



Early Detection of Bovine Respiratory Disease in Dairy Calves

Exploring visual clinical observation, automated electronic activity data, and biomarker analysis for detection of early signs of respiratory disease in dairy calves

PhD Thesis 2026

Henrik Hjul Møller



This thesis has been submitted to the Graduate School of Health and Medical Sciences,
University of Copenhagen, 15 May 2026

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1 Preface and acknowledgements

2 Detecting respiratory disease in preweaned dairy calves is associated with considerable
3 challenges. Which clinical signs appear in a calf that is truly suffering from respiratory disease?
4 Stakeholders agree that respiratory disease is a major challenge to the calf, but when is it a
5 sufficiently critical problem that the condition needs to be addressed by the farmer, calf-rearing
6 staff or veterinarian, or even requires treatment? Do we agree about these clinical signs and the
7 detection of it? Do the clinical, technical and biological toolboxes agree about this diagnosis?
8 These questions were the biggest motivation for me to start this PhD some years ago.

9
10 This process has truly been a long journey, and some would probably say that it has been in
11 progress for too long! However, this work has encouraged many great thoughts and discussions,
12 as well as some frustrations about the discipline of academic work, which includes generating
13 ideas, planning a study, planning and performing data collection, retaining a full overview of
14 sample handling, staff capacity, economy and funding applications, communication and legal
15 contracting – alongside finding a work-life balance.

16
17 I started this work two months before the COVID-19 pandemic hit the world in the spring of
18 2020, and this affected the project to some extent. I had never attended an online meeting before
19 the pandemic, and this is a skill that I have learned during the years of my PhD work. During the
20 project I took leave a couple of times to return to work as a practising veterinarian. I also moved
21 to another part of Denmark and worked on the distance to University of Copenhagen at Aarhus
22 University for the last two years of my study. Finally, I became a father of three boys during my
23 PhD project. On the journey, I have tried to remind myself that conducting a PhD is an
24 educational programme – and it has certainly also been a life education. I have gained a lot of
25 skills, attended many courses, taught a clinical course to approximately 250 third-year veterinary
26 students, participated in working groups and workshops and been affiliated with two different
27 work cultures at two different university departments in Denmark. Unfortunately, my PhD
28 programme lacked an international angle due to the pandemic, but it was also a priority of mine
29 to stay in Denmark with my family.

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31 my supervisor group, who stayed and supported and helped with writing and proofreading the
32 articles alongside probably around 100 supervisor meetings. Thank you for your flexibility in
33 helping me finish this project. I would also like to thank those who helped during the very busy
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38

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41 University of Copenhagen. In addition, Lulus Dyrefond (2022) and the Danish Milk and Cattle
42 Levy boards contributed to expenditure relating to the field and laboratory work through the
43 Robust Calves project (2018–2021).

44

45 It has been a great pleasure and a privilege to be able to conduct a PhD and gain insights into a
46 narrow spectrum of the fascinating world of veterinary sciences. I hope you will enjoy reading
47 this thesis and having a look into my thoughts, ideas and the results of three studies discussing
48 the detection of signs of bovine respiratory disease in dairy calves.

49

50

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52

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54 Viborg, Denmark, 15 May 2026

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56

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58 Henrik Hjul Møller

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104 **Summary**

105 Bovine respiratory disease (BRD) in calves is a disease complex that poses a major challenge to
106 cattle farming. It is a multifactorial disease associated with different combinations of potential
107 pathogens that are frequently present in and around dairy calves. The treatment of BRD can lead
108 to a high consumption of often broad-spectrum antimicrobials locally within a farm, nationally,
109 and globally. At the same time, BRD affects the welfare, growth, and future production of the
110 individual calf. It is crucial that optimal examination and test procedures are carried out as early
111 in the disease course as possible to achieve the best effect of an initiated treatment. However,
112 hitherto most research has focussed on the validity of different diagnostics of clinical BRD.

113
114 The overall hypothesis behind this thesis is that calves can go through three stages in the disease
115 development upon the initial infection: a subclinical stage with no measurable signs of the calf
116 being affected, even if pathogens might already be detectable in the calf; a preclinical stage with
117 measurable signs of immune reactions and/or changed behaviour and/or mild clinical signs
118 starting to appear; and a clinical stage with clearly observable clinical signs of disease from the
119 upper and/or lower respiratory system. Which diagnostics are most relevant will differ during
120 these stages. The work described in this thesis focussed on identifying the preclinical stage to
121 enable early detection of BRD allowing for early treatment with alternatives to antibiotics, such
122 as non-steroidal anti-inflammatory drugs (NSAIDs), in preweaned dairy calves under practical
123 on-farm conditions. Three diagnostic approaches were assessed: visual clinical assessment,
124 scoring (VAS), analysis of biomarkers from blood serum, and automated activity recordings
125 using electronic ear tags.

126
127 To optimise the use of resources according to the “reduce, refine, replace”-principles in research
128 using animals, one longitudinal field activity was organised with repeated visits (3–4 times per
129 week for 5 months) to one dairy farm for clinical examinations collection of blood samples. The
130 clinical examination data, laboratory results of blood sample tests, and electronic ear-tag data
131 from this field activity were used for three distinct studies with specific objectives and
132 hypotheses.

133
134 In the first study, a Visual Analogue Scale (VAS) was developed and compared with the widely
135 used Wisconsin Calf Health Scoring Chart (WCHSC) as a method for clinical detection of BRD.

136 The VAS is a continuous 0–10 unit scale, where specific anchor points on the scale were used to
137 represent the subclinical, preclinical, and clinical stages of disease. The results showed an
138 acceptable level of agreement between the VAS and the WCHSC, indicating that the VAS can
139 reflect clinically relevant disease states. However, interobserver reliability was moderate to low,
140 both among experienced observers and veterinary students, highlighting that the application of
141 the VAS requires training and calibration to ensure consistent and reliable use, particularly when
142 aiming to detect subtle clinical signs.

143

144 In the second study, activity data obtained from ear-attached accelerometers were analysed
145 together with longitudinal clinical observations to investigate whether changes in activity could
146 be detected prior to clinical identification of BRD. Calves developing BRD showed a significant
147 reduction in activity prior to clinical detection, indicating that behavioural deviations occur
148 before observable clinical signs. The detection window observed in this study was shorter than
149 reported in some previous studies, which may be explained using the VAS allowing earlier
150 identification of calves with weaker clinical signs. These findings support the potential of
151 activity measurements as an early-warning component in BRD detection systems.

152

153 In the third study, serum concentrations of acute phase proteins, including serum amyloid A
154 (SAA) and haptoglobin (Hp), were evaluated as biomarkers for BRD and for predicting recurrent
155 disease events. The results showed generally low concentrations of these biomarkers, suggesting
156 that sampling occurred early in the disease process before a measurable systemic inflammatory
157 response had developed. Consequently, SAA and Hp were not found to be useful for detecting
158 preclinical disease or predicting recurrent BRD events under the conditions of this study.

159

160 Across the three studies, the findings demonstrate that the investigated diagnostic approaches
161 capture different aspects and stages of BRD development rather than providing overlapping
162 measures of the same condition. Activity measurements are associated with subsequent BRD
163 development and likely corresponding to the transition from subclinical to preclinical disease,
164 while the VAS captures early clinical signs. In contrast, biomarker responses were not detectable
165 in preclinical stage of BRD, indicating that they reflect later stages of disease progression.

166

167 Overall, combining behavioural monitoring with structured clinical assessment offers a
168 promising approach for improving early identification of diseased calves. Such integrated

169 detection strategies may support more timely and targeted interventions, improve animal welfare,
170 and contribute to reducing the use of antimicrobials in dairy production systems.

171 **Sammendrag**

172 Bovin respiratorisk sygdom (BRD) hos kalve er et sygdomskompleks, der udgør en væsentlig
173 udfordring i kvægproduktionen. Sygdommen er multifaktoriel og er forbundet med forskellige
174 kombinationer af potentielle patogener, som ofte er til stede i og omkring kalve.

175

176 Behandling af BRD kan medføre et højt forbrug af ofte bredspektret antibiotika både på
177 bedriftsniveau, nationalt og globalt. Samtidig påvirker BRD den enkelte kalvs velfærd, tilvækst
178 og fremtidige produktivitet negativt. Det er derfor afgørende, at undersøgelser og diagnostiske
179 procedurer udføres så tidligt som muligt i sygdomsforløbet for at opnå den bedst mulige effekt af
180 en iværksat behandling. Hidtil har hovedparten af forskningen dog haft fokus på diagnostik af
181 klinisk manifesteret BRD.

182

183 Den overordnede hypotese bag denne afhandling er, at kalve gennemgår tre faser i
184 sygdomsudviklingen efter en initial infektion: en subklinisk fase uden målbare tegn på sygdom,
185 selvom patogener allerede kan være til stede; en præklinisk fase karakteriseret ved målbare
186 immunreaktioner og/eller ændret adfærd og/eller milde kliniske tegn; samt en klinisk fase med
187 tydeligt observerbare kliniske tegn fra de øvre og/eller nedre luftveje. Relevansen og
188 anvendeligheden af forskellige diagnostiske metoder forventes at variere mellem disse faser.

189

190 Arbejdet i denne afhandling har derfor haft fokus på at identificere den prækliniske fase med
191 henblik på at muliggøre tidlig detektion af BRD og dermed tidlig intervention med alternativer
192 til antibiotika, såsom non-steroide antiinflammatoriske lægemidler (NSAID), hos mælkefodrede
193 kalve i praksis. Tre diagnostiske tilgange blev undersøgt: visuel klinisk vurdering, scoring ved
194 hjælp af en visuel analog skala (VAS), analyse af biomarkører i blodserum samt automatiserede
195 aktivitetsmålinger via elektroniske øremærker.

196

197 For at optimere anvendelsen af ressourcer i overensstemmelse med principperne om “reduce,
198 refine, replace” i dyreforsøg blev der gennemført et longitudinelt feltstudie med gentagne besøg
199 (3–4 gange ugentligt over 5 måneder) på én malkekvægsbedrift, hvor der blev udført kliniske
200 undersøgelser, observationer og blodprøvetagning. Data fra kliniske observationer,
201 laboratorieanalyser af blodprøver samt aktivitetsdata fra elektroniske øremærker blev anvendt i
202 tre delstudier med specifikke formål og hypoteser.

203 I det første studie blev en visuel analog skala (VAS) udviklet og sammenlignet med det bredt
 204 anvendte Wisconsin Calf Health Scoring Chart (WCHSC) som metode til klinisk detektion af
 205 BRD. VAS er en kontinuert skala fra 0–10 cm, hvor foruddefinerede intervaller repræsenterede
 206 de tre sygdomsfaser. Resultaterne viste en acceptabel overensstemmelse mellem VAS og
 207 WCHSC, hvilket indikerer, at VAS kan afspejle klinisk relevante sygdomstilstande.
 208 Interobservatør-overensstemmelse var imidlertid moderat til lav, både blandt erfarne
 209 observatører og veterinærstuderende, hvilket understreger, at anvendelsen af VAS kræver
 210 træning og kalibrering for at sikre konsistente og pålidelige vurderinger, særligt ved detektion af
 211 tidlige og underlæggende sygdomstegn.
 212
 213 I det andet studie blev aktivitetsdata fra øreinstallerede aktivitetsmålere analyseret sammen med
 214 longitudinelle kliniske observationer med henblik på at undersøge, om adfærdsændringer kunne
 215 detekteres før klinisk identifikation af BRD. Kalve, der udviklede BRD, udviste en signifikant
 216 reduktion i aktivitet forud for klinisk detektion, hvilket indikerer, at adfærdsændringer opstår før
 217 observerbare kliniske tegn. Den observerede detektionsperiode var kortere end rapporteret i
 218 nogle tidligere studier, hvilket sandsynligvis kan forklares ved, at anvendelsen af VAS
 219 muliggjorde tidligere identifikation af mildere sygdomstilfælde. Resultaterne understøtter
 220 potentialet for automatiseret aktivitetsmonitorering som en tidlig varslingskomponent i systemer
 221 til detektion af BRD.
 222
 223 I det tredje studie blev serumkoncentrationer af akutfaseproteiner, herunder serum amyloid A
 224 (SAA) og haptoglobin (Hp), undersøgt som biomarkører for BRD samt for at forudsige
 225 tilbagevendende sygdomstilfælde. Resultaterne viste generelt lave koncentrationer af disse
 226 biomarkører, hvilket tyder på, at prøvetagningen fandt sted tidligt i sygdomsforløbet, før et
 227 målbart systemisk inflammatorisk respons var udviklet. SAA og Hp blev derfor ikke fundet
 228 egnede til tidlig detektion af sygdom eller til at forudsige tilbagevendende BRD-episoder under
 229 de givne betingelser.
 230
 231 Samlet set viser resultaterne fra de tre studier, at de undersøgte diagnostiske metoder ikke måler
 232 de samme aspekter af sygdommen, men derimod forskellige stadier af sygdomsudviklingen.
 233 Aktivitetsmonitorering synes at identificere tidlige adfærdsændringer, sandsynligvis svarende til
 234 overgangen fra subklinisk til præklinisk sygdom, mens VAS opfanger tidlige kliniske tegn.

235 Biomarkørresponset var derimod ikke målbart på dette tidspunkt, hvilket indikerer, at disse
236 afspejler senere stadier af sygdomsforløbet.
237
238 Overordnet viser afhandlingen, at ingen enkelt metode kan stå alene som en fuldstændig løsning
239 til tidlig detektion af BRD. I stedet peger resultaterne på, at en kombination af
240 adfærdsmonitorering og struktureret klinisk vurdering er en lovende tilgang til at forbedre den
241 tidlige identifikation af syge kalve. Sådanne integrerede detektionsstrategier kan understøtte
242 mere rettidige og målrettede behandlinger, forbedre dyrevelfærden og bidrage til at reducere
243 anvendelsen af antibiotika i malkekvægsproduktionen.

244 **List of manuscripts**

245 List of manuscripts included in the thesis:

246

247 I. Henrik H. Møller, Mogens A. Krogh, Mette B. Petersen, Liza R. Nielsen and
248 Nynne Capion (2024). **Comparison and interobserver reliability between a**
249 **visual analogue scale and the Wisconsin Calf Health Scoring Chart for**
250 **detection of respiratory disease in dairy calves.** In Journal of Dairy Science,
251 107:1102-1109. <https://doi.org/10.3168/jds.2023-23554>

252

253 II. H.H. Møller, M.A. Krogh, M.B. Petersen, L.R. Nielsen, N. Capion (2026).
254 **Reduced activity measured by ear-tag sensors indicate early signs of bovine**
255 **respiratory disease 2-3 days prior to clinical onset in preweaned dairy calves**
256 Manuscript under preparation.

257

258 III. H.H. Møller, M.B. Petersen, M.A. Krogh, L.R. Nielsen and N. Capion (2024).
259 **Serum Amyloid A and Haptoglobin concentrations in calves at first event of**
260 **respiratory disease were not associated with subsequent risk of respiratory**
261 **disease during the preweaning period.** In The Veterinary Journal, 306, 106188.
262 <https://doi.org/10.1016/j.tvjl.2024.106188>

263 **Abbreviations**

264	APP	Acute phase protein
265	BRD	Bovine respiratory disease
266	BRSV	Bovine respiratory syncytial virus
267	CT	Computed tomography
268	DAPD	Daily Doses per 1000 animals per day
269	<i>H. somni</i>	<i>Histophilus somni</i>
270	Hp	Haptoglobin
271	<i>M. bovis</i>	<i>Mycoplasma bovis</i>
272	<i>M. haemolytica</i>	<i>Mannheimia haemolytica</i>
273	NSAID	Non-steroidal anti-inflammatory drug
274	PCR	Polymerase chain reaction
275	PI-3	Bovine parainfluenza virus 3
276	<i>P. multocida</i>	<i>Pasteurella multocida</i>
277	SAA	Serum amyloid A
278	TR	Thoracic radiography
279	<i>T. pyogenes</i>	<i>Trueperella pyogenes</i>
280	TUS	Thoracic ultrasonography
281	VAS	Visual analogue scale
282	WCHSC	Wisconsin Calf Health Scoring Chart

1. Introduction

Bovine Respiratory Disease (BRD) causes major challenges in dairy calves, affecting health and production and with an economic impact for the dairy farmer. Overton (2020) reported that approximately 36% of all replacement heifers across the US had at least one episode of BRD within the first 120 days of life, and each episode potentially costing up to \$282 in additional expenses and lost production.

An episode of BRD can have several negative consequences for the individual calf. In one study, heifers detected with BRD as calves had 2.85 times higher odds of dying, and 2.3 times higher odds of being removed from the herd before the first calving (Buczinski *et al.*, 2021). In the same study, a lower daily weight gain of -0.067 kg was found in heifers being detected with BRD compared to calves that had remained healthy (Buczinski *et al.*, 2021). Hurst *et al.* (2022) reported that Holstein heifer calves treated once for BRD had a lower rate of becoming pregnant by 483 days of age (hazard ratio = 0.89) compared with calves that were never treated for BRD (hazard ratio = 1.0).

Large quantities of antimicrobials are used every year for treatment of BRD in dairy and slaughter calves. In Denmark, the prescription of antimicrobials used in young cattle (< 1 year old) has increased from 5.93 DAPD (Daily Doses per 1000 animals per day) in 2015 to 8.13 DAPD in 2024, where BRD is the primary indication for prescribing antimicrobials for systemic treatment (DANMAP, 2024). Many of these antimicrobials are broad-spectrum, with amphenicols (primarily florfenicol) and macrolides accounting for approximately 60-65% of the antimicrobial use in standardised doses in young cattle, which adds to concerns about development of antimicrobial resistance. It is highly relevant to assess the antimicrobial use for the treatment of e.g. BRD by investigating the stakeholders involved in treatment decisions for the individual dairy calf. In Denmark the decision to treat calves with signs of respiratory disease are often made by the farmer, based on formalised herd health agreements with the veterinarian. A study of the farm practice in Denmark reported that 76% of the decisions were made by the farmer/farm personnel alone, without veterinary consultation (Olson *et al.*, 2019). This finding emphasises the importance of calibrating decision-making to identify BRD as early and correctly as possible to be able to initiate mitigation or treatment of the disease early. With the practices

314 currently performed on many Danish dairy farms, the treatment effect is often poor, and the
315 calves that survive are affected by the sequelae of BRD throughout their productive life. Calves
316 diagnosed with BRD have been shown to produce carcasses weighing approximately 8.2 kg less
317 compared to unaffected calves (Pardon *et al.*, 2013), and early-life respiratory disease has also
318 been associated with reduced milk yield in the first lactation, with reported losses of around 150-
319 300 kg of milk (Buczinski *et al.*, 2021). Therefore, a different approach is needed.

320
321 A major challenge in the field is the early detection of BRD in preweaned dairy calves to enable
322 early intervention preventing progression to the clinical stage. The early detection is particularly
323 challenged by lack of a well-defined gold standard for identifying the preclinical stage of the
324 disease. This lack of standardisation complicates both the comparison of studies and the
325 evaluation of diagnostic tools. A recent review by Churchill *et al.* (2025) highlights this issue,
326 reporting that only 36% of studies investigating BRD detection in dairy calves provided a clearly
327 referenced case definition. Furthermore, only approximately 1% of the reviewed studies included
328 validation of the diagnostic method used. When BRD detection is based on clinical scoring
329 protocols, the performance of these systems is highly dependent on the underlying case
330 definition. Changes in the case definition can therefore lead to substantial differences in the
331 apparent performance of the same scoring protocol (Donlon *et al.*, 2024).

332
333 In this study we assume that the disease process of BRD in preweaned dairy calves consists of a
334 transition between three different stages: A *subclinical stage* that include pathological changes in
335 the lower respiratory tract due to invading pathogens and impaired clearance in the upper
336 respiratory tract but with absence of obvious clinical signs (Storoni *et al.*, 2026). For example,
337 Ollivett *et al.* (2015) demonstrated that a considerable proportion of preweaned dairy calves with
338 ultrasonographic lung consolidation did not exhibit clinical signs of BRD and Buczinski *et al.*
339 (2014, 2015) reported that thoracic ultrasound improved detection of bronchopneumonia and
340 detected lung lesions missed by clinical examination.

341
342 A *preclinical stage* may follow the *subclinical stage* of BRD, during which pathogens have
343 already established an infection in the lower respiratory tract, but specific clinical signs are not
344 yet detectable. Experimental studies have shown that pathogens can be present in lung tissue as
345 early as 2 hours after infection, whereas specific clinical signs may not appear until at least 6
346 hours post-inoculation (Berman, 2024). In natural infected calves, this interval is likely longer

347 due to variation in pathogen load, host immunity, and environmental stressors. Field
348 observations suggest that this stage may extend over approximately 1-4 days prior to the onset of
349 visible disease. During this stage, subtle behavioural changes such as decreased activity, reduced
350 feeding behaviour, and altered movement patterns may become evident. Sensor-based studies
351 have demonstrated that such changes can be detected using automated systems and may allow
352 for identification of BRD prior to clinical diagnosis (Cantor *et al.*, 2022b; Casella *et al.*, 2023).

353
354 During this period, pathological changes such as inflammation and impaired pulmonary function
355 may already be developing, potentially affecting calf performance and welfare. Precise
356 identification of preclinical calves remains challenging, as traditional clinical scoring systems are
357 not sensitive to these initial changes (Timsit *et al.*, 2016; Berman, 2024) and improved methods
358 are called for.

359
360 A *clinical stage* of BRD is characterized by specific clinical signs involving both the upper and
361 lower respiratory tract. Common manifestations include coughing, nasal and ocular discharge,
362 abnormal ear position, depression, and elevated rectal temperature (Smith, 2015). These signs
363 are widely described in the literature and are typically integrated into standardized clinical
364 scoring systems used for on-farm assessment. Scoring protocols, such as those developed by
365 McGuirk and Peek (2014) and Love *et al.* (2014), assign values to individual clinical variables,
366 which are then combined into an overall score to guide diagnosis and treatment decisions. Such
367 tools improve consistency in disease detection, although their sensitivity (Se) and specificity (Sp)
368 can vary depending on observer experience and disease stage (McGuirk and Peek, 2014; Love *et al.*, 2014; Timsit *et al.*, 2016).

370
371 The aim of this PhD project was to investigate the detection of a preclinical stage of BRD in
372 preweaned dairy calves, with focus on the potential for early detection using visual clinical
373 assessment and ear-tag-based activity measurements. In addition, the thesis aimed to evaluate the
374 predictive value of serum biomarkers for detecting subsequent events of BRD.

