathology detectable by ultrasound or auscultation at the time of examination. Such calves remained eligible to be sampled in future visits. Calves randomly identified for evaluation that had no abnormal lung sounds or ultrasound findings were enrolled in the study as healthy calves and labeled as “controls.” Randomly identified calves with thoracic ultrasound or auscultation abnormalities were labeled “random cases.” Previously enrolled cases were not eligible for random selection at a later time. Previously enrolled healthy calves remained eligible for examination if they presented with signs consistent with BRDC at a later time. Figure 1 summarizes the frequency of calves enrolled by BRD status.

Nasopharyngeal and pharyngeal recess culture samples were collected when calves were enrolled in the study and tested for viruses and bacteria as described by others.^{13,14,21} Briefly, nasopharyngeal and pharyngeal recess samples were tested for viral organisms using a qPCR panel that included

primers for IBR,⁶ BRSV,⁴ BVDV,²² and bovine coronavirus (BCoV; K Kurth, Wisconsin Veterinary Diagnostic Laboratory, Madison, Wisconsin, unpublished data). Aerobic and mycoplasma cultures were performed on pharyngeal recess samples. Aerobic cultures were performed by plating onto blood and chocolate agar plates at 37°C for 48 hr. Individual colonies were replated and cultured for identification based on morphology and confirmed by biochemical tests.²¹ Aerobic cultures were considered positive for BRDC if *Histophilus somni*, *P. multocida*, *M. haemolytica*, or *Bibersteinia trehalosi* were identified, and negative otherwise. Samples for *Mycoplasma* spp. were cultured in enrichment broth for 48 hr, then plated on modified Hayflick agar, incubated in CO₂ for up to 7 days, and colonies identified by their characteristic fried-egg appearance and confirmed with digitonin test.^{25,30} Mycoplasma culture was considered positive for BRDC if colonies characteristic of *Mycoplasma* spp. were identified from a sample and showed a digitonin inhibition zone of 5–15 mm. Viral qPCR was performed by the Davis branch of the California Animal Health and Food Safety (CAHFS) laboratory system; aerobic cultures were performed by the Tulare branch of CAHFS; and mycoplasma cultures and digitonin tests were performed by the Milk Quality Laboratory at the Veterinary Medicine Teaching and Research Center in Tulare, CA. All nasal samples were collected, handled, and stored as previously described,²¹ except as noted above.

Statistical analysis

The total scores for both scoring systems were calculated and interpreted as described in their respective references.^{21,23} The mean number of calves eligible to be sampled per day (N_j , for the j th location) during the study was estimated based

Table 2. Summary of 536 preweaned calves sampled from 5 locations in California southern San Joaquin Valley enrolled in a study to estimate the sensitivity and specificity of 2 clinical scoring systems and 2 microbial cultures to detect bovine respiratory disease (BRD) complex performed from April through September 2013, including total number of calves sampled (n_j), estimated average number of calves eligible to be sampled from each location (N_j), sampling fraction (f_j), and survey weight assigned to randomly selected calves (w_j).

Location	n_j	N_j	f_j (%)	w_j	BRD status			Sex		Breed			Age (days)		
					Clinically apparent cases	Random selected cases	Healthy controls	Female	Male	Holstein	Jersey	Other	Min.	Median	Max.
1	90	90	100	1.00	4	6	80	81	9	90	0	0	17	37	62
2	76	267	24.7	4.05	13	13	50	74	1	0	75	2	16	49	138
3	72	216	23.8	4.20	27	9	36	43	29	71	1	0	44	64	94
4	251	310	74.6	1.34	77	50	124	169	82	153	97	1	21	65	110
5	47	200	17.7	5.65	14	8	25	13	34	46	0	1	54	62	64

on the records and vaccination protocols from each location. The sampling fraction for each location (f_j) was approximated by the proportion of eligible calves that were randomly sampled, corrected for the rate of sampling of clinically apparent cases (across study locations).

The Sp and Se of the tests to detect BRDC in calves were calculated as stratified, survey-weighted proportions using a commercial software package.^b Individual observations from each location were assigned weights (w_{ij} , for the i th individual observation from the j th location) based on the method used to identify the calf for evaluation. Calves selected for evaluation due to presenting signs were assigned a weight of 1 ($w_{ij} = 1$), and calves that were randomly identified and enrolled were assigned a weight equal to the inverse of the sampling fraction ($w_{ij} = 1/f_j$). Observations were stratified by location. A finite population correction was made using f_j . Variance estimates were calculated using a Taylor series linearization method.³⁴ Similarly, survey-adjusted proportions of cases that were nasal swab culture positive for *Mycoplasma* spp. or aerobic bacteria were estimated.

Two estimates of Se were reported for each test. The DSe was the estimated proportion of clinically apparent cases that had positive test results; hence, estimates of DSe excluded randomly selected cases. The SSe was the estimated proportion of all BRDC cases that had positive test results and was estimated using the results from all randomly selected and clinically apparent cases. A single estimate of Sp was reported for each test and was estimated using test results from all enrolled healthy calves (controls).

McNemar test was used to compare Se and Sp pairwise between tests.²⁴ The SSe, DSe, and Sp estimates for the CA system were compared with the respective estimates for the WI system, aerobic nasal culture, mycoplasma culture, and aerobic and mycoplasma cultures interpreted in parallel. In addition, SSe, DSe, and Sp estimates of the WI system were compared with aerobic nasal culture, mycoplasma culture, and aerobic and mycoplasma cultures interpreted in parallel. Sensitivity and Sp of viral qPCR results were not estimated due to the small number of positive results.

Results

Study calves

A total of 536 calves were enrolled, of which 360 (67.2%, standard error [SE]: 2.0) were Holstein, 172 (32.1%, SE 2.0) were Jersey, and 4 (0.7%, SE 0.4) were other breeds. Heifers made up the majority of enrolled calves (380, 70.9%, SE 2.0). Study calves ranged between 16 and 138 days of age (median 58 days). Healthy calves ranged in age from 17 to 138 days (median 55 days) and BRDC cases from 16 to 110 days of age (median 62 days). The distributions of BRDC status, sex, breed, and age of calves across the study premises are summarized in Table 2.

A total of 401 calves were randomly selected and enrolled (Fig. 1). Of the randomly selected calves, 315 (78.6%, SE 2.1) were healthy, and 86 (21.4%, SE 2.1) were BRDC cases. Fifty (58.1%, SE 5.4) of the cases identified after examining the randomly selected calves had abnormal ultrasound findings only, 13 (15.1%, SE 3.9) had abnormal respiratory sounds on auscultation only, and 23 (26.7%, SE 4.8) had both abnormalities. In addition to the randomly selected calves, 135 clinically apparent cases were enrolled, of which 7 (5.2%, SE 1.9) had abnormal ultrasound findings only, 37 (27.4%, SE 3.9) had abnormal respiratory sounds on auscultation only, and 91 (67.4%, SE 4.0) had both abnormalities. Of all 221 cases (86 + 135), 57 cases (25.8%, SE 2.9) of cases had evidence of pulmonary disease on ultrasound but no abnormal auscultation, 50 cases (22.6%, SE 2.8) had abnormal auscultation without abnormal ultrasound findings, and 114 had both (51.6%, SE 3.4). Six healthy calves were first enrolled as such and then again as clinically apparent cases at a later date when they were identified with clinical illness and confirmed on auscultation, ultrasound, or both. Hence, our study included 536 observations from 530 unique calves. All eligible calves were enrolled at location 1; hence, the sampling fraction was 100%. Sampling fractions for other dairies ranged from 17.7% to 74.7% (Table 2).