375 2. Background

376 This chapter describes the theoretical background relevant to the BRD diagnostic tools used in
377 the studies included in this thesis. It includes a review of the pathogenesis and pathophysiology
378 of BRD, with particular focus on aspects relevant to the detection of clinical signs related to the
379 preclinical stage. In addition, the prognostic potential of the applied detection tools, is discussed.
380 Finally, supplementary material and examples of the calf activity data are presented to improve
381 the understanding of the data used in the analyses.

382 2.1 Definition of bovine respiratory disease

383 BRD is a multifactorial disease in cattle. The term BRD is sometimes called *enzootic*
384 *pneumonia*. It includes multiple specific diseases, for example *pleuropneumonia* and
385 *bronchopneumonia*. The term *shipping fever* has also been used, especially when BRD is
386 associated with the movement of bull calves from dairy herds into a fattening herd (López and
387 Masvidal, 2025). The clinical signs of BRD can be located and related to both the upper and
388 lower respiratory tract, and the pathogens include viruses (e.g. *Bovine respiratory syncytial virus*
389 (*BRSV*), *Parainfluenza-3 (PI-3)*, and *Bovine coronavirus*), mycoplasmas (e.g. *Mycoplasma*
390 *bovis*), and bacterial agents (*Mannheimia haemolytica*, *Pasteurella multocida*, *Histophilus somni*
391 and *Trueperella pyogenes*) (Confer, 2009; Smith, 2015; Cummings *et al.*, 2022, Otten *et al.*,
392 2024). BRD is therefore a broad term that covers a mix of different diagnoses and aetiologies.

393 2.1.1 Pathogenesis and pathophysiology and clinical signs

394 Stress combined with viral agents can cause direct damage to the lung parenchyma and the
395 respiratory tract, which can change the immunity and clearance mechanisms and thereby allow
396 commensal bacteria in the upper respiratory tract to proliferate and be inhaled into the lungs. The
397 commensal bacteria can thereby invade the mucosa of the lower respiratory tract and cause
398 infection (Martin and Bohac, 1986; Czaprynski *et al.*, 2004; Taylor *et al.*, 2010). There are
399 several disease defence mechanisms including mucociliary clearance, antimicrobial peptides in
400 the epithelial lining fluid, proinflammatory responses of respiratory epithelium and recruitment
401 of alveolar macrophages, neutrophils and monocytes. These defence mechanisms can fail, which
402 makes the lower respiratory tract more susceptible to invading pathogens (Caswell, 2014).

403 The bacteria found in the respiratory tract are omnipresent and probably part of the normal
404 nasopharyngeal microbiome. The bacteria have a wide variation of virulence factors (biofilm
405 formation, adhesins, toxins etc.) that enables them to evade the immune system, destroy tissues,
406 and initiate a potentially damaging inflammatory response (Panciera and Confer, 2010). It is
407 suggested that some of the pathological changes, such as irreversible consolidation and
408 atelectasis, seen in diseased lung tissue are due to the host immune response to virus infection
409 (Valarcher and Taylor, 2007; Carvallo Chaigneau *et al.*, 2021). Hence, an important part of
410 treatment is to modulate inflammation, e.g. by using non-steroidal anti-inflammatory drugs
411 (NSAIDs) (Scahill *et al.*, 2026, under review).

413 The most common form of pneumonia associated with BRD in dairy calves is suppurative
414 bronchopneumonia (Gordon and George 1997, Caswell and Williams, 2007; Dabo *et al.*, 2008)
415 that occurs when the bronchia are colonized by virulent bacteria initiating a suppurative
416 bronchitis spreading in the airways. In the acute stages, the lungs feel uniform at palpation
417 during necropsy, and lesions are centred around the bronchioles within each lung lobule, often
418 bilateral in the cranioventral parts of the lungs. There is bronchiolar and peribronchiolar
419 inflammation, with varying degrees of congestion and atelectasis and there may be purulent
420 exudate in the bronchia. Some of these pathological changes are irreversible already in the acute
421 stages of BRD, even with antibiotic and anti-inflammatory treatments (Miró *et al.*, 2025;
422 O'Donoghue *et al.*, 2025; Storoni *et al.*, 2026).

424 In the chronic stages of BRD, macroscopic pathological findings include that the lungs are firm,
425 and consolidation results in a lumpy texture. Purulent bronchitis, abscess formation and
426 bronchiectasis can in some circumstances be developed as well as interlobular, intralobular and
427 peri-bronchial fibrosis. There may be pleuritis with focal fibrinous to fibrous adhesions. Most of
428 the pathological changes in the chronic stages are irreversible and depending on the proportion
429 of the lung tissue affected, it may not support a productive life (Gordon and George 1997,
430 Caswell and Williams, 2007; Dabo *et al.*, 2008; Panciera and Confer, 2010).

432 As both bacterial and viral pathogens can cause BRD, it is natural to use bacterial cultivation and
433 detection of viral pathogens (e.g. by PCR) as diagnostic tools. However, this is not always
434 straight forward. Several of the pathogens involved in BRD can be isolated from the upper
435 respiratory tract in healthy calves (Angen *et al.*, 2009, McMullen *et al.*, 2020, Studer *et al.*, 2021,

436 Timsit, *et al.*, 2018). Approximately 63% of apparently healthy calves were found to have
437 pathogenic bacteria isolated from trans-tracheal aspirations in both pure and mixed cultures
438 (Angen *et al.*, 2009). Therefore, detection of pathogens does not imply disease and should
439 always be interpreted in relation to the clinical status of the calf.

440
441 The time from inoculation of calves experimentally infected with BRSV until clinical signs can
442 be detected was found to be 3-4 days in a study by Klem *et al.* (2019). The first clinical signs in
443 that study included mild depression, spontaneous coughing, hyperpnoea, tachypnoea and
444 anorexia. The clinical stage of BRD with signs such as a raise in rectal temperature above
445 39.3°C, accompanied of enhanced vesicular or bronchial respiration, nasal discharge, coughing,
446 and varying degrees of anorexia and depression were detected from day 7 post-infection in
447 calves infected with bovine respiratory syncytial virus. (Viuff *et al.*, 2002; Tjørnehøj *et al.*, 2003,
448 Valarcher and Taylor, 2007).

449
450 In a study of young bulls, hyperthermia was detected approximately 19 hours before the onset of
451 mild clinical signs of BRD and 51 hours before more specific signs such as depression,
452 coughing, and ocular discharge (Timsit *et al.*, 2011). This indicates a window of more than 48
453 hours between the initial physiological response and the appearance of specific clinical signs,
454 meaning that disease detection may be delayed when relying solely on conventional clinical
455 scoring systems such as the Wisconsin calf health score chart (WCHSC) (McGuirk and Peek,
456 2014).

457
458 This means that is it plausible that there is a subclinical stage of (at least) 2-5 days upon initial
459 infection where the calves appear unaffected, followed by a preclinical stage of (at least) 1-3
460 days where the clinical signs are subtle and may include reduced or changed activity, a slight
461 temperature increase, and decreased appetite. Both stages can easily go undetected by farmers
462 and farmworkers under current dairy farming practices, if targeted diagnostic tools are not used.

2.2 Tools and concepts generally used to detect bovine respiratory disease in calves

2.2.1 Clinical detection of bovine respiratory disease and performance of different scoring protocols

The generally recommended valid way of diagnosing BRD is by performing a full clinical examination. This could include measuring rectal temperature and observing visual signs as coughing, nasal and eye discharge, ear and head position, measuring pulse and respiratory frequency, characterisation of respiration, palpation of pharynx and larynx and regional lymph nodes, auscultation of thorax, and thoracic percussion (Smith, 2015). However, this thorough examination is not always feasible in Danish dairy herds. In Denmark, it is most often the farm personnel that identifies diseased calves, and the personnel initiate treatment based on well-defined protocols from the herd veterinarian. Therefore, different diagnostic tools for BRD, such as the Wisconsin Calf Health Score Chart (WCHSC) (McGuirk and Peek, 2014) and California BRD3 protocol (Love *et al.* 2014) have been developed to facilitate a more systematic approach to diagnosis and quantification.

The clinical signs include categorized descriptions of nasal discharge, ocular discharge, coughing, rectal temperature, ear score and faecal scores. These scoring protocols includes some of the pivotal clinical signs that are easy to visualise, and the scoring of calves are fast. The clinical signs are categorised into a total score that can be used to decide whether the calf needs treatment (Love *et al.* 2014; McGuirk and Peek, 2014). The WCHSC protocol can be found in detail in Appendix I. Reported sensitivities (Se) and specificities (Sp) differ between producer-based detection that is defined as the farmer or farm personnel performing the clinical observation (Se: 64.5% and Sp: 69.1%; White *et al.*, 2016), the WCHSC (Se: 62.4% and 71.1%, and Sp: 74.1% and 91.2%; Buczinski *et al.*, 2015; Love *et al.*, 2014), and the California BRD3 scoring system (Se: 72.6% and Sp: 87.4%; Love *et al.*, 2014).

In addition to clinical scoring protocols, several studies have investigated their diagnostic performance relative to alternative reference methods, particularly thoracic ultrasonography (TUS), which has been proposed as a proxy for detecting lung lesions associated with BRD. Ollivett *et al.* (2015) demonstrated that a considerable proportion of calves with ultrasonographic evidence of lung consolidation did not exhibit corresponding clinical signs, indicating that clinical scoring systems may lack sensitivity when detecting BRD in the subclinical and

495 preclinical stage. Similar discrepancies between clinical scores and ultrasonographic findings
496 have been reported by van Leenen *et al.* (2020), Rhodes *et al.* (2021), and Decaris *et al.* (2022),
497 suggesting that reliance solely on clinical observations may underestimate the respiratory
498 pathology in calves. More recent studies by Hinnant *et al.* (2024), Donlon *et al.* (2024), and
499 Lindley *et al.* (2025) have further explored the relationship between clinical scoring systems,
500 imaging techniques, and health outcomes, reinforcing that no single diagnostic approach
501 provides a complete assessment of BRD status. Although TUS offers improved Se for detecting
502 lung lesions, its application is limited by the requirement for specialised equipment, training, and
503 time, which may restrict its use under field conditions.

504
505 The most important thing to consider is that a scoring system must be easy to implement and
506 have a high Se and Sp in correctly distinguishing diseased from non-diseased calves and
507 education and calibration of personnel are important to achieve a better health outcome in the
508 farms using these protocols. A challenge is that there is no agreement about a gold standard
509 definition and a gold standard protocol for BRD, and studies evaluating Se, Sp and agreement
510 between scoring protocols are therefore difficult to compare and summarise. An overview of
511 studies reporting on clinical detection methods is provided in Table 1. Aly *et al.* (2014) found an
512 excellent agreement between the California BRD3 scoring system (Love *et al.*, 2014) and the
513 WCHSC system (McGuirk and Peek, 2014) for detecting signs of BRD in preweaned dairy
514 calves. A latent class analysis study found that if only two clinical signs (cough, unilateral or
515 bilateral ear droop or head tilt, or rectal temperature $\geq 39.7^{\circ}\text{C}$) were present in a calf, it had a 31%
516 chance of having BRD, while if none of these signs were present, the calf had a 100% chance of
517 not having BRD (Berman *et al.*, 2022).

518 Table 1. Overview of clinical scoring systems and key validation/application studies for BRD detection in preweaned dairy calves

Study	Scoring system / application	Very short strengths	Very short limitations	Reference method / comparator
McGuirk and Peek, 2014	Wisconsin Calf Health Scoring Chart (WCHSC/CRSC); 5 signs scored 0–3 and summed for case identification.	Clear descriptions of categories 0, 1, 2 Easy implementable. Widely used; standardized descriptors; easy to compare across studies.	Requires calf handling, rectal temperature and cough induction; temperature can dominate the total score.	The score itself functions as the case definition; no independent external reference test in the protocol paper.
Love <i>et al.</i>, 2014	California BRD score; binary/streamlined signs with model-based weighting for on-farm diagnosis in preweaned calves.	Simpler than Wisconsin; less handling; designed for rapid on-farm use.	Developed in a specific calf-ranch setting; weighting is less intuitive than simple category sums.	Matched case-control derivation with model-based diagnosis rather than a single perfect reference test.
Aly <i>et al.</i>, 2014	Direct on-farm agreement study comparing Wisconsin and California systems in preweaned calves.	Showed excellent agreement between the 2 systems; supports use of a simpler California approach.	Single-herd cross-sectional design; agreement does not necessarily equal diagnostic accuracy.	Calves were further examined by thoracic auscultation and ultrasonography; focus was agreement, not a true gold standard.
Buczinski <i>et al.</i>, 2014	Wisconsin score compared with producer treatment history, thoracic auscultation and TUS.	Key field comparison showing that clinical scoring alone can miss calves with lung consolidation.	Ultrasound adds time, equipment and training; study was not designed as a simple farmer-applied protocol.	Thoracic ultrasonographic lung consolidation used as the principal reference outcome.
Buczinski <i>et al.</i>, 2015	Bayesian estimation of the accuracy of the calf respiratory scoring chart and TUS in preweaned dairy calves.	Avoids assuming a perfect test; quantifies sensitivity/specificity of clinical scoring and TUS.	Methodologically complex and less accessible for routine on-farm decision-making.	Bayesian latent class model: explicitly no perfect gold standard assumed.
Ollivett <i>et al.</i>, 2015	TUS and bronchoalveolar lavage fluid (BALF) analysis in calves with low respiratory scores.	Highlights subclinical lung lesions that may be missed by low clinical scores.	Small research study; not a practical routine herd-screening protocol.	Postmortem examination used to evaluate lesion detection; BALF and ultrasonography compared with pathology.

Study	Scoring system / application	Very short strengths	Very short limitations	Reference method / comparator
van Leenen <i>et al.</i>, 2020	Combined clinical scoring and TUS used to classify calves before BALF bacteriology/cytology comparison.	Moves beyond clinical signs alone and links score-based classification to lower-airway sampling.	Combined protocol is less feasible for routine farm use; pathogen isolation did not associate directly with score categories.	Wisconsin Calf Health Scoring Chart. BALF. Bacteriology and cytology used as comparator methods.
Decaris <i>et al.</i>, 2022	External validation of Wisconsin and California scores under subtropical conditions; compared with TUS, auscultation and haptoglobin.	Useful external validation outside North America; directly compares multiple field tests.	Performance remained imperfect and estimates depended on latent-class model assumptions.	Bayesian latent class model using Wisconsin, California, TUS, auscultation and serum haptoglobin.
Hinnant <i>et al.</i>, 2024	Sequential comparison of clinical respiratory scoring and TUS during BRD progression in preweaned dairy calves.	Useful for showing where clinical and ultrasound diagnoses align or diverge over time.	Research-intensive repeated measurements; not a simple protocol-validation design.	Repeated TUS served as the main comparator to clinical respiratory scoring.
Donlon <i>et al.</i>, 2024	Bayesian assessment of different interpretations and cut-offs of Wisconsin and California scores in a temperate climate.	Very useful for reviewers because it shows that score interpretation strongly affects test performance.	Does not create a new score; conclusions depend on chosen latent-class structure and interpretation rules.	Hierarchical Bayesian latent class model with thoracic ultrasonography and clinical scores.
Lindley <i>et al.</i>, 2025	Prospective UK cohort using repeated Wisconsin and California respiratory scoring together with TUS.	Recent large longitudinal field study; distinguishes new, repeat and chronic cases and contrasts clinical vs ultrasound findings.	More an application/progression study than a new score validation paper; repeated TUS limits farm simplicity.	Wisconsin and California scores interpreted alongside repeated thoracic ultrasonography.

519 TUS = thoracic ultrasonography; BALF = bronchoalveolar lavage fluid. Because no universally accepted gold standard exists for calf BRD, the last column
520 reports the reference method or comparator used in each study.

2.2.2 Behavioural changes as an indicator of BRD

Many aspects of the assessment of behavioural changes as a clinical tool for detecting BRD have been described. Cramer and Stanton (2015) found that calves with BRD were less likely to approach both a novel object and a stationary person on the day of BRD diagnosis compared to healthy controls. In addition, an association between a BRD diagnosis in calves and other behavioural changes like lying posture, standing posture, isolation, an approach test (where the calf takes steps towards the observer) and encouragement to rise have been reported (Cramer *et al.*, 2016). Furthermore, they found that a combination of behavioural changes (i.e. behavioural attitude scores (normal = bright, alert, responsive; depressed = dull but responds to stimulation, slow to stand, or reluctant to lie down)) lacked sensitivity ($Se = 0.23$) when held up against TUS combined with WCHSC as the reference test for BRD. At the same time, they found a $Sp = 0.95$. It suggests that either the comprehensive evaluation of behavioural changes is not fully specific for BRD, or TUS combined with WCHSC is not a perfect gold standard for BRD and some calves may be detected with the behavioural changes alone. All in all, behavioural assessment can be used in combination with clinical assessment to increase detection of BRD in calves with feasible on-farm observations (Cramer and Ollivett, 2020).

Overall, it may be possible to develop a clinical scoring tool combining observation of behaviour, and specific clinical signs to identify calves with BRD in the preclinical stage than it is possible with protocols that categorize more severe clinical signs occurring later in the clinical stage. A scoring protocol that could be used for detecting calves in the beforementioned preclinical stage of BRD might therefore, in addition to looking for specific clinical signs, include the calves' interest in the surroundings, brightness, alertness and responsiveness, brightness of the eyes, posture, head position, and activity, which is not included in the "traditional" clinical scoring protocols such as the WCHSC.

Such a protocol could for example be a visual analogue scale (VAS) that is widely used as continuous measures to quantify clinical status and disease severity across medical and veterinary contexts. Typically represented as a scale from one extreme to another (e.g. healthy to severely affected), VAS enables the observer to integrate multiple clinical and behavioural observations into a single score. Although inherently subjective, VAS has been shown to be sensitive to subtle changes that may not be captured by other scoring systems, making it

553 particularly useful in conditions with gradual onset. In cattle, VAS has been applied to assess
554 pain and clinical condition, including in studies of BRD, where it has been used to monitor
555 disease progression and treatment response (Martin *et al.*, 2022a; 2022b). The applications
556 highlight its value as a descriptive tool capable of capturing the progress of clinical expression.
557 Importantly, the sensitivity of VAS may be advantageous for identifying preclinical stages of
558 BRD, where clinical signs are mild and non-specific. Overall, VAS represents a flexible and
559 sensitive approach for assessing complex clinical conditions under both experimental and field
560 setting.

561 **2.3 Automated electronic surveillance sensors as a tool for detecting BRD**

562 The implementation of automated electronic sensor systems represents an emerging approach for
563 detecting diseases in cattle, including BRD. The literature in this field primarily focuses on
564 describing sensor techniques, data interpretation, information integration, and decision support,
565 including treatment decisions (Sun *et al.*, 2021). Such electronic disease detection tools have
566 shown promising performance, when treatment and health control were primarily based on
567 automated health alarms followed by clinical examination, as opposed to systematic clinical
568 examination of all animals within the herd. The automatic screening should be combined with
569 visual observation detecting animals not found by the alarm (Perez *et al.*, 2023). A review study
570 concluded that the use of sensor-based systems including activity measurements, behaviour, and
571 feeding data provide continuous monitoring of the individual and allow early detection of BRD
572 before obvious clinical signs appear (Prosser *et al.*, 2025).

573
574 Other alarm systems, such as ear thermometers that indicate elevated ear temperature via a
575 flashing light on the ear tag, have been used to detect BRD with varying and limited success
576 (McCorkell *et al.*, 2014; Mahendran, 2020). Recording cough sounds in calf group pens has been
577 evaluated as an early detection tool, with the method demonstrating Se = 50.3%, Sp = 99.2%,
578 and a precision of 87.5% overall, and these measurements were taken in calves in a clinical
579 outbreak of BRD requiring treatment (Vandermeulen *et al.*, 2016). However, the use of cough
580 sounds may be of limited value due to the timing of the coughing in relation to the disease stages
581 of BRD, as Timsit *et al.* (2011) found that the onset of clinical signs after a reticulo-ruminal-
582 measured hyperthermia event occurred, were approximately 19 hours for nasal discharge, 39
583 hours for abnormal pulmonary sounds, 51 hours for depression, 64 hours for coughing, and 79

584 hours for ocular discharge. This indicates that many clinical signs used for BRD detection may
585 appear too late for early detection in the preclinical stage of BRD in calves.

586

587 Behavioural changes measured by electronic devices might also be useful for detecting disease at
588 an early stage. Promising results have been reported in the use of activity measured in different
589 ways and with different sensor systems, such as specific lying time, lying bouts, and step count
590 as measured by the *IceQube* system, which is a pedometer attached to the tarsal area (Chisholm
591 *et al.*, 2021; Wottlin *et al.*, 2021; Cantor *et al.*, 2022a; Cantor and Costa, 2022).

592

593 Feeding behaviour recorded by automated milk feeders has been explored for early disease
594 detection. In fattening calves, BRD cases showed approximately 3.5 minutes shorter feeding
595 time, and one fewer daily feeder visit than healthy calves (Duthie *et al.*, 2021). Milk feeder data
596 also showed significantly fewer visits in calves that were developing or had developed clinical
597 signs of BRD defined from the WCHSC in combination with TUS (Cantor and Costa, 2022).
598 Another study used milk feeder data to evaluate if calves detected with BRD by use of TUS
599 recovered satisfactorily after antimicrobial treatment or relapsed. In that study, the milk intake –
600 among many other parameters – was 1 L of milk higher per day in recovered calves compared to
601 calves that had a relapse of BRD and thereby did not recover satisfactorily. This showed
602 potentials for detecting calves at risk of or with recurrent BRD (Cantor *et al.*, 2022a).

603

604 An alternative approach using milk feeder data was investigated by Perttu *et al.* (2023). They
605 found that diseased calves exhibited lower milk intake, fewer feeder visits, slower drinking
606 speed, and longer intervals between visits. In that study, calves were clinically assessed using
607 categorical scoring systems. Health status was scored from 0 to 4 (0 = healthy, 4 = severely
608 diseased), and behavioural state was similarly score from 0 to 4 (0 = active, 4 = non-responsive
609 or dead). In addition, clinical signs based on the WCHSC were recorded, and calves with a
610 cumulative score ≥ 3 were classified as diseased.

611

612 Building on this framework, Perttu *et al.* (2024) demonstrated that milk feeder data can be used
613 to predict disease status, as defined by clinical scoring protocols. However, the model was
614 developed and evaluated using data from a single study population on one farm, without
615 validation on an independent external dataset, limiting conclusions regarding generalisability.
616 Importantly, they also reported that weekly clinical assessments alone were insufficient for early

disease detection. Instead, combining daily treatment records with high-resolution milk feeder data in machine-learning models, including random forest and gradient boosting algorithms, improved the identification of diseased calves, including cases of BRD.

The area of using activity data for machine-learning algorithms to predict especially early cases of BRD was part of the work published by Casella *et al.* (2023), where data from automated feeders, accelerometers, and scales had an accuracy of 88% for correctly labelling healthy calves from calves with BRD based on the WCHSC as reference test. Such use of machine-learning modelling has also been discussed with primary focus on the changes in behavioural patterns, including feeding behaviour, along with pedometer data, but also calves clinically detected with BRD by the WCHSC system, where especially the behavioural changes were reported being useful to detect BRD in calves with accuracies around 95% (Cantor *et al.*, 2022b). Once the performance of the sensor systems has been validated for detection of clinical cases of BRD, it may be possible to include available systems in machine-learning algorithms, to be able to detect BRD in the subclinical or preclinical stage of BRD.

2.4 Biomarker analysis in relation to BRD

Another possible way of identifying calves with early signs of BRD is by detection of inflammatory biomarkers such as acute phase proteins (APPs) in blood serum. Biomarker analysis of e.g. APPs might be highly relevant as a tool supporting the severity of disease and might provide information for predicting BRD outcome (Aich *et al.*, 2009). The APP Serum amyloid A (SAA) is a protein that is present in all tissues in both healthy and diseased animals (Berg *et al.*, 2011), but it is mostly isolated from liver tissue because hepatocytes in the liver have been found to be the primary site of synthesis in all mammalian species investigated (Uhlir and Whitehead, 1999). SAA plays a role in the immunity as it binds to Gram-negative bacteria and facilitates phagocytosis (Orro *et al.*, 2011; Joshi *et al.*, 2018). SAA has been found to have a relatively fast and potent response to acute stages of inflammation through a marked upregulation and therefore it is defined as a useful biomarker for indicating a generalised inflammation in cattle (Alsemgeest *et al.*, 1995). SAA increased in lipopolysaccharide (LPS) challenged calves within the first 24-48 hrs (Jacobsen *et al.*, 2004). Because the synthesis might be sensitive in a preclinical stage of BRD due to the potent and fast response after inflammation, SAA might be a potentially relevant biomarker for detecting early signs of BRD in dairy calves or be able to predict future events of BRD.

649 It has been shown that the SAA response increases with increasing clinical severity and
650 decreases following treatment of BRD (Joshi *et al.*, 2018). In more severe cases, SAA
651 concentrations have been reported to increase approximately fourfold in viral infections and up
652 to fivefold in mixed viral and bacterial infections (Akgul *et al.*, 2019), although clinical severity
653 was not stratified in that study. In contrast, evidence from Joshi *et al.* (2018) suggests that in
654 earlier or less severe stages of BRD, the increase in SAA may be more moderate, typically less
655 than threefold. Another study found that SAA was significantly elevated in group-housed calves
656 diagnosed with BRD, supporting its utility as a biomarker of both general infection pressure and
657 individual disease status (Kaura *et al.*, 2026).

658

659 Haptoglobin (Hp) is another APP that is synthesised by hepatocytes in the liver. The synthesis is
660 mostly induced by cytokines such as IL-6 and TNF- α (Nakagawa-Tosa *et al.*, 1995) and has been
661 isolated from granulocytes in the blood of healthy cattle (Cooray *et al.*, 2007). After an
662 experimental infection with BRSV, Hp increased in concentration after 5 days and peaked at 6-7
663 days after infection (Heegaard *et al.*, 2000). In LPS-challenged calves Hp increased around 72
664 hrs (Jacobsen *et al.*, 2004), and in natural infections there was a 14-fold increase in Hp in
665 diseased calves (Joshi *et al.*, 2018). A clear positive correlation between clinical signs and Hp
666 concentration has also been described (Godson *et al.*, 1996; Prohl *et al.*, 2015; Joshi *et al.*, 2018;
667 Moisé *et al.*, 2019). The Hp response varies depending on the pathogens associated with the
668 BRD cases and with the severity of the disease across detected pathogens (Svensson *et al.*, 2007;
669 Orro *et al.*, 2011; Idoate *et al.*, 2015). The changes in the Hp response were a twofold increase in
670 relation to a viral BRD case, and a factor 5 increase in mixed viral and bacterial BRD cases
671 (Akgul *et al.*, 2019).

672

673 In general, Hp has been valuable to use in treatment decisions due to its high specificity and
674 distinction between healthy and sick (Humblet *et al.*, 2004) and thereby the ability to rule-out
675 non-affected animals as well as evaluating treatment effect as Hp declines when the animal
676 recovers (Prohl *et al.*, 2015, Joshi *et al.*, 2018). Few studies have evaluated APPs as a tool to
677 predict BRD outcome in calves (El-Deeb *et al.*, 2020; Kamel *et al.*, 2024), and this might
678 therefore be an under-investigated area of BRD research. Whether APP can be useful for
679 detection of preclinical BRD or for prognosis of recurrence of BRD in dairy calves has not yet
680 been clarified according to the author's knowledge.

681 **2.5 Treatment of bovine respiratory disease**

682 Identifying the right animals and deciding the right moment to initiate treatment are key
683 elements to successful treatment outcome together with the choice of treatment. Several studies
684 have focused on defining parameters or clinical signs that can be observed by the caretakers and
685 created score systems to aid the decision process. In most scoring systems, there is a defined cut-
686 off for initiation of antimicrobial treatment. However, questions emerge: Have we found the
687 right calves to treat? Is this the right moment to treat? And are we choosing the right treatment
688 for the given timing in the BRD development?

689

690 In recent years, treatment protocols and strategies for treating BRD have increasingly
691 incorporated NSAIDs into common treatment protocols primarily using antimicrobials. In theory
692 the treatment effect should be improved with a combination of antimicrobials to reduce the
693 number of bacteria causing the inflammatory response and NSAIDs to reduce the tissue damage
694 caused by the inflammation itself. This improvement is attributed to enhanced immunologic
695 responses, particularly the increase in white blood cell count in calves treated with NSAIDs
696 alongside antimicrobials (Bednarek *et al.*, 2003; Masebo *et al.*, 2024). Additionally, studies show
697 that calves treated for BRD with a combined NSAID/antimicrobial protocol exhibited reduced
698 pain and a 3.8–4.0% lower relapse rate compared to those treated with antimicrobials alone
699 (Martin *et al.*, 2022a; De Koster *et al.*, 2022). However, those studies focused on calves in the
700 clinical stage of BRD. The benefit of NSAID treatment is likely to be higher, if given in the
701 subclinical or preclinical stages where the tissue-damaging inflammation processes begin.

702

703 The acute inflammatory response and the inflammatory induced tissue damage could potentially be
704 relieved with NSAID alone in viral infections (Blanco *et al.*, 2005; Walsh *et al.*, 2016; Walsh *et al.*,
705 2018). The treatment has been found most effective, if it was initiated no later than five days after
706 the onset of experimental infection with BRSV for optimal prognostic outcomes (Carvallo
707 Chaigneau *et al.*, 2021). This very well could be in the preclinical stage of BRD. Some of the
708 NSAID-only treatment studies are challenging to compare/transfer to Danish settings, as the study
709 design includes calves receiving prophylactic or metaphylactic antimicrobial treatments, which is
710 common practice in some countries, particularly in slaughter calf production (Toaff-Rosenstein *et*
711 *al.*, 2016; Thesing *et al.*, 2016). Hence, it might be possible to implement NSAID-only treatment
712 protocols in calves that are detected with preclinical BRD. This could help reduce the inflammatory
713 processes and thereby prevent secondary bacterial infections that require antimicrobial treatment.

714 To be able to evaluate the effect of treating BRD with NSAIDs in the preclinical stage (or if
715 possible, already in the subclinical stage), new methods for early detection of BRD are needed.

716

717 3. Aim and hypotheses

718 The overall purpose of the project was to contribute to reducing use of antimicrobials for
719 treatment of preweaned dairy calves through improved, earlier detection of BRD.

720

721 The overarching hypothesis is that BRD can be detected earlier than clinical signs will appear
722 due to the above-mentioned hypothesized stages of the BRD; a subclinical stage, a preclinical
723 stage and a clinical stage.

724

725 The aim of this PhD project was to investigate the detection of a preclinical stage of bovine
726 respiratory disease (BRD) in preweaned dairy calves, with focus on early detection using visual
727 clinical assessment and ear-tag-based activity measurement.

728

729 Before presenting specific objectives and hypotheses, the overall hypothesis is clarified together
730 with an elaborate description of how ‘early-stage BRD’ is understood in this thesis. The overall
731 hypothesis is developed based on the state-of-the-art scientific knowledge about BRD described
732 in the background in Chapter 2. It suggests that three distinct stages of BRD are relevant upon
733 exposure of preweaned dairy calves to a combination of potential respiratory pathogens and risk
734 factors in their environment.

735

736 In the first (subclinical) stage, the calf exhibits no observable signs of being affected, but
737 pathogens may be detectable in diagnostic tests on respiratory organs or fluids. The duration of
738 this stage may vary under farm conditions depending on the dominating pathogens or the mix of
739 pathogens present and depending on the risk factors present but likely lasts ½-2 weeks.

740 Moreover, the duration of this stage is also difficult to describe based on literature, as reported
741 studies are often based on experimental studies where the initial inoculation dosage may be
742 higher than under field conditions, and where risk factors commonly present on farm might not
743 be present (e.g. high stocking-density, cold draft, lack of dry and clean bedding,
744 undernourishment).

745

746 In the second (preclinical) stage lasting approximately 2-5 days, calves may show mild signs of
747 BRD, including reduced activity, behavioural changes, slight fever, dull hair coat, early initial

748 ear droop, and reduced feeding activity. Acute phase proteins may also begin to increase if
749 measured.

750

751 In the third (clinical) stage, specific clinical signs are typically present, including rectal
752 temperature ≥ 39.6 C°, nasal discharge (serous or purulent), coughing, uni- or bilateral ear drop,
753 dull hair coat, reluctance to get up, and anorexia. Activity is markedly reduced, and serum APPs
754 are clearly elevated.

755

756 In this thesis, subclinical, preclinical, and clinical will be used as analytical constructs defined by
757 VAS-derived criteria, not directly observable biological states as those used in e.g. the WCHSC.

758

759 **Specific objectives**

760 Therefore, to address this aim, the following specific objectives were established:

761

762 1. The first objective was to determine the agreement between the VAS compared with the
763 WCHSC to detect signs of respiratory disease.

764

765 2. The second objective was to estimate interobserver reliability between 2 experienced
766 observers in calf health and between an experienced observer in calf health and a group of
767 veterinary students using VAS and the WCHSC to detect signs of respiratory disease.

768

769 3. The third objective was to examine differences in ear-tag-based activity measurements
770 between preweaned dairy calves with BRD and healthy age-matched controls prior to clinical
771 detection, and to quantify the timing at which these differences become statistically detectable.

772

773 4. The fourth objective was to evaluate the prognostic value of VAS scores below the treatment
774 threshold for NSAIDs, together with serum concentrations of serum amyloid A and haptoglobin,
775 following detection of a mild BRD event under commercial dairy farm conditions.

776

777 **Hypotheses**

778 The following hypotheses were formulated and tested across three studies undertaken within this
779 project:

- 780 1. A VAS can detect the *preclinical stage* of BRD and shows acceptable agreement with the
781 WCHSC as a reference test for clinical BRD.
782
- 783 2. Agreement with a reference observer using the VAS is comparable between experienced and
784 inexperienced observers.
785
- 786 3. Calves developing BRD exhibit reduced activity prior to clinical detection compared with
787 healthy age-matched control calves.
788
- 789 4. Serum concentration of the acute phase proteins SAA and Hp can predict future cases of both
790 preclinical and clinical stages of BRD in preweaned dairy calves.
791
- 792 The hypotheses were evaluated across three different studies, with Hypotheses 1 and 2 in Study
793 1, Hypothesis 3 in Study 2, and Hypothesis 4 in Study 3.
794

795 **4. Materials and methods**

796 The research protocol was reviewed and approved by the Ethical Committee at the Department
797 of Veterinary Clinical Sciences, University of Copenhagen (Project No. 2020-023). Additionally,
798 the Animal Experiments Inspectorate, Ministry of Food, Agriculture and Fisheries of Denmark,
799 approved the study (Project No. 2020-15-0201-00677). The research aims, objectives, and
800 hypotheses were tested, evaluated and conducted across the three studies included in this thesis.
801 The following section outlines the general study design and methods used. Specific
802 methodological approaches for each study are detailed in Papers I–III.

803 **4.1 Study herd and population**

804 This PhD thesis builds on one longitudinal field data collection activity that took place from 2
805 September 2020 until 30 January 2021 in a single conventional dairy herd in Zealand, Denmark,
806 with approximately 650 dairy cows and 800 heifers. The breeds included were Holstein (90%)
807 and Red Holstein (10%). After calving, all calves were fed 4 litres of colostrum via bottle and
808 subsequently moved to outdoor single “igloo” hutches with wheat and barley straw bedding.
809 Calves were bucket-fed 3 to 4 litres of milk replacer twice daily and had *ad libitum* access to a
810 calf starter grain mix and hay. At 35–40 days of age, calves were moved to group pens (either
811 outdoor group hutches or indoor pens with 6–14 calves per pen), where they were fed milk
812 replacer in feed troughs and had *ad libitum* access to hay and grain mix. Within a week of
813 moving to group housing, calves were dehorned and vaccinated against ringworm (*Trichophyton*
814 *spp.*), but not against respiratory disease pathogens.

815 **4.2 Study design and general inclusion criteria**

816 The longitudinal field data collection ended up including a cohort of 126 preweaned dairy heifer
817 calves. The herd was visited the first time 2 September 2020, and then every other day until 30
818 January 2021, resulting in a total of 76 herd visits. At the first herd visit calves aged 17–24 days
819 were included, and only heifers were selected, as bulls were sold for fattening and removed from
820 the herd at this age. New calves aged 17–24 days were enrolled every eighth day (the inclusion
821 days) throughout the data collection period, and all included calves were assessed every other
822 day. The maximum number of calves monitored simultaneously was approximately 50, to ensure

that all observations and blood samples could be completed between morning and afternoon feedings on data collection days. If too many calves were born between two inclusion days to include them all, calves within this age group were randomly selected using the random selection function in Microsoft Excel. When the number of new available calves was limited, all calves within the specified age range were enrolled. Calves were selected 10 days prior to the start of data collection from each calf, and on the same day, a Sensehub™ ear-tag activity recorder (SCR Engineers Ltd., Netanya, Israel) was attached to the left ear of each calf. According to the manufacturer, the ear tags required approximately 7 days after attachment to calibrate to the baseline activity level of each individual calf. Therefore, a 10-day calibration period was applied prior to data collection. The sample size of enrolled calves was limited to 126 calves by the availability of the ear-tag activity recorders.

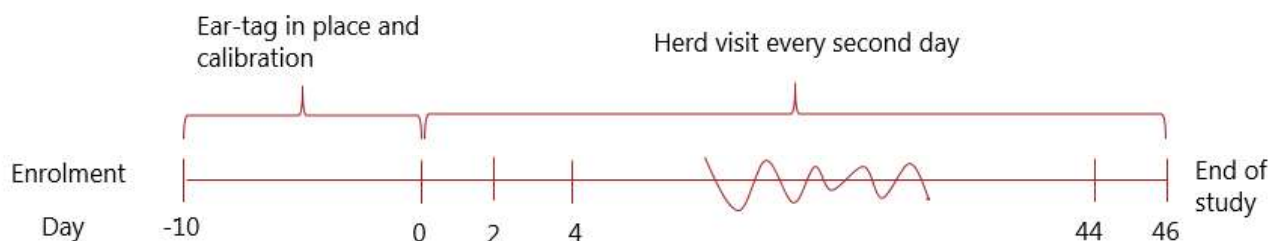


Figure 1. Timeline of the study design for each individual calf enrolled in the study. All calves were fitted an ear-tag activity recorder on day -10 leaving time for calibration. From day 0, the calves were visited every other day until the end of their participation in the study on day 46.

4.3 The herd visit

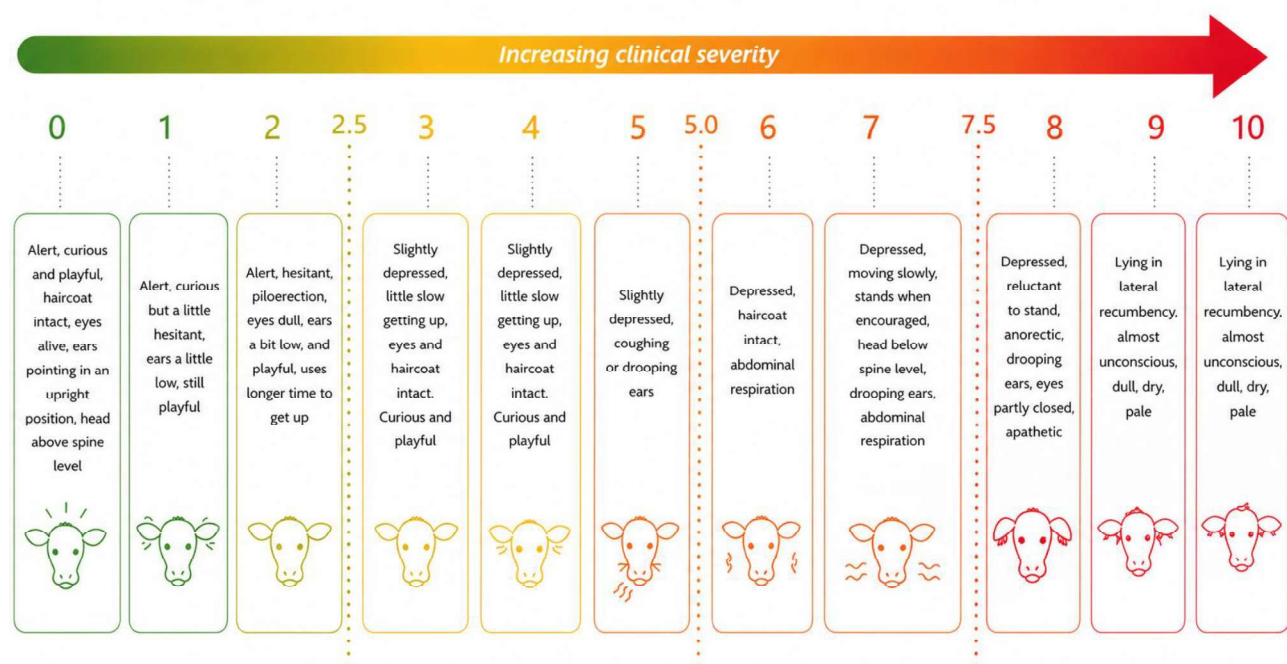
Each herd visit commenced with an initial walk around the calf-rearing area to evaluate all calves using a VAS score (further described in section 4.3.1) without touching the calves or entering their hutches/pens, followed by a second walk where the visually assessable variables of the WCHSC protocol were evaluated. The two rounds were carried out separately to prevent bias as these results were used for comparison of the two protocols in Study 1. After the visual observations, with minimal disturbance of the calves, the third round involved entering the single hutches or group pens, restraining the individual calf with a rope or with assistance from helpers, measuring the rectal temperature, manipulating the larynx if the calf did not already cough spontaneously during the visual clinical scoring, and collecting blood samples depending on the VAS score result (explained further in section 4.4.3).

849 **4.3.1 Development of a Visual Analogue Scale Score (VAS)**

850 The VAS relies on the observer's overall clinical impression, however, it allows for quantification
851 of subtle behavioural and clinical changes that may not be captured by categorical scoring systems
852 based on specific clinical signs. This was considered particularly relevant for identifying the
853 preclinical stage of BRD, where deviations from normal behaviour and signs of infection are mild
854 and gradually progressing. A VAS was therefore developed to assess the clinical status of each calf.

855 The use of VAS in the present study was inspired by previous applications in cattle, where similar
856 scales have been used to assess pain and clinical condition associated with BRD under experimental
857 conditions (Martin *et al.*, 2022a, 2022b). In these studies, VAS was primarily applied as a
858 descriptive measure to evaluate disease severity and treatment effects. In contrast, the VAS in the
859 present study was adapted for field conditions and designed to capture the full spectrum from
860 healthy to severely clinically affected calves over time, with particular emphasis on enabling
861 detection of preclinical disease.

862 The VAS ranged from 0 (clinically healthy) to 10 (severely affected, moribund), and to improve
863 interobserver reliability, a guide to use the VAS was developed (Figure 2). In addition, anchor
864 points (2.5, 5.0, and 7.5) were incorporated. These anchor points were selected to reflect clinically
865 meaningful transitions in disease progression. A score ≥ 2.5 was interpreted as the onset of definitive
866 detectable deviation from normal behaviour, corresponding to a preclinical stage, whereas a score
867 ≥ 5.0 indicated obvious clinical disease with specific signs of respiratory disease. In addition, the
868 anchor points were operationalized as decision thresholds for treatment, linking VAS scores directly
869 to clinical management.



871
872 *Figure 2. Visual analogue scale used for clinical assessment of calves, ranging from 0 (clinically*
873 *healthy) to 10 (severely affected). The scale is continuous, and the descriptive categories serve as*
874 *guidance for scoring based on the observer's overall clinical impression, including behaviour,*
875 *posture, and respiratory signs. Anchor points at 2.5, 5.0, and 7.5 represent clinically relevant*
876 *transitions from normal/subclinical to preclinical changes and from preclinical to obvious clinical*
877 *disease. Vertical dotted lines at these anchor points indicate the boundaries between these stages*
878 *and are included for more standardised scoring.*

879
880 The VAS scores were applied consistently across all studies in this thesis, including comparison
881 with the WCHSC (Study 1), evaluation of interobserver agreement (Study 1), to classify BRD cases
882 in the activity-based analyses (Study 2), and in the assessment of APP responses predicting future
883 BRD (Study 3).