Table 3. Estimated screening sensitivity (SSe), diagnostic sensitivity (DSe), and specificity (Sp) of the California (CA) scoring system, Wisconsin (WI) scoring system, aerobic and mycoplasma culture of nasal swabs, as well as both cultures interpreted in parallel to detect bovine respiratory disease complex. Aerobic cultures were positive if *Pasteurella multocida*, *Mannheimia haemolytica*, or *Bibersteinia trehalosi* was isolated. Mycoplasma cultures were positive if colonies of appropriate morphology suggestive of *Mycoplasma* spp. were isolated and confirmed by digitonin test. Estimates are based on 536 preweaned calves sampled from 5 locations in California southern San Joaquin Valley between April and September 2013.*

Test	Screening sensitivity (%)			Diagnostic sensitivity (%)			Specificity (%)		
	SSe	SE	95% CI	DSe	SE	95% CI	Sp	SE	95% CI
CA scoring system	46.8	3.8	(39.5, 54.3)	72.6	3.5	(65.2, 78.9)	87.4	2.1	(82.6, 91.1)
WI scoring system	46.0	3.7	(38.9, 53.3)	71.1	3.6	(63.5, 77.7)	91.2	1.7	(87.2, 94.0)
Aerobic culture	43.4	3.1	(37.9, 49.7)	52.6	3.2	(46.2, 58.9)	71.3	2.5	(66.3, 75.9)
<i>Mycoplasma</i> spp. culture	57.5	3.8	(49.9, 64.7)	68.9	3.1	(62.4, 74.7)	59.7	2.8	(54.1, 65.0)
Aerobic and <i>Mycoplasma</i> spp. culture†	65.6	3.8	(57.8, 72.7)	77.8	2.8	(71.9, 82.7)	48.7	2.8	(43.2, 54.2)

* SE = standard error; CI = confidence interval.

† Parallel interpretation.

Survey-adjusted prevalence estimates

Overall prevalence of BRDC in calves across the study dairies and calf ranches was 31.7% (SE 1.9), specifically, 11.1% (SE 3.3), 24.5% (SE 4.3), 30.0% (SE 4.6), 46.4% (SE 1.4), and 29.5% (SE 6.3) in locations 1–5, respectively. Prevalence of aerobic bacterial pathogens isolated from nasal swabs from the study calves was 33.3% (SE 1.7). *Pasteurella multocida* was the most frequently isolated pathogenic aerobe, with a prevalence of 27.3% (SE 1.6), and ranged from 2.9% (SE 1.7; location 2) to 73.5% (SE 1.2; location 4). *Mannheimia haemolytica* and *B. trehalosi* prevalences were 6.6% (SE 0.7) and 1.8% (SE 0.6), respectively. *Histophilus somni* was not isolated from any calves in our study. Prevalence of *Mycoplasma* spp. was 45.8% (SE 2.0). Prevalence of calves with nasal swab cultures positive for *Mycoplasma* spp. or an aerobic bacterial pathogen was 55.8% (SE 2.1) and ranged from 17.8% (SE 4.1; location 1) to 92.0% (SE 1.7; location 4). In contrast, the prevalence of both *Mycoplasma* spp. and aerobic bacteria isolated was 23.3% (SE 1.3).

Viral pathogens were isolated from <2% of the study calves at locations 4 (3 calves) and 5 (3 calves) only. *Mycoplasma* spp. were isolated from 3 calves with BRSV detected on PCR; 2 were healthy and 1 was a clinically apparent case. *Mannheimia haemolytica* was isolated from one of the healthy viral-positive calves without *Mycoplasma* spp., and *P. multocida* was isolated from the viral-positive clinically apparent case. The latter and one of the healthy calves positive for BRSV had positive CA and WI scores and rectal temperatures >39.2°C, while the other 4 BRSV-positive calves had negative CA and WI scores and were not febrile. Six calves tested positive for BRSV on PCR, 5 of which were enrolled as healthy calves while the remaining BRSV-positive calf was a clinically apparent BRDC case. No calves tested positive for IBR or BVDV by PCR.

The survey-adjusted proportion of cases positive for *Mycoplasma* spp. or aerobic bacteria was 65.6% (SE 3.8), and the proportion positive for both was 35.3% (SE 2.7). In

contrast, of the 135 clinically apparent cases, 105 (77.8%, SE 2.8) were positive for either *Mycoplasma* spp. or an aerobic bacteria, and 59 (43.7%, SE 3.1) were positive for both. The survey-adjusted proportion of healthy calves positive for aerobic bacteria was 28.7% (SE 2.5) and for *Mycoplasma* spp. was 40.3% (SE 2.8).