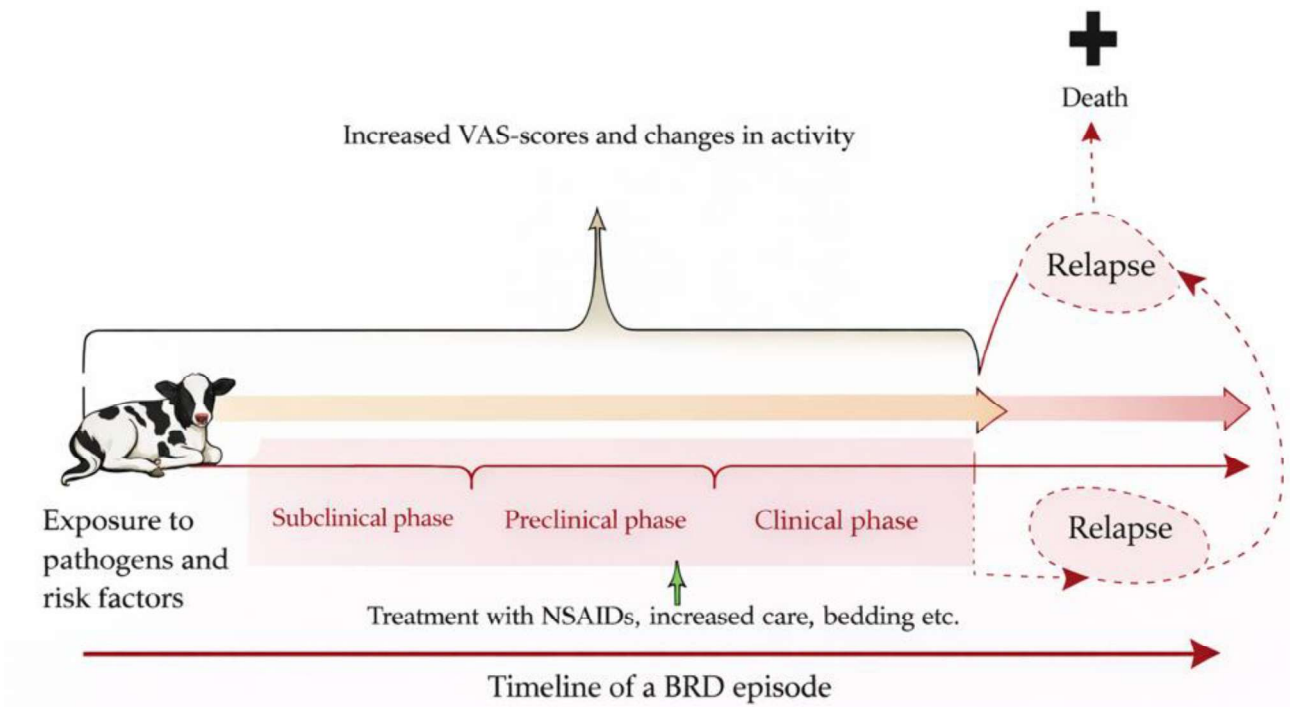
884 **4.3.2 Definition of a subclinical and a preclinical BRD event**

885 As described in the Introduction, the preclinical stage is defined as the period during which the
886 calf begins to show the first indications of compromised health. These changes are subtle and are
887 general unspecific clinical signs and include primarily behaviour, including slightly reduced
888 activity, longer latency getting up, slower suckling behaviour, and reduced curiosity. This stage
889 may progress into specific clinical signs, such as slightly depressed demeanour, lowered head
890 position, duller eyes and serous nasal discharge, before specific clinical signs become evident.
891 The clinical stage is characterized by specific clinical signs including depression, coughing,

892 mucopurulent nasal discharge, ear droop, and ocular discharge. The transition between healthy,
893 subclinical, preclinical, and clinical stages is gradual and often indistinct, requiring frequent and
894 careful observation.

895

896 In the clinical stage (defined as VAS scores of 5.0-10.0) the calf most likely needs an
897 intervention that requires treatment with a NSAID and antimicrobials. The VAS score was
898 developed to detect/identify these preclinical stages (Figure 3), which is not directly identified in
899 any of the existing calf scoring protocols. A BRD event was defined as a VAS score ≥ 2.5 , with
900 time 0 corresponding to the first observation at which this threshold was reached. Based on the
901 VAS scoring system, scores between 2.5 and 4.9 were classified as representing early or
902 preclinical changes, whereas scores ≥ 5.0 were considered indicative of clinical disease. The
903 VAS scale was developed for the purpose of this study to capture early behavioural and clinical
904 changes preceding overt disease. The conceptual progression of BRD, including subclinical,
905 preclinical, and clinical stages, is illustrated in Figure 3.



906

907 *Figure 3. Conceptual illustration of the progression of a BRD episode. Following exposure to*
908 *pathogens, calves may progress through subclinical, preclinical, and clinical stages, reflected by*
909 *increasing VAS scores and changes in activity. Following the clinical stage, VAS scores may*
910 *decrease during recovery; however, in cases of relapse, VAS scores may increase again. The*
911 *figure illustrates potential disease trajectories and is intended as a conceptual framework.*

912 **4.4 Methods for testing specific research hypotheses**

913 Different data from the data collection were used to answer the different research objectives in
914 this thesis. The data originate from intensive monitoring and repeated sampling of 126 calves in
915 one dairy herd in Denmark, described in detail above. Table 2 provides an overview of which
916 data are included in the different studies in this thesis. All clinical scores including the VAS and
917 the WCHSC data were used for all study elements and were used to both make interobserver
918 agreement, agreement with the WCHSC (Study 1). The clinical data were also used for treatment
919 decisions that were used to divide the calves in subclinical, preclinical, or clinical disease
920 processes in both Study 2 and 3.

Table 2. Overview of materials and methods used in the present thesis studies

Study	Element	No. of calves	Study design & population	Clinical assessment method	Additional measurements / outcome
Study 1	Comparison study	126	Observational field study in preweaned dairy calves in Danish dairy herds	Visual Analogue Scale (VAS) and Wisconsin Calf Health Scoring Chart (WCHSC)	BRD defined based on clinical scoring thresholds and overall clinical assessment
	Agreement (experienced observers)	53	Subgroup of calves assessed on a single day by experienced observers	VAS and WCHSC scoring performed independently by observers	Agreement between observers evaluated
	Agreement (students)	47	Randomly selected calves assessed on two different days by student observers	VAS and WCHSC scoring performed independently	Inter-observer agreement evaluated
Study 2	Activity	107	Calves detected with BRD followed retrospectively hourly measurements from 240 hours before and 96 hours after the BRD event	Visual Analogue Scale (VAS), repeated clinical scoring of respiratory disease events.	Calves classified as BRD cases were retrospectively analysed using hourly activity measurements spanning from 240 hours before to 96 hours after the BRD event.
Study 3	Acute phase protein study	65	Longitudinal observational study in preweaned dairy calves. Calves followed over time after initial mild BRD event	Repeated clinical scoring of respiratory disease events and recurrent disease episodes	Measurement of acute phase proteins (haptoglobin, serum amyloid A); association with BRD events defined as recurrence of BRD and comparison with acute phase response

923 4.4.1 Evaluating the performance of visual clinical detection of BRD

924 In Study 1, all 126 enrolled calves (Table 2) were included, and the aim was to compare the VAS
925 and the WCHSC scoring protocols used during the herd visits, as described in Section 4.3.

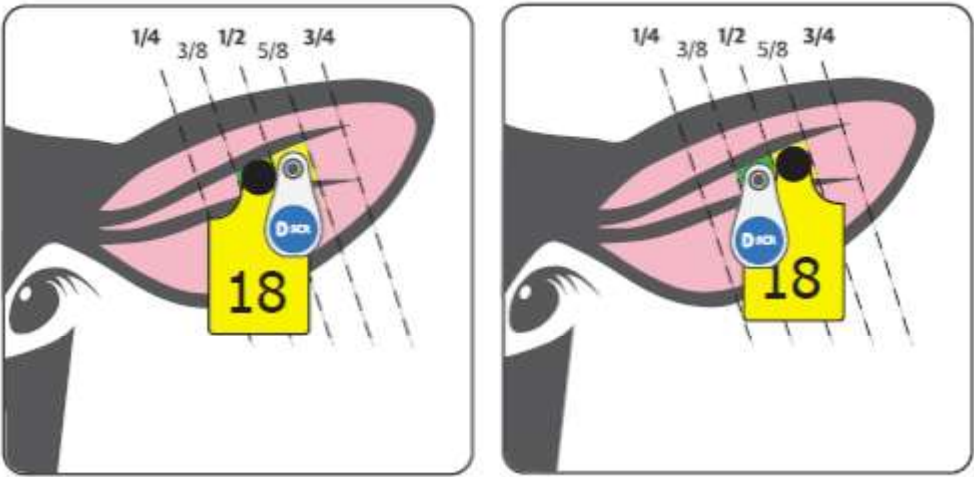
926 The reporting on Study 1 included an analysis of the overall agreement between the two scoring
927 protocols. The WCHSC score was divided into 2 groups to describe high (≥ 5) versus low (< 5)
928 probability that the calf had respiratory disease. We calculated agreement between the 2 scoring
929 systems based on conditional probability to score higher or lower than a cutoff in the VAS
930 compared with a specified cutoff in WCHSC used as reference. The data were analysed using a
931 generalised mixed-effects regression model, with the individual calf as a random effect due to
932 repeated observations of the same calf. The best agreement cut-off on the VAS with the specified
933 cut-off in WCHSC dividing clinical signs into healthy versus affected calves was calculated in the
934 study.

935 The second part of Study 1 analysed the agreement between observers using the VAS and WCHSC
936 protocols. An evaluation of interobserver agreement between two experienced observers and
937 between an experienced observer and 19 fourth-year veterinary students was included. We used the
938 intraclass correlation coefficient (ICC) as described by Koo and Li (2016) to evaluate agreement
939 between observers, who scored a varying number of calves. Only calves scored by all or most
940 observers in the group were included in the study, with details provided in Table 2. The inclusion of
941 veterinary students aimed to assess the feasibility of introducing the VAS protocol to relatively
942 inexperienced observers, comparable to introducing a scoring system to calf-rearing workers and
943 farmers with limited knowledge of systematic calf scoring.

944 4.4.2 Methods used in ear-tag sensor study that indicated early signs of BRD

945 Study 2 aimed to investigate differences in ear-tag-based activity measurements between preweaned
946 dairy calves with BRD and healthy age-matched controls prior to clinical detection and quantifying
947 the time where this difference become statistically detectable. This study was conducted on the
948 same population of calves as used in Study 1 and 3, however, challenges during extraction of data
949 from the activity database resulted in a total of 107 calves having a complete dataset in the period of
950 interest. Activity data from 19 calves were therefore excluded from the analysis.

951 A crucial factor in using activity recorders in calves is the correct installation and
952 implementation of the device. According to the Sensehub™ ear-tag activity recorder user guide,
953 the ear tag should be installed with a universal ear-tag applicator, commonly used for applying
954 the standard yellow ID tags. The ear tag should ideally be placed in the left ear, either on the
955 base side or outside of the ID tag, as illustrated in Figure 4. The main challenge in applying the
956 ear tags occurs when the yellow ID tag was positioned precisely in the middle, necessitating the
957 placement of the ear tag further to one side of the ID tag. Further details on ear tag installation
958 and data output can be found in the materials and methods section of Paper II. The data output,
959 as visualised on the user interface available on the farm, is shown in Figure 5 below.
960



961
962 *Figure 4. The ideal placement of the Sensehub™ activity ear tag according to the manufacturer.*
963 *The small green area was recommended as the optimal area to install the ear tag. Source:*
964 *<https://www.osid.no/wp-content/uploads/2020/08/SenseHub-Dairy-User-Guide-Ver-4.3.0.pdf>, P.*
965 *11.*

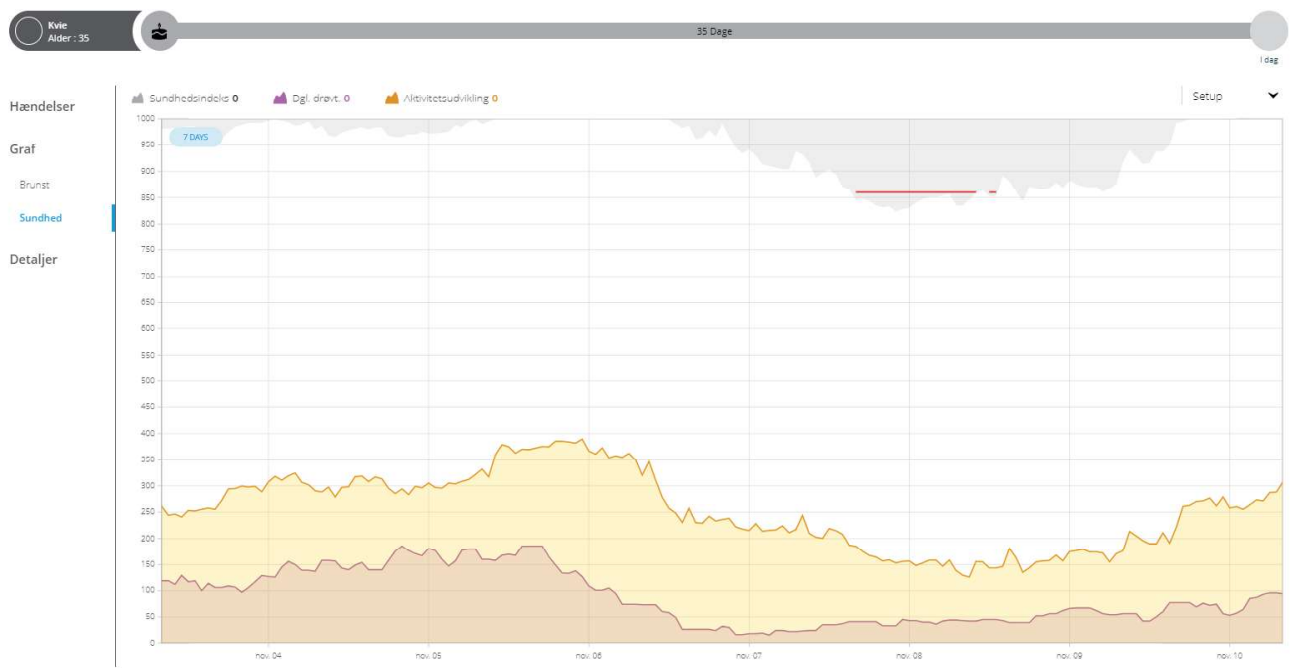


Figure 5. An example of a user interface platform (in Danish) from the Sensehub™ ear tag activity recorder system used during this project, with activity data including activity (orange line), rumination (purple line) and a health index (the grey clouded area at the top). The rumination and health index data were not further analysed in this study. (Source: Screenshot of the user interface installed for the Sensehub™ ear-tag system from one randomly selected calf enrolled in the study, obtained with permission from the farmer).

The activity data measured by the ear tag were available through the Danish Dairy Management System (DMS), which the farmer used for herd data handling. The Sensehub™ system was integrated by registration of the individual calf's ID number into DMS. To avoid clinical scoring bias during Study 1, the activity data were neither available to nor known by the primary observer until after the clinical data collection period was completed in January 2021. Instead, the data were retrospectively retrieved from the Sensehub™ data platform after the clinical study were ended, allowing for subsequent analysis. The data were extracted at the central office at Allflex, Sensehub™, and sent to the research group in an encrypted folder via email. The DMS provided data output only as visual graphs, whereas the numeric values of activity measured hourly throughout the study period were available exclusively through the Sensehub™ data platform. A calf with BRD was defined as $VAS \geq 2.5$, and calves that remained below this threshold throughout the study were considered healthy controls. Data analysis involved using hourly measurements from 240 hours before a calf had a BRD diagnosed until 96 hours post-detection. All calves with BRD were then analysed against healthy age-matched control calves. One control calf could serve as a control for more than one case. A linear mixed-effects model was fitted with activity level as the outcome variable. Fixed effects included BRD status, time

989 relative to detection, their interaction, and calf age. Calf identity was included as a random effect
990 to account for repeated measurements within individuals. A detailed description of the analytical
991 methods is provided in Paper II.

992 **4.4.3 Methods used in evaluating acute phase proteins as biomarkers for the recurrence of** 993 **BRD in dairy calves**

994 Study 3 aimed to investigate the prognostic value of the APPs SAA and Hp on the probability of a
995 calf having recurrent episodes of BRD. Calves that experienced a preclinical event of BRD, which
996 was defined as a VAS score between 2.5 and 4.9, were blood sampled in serum tubes and the
997 samples were analysed for concentrations of SAA and Hp. Blood samples were collected from the
998 *Vena jugularis* by venipuncture into serum tubes, processed by centrifugation in the laboratory,
999 frozen at -80°C, and subsequently transported to the analytical laboratory, as detailed in Paper III.

1000 During this study, the calves received treatment with NSAIDs only. If the calves received a VAS
1001 ≥ 5 , they received treatment with an antimicrobial agent and were excluded for this study.
1002 Photographs from data collection and blood sampling are available in Appendix III. Detailed
1003 methods for the analysis of serum SAA and Hp are provided in the materials and methods section of
1004 Paper III.

1005 A logistic regression analysis was used and the predictors in the final model were subsequently
1006 adjusted to include values for SAA, Hp, VAS, and the “follow-up time” defined as the period
1007 within the 46-day study from the calf’s first preclinical BRD event to any subsequent (preclinical or
1008 clinical) BRD event. The numerical changes in values included a 0.5 cm change in VAS score, a
1009 10-day change in follow-up period, a 5 mg/L change in SAA concentration, and a 50 mg/L change
1010 in Hp concentration, chosen as biologically meaningful values to estimate the odds ratios of
1011 recurrent BRD events in the final model. Employed to determine whether serum SAA and Hp levels
1012 could predict whether a calf with one BRD event would experience recurrent events during the
1013 study period, which could either result in treatment with NSAIDs only or develop into a more
1014 severe BRD event requiring antimicrobial treatment.

1015 **4.4.4 Follow-up data collection and outcome variables**

1016 A follow-up dataset was established on 27 April 2026, corresponding to approximately 5.5 years
1017 after the initiation of the original data collection. The purpose of this dataset was to enable
1018 assessment of long-term outcomes for the calves included in the study cohort.

1019 Individual-level data were obtained for all 126 calves enrolled in the original study. The data
1020 were manually extracted from the Danish Cattle Database (CHR) where all historically data of
1021 the individual cow are available (<https://chr.fvst.dk/chri/faces/frontpage>) by searching of the
1022 unique CKR number (Animal ID) after searching for the specific herd number of interest. All
1023 data were manually added to an Excel document and handled in R for overview of data. Each
1024 animal contributed one observation to the dataset. Calves were classified as BRD cases or
1025 controls based on their classification during the preweaning period in the original study.
1026
1027 The follow-up dataset included information on days in herd, exit status (dead, exported, still in
1028 herd, or slaughtered), exit before first calving, age at first calving, and number of calvings. These
1029 variables were extracted to describe survival, culling patterns, and reproductive performance.
1030 The follow-up analysis was descriptive in nature and aimed to provide contextual information on
1031 potential long-term consequences associated with early-life BRD classification.

1032 **5. Results**

1033 This chapter presents the results obtained from three studies conducted as part of the PhD
1034 project, each addressing different aspects of detection and characterisation of BRD in preweaned
1035 dairy calves. The main findings from each study are summarised in the following sections, while
1036 an overview of the study designs and data used is provided in Table 3. Study 1 focused on the
1037 development and validation of a VAS for clinical assessment of BRD, including comparison
1038 with the WCHSC and evaluation of interobserver reliability. Study 2 investigated whether
1039 behavioural changes, measured as activity using ear-tag accelerometers, differed between calves
1040 developing BRD and healthy controls prior to clinical detection. Study 3 examined whether
1041 concentrations of APPs, specifically SAA and Hp, measured at the time of a preclinical BRD
1042 event were associated with the subsequent risk of recurrent disease.

1043 Table 3. Includes information about the number of calves enrolled in each study, the number of
 1044 observers and number of observations analysed in each part of the study. The table is modified
 1045 from Table 1 in Paper I, with information about study elements for Study 2 and Study 3 added.
 1046 All calves referenced for each study are among the total calves enrolled (n=126) and therefore
 1047 each calf is enrolled in at least one or more of the study elements.

Study	Study period	No. calves	No. observers	No. observations
Study 1				
WCHSC-VAS agreement	Sep 2020 – Jan 2021	126	2	3,011
Interobserver reliability – experienced observers	Nov 2020	53	2	106
Interobserver reliability – veterinary students	Nov 2020	47	19	224
Study 2				
Activity data as an early detection tool for BRD	Sep 2020 – Jan 2021	107	2	41,616
Study 3				
Serum Amyloid A and Haptoglobin concentration's subsequent risk of BRD	Sep 2020 – Jan 2021	65	2	65

1048 **5.1 Results obtained from Study 1 comparison of a Visual Analogue Scale and the**
 1049 **Wisconsin Calf Health Score Chart and interobserver reliability.**
 1050 The main results from this study are presented in Paper I, which evaluated the agreement
 1051 between a VAS and the WCHSC for detection of respiratory disease in dairy calves, as well as
 1052 the interobserver reliability of the VAS method.
 1053
 1054 A total of 126 calves were included in the study, resulting in 3,011 clinical observations. Based
 1055 on the WCHSC, 380 observations were classified as having a high probability of respiratory
 1056 disease, corresponding to an estimated prevalence of 12.5% after accounting for repeated
 1057 measurements. The VAS scores were significantly higher in observations classified as diseased
 1058 compared with non-diseased observations ($P < 0.0001$).

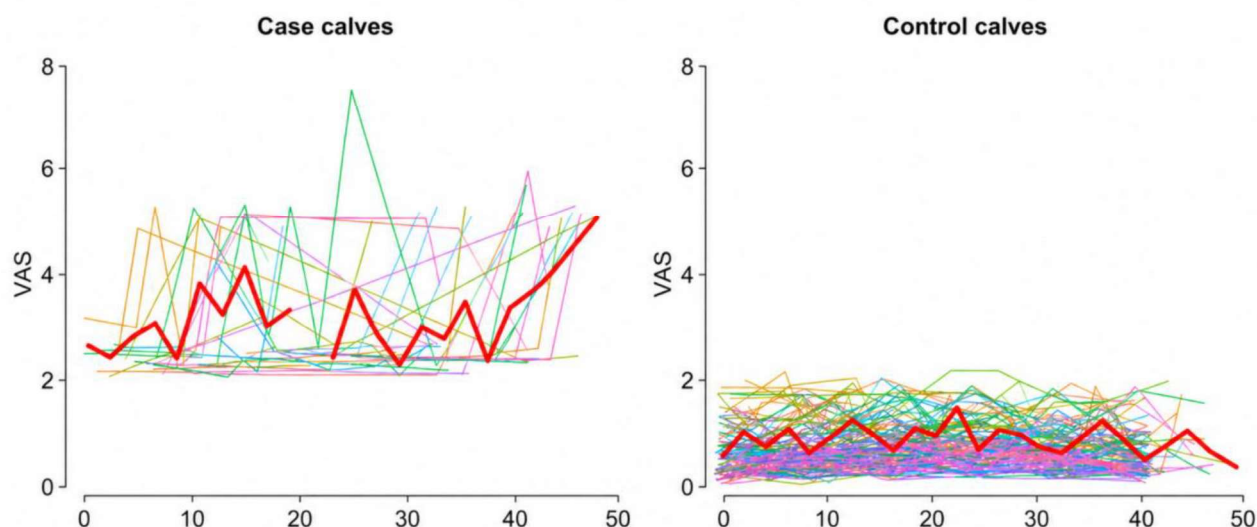


Figure 6. Individual trajectories of VAS scores over time (days in study) for calves classified as BRD cases (left) and healthy controls (right). Thin coloured lines represent individual calves, while the red line indicates the mean VAS score within each group. VAS scores vary over time in both groups; however, case calves show greater variability and a higher overall mean level compared with control calves.

The overall agreement between the VAS and the WCHSC was 89.6%, with the highest agreement observed at a VAS cutoff value of ≥ 2.8 corresponding to the WCHSC threshold of ≥ 5 . This indicates that the VAS was able to classify calves with a high or low probability of respiratory disease similarly to the WCHSC in the majority of observations.

Interobserver reliability of the VAS was evaluated between two experienced observers and between an experienced observer and a group of veterinary students. The agreement between experienced observers was moderate (ICC = 0.58), whereas agreement between the experienced observer and veterinary students was low (ICC = 0.34). In addition, veterinary students assigned significantly higher VAS scores compared with the experienced observer ($P < 0.0001$), indicating variability in scoring between observers with different levels of experience.

These results demonstrate that the VAS shows a high level of agreement with the WCHSC for classification of respiratory disease, although interobserver reliability is moderate to low, particularly among less experienced observers. Building on this clinical classification framework, the subsequent study investigated differences in ear-tag-based activity measurements between calves classified as BRD cases and age-matched healthy controls, with particular focus

1083 on the period preceding clinical detection and the timing at which these differences became
1084 detectable.

1085

1086 **5.2 Results obtained from the study investigating activity differences between BRD cases** 1087 **and healthy controls.**

1088 The results from Study 2 focused on the comparison of activity data obtained from the
1089 SensehubTM ear-tag recorders applied to all calves. Activity data were analysed relative to the
1090 time of clinical BRD detection, allowing comparison between calves developing BRD and
1091 healthy age-matched controls across time.

1092

1093 In this study, calves developing BRD showed a reduction in activity compared with healthy
1094 controls prior to clinical detection. Differences in activity between the two groups became
1095 apparent approximately 30 hours before the BRD event, with the first statistically significant
1096 difference identified at 31 hours prior to clinical detection. A gradual divergence in activity
1097 patterns was observed approaching the time of diagnosis.

1098

1099 At the daily level, statistically significant differences in activity were identified from 2 days
1100 before clinical detection and onwards. The model included calf as a random effect and BRD
1101 status, age, and time relative to the BRD event as fixed effects, including their interaction. The
1102 interaction between BRD status and time was found to be statistically significant, indicating that
1103 activity patterns over time differed between calves developing BRD and healthy controls. Age
1104 was also identified as a significant predictor of activity.

1105

1106 Post hoc comparisons showed that activity levels in calves with BRD were significantly lower
1107 than in controls in the period leading up to clinical detection, with the most pronounced
1108 differences observed close to the time of diagnosis.

1109

1110 These findings demonstrate that activity differ between diseased and healthy calves prior to
1111 clinical detection, although statistically detectable differences were observed within the last 48
1112 hours before the BRD were detected. To further investigate whether early disease processes
1113 could be identified through biological markers, the final study evaluated APP responses in
1114 relation to subsequent disease risk.

1115 **5.3 Results obtained from the study investigating levels of Acute Phase Protein's**
1116 **subsequent risk of having BRD**

1117 The main results from this study are presented in Paper III, which investigated whether serum
1118 concentrations of SAA and Hp measured at the time of a first mild/preclinical BRD were
1119 associated with the subsequent risk of developing recurrent BRD during the preweaning period.
1120

1121 A total of 65 calves experiencing first preclinical BRD were included in the analysis. Of these,
1122 26 calves experienced only one preclinical BRD, whereas 39 calves developed recurrent
1123 preclinical BRD during the follow-up period. Descriptive statistics of follow-up time, age, VAS
1124 score, and concentrations of SAA and Hp are presented in Table 4. The mean concentrations of
1125 both SAA and Hp at the time of the first preclinical BRD were similar between calves with one
1126 preclinical BRD and those with recurrent BRD, indicating no apparent difference in APP
1127 response between the groups.
1128

1129 Logistic regression analysis showed no evidence of an association between serum concentrations
1130 of SAA or Hp at the time of the first preclinical BRD and the odds of developing recurrent BRD.
1131 Likewise, the clinical severity of the initial BRD, measured by VAS, was not associated with
1132 recurrence. In contrast, the length of the follow-up period was significantly associated with the
1133 odds of recurrent BRD, with an estimated odds ratio of approximately 2.2 for each 10-day
1134 increase in follow-up time.
1135

1136 These findings indicate that serum concentrations of SAA and Hp measured at the time of
1137 preclinical BRD do not provide prognostic information regarding the risk of subsequent disease
1138 events under the conditions of this study. This suggests that APP responses at early stages of
1139 BRD may have limited value for predicting disease recurrence in preweaned calves.

1140 Table 4. Descriptive results of the concentrations of SAA and Hp and VAS on the day where the
 1141 preclinical BRD event were detected divided into two groups of calves: either having one BRD
 1142 event during the total 46-day study period or having two or more events detected during the
 1143 study period, derived from Results section in Paper II.

Parameter	One BRD event (n = 26)	Two or more BRD events (n = 39)
Follow-up time (days)	25 ± [SD: 9.42]	32.7 [SD: 11.4]
Mean age (days)	40.9	34.9
Mean VAS	2.8	2.9
Mean SAA (mg/L)	30.3 [SD: ±17.3]	30.5 [SD: ± 10.3]
Hp (mg/L)	381 [SD: ± 265]	403 [SD: ± 311]

1144
 1145 The results of the three studies demonstrate a progressive understanding of BRD detection and
 1146 characterisation in preweaned dairy calves across clinical, behavioural, and biological levels.
 1147
 1148 Study 1 established that the visual analogue scale (VAS) provides a valid and practical method
 1149 for clinical classification of BRD, showing high agreement with the WCHSC, although with
 1150 some limitations in interobserver reliability. Building on this clinical framework, Study 2
 1151 demonstrated that changes in activity patterns occur prior to clinical detection, with statistically
 1152 significant reductions identified approximately 31 hours before diagnosis, indicating potential for
 1153 early detection of disease using behavioural traits. In contrast, Study 3 showed that APP
 1154 concentrations measured at the time of a mild BRD event were not associated with subsequent
 1155 disease risk, suggesting limited prognostic value of these biomarkers under the conditions of this
 1156 study.
 1157
 1158 Collectively, these findings indicate that while clinical scoring and activity measurement provide
 1159 useful tools for identifying disease presence and early changes, the evaluated biological markers
 1160 did not improve prediction of disease progression, highlighting the importance of combining
 1161 complementary approaches for effective BRD detection and management.

1162 **5.4 Long-term follow-up of calves included in the study cohort**

1163 A follow-up dataset was established on 27 April 2026, corresponding to approximately 5.5 years
1164 after the initial data collection period. This enabled inclusion of longitudinal information on
1165 survival, exit patterns, and reproductive performance for the calves originally enrolled in the
1166 study.

1167
1168 The dataset included all 126 calves from the original cohort, of which 73 had been classified as
1169 BRD cases and 53 as controls based on early-life detection. Each animal contributed one
1170 observation.

1171
1172 The median number of days in herd was 859 days for BRD cases and 1077 days for control
1173 calves. The distributions showed substantial overlap between groups. The median age at first
1174 calving was 659 days for BRD cases and 682 days for controls. The median number of calvings
1175 was 1 in both groups.

1176
1177 Exit outcomes were similar between groups. Among BRD cases, 8 calves were recorded as dead,
1178 9 were exported, 10 remained in the herd as dairy cows still producing, and 46 were slaughtered.
1179 In the control group, 6 calves were recorded as dead, 6 were exported, 6 remained in the herd as
1180 dairy cows still producing, and 35 were slaughtered. The proportion of calves exiting the herd
1181 before first calving appeared comparable between groups.

1182
1183 Overall, no clear differences were observed between BRD cases and controls in terms of
1184 survival, exit outcomes, or reproductive performance at the descriptive level.

1185 **6. General discussion**

1186 Working under the overarching hypothesis that BRD can be detected earlier than clinical signs
1187 will appear, we needed to identify and evaluate new approaches closing the gap between healthy
1188 calves and calves detected too late to prevent permanent damage to/loss of respiratory function.

1189
1190 The aim of the work presented in this PhD thesis was to investigate tools capable of detecting a
1191 preclinical stage of BRD in preweaned dairy calves using clinical assessment, ear-tag-based
1192 activity measurements, and biomarker analysis. A central premise of the thesis is that BRD
1193 develops gradually, and that different detection tools capture different stages of this progression.

1194
1195 A key methodological consideration is that all three studies included in this thesis were
1196 conducted on the same cohort of calves within a single herd and time period. This means that the
1197 studies are not independent in a strict epidemiological sense. This design was, however,
1198 deliberate and reflects a longitudinal cohort approach rather than three separate experimental
1199 studies. The intention was to investigate different dimensions of the same biological process—
1200 clinical expression, behavioural change, and physiological response—within the same animals
1201 and under identical environmental and management conditions.

1202
1203 This integrated approach strengthens internal validity by reducing between-herd and between-
1204 animal variation. It allows direct comparison of detection methods in relation to the same disease
1205 events and across the same temporal framework. In contrast, conducting the studies in separate
1206 populations would have introduced heterogeneity in pathogen exposure, management, and
1207 environmental conditions, making it more difficult to interpret differences between detection
1208 methods. Although the studies are data-linked, they address distinct research questions, apply
1209 different analytical models, and use different outcome variables. They can therefore be
1210 considered analytically independent but biologically and temporally connected. The non-
1211 independence of repeated observations within calves was accounted for through mixed-effects
1212 modelling. The main limitation of this design is reduced external validity, and the findings
1213 should therefore be interpreted as reflecting patterns observed within this specific herd.

1214
1215 The exploration of early detection of BRD was partly inspired by observations from experienced
1216 farmers, who are often able to identify calves at risk several days before specific clinical signs

1217 become apparent. These observations are typically based on subtle changes in behaviour and
1218 demeanour, allowing early intervention that may reduce disease duration and severity. The VAS
1219 approach offers a potential framework to quantify this clinical intuition by capturing gradual
1220 deviations from normal behaviour. However, an inherent challenge is defining when such
1221 deviations reflect meaningful disease rather than normal variation. In this study, thresholds were
1222 pragmatically set at VAS ≥ 2.5 for early intervention with NSAIDs and ≥ 5.0 for clinical disease
1223 requiring antimicrobial treatment, reflecting increasing severity of clinical signs.

1224
1225 The question remains whether these thresholds were optimally defined. Based on the results
1226 from Study 1, the first threshold corresponding to alignment with the WCHSC would be closer
1227 to 2.8. However, observational and activity data suggested that behavioural changes may occur
1228 at even lower levels, potentially around VAS 2.0. At this stage, APPs did not provide additional
1229 support for defining disease onset, limiting their utility in early detection. It is also possible that
1230 calves should remain above a given threshold for a period before intervention is initiated, to
1231 distinguish true disease from transient variation. Due to the study design, it was not possible to
1232 systematically evaluate alternative cut-offs. Notably, few studies have addressed this early stage
1233 of the disease spectrum, as most BRD research focuses on specific clinical signs corresponding
1234 to VAS levels above 5.0.

1235
1236 Taken together, the results suggest that the investigated methods capture different aspects of the
1237 disease process rather than overlapping measures of the same disease stage. Activity
1238 measurements appear to detect the earliest deviations, likely reflecting the transition between
1239 subclinical and preclinical stages. The VAS captures early clinical or preclinical signs, while
1240 categorical scoring systems such as the WCHSC primarily detect more specific clinical signs. In
1241 contrast, the APPs evaluated in this study did not show measurable changes at the time of
1242 detection, suggesting limited utility for identifying early or mild disease under field conditions.

1243
1244 These findings may also indicate that the applied VAS threshold of 2.5 could be positioned too
1245 high to capture the earliest signs of disease, and that lower values, potentially around 2.0, may
1246 better reflect the initial effects of systemic inflammation. However, interpretation at these lower
1247 levels is challenging, as subtle changes in behaviour may also reflect individual variation in
1248 temperament rather than disease processes (Neave *et al.*, 2018).

1249 This interpretation is consistent with previous findings showing that pulmonary lesions can be
1250 present several days before clinical signs are detectable, as demonstrated using TUS (Cuevas-
1251 Gomez *et al.*, 2021). Furthermore, commonly used clinical scoring systems have been shown to
1252 have limited sensitivity, particularly in early disease stages (Berman, 2024). Together, this
1253 supports the need for detection approaches that capture earlier and more subtle indicators of
1254 disease.

1256 The VAS was chosen as a clinical tool to capture subtle changes in behaviour and general
1257 appearance that are not included in traditional categorical scoring systems such as the WCHSC
1258 (McGuirk and Peek, 2014; Love *et al.*, 2014). This approach aligns with how farmers assess
1259 calves in practice, integrating multiple observations into an overall judgement of health status.
1260 While this flexibility is a strength, it may also introduce subjectivity, which was reflected in the
1261 low to moderate interobserver agreement observed in Study 1. Similar challenges in observer
1262 agreement have been reported for clinical signs such as coughing and ear droop, where
1263 agreement varies depending on observer experience (Berman *et al.*, 2021a). This indicates that
1264 although the VAS has potential as an early detection tool, its practical implementation requires
1265 training and calibration of observers.

1267 The comparison with WCHSC highlights an important conceptual difference between the two
1268 approaches. While WCHSC relies on predefined categorical signs—many of which are
1269 associated with later stages of disease—the VAS allows earlier and more subtle deviations to be
1270 captured. In that way, if the goal is early treatment an alternative to WCHSC is needed.
1271 However, WCHSC was used as a pragmatic reference rather than a true gold standard, as no
1272 validated gold standard exists for early BRD detection.

1274 Rectal temperature represents a specific limitation within categorical scoring systems. The
1275 finding that healthy calves may have temperatures close to thresholds used in WCHSC suggests
1276 that temperature may disproportionately influence scoring outcomes. This supports the argument
1277 that reliance on single parameters may reduce the accuracy of early detection and reinforces the
1278 need for more holistic assessment approaches.

1280 The results from the activity measurement study further support the concept of early behavioural
1281 change preceding clinical detection. Reduced activity was observed 31 hours prior to VAS-

defined disease detection, indicating that behavioural deviations occur before they are captured clinically. Other studies have reported behavioural changes, including reduced feeding behaviour and activity, several days prior to clinical detection using categorical scoring systems (Duthie *et al.*, 2021; Cantor and Costa, 2022), and machine-learning approaches have demonstrated predictive potential up to four days before diagnosis (Casella *et al.*, 2023). The shorter detection window observed in this study may be explained by the earlier clinical detection threshold applied using the VAS, highlighting that the apparent performance of detection tools is strongly influenced by the chosen case definition.

The analysis of APPs showed no association with recurrent BRD events and generally low concentrations at the time of sampling. This likely reflects that calves were sampled early in the disease process, before a SAA and Hp response could be measured. However, measurement of other APPs or cytokines may have been more sensitive to the earliest disease stages. Measuring the known bovine APPs enabled comparison to other studies of BRD in calves. The kinetics of APP responses support this interpretation, as Hp typically peaks several days after the onset of inflammation, while SAA shows a faster but still time-dependent response (Jacobsen *et al.*, 2004; Orro *et al.*, 2011; Seppä-Lassila *et al.*, 2013). Studies investigating clinically affected calves have reported significantly higher levels of these proteins in more advanced disease stages (Akgul *et al.*, 2019). Together, these findings suggest that SAA and Hp are not suitable as stand-alone tools for early detection of mild or preclinical BRD under field conditions.

To further explore the potential implications of early detection, a longitudinal follow-up of the study cohort was conducted. A follow-up analysis of the calves included in this thesis did not indicate clear differences in long-term production outcomes between animals classified as BRD cases and controls during the preweaning period. Although control calves tended to remain longer in the herd (median 1077 days) compared with BRD cases (median 859 days), the distributions overlapped substantially. Similarly, only minor differences were observed in age at first calving, with BRD cases reaching a median age of 659 days compared with 682 days in controls, and both groups having a median of one calving. Exit outcomes were also comparable, with slaughter representing the most frequent outcome in both groups (46/73 cases and 35/53 controls), and similar distributions observed for death, export, and animals remaining in the herd. These findings suggest that the BRD cases identified in the present study were likely detected at an early or mild stage of disease, where progression and long-term impact may have been

1315 limited. This interpretation is consistent with the overall study design, where detection methods
1316 were aimed at identifying preclinical or early clinical deviations rather than severe disease. In
1317 contrast to studies reporting negative long-term consequences of BRD, the present results
1318 indicate that early detection and management may mitigate such effects. However, the
1319 descriptive nature of the analysis, the relatively small sample size, and the single-herd design
1320 should be considered when interpreting these findings.

1322 From a practical perspective, the findings support a combined detection strategy. Activity
1323 measurements provide continuous, objective data and may serve as an early warning system,
1324 particularly in larger herds where individual observation is limited. Clinical assessment, whether
1325 through VAS or categorical scoring, remains essential for confirming disease and guiding
1326 treatment decisions. Common bovine APP's in their current form, appear to have limited value
1327 for early detection but may still have relevance in other contexts such as prognosis or disease
1328 severity evaluation.

1330 Several practical considerations emerged during the study. The installation of ear-tag activity
1331 devices introduced minor welfare concerns, including wounds and tag loss, which may have
1332 influenced calf behaviour and appearance. Although not accounted for in the analysis, this
1333 highlights the importance of correct implementation of sensor technologies in field conditions.

1335 Overall, the results demonstrate that no single tool provides a complete solution for early BRD
1336 detection. Instead, the findings support a systems-based approach, where different tools
1337 contribute complementary information at different stages of disease progression. Early detection
1338 of BRD is therefore likely to depend on integrating behavioural monitoring with clinical
1339 assessment, supported by appropriate training and calibration of personnel.

1340 **7. Conclusions and Perspectives**

1341 This PhD thesis investigated three complementary approaches for detecting BRD in preweaned
1342 dairy calves: clinical assessment using a VAS and automated activity measurements using ear-
1343 tag-based sensors were assessed regarding their potential for detection in the preclinical stages of
1344 BRD, and biomarker analysis of two APP's were assessed regarding their prognostic value with
1345 respect to recurrence of BRD.

1346
1347 The findings demonstrate that these approaches do not all provide overlapping measures of BRD
1348 but rather capture different stages of the disease process. Activity measurements identified
1349 behavioural changes up to 31 hours prior to clinical detection, suggesting a potential for early
1350 identification of disease-related deviations in calf activity level already in the sub- and
1351 preclinical stages. The VAS proved to be a practical clinical tool capable of detecting early or
1352 mild clinical signs, i.e. the preclinical stage, although its application was associated with
1353 moderate to low interobserver reliability. This highlights the importance of training and
1354 calibrating observers when implementing such approaches in practice. In contrast, the evaluated
1355 APPs (SAA and Hp) showed limited value for predicting recurrent BRD events under the
1356 conditions of this study.

1357
1358 Taken together, these findings support an adaptive approach for monitoring of signs of BRD
1359 where different tools contribute complementary information at different stages of disease
1360 development. No single method was sufficient as a stand-alone solution for early detection.
1361 Instead, the combination of automated activity measurements and structured clinical assessment
1362 of preweaned calves appears to offer a practical and effective strategy under field conditions.

1363
1364 A follow-up of the study cohort, conducted approximately 5.5 years after the initial data
1365 collection, did not indicate clear differences in survival, exit patterns, or reproductive
1366 performance between calves classified as BRD cases and controls. Although control calves
1367 tended to remain slightly longer in the herd, the overall distributions overlapped substantially,
1368 and similar patterns were observed for age at first calving, number of calvings, and exit status.
1369 These findings suggest that the BRD cases identified in this study were likely detected and
1370 treated at an early stage of disease, limiting progression and long-term health and performance
1371 impacts.

1372 In conclusion, this thesis contributes to the understanding of how different BRD detection tools
1373 can be applied in relation to the progression of the disease. The results indicate that integrating
1374 activity measurements with clinical assessment may improve the timeliness and precision of
1375 disease detection in dairy calves and support more targeted and responsible treatment strategies
1376 in the future, while early-stage detection allowing for improved care and targeted treatment may
1377 limit the long-term impact of BRD under practical field conditions.

1378
1379 As highlighted throughout this thesis, a validated gold standard for detecting BRD in its early
1380 stages is still lacking. The findings presented here suggest that different detection tools capture
1381 different stages of disease progression, and future research should therefore focus on integrating
1382 and optimising these tools rather than seeking a single standalone solution. Further work is
1383 needed to refine visual clinical assessment methods so that approaches such as the VAS can be
1384 implemented reliably in routine on-farm practice.

1385
1386 In addition, the results indicate that currently available biomarkers, such as APPs, have limited
1387 utility for detecting early or mild disease under field conditions. Future research should therefore
1388 focus on identifying biomarkers that respond earlier in the disease process or on combining
1389 biomarker data with clinical and behavioural observations. Development of rapid, on-farm
1390 diagnostic tests based on such markers could strengthen decision-making in the field, if they are
1391 aligned with clinically relevant stages of disease.

1392
1393 The activity measurements support the potential of automated systems to detect early deviations
1394 in calf behaviour. However, further validation of electronic surveillance systems under field
1395 conditions is required before they can be widely implemented as reliable disease detection tools.
1396 Future studies should focus not only on improving detection algorithms, but also on
1397 understanding how these systems can be integrated with clinical decision-making processes on
1398 farms.

1399
1400 Increasing demands on the dairy sector to reduce antimicrobial use further emphasise the
1401 importance of early and precise disease detection. If cases of BRD can be identified at an early
1402 stage, it may be possible to implement treatment strategies based primarily on NSAIDs, thereby
1403 reducing the need for antimicrobial interventions.

1404 However, such treatment protocols require validation through controlled clinical studies,
1405 including evaluation of long-term outcomes for calf health and productivity.
1406 As demonstrated in this thesis, clinical assessment remains a central component of disease
1407 detection, but its effectiveness is highly dependent on observer experience and training.
1408 Standardisation of clinical evaluation and continuous training of farm personnel are therefore
1409 essential. Practising veterinarians could play a more active role in this process by providing
1410 structured training in clinical assessment and treatment decision-making, particularly in herds
1411 where multiple individuals are responsible for animal care.
1412
1413 Future dairy production systems are likely to become increasingly diverse, with some farms
1414 intensifying production and others moving towards more extensive and welfare-oriented
1415 systems. These developments will place different demands on disease detection strategies. In
1416 larger herds with fewer staff per animal, automated surveillance systems and artificial
1417 intelligence are likely to play a central role. In such systems, activity measurements and other
1418 sensor-based data may contribute not only to individual disease detection but also to broader
1419 herd health management, supporting data-driven decision-making in collaboration between
1420 farmers, veterinarians, and advisors.
1421
1422 However, the successful implementation of these technologies depends on the ability of
1423 stakeholders to interpret and act on the generated data. Without adequate training and clear
1424 decision-support frameworks, the value of electronic surveillance systems may be limited.
1425 Therefore, future work should also focus on how to integrate technological solutions with human
1426 expertise, ensuring that both clinical skills and digital tools are aligned.
1427
1428 Overall, this thesis contributes to a more nuanced understanding of BRD detection by
1429 demonstrating that early identification of disease is best approached through a combination of
1430 behavioural, clinical, and, potentially, physiological indicators. Continued development and
1431 integration of these approaches may improve the precision of BRD detection in dairy calves and
1432 contribute to reducing antimicrobial use in the future.