Sensitivity and specificity

Table 3 summarizes the SSe, DSe, and Sp of the CA and WI systems, mycoplasma culture, aerobic bacterial pathogen culture, and both mycoplasma and aerobic bacteria culture interpreted in parallel.

Screening sensitivity. The SSe of the tests ranged from 43.4% for aerobic culture to 65.6% for aerobic and mycoplasma cultures interpreted in parallel. The SSe values of the CA and WI systems were not significantly different ($P = 0.78$); however, both differed significantly from the SSe of mycoplasma culture, and the SSe of both mycoplasma and aerobic culture interpreted in parallel. (Tables 4, 5).

Diagnostic sensitivity. The DSe ranged from 52.6% for aerobic culture to 77.8% for both aerobic and mycoplasma culture interpreted in parallel. The DSe values for the scoring systems were not significantly different ($P = 0.70$) from each other, mycoplasma culture, or mycoplasma culture and aerobic culture interpreted in parallel, but were significantly higher than the DSe of aerobic culture (Tables 4, 5).

Specificity. The Sp of the WI system (91.2%, SE 1.7) was significantly higher than that of the CA system (87.4%, SE 2.1, $P = 0.04$). The Sp of each scoring system differed significantly from the Sp of mycoplasma culture, aerobic bacteria culture, and parallel interpretation of both mycoplasma culture and aerobic bacteria culture (Tables 4, 5).

Table 4. McNemar test results for paired proportions to compare the estimated screening sensitivity (S_{Se}), diagnostic sensitivity (D_{Se}), and specificity (Sp) of the California scoring system to the Wisconsin (WI) scoring system, aerobic and mycoplasma culture of nasal swabs, as well as both cultures interpreted in parallel to detect bovine respiratory disease complex in 536 dairy calves. The California scoring system had the following estimated test parameters: S_{Se} = 46.8%, D_{Se} = 72.6%, Sp = 87.4%.

Comparison test	Screening sensitivity		Diagnostic sensitivity		Specificity	
	%	<i>P</i> value	%	<i>P</i> value	%	<i>P</i> value
WI scoring system	46.0	0.78	71.1	0.70	91.2	0.04
Aerobic culture	43.4	0.42	52.6	<0.01	71.3	<0.01
<i>Mycoplasma</i> spp. culture	57.5	0.03	68.9	0.49	59.7	<0.01
Aerobic and <i>Mycoplasma</i> spp. culture in parallel	65.6	<0.01	77.8	0.32	48.7	<0.01

Table 5. McNemar test results for paired proportions to compare the estimated screening sensitivity (S_{Se}), diagnostic sensitivity (D_{Se}), and specificity (Sp) of the Wisconsin scoring system to the California (CA) scoring system, aerobic and mycoplasma culture of nasal swabs, as well as both cultures interpreted in parallel to detect bovine respiratory disease complex in 536 dairy calves. The Wisconsin scoring system had the following estimated test parameters: S_{Se} = 46.0%, D_{Se} = 71.1%, Sp = 91.2%.

Comparison test	Screening sensitivity		Diagnostic sensitivity		Specificity	
	%	<i>P</i> value	%	<i>P</i> value	%	<i>P</i> value
CA scoring system	46.0	0.78	72.6	0.70	87.4	0.04
Aerobic culture	43.4	0.54	52.6	<0.01	71.3	<0.01
<i>Mycoplasma</i> spp. culture	57.5	0.02	68.9	0.66	69.7	<0.01
Aerobic and <i>Mycoplasma</i> spp. culture in parallel	65.6	<0.01	77.8	0.17	48.7	<0.01