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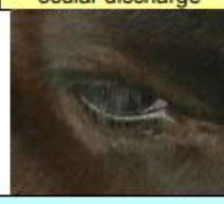
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1899 **Appendix**

1900 **Appendix I:**

1901 The WCHSC protocol used for the clinical scoring of all calves during the study period. Rectal
1902 temperature was collected as degrees Celsius in our dataset.

Calf Health Scoring Criteria			
0	1	2	3
Rectal temperature			
100-100.9	101-101.9	102-102.9	≥103
Cough			
None	Induce single cough	Induced repeated coughs or occasional spontaneous cough	Repeated spontaneous coughs
Nasal discharge			
Normal serous discharge	Small amount of unilateral cloudy discharge	Bilateral, cloudy or excessive mucus discharge	Copious bilateral mucopurulent discharge
			
Eye scores			
Normal	Small amount of ocular discharge	Moderate amount of bilateral discharge	Heavy ocular discharge
			
Ear scores			
Normal	Ear flick or head shake	Slight unilateral droop	Head tilt or bilateral droop
			
Fecal scores			
Normal	Semi-formed, pasty	Loose, but stays on top of bedding	Watery, sifts through bedding
			

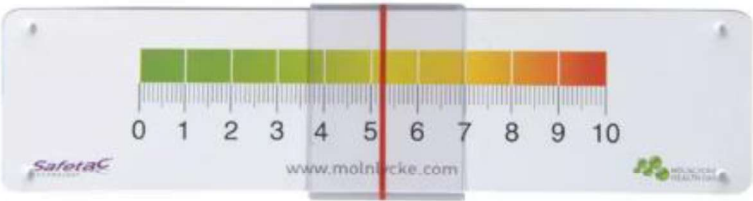
http://www.vetmed.wisc.edu/dms/fapm/fapmtools/8calf/calf_health_scoring_chart.pdf

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1905 **Appendix II**

1906 Visual Analogue Scale ruler, used as an add-on tool in the VAS scoring procedure.



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1909 **Appendix III**

1910 Photographs taken by the research group during the data collection period.

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1912 Blood sampling of calves



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1920 Outdoor single igloo hutches



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1923 Indoor group pens



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Paper I

Comparison and interobserver reliability between a visual analogue scale and the Wisconsin Calf Health Scoring Chart for detection of respiratory disease in dairy calves

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Comparison and interobserver reliability between a visual analog scale and the Wisconsin Calf Health Scoring Chart for detection of respiratory disease in dairy calves

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ABSTRACT

Respiratory disease is an ongoing challenge for calves in the dairy sector with a relatively high prevalence and impact on welfare and economics. Applying scoring protocols for detecting respiratory disease requires that they are easily implemented, consistent between observers and fast to use in daily management. This study was conducted in one Danish dairy farm from September 2020 through January 2021. The study included 126 heifer calves enrolled in the age of 17 to 24 d. All calves were observed every second day for a period of 46 d. At each visit all calves were scored with a new visual analog scale (VAS) and the Wisconsin Calf Health Scoring Chart (WCHSC). We calculated agreement between the 2 scoring systems based on conditional probability to score higher or lower than a cutoff in the VAS compared with a specified cutoff in WCHSC used as reference test. A generalized mixed effects regression model was developed to estimate the prevalence of respiratory disease and the overall agreement between the 2 scoring systems. The overall agreement between the VAS and WCHSC was 89.6%. The second part of the study assessed interobserver reliability between 2 experienced observers and between an experienced observer and veterinary students. The interobserver reliability was calculated by intraclass correlation coefficient and was 0.58 between experienced observers and was 0.34 between an experienced observer and veterinary students indicating a moderate to poor reliability between the observers. It was possible to use VAS as an alternative clinical scoring method, which primarily focuses on the general condition of the individual calf rather than specific categories of clinical signs. Our study set up lacked a comparison to other diagnostic tools i.e., thoracic ultrasound to confirm the

findings which should be considered in future studies when exploring VAS as a screening tool for detection of respiratory disease in dairy calves.

Key words: visual analog scale, Wisconsin Calf Health Scoring Chart, respiratory disease, interobserver reliability

INTRODUCTION

Respiratory disease is an ongoing challenge in dairy calves worldwide and affects more than 18% of pre-weaning dairy replacement heifers with estimated economic losses ranging between \$250 and \$280 per heifer (Cummings et al., 2022). Respiratory disease accounts for up to 61% of antimicrobial treatments in calves in the United States (Zhang et al., 2022), which makes it a target disease syndrome with a potential to reduce antimicrobial use. Detection and quantification of respiratory disease using standardized methods are needed so only calves in need of it, are given treatment.

According to McGuirk and Peek (2014), detection of respiratory disease is often based on pattern recognition performed by farm staff with different prior experiences. Poor standardization of the diagnostic methods can lead to imprecise estimates of disease occurrence on farm level and risk of treatment failures at the individual level. Failure to correctly diagnose respiratory disease can impair animal welfare and lead to unjustified use of antimicrobials.

The Wisconsin Calf Health Scoring Chart (WCHSC; McGuirk and Peek, 2014) is used in research of respiratory disease in calves (Buczinski et al., 2014; Mahendran et al., 2017; Lowie et al., 2022). The WCHSC consists of clinical observations and grades coughing, nasal discharge, eye and ear constitution, and rectal temperature, which is then aggregated on the individual calf. The WCHSC cutoff at ≥ 5 was developed based on paired results with broncho-alveolar fluid cytology and validated by culture in the work by McGuirk (2008), but no diagnostic performance was provided in that

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study. Recent studies calculated sensitivity (**Se**) of 30% and specificity (**Sp**) of 99% (Berman et al., 2022), and Se of 78% and Sp of 82% (Decaris et al., 2022) by Bayesian techniques, based on clinical examination, thoracic ultrasonography (**TUS**), and serum haptoglobin concentrations in veal calf respiratory scoring systems.

McGuirk and Peek (2014) estimated the additional labor associated with implementing the use of the WCHSC in full scale in the range of 3 to 4 h/d for each 100 calves. This level of time consumption can be a major barrier for implementation in commercial dairy farms. Therefore, there is a need to look for and evaluate other protocols that focus on other clinical aspects that can more easily be observed and aggregated simultaneously.

Several other relevant behavioral and clinical signs exist, which are not directly incorporated in the WCHSC. Calves are less active and consume less milk at presence of respiratory disease (Swartz et al., 2017), and behavioral changes such as abnormal posture when standing or lying, individual isolation, lethargy, reduced grooming and feeding time, fewer visits to automated feeding stations and fewer social interactions are found to appear earlier than more noticeable signs of respiratory disease such as nasal and eye discharge (Cramer et al., 2016; Hixson et al., 2018; Duthie et al., 2021). Love et al. (2016) addressed the possibility to include screening protocols to detect calves that need subsequent examination to make a precise diagnosis of respiratory disease with other tools. This could potentially lead to a more widespread on-farm practical tool in the management and handling of respiratory disease cases.

The visual analog scale (**VAS**) score is a method developed to assess the severity of pain using several behavioral signs, including alertness. It is defined as a continuous scale between 0 and 100 mm, where 0 is no pain and 100 mm is severe pain (Martin et al., 2020). The VAS has been reported as a valuable tool to assess pain in calves experimentally infected with *Mannheimia haemolytica* (Martin et al., 2022).

A requirement for a feasible health scoring chart is good inter-rater reliability and practical implementation, preferably with limited prior expertise or training. Buczinski et al. (2016) reported a slight to fair agreement with kappa ranging from 0.06 to 0.35 at different cutoffs between relatively unexperienced observers using the WCHSC for detection of respiratory disease. Berman et al. (2020) reported WCHSC agreement by kappa ranging between 0.2 and 0.8 based on different professional backgrounds of the observers and by agreement between the individual clinical signs that appear

in cases of respiratory disease. Inter-rater reliability of the VAS score has been described for other conditions in other animal species including elbow osteoarthritis in dogs with an intraclass correlation coefficient (**ICC**) >0.9 (Aulakh et al., 2020), and in qualitative behavior assessment of housed sheep (Diaz-Lundahl et al., 2019), which reported a Kendall coefficient of 0.45 to 0.91.

We hypothesized that it was possible to use VAS as an alternative system to the WCHSC for diagnosing signs of respiratory disease in dairy calves. The first objective was to determine the level of agreement of the VAS compared with the WCHSC as reference test to detect signs of respiratory disease. The second objective was to estimate interobserver reliability between 2 experienced observers in calf health; and between an experienced observer in calf health and a group of veterinary students using the VAS and the WCHSC to detect signs of respiratory disease.

MATERIALS AND METHODS

The study protocol was reviewed and accepted by the Local Ethical Committee at Department of Veterinary Clinical Sciences, University of Copenhagen (project no. 2020–023). A written consent from the farm owner to conduct the study was obtained before the start of the study. The veterinary students were informed that the data collected would be included in a research project.

Study Population

The study was performed in a commercial Danish dairy farm with approximately 650 dairy cows. The breeds were Danish Holstein (90%) and Red Danish Holstein (10%). Only heifer calves were enrolled in the study because bull calves were moved from the farm for veal production around 2 wk of age. The calves were housed in individual outdoor hutches after birth and subsequently at 35 to 40 d of age moved either to outdoor group hutches or indoor group pens with pen sizes of 6 to 14 calves. Within a week after moving to group housing, all calves were dehorned and received a *Trichophyton* spp. (ringworm) vaccination. The calves were not vaccinated against pathogens associated with respiratory disease.

Study Design and Data Collection

The study was carried out from September 2020 through January 2021 as an observational study following a cohort of dairy calves. Visits to the farm took place every second day leading to 76 visits in total dur-

Table 1. Overview of the number of calves, observers, and observations used for the analysis in this study

Study	Study period	No. of calves	No. of observers	No. of observations
WCHSC-VAS agreement ¹	Sep 2020 to Jan 2021	126	2	3,011
Interobserver reliability, experienced observers	Nov 2020	53	2	106
Interobserver reliability, veterinary students	Nov 2020	47	19	224

¹WCHSC = Wisconsin Calf Health Scoring Chart; VAS = visual analog scale.

ing the study period. The first author of this paper carried out 71 of the visits and the last author 5. The calves were enrolled between 17 and 24 d of age and assessed by the observers for development of signs of respiratory disease every second day for 46 consecutive days (6 wk). Every calf was scored 24 times in total using both the VAS and the WCHSC. Farm visits were done between 0900 and 1600 h between feedings to limit disturbances to the daily routines in the farm. All calves were first scored using the VAS system, and then all calves were scored using the WCHSC (see the Clinical Scoring of Signs of Respiratory Disease section for a detailed description).

New calves were enrolled once weekly during the study until 6 wk before the end of the study period. The 2 observers were veterinarians with substantial training in clinical research. Data for interobserver reliability evaluation between first and last author of this paper was collected on November 13, 2020.

Data from 4 days in November 2020 (November 17, 19, 23, and 25) were used for interobserver reliability between the first author and 19 veterinary students. The veterinary students were introduced to the VAS and the WCHSC by 10-min verbal instructions in the calf-rearing area in the farm, and all veterinary students got a handout template based on the work done by McGuirk and Peek (2014). Table 1 provides an overview of the number of enrolled calves, observers, and observations.

Clinical Scoring of Signs of Respiratory Disease

VAS Score Procedure. The VAS score given to each calf at each farm visit was a score that corresponded to the general clinical appearance in the moment the calf was observed. The score was given by observing the calf from a short distance (~1 to 2 m) without touching or being in physical contact with the calf and with no other interaction and intervention than the presence of the observer.

The VAS score was noted in the range between 0 and 10 cm with the possibility to use 0.1 increments and was given within approximately 10 s of observation. Based on the clinical assessment, the VAS score is decided with support from the anchor points described in Table 2. If the calf lacks some of the described clinical findings but has others within the scale anchor points, then the calf will remain in the particular part of the VAS scale but might be placed in the lower or higher end of the scale depending on the severity of the clinical findings. The description of the VAS anchor points along the scale used in this study can be seen in Table 2 and is defined as points anchored at 0, 2.5, 5, 7.5, and 10 cm at the continuous scale.

WCHSC Procedure. The WCHSC was used at each visit according to the method developed and described by McGuirk and Peek (2014). The clinical scores were given in the following order: nasal discharge score, eye score, ear score, and rectal temperature. Coughing was

Table 2. Definitions of visual analog scale (VAS) score anchor points along the numeric axis between 0 and 10 cm and grouped into the following 4 intervals on the numeric scale from 0 to 10 cm

VAS score interval	Definition
0.0; 2.4	The calf is interested in its surroundings, is very bright and alert, and has clear eyes; the ears are pointing in an upright position, and the head is held above spine level if the calf is standing.
2.5; 4.9	The calf is less interested in the surroundings, slightly depressed, less bright, alert, and responsive and has less clear eyes; the calf uses longer time to get up, the hair layer is not intact, or the ears are drooping.
5.0; 7.4	A depressed calf with obvious clinical signs of disease, including nasal or eye discharge, coughing, and drooping ears, or abdominal respiration in combination with increased frequency and head position below spine level.
7.5; 10.0	A significantly depressed or apathetic calf, unwilling to stand up, or lying in lateral recumbency and appearing very ill or terminally ill, often assessed upon the signs obtained in the lower score intervals.

assessed as either spontaneous or induced by manual laryngeal or pharyngeal provocation during the measurement of rectal temperature. The fecal score was not noted in this study. Each clinical score was noted as a whole number between 0 and 3. A calf that had a total WCHSC score of 3 could, for example, be a calf with unilateral cloudy nasal discharge, a single induced cough, and ear flick or head shake. Scores ≥ 5 could be a calf with bilateral, cloudy, or excessive mucus discharge, moderate bilateral amount of ocular discharge and single induced cough even if the calf were jumping around and seemed healthy. Using the WCHSC protocol, it is recommended that a calf with a total score ≥ 5 or that has 2 or more clinical parameters with a score of 2 or 3 be considered to have respiratory disease (McGuirk and Peek, 2014).

Statistical Analysis

Data management and analyses were conducted in the statistical platform R version 4.2.3 (R Core Team, 2022) and RStudio version 2023.03.0+386 (Rstudio Team, 2022). All variables were considered statistically significant at a level of $P < 0.05$.

The WCHSC score was divided into 2 groups to describe high (≥ 5) versus low (< 5) probability that the calf had respiratory disease as described by McGuirk and Peek (2014). The level of agreement was defined as the estimated probability of the VAS correctly classifying the low versus high probability of respiratory disease based on the WCHSC. Because observations of the VAS and WCHSC were repeated on the individual calf, this was adjusted for in a generalized mixed effects regression model, with calf as a random effect. Initially, an empty model with the dichotomized WCHSC scores as outcome variable and the random intercept was created to provide an unadjusted prevalence estimate of the calves having high probability of respiratory disease based on the WCHSC. Subsequently, the generalized mixed model analysis was repeated multiple times with a dichotomized VAS score (**dVAS**) as explanatory variable for each of the possible cutoff of the VAS score. Based on these models the conditional probabilities $p(\text{WCHSC} \geq 5 \mid \text{dVAS} = 1)$ and $p(\text{WCHSC} < 5 \mid \text{dVAS} = 0)$ were predicted for each cutoff of the VAS score. Based upon the prevalence estimate and the 2 conditional probabilities the agreement could be estimated solving 2 equations with 2 unknowns. The level of agreement was then estimated for all possible cutoff values of VAS score and the cutoff with the best possible agreement was identified.

The autocorrelation functions of the residuals were used to evaluate if the calf random effect was suffi-

ciently accounting for correlation in the repeated measurements. The parameter estimates of the calf random effects were also evaluated between the different models to assess model stability. Model control was done by assessing simulated scaled residuals from the DHARMA-package (Hartig, 2022). The generalized mixed effects regression model was analyzed using the glmer-function from the lme4-package (Bates et al., 2015) and the prediction of the conditional probabilities and prevalence was done with the emmeans-package (Lenth, 2022).

To compare the difference in mean values of VAS scored by both the experienced observers and by the author and veterinary students, a 2-sample paired t -test was performed.

The ICC was used to estimate the interobserver reliability in use of VAS for clinical scoring of the calves. The ICC was calculated and interpreted based on the work and descriptions by Koo and Li (2016). The ICC estimates and their 95% confidence intervals were calculated in the irr-package (Gamer et al., 2019) and the psych-package (Revelle, 2022) by a 2-way random effects model based on a specific rating by different observers and absolute agreement, because we wanted to assess the agreement in VAS scores from specific calves scored by a fixed set of observers. The calculations were based on a single unit level, meaning that the VAS score was compared equally between all observers participating in the reliability study.

RESULTS

Comparison of VAS and WCHSC

A total of 126 calves were enrolled in the study. This resulted in a total of 3,011 calf observations by 24 observations per calf throughout the study period. Details of the results can be seen in Table 3. Based on the WCHSC, there were 380 observations with a high probability of respiratory disease and 2,631 observations with a low probability of respiratory disease. In the low probability group, the VAS score had a mean of 0.9 (median of 0.7) and ranged between 0.1 and 6.4 whereas the mean VAS score was 1.8 (median of 1.3) and ranged between 0.2 and 7.5 in the high probability group. The means of the VAS score were significantly different in the high and low probability groups and calculated by a 2-sided paired t -test ($P < 0.0001$). The prevalence of high probability of respiratory disease across all observations, but adjusted for the repeated observations on the same calves in the generalized mixed effects regression model, was 0.125 (95% CI: 0.106, 0.146). The highest accuracy between a dichotomized VAS score and the high versus low probability of respiratory disease

Table 3. Distributions of visual analog scale (VAS) scores for each of 2 outcomes of the reference test Wisconsin Calf Health Scoring Chart (WCHSC) scores dichotomized into low or high probability of respiratory disease being present in the calf at cutoff of ≥ 5 ¹

Item	Parameter	Category	Observations	Min.	25% quartile	Median	Mean	75% quartile	Max.
Comparison study	WCHSC score < 5.0	VAS	2,631	0.1	0.5	0.7	0.9	1.0	6.4
	WCHSC score ≥ 5.0	VAS	380	0.2	0.8	1.3	1.8	2.6	7.5
	First author	VAS	53	0.3	0.3	0.8	1.1	2.8	5.3
	Last author	VAS	53	0.2	0.3	1.0	1.1	2.7	2.8
	Observer A	VAS	224	0.2	0.5	0.7	0.8	0.9	2.7
Agreement, veterinary students	Veterinary students	VAS	224	0.0	0.0	1.7	1.8	2.5	6.8

¹Distributions of VAS scores are described for 3 different parts of the study: (1) comparison study of VAS and WCHSC including all calves observed primarily by the first author; (2) interobserver reliability between 2 experienced observers; and (3) interobserver reliability between observer A and a group of veterinary students. Distributions are given by number of observations, minimum (Min.), 25% quartile, median, 75% quartile, maximum (Max.), and mean distribution of VAS scores by each category of dichotomized WCHSC scores.

based on the dichotomized WCHSC scores was 0.896 at a VAS score cutoff ≥ 2.8 . At this cutoff the conditional probability of $p(\text{high VAS} | \text{WCHSC} \geq 5)$ and $p(\text{low VAS} | \text{WCHSC} < 5)$ were 0.267 and 0.984.

Interobserver Reliability in Using the VAS Score

Two experienced observers, A and B, observed the same 53 calves on the same day. Details can be seen in Table 3. Observer A had a mean of VAS score of 1.1, which was equal to observer B's mean VAS score of 1.1. The mean difference in VAS score between observer A and observer B was nonsignificant ($t_{\text{paired}} = -0.54$, $P = 0.59$). The ICC between observers A and B was 0.58 (95% CI: 0.37, 0.73), which indicated poor to moderate reliability between observers according to Liljequist et al. (2019).

Observer A and 19 veterinary students observed the same 47 calves on the same day, which gave a total of 224 calf observations. Observer A had VAS scores ranging between 0.2 and 2.7 with a mean of 0.8 (median = 0.7). The veterinary student group had VAS scores ranging from 0.0 to 6.8 with a mean of 1.8 (median = 1.7). The veterinary students had significant higher VAS scores than observer A ($t_{\text{paired}} = 13.04$, $P < 0.0001$). The ICC between observer A and the veterinary students was 0.34 (95% CI: 0.26, 0.44), which indicated a poor reliability between the observers (Liljequist et al., 2019).

DISCUSSION

We investigated the use of VAS to evaluate signs of respiratory disease in dairy calves by comparing it to the WCHSC as reference test. The WCHSC is a well-established clinical scoring system that has been used for quantifying clinical signs of respiratory disease in calves.

The first part of the study focused on comparing 2 methods that based the assessment of the health or disease status of a calf by 2 different approaches. We found a good overall agreement between the 2 methods, which were estimated to agree approximately 90% of the time, when dichotomized into high and low disease probability, at a cutoff of ≥ 2.8 in VAS and ≥ 5 in WCHSC, respectively.

The WCHSC is based on specific clinical signs that are considered obvious to perceive when detecting respiratory disease in calves, but it takes longer time to perform, requires handling and to some extent restraining of the calves. The VAS system requires observation of the calves from a distance but does not assess clinical signs the same way, as WCHSC does. The VAS assesses the disease status of the calf by behavioral changes and changed appearance and focus less on specific clinical

signs. If VAS was considered as a clinical screening tool in this study, it might have been appropriate to pay more attention to the specific clinical signs and assess whether it was respiratory disease or other organ system diseases involved as the cause of a higher VAS score. A concept of doing follow-up diagnostics to confirm the clinical findings as serial testing could be further analyzed before and after the data collection took place. This could have improved the quality of the VAS system by using, for example, the Fagan nomogram (Fagan, 1975), but such an analysis was not performed in this study. When focusing on specific clinical signs in the calf, the observer might not emphasize on behavioral changes because the primary focus is on the specific clinical signs of disease. A major limitation in our study was that the same single observer both scored the VAS and the WCHSC with only a short time between, which could be an observer bias in the data. Martin et al. (2020) stated that the VAS score was a mark on a 100 mm line, and the distance from the mark to the ends of the line were measured manually with a ruler, which indicated the clinical score at the specific point on the line. In our study, we noted the specific number on the 10-cm scale without using a physical drawn line and noted the score with 0.1 increments. It is not clear whether this difference in how to perform the score introduced any unintended biases to our results.