Discussion

The current study provided estimates of on-farm Se and Sp for 2 clinical scoring systems and 2 nasal swab culture methods for BRDC-associated pathogens using ultrasound and auscultation, interpreted in parallel, as the antemortem reference standard test. The Se of both clinical scoring systems was ~46% to detect BRDC in all calves in the population (S_{Se}), and ~72% to detect BRDC in calves with clinically apparent illness (D_{Se}). Neither S_{Se} nor D_{Se} was significantly different between the scoring systems. The similarity in the sensitivities of the 2 systems might be expected, given that the WI system was used to define BRDC cases in the study used to design the CA system, although the CA system uniquely includes respiratory effort as a component of the scoring system.²¹ For S_{Se} estimates, both scoring systems were similar to an estimate for the Se of the WI scoring system (55.4%) in an earlier report.⁷ The higher D_{Se} (72%) may be expected given that such an estimate was based on a subset of cases with visually detectable clinical signs compared to the S_{Se} (46%), which was based on a population of cases that included subclinical calves. The CA and WI systems had significantly different specificities (87.4% and 91.2%, respectively). The difference in specificities between the 2 scoring systems is likely attributable to the WI system separating each clinical sign into more levels of severity than the dichotomy (normal or abnormal) of the CA system, thus reducing the likelihood of false-positive results in calves

with mild clinical signs. However, the simplicity, rapid time to completion, and reduced need for calf handling of the CA system may outweigh the greater Sp of the WI system. In addition to the similarities in D_{Se}, S_{Se}, and Sp, both the CA and WI systems had an excellent agreement beyond chance (kappa coefficient 0.85).²

The S_{Se} of the WI system is consistent with the Se estimate from an earlier study (55.4%), but the Sp in the current study (91.2%) was substantially greater than in the earlier study (58.0%).⁷ In the earlier report, ultrasound was utilized to detect consolidation of lung parenchyma at or caudal to intercostal space 4 to define BRDC cases.⁷ In contrast, our study used both auscultation and thoracic ultrasound of all lung fields, interpreted in parallel, to define BRDC cases. Lung parenchyma beneath the 3 most cranial intercostal spaces is the location where lung consolidation is most frequently observed,³ and the previous study may have misclassified some BRDC cases as healthy calves. In addition, the previous study did not include comet tail artifacts as possible indicators of pulmonary disease, leading to a lower reference Se. Other possible sources of the differences between estimates from the 2 studies include regional and herd differences in pathogens and age distributions among the sampled calves. A 2015 report estimated the Se and Sp of the WI scoring system to detect BRDC at 62.4% and 74.1%, highlighting further potential for variability in estimates depending on study design, choice of reference test, and analysis methods.⁸

Overall, DSe and Sp for both clinical scoring systems were statistically comparable or significantly better than aerobic and mycoplasma nasal cultures, interpreted either independently or in parallel. In addition, both clinical scoring systems had comparable screening sensitivities to aerobic culture of nasal swabs. However, the SSe of nasal swab mycoplasma culture, or parallel interpretation of aerobic and mycoplasma cultures, were significantly greater than the screening sensitivities for both scoring systems. The estimated specificities of the mycoplasma and aerobic cultures were ~60% and 71%, respectively, which may be explained by the fact that apparently healthy calves may harbor BRDC-associated pathogens in their nasopharyngeal regions without exhibiting signs of clinical respiratory disease. In our study, aerobic pathogens were isolated from 28.7% of healthy calves and *Mycoplasma* spp. were isolated from 40.3% of healthy calves, confirming the potential of such pathogens to be commensals.¹⁶

The main pathogens detected in the study calves were *Mycoplasma* spp. and aerobic bacteria, which were isolated from nasal swabs from more than half (55.8%) of all BRDC cases. The absence of pathogens detected in some cases in our study may be due to BRDC pathogens being undetectable with the microbial culturing methods used, lower respiratory tract pathogens not being present in the upper respiratory tracts, or failure of the swabs or laboratory tests (culture and PCR) to detect the pathogens present. The detection of BRSV, IBR, or BVDV was not included in the current study case definition, but was part of the criteria for defining BRDC cases in the study that first described the CA system.²¹ In our study, BRSV was isolated from 6 calves on 2 of the study locations only. Hence, BRSV was not included as a case criterion. Calves with BRSV may have been identified prior to clinical signs becoming severe enough to result in positive scores.

Comparison of BRDC studies is difficult because numerous methods and criteria have been used for antemortem disease detection.^{7,10,27,32} For example, rales, crackles, wheezing, moist lung sounds, pleural friction, alveolar snapping, or muffled lung sounds are uncommon in the absence of primary pulmonary disease.¹¹ Lung consolidation is similarly uncommon without primary lung disease, although it may persist after resolution of acute disease.²⁰ Hence, both auscultation and thoracic ultrasound should be specific to pulmonary pathology to serve as reference tests for BRDC cases with pulmonary pathology. In contrast, neither test may be sufficiently sensitive to act as reference tests alone. Early cases may not have developed sufficient lower respiratory tract pathology to cause detectable consolidation, and abnormal respiratory sounds may be transient. Of the study cases, 23% had abnormal auscultation findings only, and 26% had abnormal ultrasound findings only. The frequency of false-negative results from both ultrasound and auscultation in our study may indicate that neither test is sufficiently sensitive to act as a reference test alone. Parallel interpretation of tests, as

was done with thoracic ultrasound and auscultation in the current study, increased the Se and decreased the Sp of BRDC detection compared with when used separately. The loss of Sp may be acceptable for the increased Se to allow auscultation and ultrasound to be used in parallel as a BRDC reference standard test.

The BRDC scoring systems may be used as screening or diagnostic tests. In situations where the scoring systems are used to estimate the prevalence of BRDC in herds, the results are referent to the herd and act as a screening test. When used to confirm BRDC in animals suspected of disease as when presenting with signs of illness, the results are referent to individual animals and the scoring system acts as a diagnostic test to confirm BRDC. As a result, separate estimates of Se were provided for each of these scenarios. Disease severity is a factor that may influence test Se¹⁵; hence, it would be unreasonable to assume that the distribution of BRDC severity among clinically apparent cases was representative of disease severity among all cases in a herd. In addition, clinically apparent cases could not be assumed to be representative of all prevalent cases because they were not randomly selected. Similarly, the randomly selected cases were not representative of all prevalent cases because calves with observable clinical signs were sampled as clinically apparent cases.

In the current study, to better represent the frequency of random and clinically apparent cases in the population, the observations were weighted by selection method. Clinically apparent cases were assigned $w_{ij} = 1$ because each represented only the individual observed, and randomly selected cases were assigned $w_{ij} = 1/f_j$ because they represented multiple calves in the population. Because more importance was assigned to randomly selected calves, which tended, overall, to have less severe disease, and hence lower test Se, the overall estimate of SSe reported is conservative. Weighting did not affect the reported estimate of DSe because it was restricted to only calves with clinically apparent disease, which were assigned $w_{ij} = 1$.

Unlike for Se, a single estimate of Sp was reported for both screening and diagnostic tests. All randomly selected calves with normal ultrasound and auscultation were included in the Sp estimation. Clinically suspect calves with normal ultrasound and auscultation (8 calves) were not included because such calves may have been subclinical and may have not exhibited detectable lung pathology using ultrasound and auscultation. In addition, Sp is estimated using results from subjects free of the target disease; as a result, disease severity cannot influence Sp the way it influences Se. Other biological factors such as cross-reactivity with other pathogens and persistence of previous disease may affect Sp.

An important limitation of our study was that the researchers were not blinded to the clinical score results and how calves were selected for evaluation when ultrasound or auscultation was performed. The clinical signs were assessed before reference tests were performed because they were

more subjective and more likely influenced by the reference test results. A better method of evaluation would have involved multiple investigators, each evaluating the scoring systems and reference tests separately to avoid observational bias, but a blinded study design was not feasible. The study was also limited by the small number of calves that tested positive for viral BRDC pathogens. Additional studies, either observational or experimental, may be needed to evaluate the accuracy of the clinical scores to detect viral BRDC cases.