It is not clear whether the clinical signs obtained in the WCHSC or in the VAS truly reflect lesions in the lungs, which are an important parameter of respiratory disease in calves. Leruste et al. (2012) found that a high proportion of lungs with moderate or severe lesions detected by postmortem examination, could not be accurately detected by a protocol assessing clinical signs of respiratory disease alone (AUC = 0.77). Therefore, it could be a possibility that the signs upon which we based our analyses mostly reflect upper respiratory disease signs.

In this study, neither the WCHSC nor the VAS scoring system can be considered a perfect test, and therefore an appropriate gold standard test, for respiratory disease detection in calves. For a more precise diagnosis, the clinical signs could have been followed up by TUS, which could have added value as an alternative reference test for VAS instead of using the WCHSC even though TUS neither is a perfect gold standard test. Reported performances of TUS ranging from 82% to 99% for Se and from 81% to 95% for Sp (Amrine et al., 2013). Cuevas-Gómez et al. (2021) found that 61% of calves had lung lesions up to 10.5 d before a clinical respiratory disease diagnosis which further emphasizes that the clinical diagnosis is lacking diagnostic quality without other diagnostics to support

the diagnosis. Cramer et al. (2019) concluded that only 23% of clinical cases of respiratory disease showed signs of depression compared with controls and the authors claimed that many cases of respiratory disease will go undetected unless an additional screening tool is used. This is an argument for why clinical scoring primarily may act as a screening tool in the recommendation for on-farm usage. Love et al. (2014) developed an alternative clinical scoring protocol to detect respiratory disease based on the WCHSC and included nasal swab quantitative PCR viral pathogen detection as a follow-up diagnosis of the clinical diagnosis; even though that pathogen detection from nasal swabs is also of limited value because pathogens can act as commensals in some calves but cause disease in others (Angen et al., 2009; Klompmaaker et al., 2021).

Despite the 2 scoring systems using different approaches in identifying diseased calves, they agree overall about the disease decision in the calves when the VAS cutoff was 2.8. The conditional probability was 0.267 of $p(\text{high VAS} \mid \text{high WCHSC})$ and 0.984 of $p(\text{low VAS} \mid \text{low WCHSC})$, and thereby the agreement between the 2 methods were highest when WCHSC was below the specified cutoff. There was more disagreement when the WCHSC was high, and this also emphasizes the difference in nature of the 2 different scoring systems. The WCHSC protocol is one among several protocols that all categorize specific clinical signs into a general clinical score that are guiding decisions in treatment of respiratory disease (Love et al., 2014; McGuirk and Peek, 2014; Berman et al., 2022). The VAS might be a tool to use for detection of respiratory disease cases that hypothetically can be initially treated with NSAIDs. Only few studies have evaluated this concept and found varying effect (Carvallo Chaigneau et al., 2021; Mahendran et al., 2020), but it is a very interesting way of trying to reduce the amount of antimicrobials used for treatment of respiratory disease in calves.

In the second part of the study, we found a poor to moderate reliability between experienced observers in calf health and between an experienced observer and veterinary students (ICC = 0.58 and 0.34, respectively) in using the VAS for detecting signs of respiratory disease. Berman et al. (2020) reported that clinical signs were not detected equally between veterinarians, technicians, and producers, which we also found in our reliability assessment. Few studies have evaluated the inter-rater reliability of using VAS in calves. A behavioral study of calves with focus on specific temperament patterns, found ICC in VAS between observers ranging between 0.12 and 0.76 (Vogt et al., 2017). A study reported ICC ranging between 0.52 and 0.70 when analyzing reliabilities of raters by using TUS in

calves with and without respiratory disease (Buczinski et al., 2018). Overall, the ICC in this and other studies indicate that it can be difficult to achieve general high levels of reliabilities when assessing animal health and disease status or behavioral expressions by continuous measuring scales. Stakeholders in calf health (e.g., veterinarians, calf caretakers, farm managers, technicians, producers and consultants) shall be aware of how differences in reliability in clinical scoring can have an effect on decision-making outcomes based on observations of calves by different people. Also, it is important that the people that are involved in clinical scoring screenings receive a thorough introduction and education within the field to improve the reliability and the quality in the scoring.

More studies are needed to validate clinical scoring as a valuable tool to detect respiratory disease, including a more precise definition of the VAS so the reliability between observers can be optimized. Continuous scales might already be of high quality when doing intraobserver reliability and must be further analyzed in future studies.

CONCLUSIONS

We used a VAS to evaluate signs of respiratory disease in dairy calves and compared it to the WCHSC. We showed that it might be possible to implement another scoring protocol to detect clinical signs of respiratory disease in calves because of a relatively high level of agreement between the 2 scoring systems. As also found in previous studies, there was poor to moderate interobserver reliability in the VAS of calves with signs of respiratory disease between experienced observers and less experienced observers in calf health. This calls for better training and calibration of users of the different disease scoring systems. Further studies to define outcome-based cutoffs in using VAS for the desired purposes are needed. We also recommend making a follow-up diagnosis with other respiratory disease diagnostic tools to verify the diagnosis after using a clinical scoring protocol, such as VAS, to screen for respiratory disease in dairy calves.

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Paper II

Original Article

Reduced activity measured by ear-tag sensors indicate early signs of bovine respiratory disease 2-3 days prior to clinical onset in preweaned dairy calves

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Under preparation

Abstract

Bovine respiratory disease (BRD) remains a major health challenge in preweaned dairy calves, and methods to identify early behavioural changes are needed to support timely intervention and improve welfare outcomes. This longitudinal observational case-control study examined whether activity levels differ between BRD cases and healthy age-matched controls, including how these differences evolve prior to clinical detection. A total of 107 calves were enrolled at 17–24 days of age and monitored for 46 days with automated ear-tag activity sensors, while clinical examinations using a Visual Analogue Scale (VAS) were performed every 48 hours. BRD was defined as VAS $\geq$ 2.5, and calves that remained below this threshold throughout the study were considered healthy controls. Hourly activity data were analysed relative to the time of BRD detection, and mixed-effects linear modelling assessed the effects of BRD status, age, and time relative to detection, on the activity level of the calves. Post-hoc comparisons using estimated marginal means revealed that activity in BRD cases was significantly lower than in controls from approximately 2 days prior to BRD with the first statistically detectable difference in activity occurring 31 hours before BRD detection. Age and the interaction between BRD status and time relative to BRD detection were significant predictors of reduced activity. The findings demonstrate significant differences in activity levels between BRD cases and healthy controls prior to clinical detection, supporting the use of automated ear-tag monitoring as an early-warning surveillance tool. Incorporating such systems into calf management may enhance early detection and enable targeted clinical assessment and interventions.

Introduction

Bovine respiratory disease (BRD) remains a major challenge in the global dairy sector, with reported prevalence in preweaned dairy calves varying widely across studies. Estimates range from approximately 2% to over 20%, depending on study design, diagnostic criteria, and population, reflecting substantial variability in disease occurrence and detection. For example, Donlon *et al.* (2023) reported a prevalence of approximately 4% at the calf level, whereas other field studies have reported higher estimates, including regional prevalences of 4–9% and herd-level prevalences exceeding 20% (Cummings *et al.*, 2022; Dubrovsky *et al.*, 2019). This variability highlights the complexity of BRD epidemiology and the challenge of consistent diagnosis in commercial herds, particularly in the early stages of disease when clinical signs may be subtle.

Automated systems have been proposed as tools to support earlier identification of disease by capturing physiological and behavioural changes. Several technologies have been evaluated for BRD detection, including thermal imaging, which has shown only moderate agreement with rectal temperature ($r = 0.32$) (Bell *et al.*, 2020), and activity measurements based on accelerometers. Studies using leg-mounted accelerometers in beef calves have reported activity-based alerts up to 10 days before visual detection (Belaid *et al.*, 2020; Wottlin *et al.*, 2021). Ear-attached accelerometers in dairy calves have identified reduced activity at specific time points prior to diagnosis, including 4 and 2 days before clinical detection (Ramezani Gardaloud *et al.*, 2022). However, these studies report differences at predefined time points rather than quantifying the earliest time at which differences become statistically detectable.

In addition, behavioural data have been incorporated into predictive models aiming to identify diseased calves at the individual level. For example, Cantor *et al.* (2022) reported that combining feeder and activity data enabled detection of BRD cases up to 6 days before clinical diagnosis, while earlier work identified changes in feeding behaviour approximately 4.5 days before clinical signs (Quimby *et al.*, 2001). Similarly, Casella *et al.* (2023) developed a model integrating clinical scoring with automated data, achieving detection of a proportion of cases several days before diagnosis. However, these studies primarily focus on predictive performance and often do not quantify when activity patterns begin to differ between diseased and healthy calves at the group level.

Although these approaches demonstrate the potential of automated measurements, their performance varies across studies and conditions, and there is limited evidence describing the timing and magnitude of activity differences between BRD cases and healthy calves using ear-tag-based measurements in preweaned dairy calves.

Therefore, the objective of this study was to examine differences in ear-tag-based activity measurements between preweaned dairy calves with BRD and healthy age-matched controls prior to clinical detection, and to quantify the timing at which these differences become statistically detectable.

97 **Materials and methods**

98 The research protocol and associated ethical considerations were reviewed and approved by the
99 Local Ethical Committee at the Department of Veterinary Clinical Sciences, University of
100 Copenhagen (project no. 2020-023). Final approval was granted by the Animal Experiments
101 Inspectorate, Ministry of Food, Agriculture and Fisheries of Denmark (case no. 2020-15-0201-
102 00677).

104 *Study population*

105 The study was conducted on the same farm and included the same cohort of heifer calves as
106 described by Møller *et al.* (2024), a conventional 650-cow dairy farm located in eastern Denmark.
107 Activity data were collected from September 2020 to January 2021. The herd consisted of Holstein
108 (90%) and Red Holstein (10%) cattle.

110 All calves were fed 4 L of colostrum as soon as possible after birth via bottle feeding. Subsequently,
111 calves were fed 3-4 L of milk replacer twice daily from a bucket and had ad libitum access to hay
112 and a calf starter grain mix. Calves were initially housed outdoor in individual hutches and, at 35-40
113 days of age, were moved to either indoor or outdoor group pens containing 6 to 14 calves per pen.
114 After the group housing period at approximately 10-12 weeks of age, all heifers were transferred to
115 a heifer barn with concrete flooring and mattress bedding, where they were housed in two groups of
116 approximately 200 animals each.

118 *Study design and enrolment criteria*

119 The study was designed as a longitudinal observational case-control study. Heifer calves were
120 enrolled at 17-24 days of age and followed for a total of 46 days, during which clinical
121 examinations and activity measurements were performed as described by Møller *et al.*, (2024). The
122 herd was visited every 48 hours throughout the study period. At any given time, a maximum of
123 approximately 50 calves were included, and enrolment was adjusted to maintain this number. When
124 more calves were available than required, individuals were randomly selected using the
125 randomisation function in Microsoft Excel. When fewer calves were available, all eligible calves
126 were enrolled.

128 *Clinical scoring and definition of a BRD event*

129

130 Calves were clinically scored using a visual analogue scale (VAS) as described by Møller *et al.*
131 (2024). The VAS was defined as a continuous scale from 0 to 10 cm, where 0 represented a
132 clinically healthy calf and 10 represented a severely diseased, apathetic calf.

133

134 A BRD event was defined as a VAS score ≥ 2.5 . Calves with scores between ≥ 2.5 and <5 were
135 characterised by reduced interest in their surroundings, mild depression, decreased alertness and
136 responsiveness, and may have exhibited mild serous or seromucous ocular or nasal discharge.
137 Additional signs included a dull hair coat and slight ear droop. Calves with VAS scores from ≥ 5.0
138 to 10 were considered clinically affected to severely clinically affected, exhibiting marked
139 depression, coughing, mucopurulent ocular and/or nasal discharge, tachypnoea, uni- or bilateral ear
140 droop, sunken eyes, impaired ability to stand, and anorexia.

141

142 Healthy control calves were defined as those with VAS scores between 0 and 2.4 at all clinical
143 assessments (a total of 26 clinical observations were performed per calf every 48 hours). These
144 calves were characterised by normal behaviour, including being active and responsive, with interest
145 in their surroundings, head carriage above spine level, bright eyes, and a smooth, shiny hair coat.
146 The ears were upright, and calves showed a rapid response to human approach. Calves receiving a
147 VAS score below 2.4 were different in behaviour but did not raise a suspicion of being diseased.

148

149 *Automated electronic ear-tag and activity data*

150 All enrolled calves were fitted with automated electronic ear tags (eSense Flex Tag, Sensehub
151 Youngstock®, SCR Engineers Ltd., Netanya, Israel) in the left ear, in addition to the standard
152 identification ear tag.

153

154 Ear tags were applied using a standard ear-tag applicator according to the manufacturer's
155 instructions. Ten minutes prior to application, 1 mL of procaine hydrochloride (Pronestestic 40
156 mg/mL, 0.036 mg/mL, Fatro, Spain) was administered subcutaneously at the intended site of tag
157 placement.

158

159 Each calf's identification number was linked to the unique ear-tag number within the Sensehub™
160 software. Ear tags were installed 10 days prior to the start of data collection to allow calibration to

161 the individual calf's baseline activity level. Data from the Sensehub™ software were extracted by the
162 manufacturer (SCR Engineers Ltd.) and provided to the authors for analysis. The complete dataset
163 included three different data outputs: A health index, rumination, and activity. Only activity was used
164 for further analysis in this work.

166 *Statistical analysis*

167 Data handling and processing were performed in R version 4.5.2 (R Core Team, 2022). Activity
168 data obtained from the SenseHub™ software were aggregated, and summary statistics (median, 25th
169 and 75th percentiles) were calculated. Age (in days) of the calf at the time of clinical BRD detection
170 was also recorded.

171
172 A time variable relative to the clinical event defined as a VAS score ≥ 2.5 was created to evaluate
173 activity prior to BRD detection and to enable comparison between affected calves and matched
174 healthy controls. The time point at which a calf reached a VAS score ≥ 2.5 was defined as time 0.
175 Activity data were analysed from 240 hours before to 96 hours after detection. Observations with
176 missing values or activity equal to zero were excluded prior to analysis. Age-matched calves were
177 categorised into two groups (cases and controls), which were included as a binary explanatory
178 variable in subsequent analyses. Mean activity for cases and controls was plotted over time to
179 visualise differences in activity patterns relative to disease detection.

180
181 A linear mixed-effects model was fitted using the lme4 package (Bates *et al.*, 2015), with activity as
182 the outcome variable. Fixed effects included BRD status (case/control), time relative to clinical
183 detection, and their interaction, as well as age (in days). Calf identity was included as a random
184 effect to account for repeated measurements within individuals. To facilitate interpretation of time-
185 specific differences, time relative to detection was additionally categorised into whole-day intervals.
186 Estimated marginal means were calculated for each group at each day, and post-hoc comparisons
187 between cases and controls were performed using the emmeans package (Lenth and Piaskowski,
188 2026) with false discovery rate adjustment for multiple testing. Variance components for calf-level
189 and residual variation were extracted from the fitted model, and model assumptions were assessed
190 by visual inspection of residual plots.

191

192 Activity was analysed at the level of individual time observations, with each calf contributing
193 repeated measurements over time. Data was recorded at an hourly resolution, resulting in up to 24
194 observations per calf per day. All available observations within the defined time window were
195 included in the analysis, rather than aggregated to daily means. In total, 107 calves contributed to
196 the analysis, with an average of 389 observations of both clinical and activity measures per calf,
197 corresponding to 41,616 observations overall after exclusion of missing values and zero activity
198 recordings.

200 To further explore the temporal resolution of group differences, separate comparisons were
201 performed at each hourly time point relative to detection using repeated t-tests, independent of the
202 mixed-effects model. The earliest significant difference was defined as the first time point at which
203 the adjusted p-value was < 0.05 .

205 **Results**

206 Activity data from sensor recordings were analysed relative to the time of BRD detection, after
207 exclusion of zero activity values. Visual inspection of mean activity patterns demonstrated a gradual
208 divergence between BRD cases and control calves prior to detection, with cases showing a
209 reduction in activity approaching the time of diagnosis (Fig. 1). This trend was supported by model-
210 based estimates, indicating that activity patterns over time differed between groups.

212 Linear mixed-effects modelling revealed a significant interaction between disease status and time
213 relative to detection ($p < 0.001$), indicating that activity patterns differed between BRD cases and
214 controls over time. Activity was also significantly associated with animal age ($\beta = 3.27$, $SE = 0.34$,
215 $p < 0.001$), reflecting increasing activity levels with age. These results confirm that differences
216 between diseased and healthy calves are not constant but evolve over time.

218 Post-hoc comparisons using estimated marginal means demonstrated that activity in BRD cases was
219 significantly lower than in control calves from 2 days prior to detection onwards (Day -2: $p =$
220 0.0065 ; Day -1: $p < 0.001$; Day 0: $p < 0.001$), as illustrated by the separation in estimated marginal
221 means (Fig. 2). No significant differences were observed in earlier days. The magnitude of the
222 difference increased approaching detection, with the largest reduction observed at the day of
223 detection.

224 At the hourly level, separate comparisons between BRD cases and control calves were performed at
225 each time point using repeated t-tests. Based on these analyses, the first statistically significant
226 difference in activity was observed at 31 hours prior to clinical detection (estimate = -29.1 , $p =$
227 0.0495). The temporal distribution of p -values showed a consistent period of significant differences
228 in the hours preceding detection (Fig. 3), supporting the pattern observed in the day-level mixed-
229 model analysis.

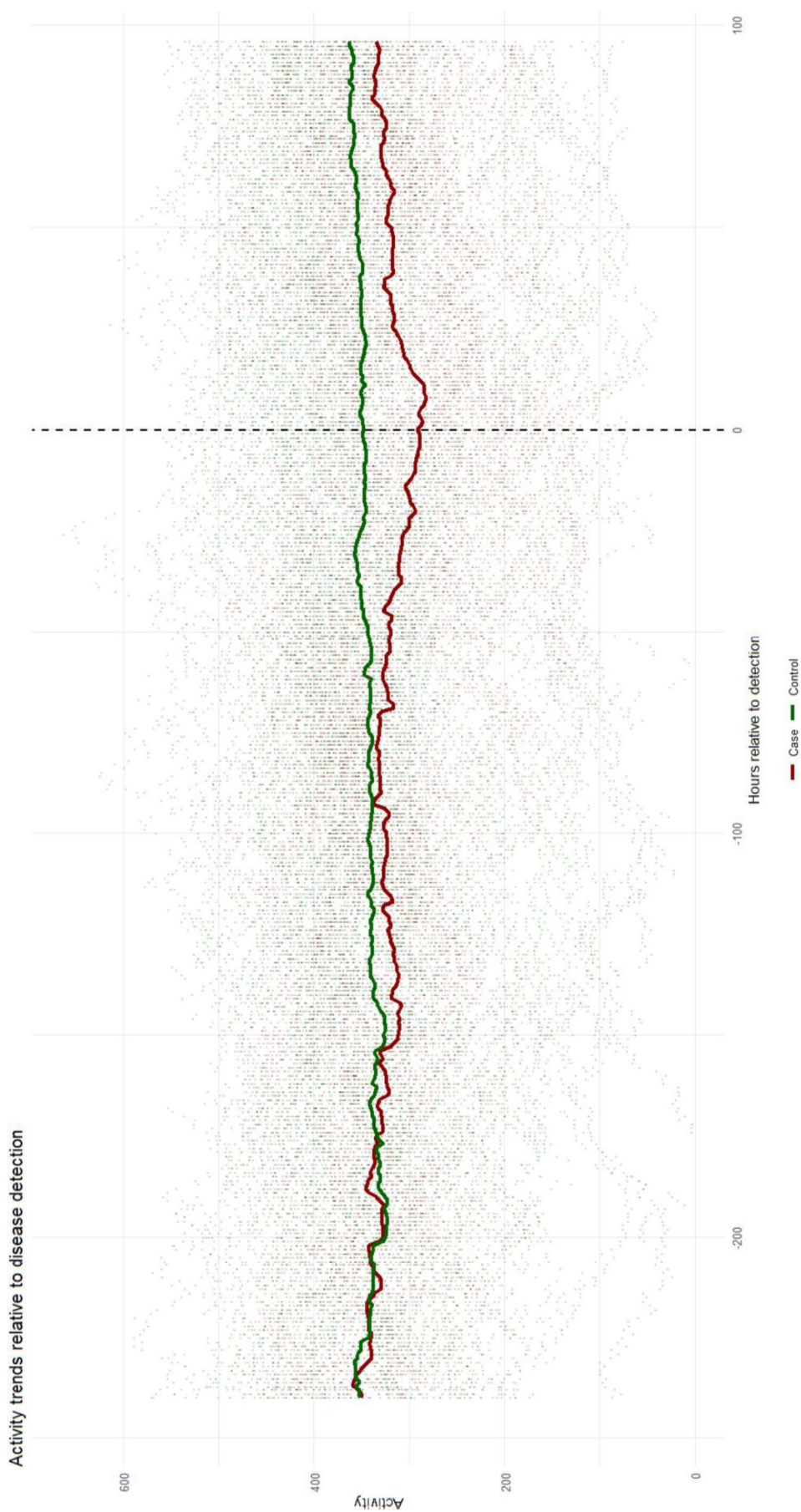
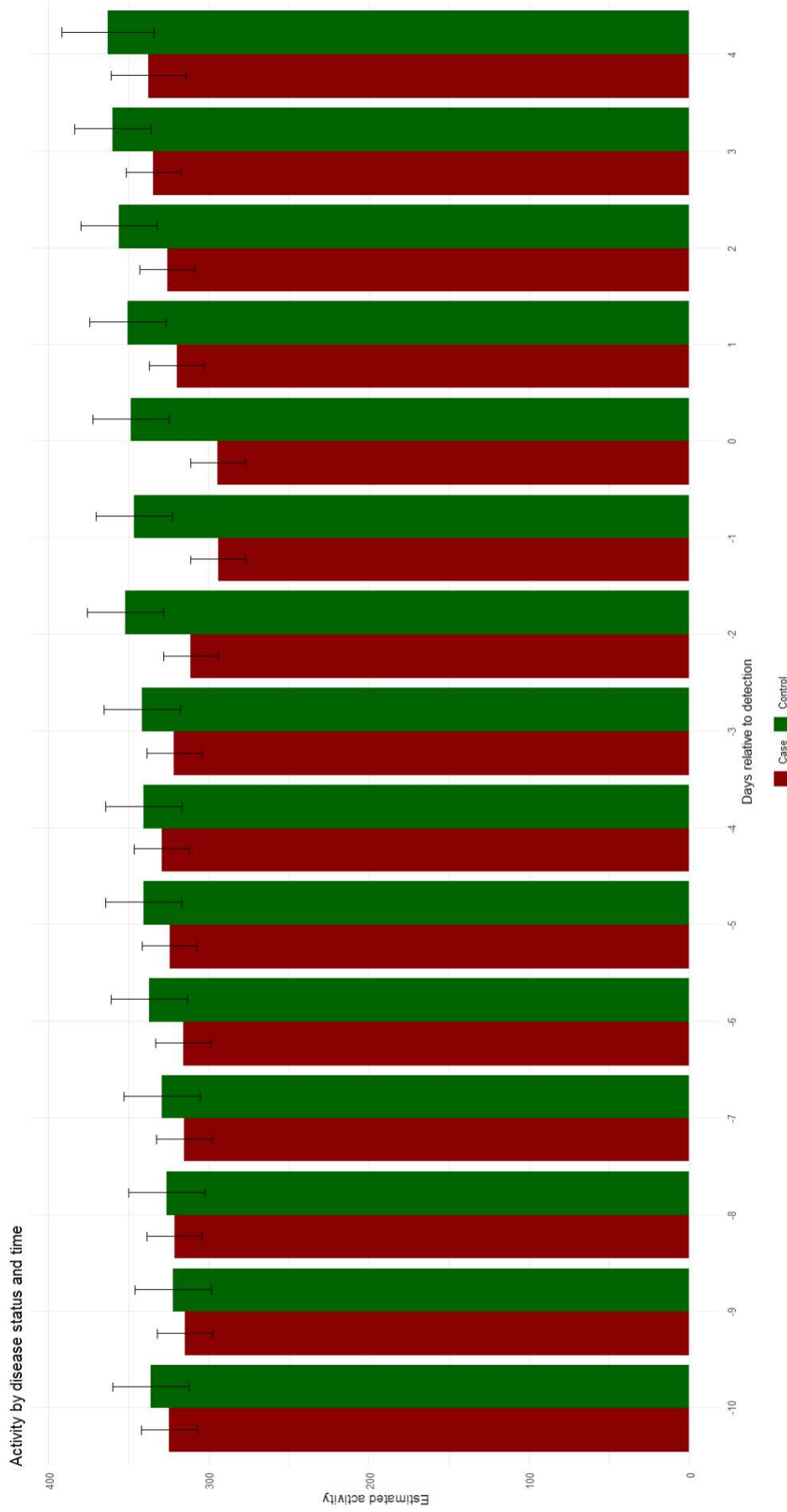


Fig. 1. Activity trends in BRD cases and control calves relative to time of disease detection. Points represent individual activity observations, while solid lines indicate mean activity for each group. The dashed vertical line marks the time of clinical detection (0 hours). Activity in BRD cases declines relative to controls prior to detection.