The current study provides estimates of Sp, SSe, and DSe for 2 on-farm clinical scoring systems and 2 nasal swab culture methods to detect BRDC in preweaned dairy calves. Reporting 2 separate Se estimates to reflect 2 expected uses of the clinical scoring tests in the field is more useful compared with a single crude Se estimate. The first Se estimate is when confirming BRDC in ill calves, the second is when estimating BRDC prevalence in a calf population. The clinical scoring systems performed as well or better than the cultures interpreted alone or in parallel in terms of Sp, and SSe and DSe, with the exception of mycoplasma and parallel interpretation of mycoplasma and aerobic nasal swab cultures, which both had higher SSe. The estimated SSe and DSe of the WI system were not significantly different than the CA system, but the Sp of the WI system was significantly greater than the CA system. Although the biological significance of the difference in Sp is not known, a 3.8% difference may be an acceptable compromise given the reduced calf handling requirement and ease with which the CA system can be used compared with the WI system.

Acknowledgments

We thank the study herds' owners, managers, and staff for their cooperation, and Paul Rossitto, Casey Glenn, Justin Tonooka, Roman Podolkhov, and Dr. Deniece Williams for technical assistance.

Authors' contributions

WJ Love contributed to design of the study; contributed to acquisition, analysis, and interpretation of data; and drafted the manuscript. TW Lehenbauer and AL Van Eenennaam contributed to conception and design of the study, and contributed to interpretation of data. CM Drake, PH Kass, and TB Farver contributed to design of the study, and contributed to analysis and interpretation of data. SS Aly contributed to conception and design of the study, and contributed to acquisition, analysis, interpretation of data, and manuscript write up. All authors critically revised the manuscript; gave final approval; and agreed to be accountable for all aspects of the work in ensuring that questions relating to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Authors' note

This article is part of the dissertation by WJ Love to the University of California at Davis, Graduate Group in Epidemiology in partial fulfillment of the requirements for the Doctor of Philosophy Degree.

Sources and manufacturers

- a. Ibex Pro, E.I. Medical Imaging, Loveland, CO.
- b. Stata 13.1, StataCorp LP, College Station, TX.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The research was funded by competitive grant #1753 provided by the University of California Division of Agriculture and Natural Resources (Principal Investigator: SS Aly).

References

1. Abutarbush SM, et al. Evaluation of the diagnostic and prognostic utility of ultrasonography at first diagnosis of presumptive bovine respiratory disease. *Can J Vet Res* 2012;76:23–32.
2. Aly SS, et al. Agreement between bovine respiratory disease scoring systems for pre-weaned dairy calves. *Anim Health Res Rev* 2014;15:148–150.
3. Ames TR, et al. The bronchopneumonias (respiratory disease complex of cattle, sheep, and goats). In: Smith BP, ed. *Large Animal Internal Medicine*. 3rd ed. Philadelphia PA: Mosby, 2002:551–570.
4. Boxus M, et al. Real Time RT-PCR for the detection and quantitation of bovine respiratory syncytial virus. *J Virol Methods* 2005;125:125–130.
5. Brodersen BW. Bovine respiratory syncytial virus. *Vet Clin North Am Food Anim Pract* 2010;26:323–333.
6. Brower A, et al. Encephalitis in aborted bovine fetuses associated with Bovine herpesvirus 1 infection. *J Vet Diagn Invest* 2008;20:297–303.
7. Buczinski S, et al. Comparison of thoracic auscultation, clinical score, and ultrasonography as indicators of bovine respiratory disease in preweaned dairy calves. *J Vet Intern Med* 2014;28:234–242.
8. Buczinski S, et al. Bayesian estimation of the accuracy of the calf respiratory scoring chart and ultrasonography for the diagnosis of bovine respiratory disease in pre-weaned dairy calves. *Prev Vet Med* 2015;119:227–231.
9. Caswell JL, et al. *Mycoplasma bovis* in respiratory disease of feedlot cattle. *Vet Clin North Am Food Anim Pract* 2010;26:365–379.
10. Curtis CR, et al. Descriptive epidemiology of calfhoo morbidity and mortality in New York holstein herds. *Prev Vet Med* 1988;5:293–307.
11. Curtis RA, et al. Lung sounds in cattle, horses, sheep and goats. *Can Vet J* 1986;27:170–172.
12. Dohoo IR, et al. Screening & diagnostic tests. In: *Veterinary Epidemiologic Research*. 2nd ed. Charlottetown, Prince Edward Island: VER Inc., 2010:91–127.
13. Fulton RW, Confer AW. Laboratory test descriptions for bovine respiratory disease diagnosis and their strengths and weaknesses: gold standards for diagnosis, do they exist? *Can Vet J* 2012;53:754–761.
14. Godinho KS, et al. Use of deep nasopharyngeal swabs as a predictive diagnostic method for natural respiratory infections in calves. *Vet Rec* 2007;160:22–25.

15. Greiner M, Gardner IA. Epidemiologic issues in the validation of veterinary diagnostic tests. *Prev Vet Med* 2000;45:3–22.
16. Griffin D, et al. Bacterial pathogens of the bovine respiratory disease complex. *Vet Clin North Am Food Anim Pract* 2010;26:381–394.
17. Guterbock WM. The impact of BRD: the current dairy experience. *Anim Health Res Rev* 2014;15:130–134.
18. Jones C, Chowdhury S. Bovine herpesvirus type 1 (BHV-1) is an important cofactor in the bovine respiratory disease complex. *Vet Clin North Am Food Anim Pract* 2010;26:303–321.
19. Lago A, et al. Calf respiratory disease and pen microenvironments in naturally ventilated calf barns in winter. *J Dairy Sci* 2006;89:4014–4025.
20. Leruste H, et al. The relationship between clinical signs of respiratory system disorders and lung lesions at slaughter in veal calves. *Prev Vet Med* 2012;105:93–100.
21. Love WJ, et al. Development of a novel clinical scoring system for on-farm diagnosis of bovine respiratory disease in pre-weaned dairy calves. *PeerJ* 2014;2:e238.
22. Mahlum CE, et al. Detection of bovine viral diarrhoea virus by TaqMan reverse transcription polymerase chain reaction. *J Vet Diagn Invest* 2002;14:120–125.
23. McGuirk SM. Disease management of dairy calves and heifers. *Vet Clin North Am Food Anim Pract* 2008;24:139–153.
24. McNemar Q. Note on the sampling error of the difference between correlated proportions or percentages. *Psychometrika* 1947;12:153–157.
25. Razin S. Identification of mycoplasma colonies. In: *Mycoplasma Characterization*. (Vol. 1, Methods in Mycoplasmaology). New York: Academic Press, 1983:83–88.
26. Ridpath J. The contribution of infections with bovine viral diarrhoea viruses to bovine respiratory disease. *Vet Clin North Am Food Anim Pract* 2010;26:335–348.
27. Sivula NJ, et al. Management practices and risk factors for morbidity and mortality in Minnesota dairy heifer calves. *Prev Vet Med* 1996;27:173–182.
28. Snowden G. Genetics, environment and bovine respiratory disease. *Anim Health Res Rev* 2009;10:117–119.
29. Szklo M, Nieto FJ. *Epidemiology: Beyond the Basics*. 2nd ed. Sudbury, MA: Jones & Bartlett, 2007.
30. Thurmond MC, et al. Evaluation of a digitonin disk assay to discriminate between *Acholeplasma* and *Mycoplasma* isolates from bovine milk. *Cornell Vet* 1989;79:71–81.
31. U.S. Department of Agriculture (USDA). *Dairy 2007, Heifer Calf Health and Management Practices on U.S. Dairy Operations, 2007*. Fort Collins, CO: USDA:APHIS:VS:CEAH, National Animal Health Monitoring System, 2007. No.: 550.0110.
32. Van Donkersgoed J, et al. Epidemiological study of enzootic pneumonia in dairy calves in Saskatchewan. *Can J Vet Res* 1993;57:247–254.
33. Virtala AM, et al. The effect of maternally derived immunoglobulin G on the risk of respiratory disease in heifers during the first 3 months of life. *Prev Vet Med* 1999;39:25–37.
34. Williams RL. A note on robust variance estimation for cluster-correlated data. *Biometrics* 2000;56:645–646.
35. Wittum TE, et al. Relationships among treatment for respiratory tract disease, pulmonary lesions evident at slaughter, and rate of weight gain in feedlot cattle. *J Am Vet Med Assoc* 1996;209:814–818.