236
 237 Fig. 2. Estimated activity in BRD cases and control calves by day relative to disease detection. Bars represent model-based estimated
 238 marginal means (lsmeans), with error bars indicating 95% confidence intervals. Activity in BRD cases was significantly lower than in
 239 controls from 2 days prior to detection onwards ($p < 0.05$), as indicated by post-hoc comparisons adjusted for multiple testing.

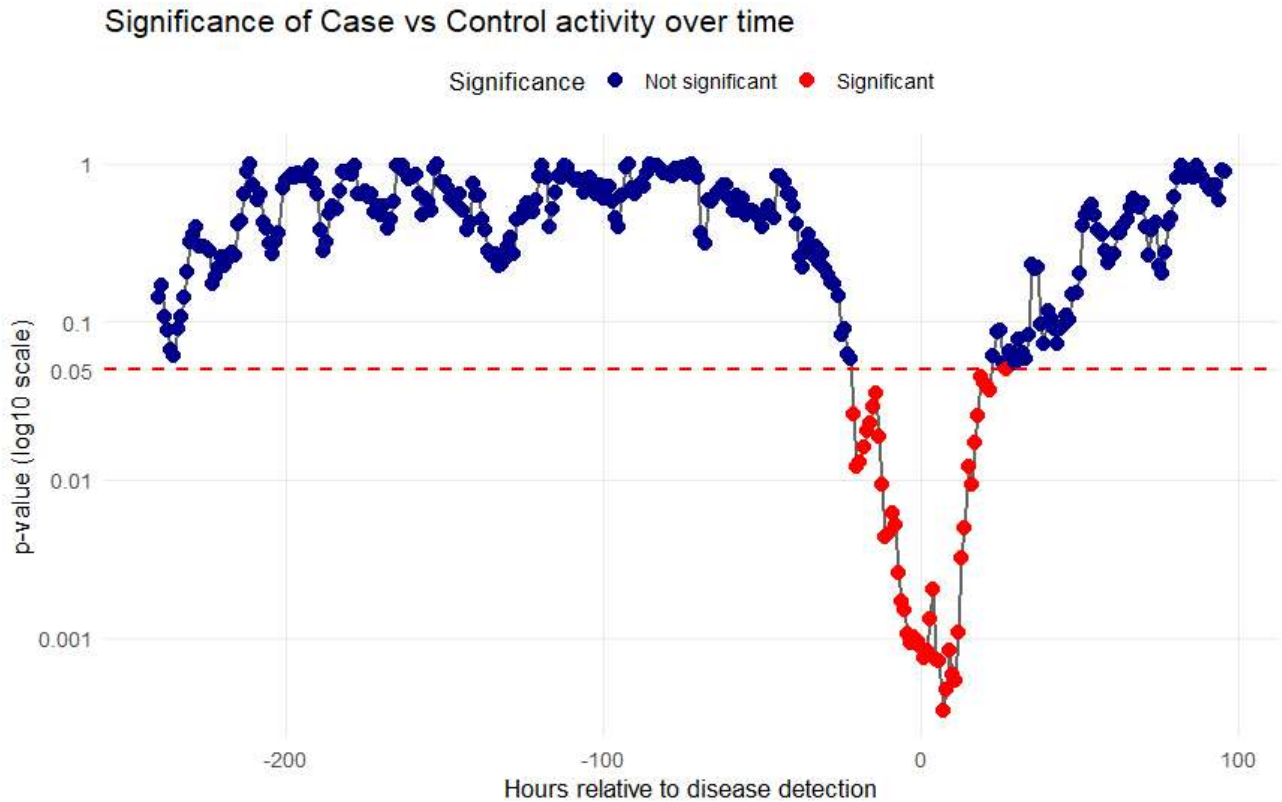


Fig. 3. Significance of differences in activity between BRD cases and control calves across time relative to clinical detection. Points represent p-values obtained from separate t-tests performed at each hourly time point, displayed on a log scale. The dashed horizontal line indicates the significance threshold ($p = 0.05$), with red points indicating significant differences and blue points indicating non-significant differences. A consistent period of significant differences is observed in the hours preceding detection, indicating reduced activity in BRD cases relative to controls.

Table 1. Linear mixed-effects model of activity prior to BRD detection. Fixed effects are presented as estimates (β) with standard errors (SE) and p-values. Only key interaction terms are shown for clarity. Variance components describe between-calf variability (random effect) and within-calf residual variability.

Fixed effects			
Variable	Estimate (β)	SE	P-value
Age (days)	3.27	0.34	<0.001
BRD $\times$ Day -2	38.83	22.31	0.082
BRD $\times$ Detection day	63.26	22.14	0.004

Variance components	
Component	Variance
Calf-level (random effect)	3751.71
Residual	4864.54

Discussion

The objective of this study was to assess whether activity patterns differ between preweaned dairy calves with bovine respiratory disease (BRD) and healthy age-matched controls prior to clinical detection.

In the present study, activity patterns differed between BRD cases and control calves over time, with statistically significant differences emerging from 31 hours prior to clinical detection, while consistent differences were observed from 2 days prior at the day level. These findings indicate that behavioural changes develop gradually and only become distinguishable from normal variation relatively close to the time of diagnosis. The significant interaction between time relative to detection and BRD status further supports that differences between diseased and healthy calves evolve over time rather than remaining constant. In addition, activity was positively associated with age, reflecting increasing activity levels as calves matured which perhaps reflects the calves being moved from single hutches and into group pens and thereby higher movement- and activity level.

290 Reduced activity prior to clinical detection of BRD is biologically plausible, as sickness behaviour
291 in calves is characterized by decreased movement, reduced feeding activity, and energy
292 conservation in response to infection (Cantor *et al.*, 2022). However, the relatively short time
293 window in which statistically significant differences were detected in the present study suggests that
294 these changes in activity may be subtle and difficult to distinguish from normal behavioural
295 variation until shortly before clinical detection.

296

297 Cantor and Costa (2022) reported reduced activity in BRD-affected calves up to five days prior to
298 clinical detection, and Marchesini *et al.* (2018) reported reductions 3–6 days before the onset of
299 clinical signs. Compared to these studies, the later onset of statistically detectable differences
300 observed in the present study suggests either a weaker early signal or greater variability in the data.
301 One possible explanation is the use of a VAS cut-off of 2.5, which may include milder or more
302 heterogeneous cases and thereby reduce contrast between groups at earlier time points. Although
303 Møller *et al.* (2024) reported substantial agreement between VAS and the Wisconsin Calf Health
304 Score Chart (WCHSC) scoring systems, small differences in classification criteria may still
305 influence the timing at which behavioural differences become detectable.

306

307 Another important consideration is the temporal resolution of clinical observations. Calves in the
308 present study were only clinically evaluated every 48 hours, which introduces uncertainty in the
309 exact timing of disease onset. As highlighted by Perttu *et al.* (2024), accurate identification of
310 disease onset is critical when evaluating early detection using automated data. This limitation may
311 have attenuated associations between activity and disease status, particularly at earlier time points,
312 and may partly explain why significant differences were not observed earlier than 31 hours before
313 detection.

314

315 From a practical perspective, the results indicate that activity-based monitoring systems can detect
316 group-level differences in behaviour approximately 1–2 days prior to clinical detection of BRD.
317 However, these findings should not be interpreted as evidence of reliable individual-level
318 prediction. As previously reported by Bowen *et al.* (2021), automated detection systems may
319 achieve relatively high sensitivity but lower specificity, suggesting that they are better suited as
320 screening or early-warning tools rather than definitive diagnostic methods. Reduced activity is a
321 non-specific indicator that may reflect multiple underlying conditions and therefore requires follow-

322 up clinical assessment. Nevertheless, such activity measurements may still provide value by
323 identifying calves at risk and enabling earlier targeted intervention, as well as improving labour
324 efficiency and monitoring consistency on farms (Perttu *et al.*, 2023).

325

326 In conclusion, this study demonstrates that activity differences between BRD cases and control
327 calves become statistically detectable within approximately 24–48 hours prior to clinical detection,
328 with the earliest detectable difference identified at 31 hours before diagnosis. While these findings
329 support the potential of automated activity as an early indicator of disease, the relatively short
330 detection window and substantial within-animal variation highlight the need for cautious
331 interpretation and further validation under commercial conditions.

332

333 **Conflict of interest statement**

334 None of the authors has any financial or personal relationships that could inappropriately influence
335 or bias the content of the paper.

336

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Paper III

Serum Amyloid A and Haptoglobin concentrations in calves at first event of respiratory disease were not associated with subsequent risk of respiratory disease during the preweaning period

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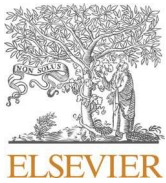
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Serum Amyloid A and Haptoglobin concentrations in calves at first event of respiratory disease were not associated with subsequent risk of respiratory disease during the preweaning period

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ABSTRACT

Bovine respiratory disease (BRD) causes decreased welfare and production losses and is a major reason for use of antimicrobials in dairy calves. Inflammatory markers released into the blood stream during BRD include acute phase proteins such as Serum Amyloid A (SAA) and Haptoglobin (Hp). This longitudinal observational study aimed to investigate whether the serum concentrations of SAA and Hp measured on the day of a detected mild clinical event of BRD, were associated the odds of developing recurrent BRD events requiring additional treatments in up to a 46-day follow-up period after the first event. A total of 65 preweaned dairy calves were observed for 46 days each in one Danish dairy herd. They were enrolled in this study in the age between 17 and 24 days of age and were followed for the following 46 days in total in which the calves potentially could develop an event of BRD. The calves were clinically assessed every other day using a Visual Analogue Scale (VAS), where a mild BRD event was defined as a calf that deviated from a normal and non-affected calf. The clinical signs included that the calf was less interested in its surroundings, slightly depressed, less bright, alert, and responsive with less clear eyes and using longer time to get up. The calf could have scruffy hair coat and drooping ears. Blood samples were collected on the day of the first mild BRD event that was only treated with a non-steroidal anti-inflammatory drug. A logistic regression model was performed to detect associations between having recurrent events of BRD and VAS, serum SAA and Hp concentrations at the day of the first BRD event and the follow-up period after the BRD event. Only the follow-up period after the first BRD event had a significant association with the odds ratio of having recurrent events of BRD of 2.3 for a 10-day difference in follow-up time after the BRD event.

Introduction

Bovine respiratory disease (BRD) is continuously causing health and welfare challenges to dairy calves. Dairy heifer calves diagnosed with BRD have been shown to have 2.8 times higher odds of dying and 2.3 times higher odds of leaving the herd before the first calving compared to calves without a BRD diagnosis (Buczinski et al., 2021). Measurement of the concentrations of serum acute phase proteins (APPs) has been investigated as biomarkers for BRD for years and it has been stated that Serum Amyloid A (SAA) and Haptoglobin (Hp) are among the most relevant for detection of inflammation in cattle (Saco and Bassols, 2023). The serum concentration of APPs can be used to quantify the inflammatory response and the level of tissue damage (Heegaard et al.,

2000; Jacobsen et al., 2004). Furthermore, the successful treatment has been shown to be associated with a decrease in serum APP concentrations in the first couple of days following the treatment (Hultén and Demmers, 2002).

In calves with naturally occurring BRD, both serum SAA and Hp concentrations were increased in affected compared to healthy calves (Joshi et al., 2018), especially when *Pasteurella multocida* was isolated from calves with BRD (Nikunen et al., 2007). Furthermore, an association between a high clinical BRD score (with coughing and increased rectal temperature) and the concentration of Hp were found in calves diagnosed with naturally occurring BRD (Ramos et al., 2021).

Increased serum Hp has been reported as a useful indicator of whether calves with BRD need treatment with a non-steroidal anti-

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inflammatory drug (NSAID) in combination with an antimicrobial (Humblet et al., 2004). Early treatment of BRD with an NSAID alone without use of an antimicrobial, has been studied with varying results. One study found decreased lung injury at day 3 post infection in calves experimentally infected with Bovine Respiratory Syncytial Virus after treatment with NSAID alone (Carvalho Chaigneau et al., 2021) but they did not assess BRD cases with a combination of viral- and bacterial pathogens. However, Mahendran (2020) found that calves treated for BRD (defined as fever with no other cause) with an NSAID alone, were more likely to require repeated treatments. El-Deeb et al. (2020) found that lower SAA and Hp concentrations in serum were useful to predict whether calves with BRD would recover from the disease in 79 % and 85 % of the cases, respectively. In this study, the threshold of SAA was higher than or equal to 24 mg/L and the threshold of Hp were at 590 g/L in which the samples were considered positive. This indicates that APPs are potentially valuable for detection and prognosis of BRD cases.

We hypothesize that calves experiencing recurrent BRD events would have more injured lung tissue during their first BRD event, resulting in a higher clinical score using a Visual Analogue Scale (VAS), higher serum concentrations of SAA and Hp, and be more susceptible to recurrent infections afterwards. Therefore, the objective of this study was to investigate the prognostic value of the VAS score below the threshold level for NSAID-treatment and serum concentrations of SAA and Hp after the detection of a mild BRD event under commercial dairy farm conditions.

Materials and methods

The Local Ethical Committee at Department of Veterinary Clinical Sciences, University of Copenhagen reviewed and accepted this study (project no. 2020–023) following the final permission by The Animal Experiments Inspectorate, Ministry of Food, Agriculture and Fisheries of Denmark, by case number 2020–15–0201–00677.

Study population and design

This study was a longitudinal observational study where one dairy herd in Eastern Denmark was recruited to participate in the study. The herd was visited every 2nd day in the period between September 2020 through January 2021. Initially, we included a cohort of 126 preweaned dairy heifer calves consisting of Holstein (90 %) and Red Holstein (10 %). The calves were enrolled at the age of 17–24 days and were followed for 46 days in total with 24 observations. Every 8th day a new group of calves were randomly selected and enrolled but a maximum of approximately 50 calves were followed at the same time due to capacity limits. The calves were housed in individual outdoor hutches for the first 35–40 days of age – and were afterwards moved to either indoor or outdoor group pens containing between 6 and 14 calves. After moving to group pens, all calves received a *Trichophyton* spp. (ringworm) vaccination but they were not vaccinated against pathogens associated with BRD. All calves in single pens were bucket-fed twice daily with 3–4 liters of milk replacer whereas calves housed in group pens were fed milk replacer in feed troughs twice a day. All calves had access to ad libitum homemade calf starter and hay.

Clinical scoring

At all herd visits every 2nd day, all included calves were clinically scored for signs of BRD using a Visual Analogue Scale (VAS) that included a numeric scale between 0 and 10 cm, as described in detail in Møller et al. (2024). If the calves received a VAS score between 0 and 2.4 the calf was classified as healthy. The VAS score between 2.5 and 4.9 was obtained when the calf were little interested in its surroundings, slightly depressed, less bright, alert, and responsive and has less clear eyes, uses longer time to get up, had a scruffy hair coat, and drooping ears which then classified the calf having mild BRD and was treated with

NSAID only (hereafter referred to as a mild BRD event). A VAS score between 5 and 10 was indicated by a calf that was depressed with obvious clinical signs of disease including large amounts of nasal and eye discharge, were coughing, had drooping ears, abdominal respiration in combination with increased frequency, head position below spine level and in the upper parts of the scale the calf might have been lying in lateral recumbency and appeared very ill or apathetic. In these cases, the calf was classified having severe BRD and a NSAID treatment was combined with an antimicrobial treatment (hereafter referred to as a severe BRD event).

The blood sampling and intervention is described in detail below. Calves that had clinical signs of other diseases that were not related to BRD or calves that were disbudded within 8 days before the mild BRD event were excluded from the study. The follow-up period after the first mild BRD event was the rest of the study period for the individual calf, i. e. up to a maximum of 46 days during which the calves were considered susceptible to recurrent BRD events. The follow-up period for the individual calf therefore varied.

Blood sampling and intervention

Blood was collected from vena jugularis in serum tubes (BD Vacutainer, SST™ II Advance, BD, USA). If the calves were having a mild BRD event, a blood sample were collected before a subcutaneous treatment with 0.5 mg/kg Meloxicam (Loxicom 20 mg/ml, Norbrook, Ireland). If the calf had a severe BRD event, a blood sample was collected and the calf was treated subcutaneously with 2.5 mg/kg Tulathromycin (Draxxin 100 mg/ml, Zoetis, Belgium) and 0.5 mg/kg Meloxicam (Loxicom 20 mg/ml, Norbrook, Ireland). If the first BRD event was classified as severe the calf was excluded from the analysis.

Blood sample preparation and laboratory analysis

Within 8 hours after collection, the serum tubes were centrifuged by 1800 G at 20 °C for 10 minutes, and serum was pipetted into 2 ml cryo tubes and stored at – 80 °C until analysis. All analyses were performed at The Veterinary Diagnostic Laboratory, University of Copenhagen. The samples were analyzed for SAA (VETSAA, Eiken Chemicals Co., Ltd, Tokyo, Japan, with a VETSAA control from the same company) and Hp (Tridelta PHASE Haptoglobin Assay cat. No. TP-801, with Haptoglobin control (cat. No. TP-801-CON)) on a Atellica solution CH from Siemens (Atellica® CH 930 Analyzer, Erlangen, Germany). All analyses were done according to the manufacturers' manuals.

Statistical analysis

Data management, graphical outputs and statistical analyses were done in R version 4.3.2 (R Core Team, 2022). The outcome in the analysis was defined as 0 = one mild BRD event, i.e. a calf having only one mild BRD event treated with NSAID (non-recurrent BRD), and 1 = one or more BRD events after the first mild BRD event needing additional NSAID or antimicrobial treatment (recurrent BRD). Descriptive statistics are reported as the distribution of the follow-up period and SAA and Hp concentrations on the day of the mild BRD event. A multiple logistic regression analysis with the binary outcome (0/1) was performed using the “GLM” function in R. The predictors in the model were the numeric clinical score (VAS), serum concentration of SAA and Hp and the follow-up period in days of the calf at the day of the mild BRD event. Preliminary results showed that age and follow-up time were highly correlated which was the reason not to include age in the final model. Non-significant interactions were omitted at a significance level of 0.05. Odds ratios for each of the included continuous variables were calculated as the exponential to the log-odds of a predefined numeric difference as described by Dohoo et al. (2009). A 0.5-cm change in VAS, a 10-day change in follow-up period, a 5-mg/L change in SAA concentration, and a 50-mg/L change in Hp concentration were chosen as a

biological meaningful change for estimation of the odds ratios.

Results

A total of 126 calves were observed in the study period. Three calves were excluded due to other clinical signs that were not related to BRD. A total of 36 calves did not have any clinical signs detected and were excluded from the analysis. Calves with a severe BRD event before a mild BRD event (n = 22) were excluded due to risk of them having affected lung tissue from previous infections and thereby being more susceptible to new infections. Calves having a first BRD event in study period classified as mild (n = 65) were included in the study and out of these 65 calves, 26 calves had one mild BRD event and the mean follow-up time after the first day of their mild BRD events was 25 days [SD: ±9.42] with a mean age on 40.9 days and a mean VAS score on 2.8. Thirty-nine calves had recurrent BRD events after having their first mild BRD event and the mean follow-up time after their first events was 32.7 days (SD ± 11.4), a mean age on 34.9 days, and a mean VAS score on 2.9. The mean SAA concentration on the day of the BRD event in calves with only one mild BRD event was 30.3 mg/L (SD: 17.3) and for calves with recurrent BRD events it was 30.5 mg/L (SD: 10.3 mg/L). In the calves with one BRD event the mean Hp concentration was 381 mg/L (SD: 265 mg/L) and 403 mg/L (SD: 311 mg/L) in calves with recurrent BRD events.

Table 1 shows the results of the logistic regression analysis of the association between serum SAA and Hp concentrations on the day of detection of the first mild BRD event and the binary outcome of having one mild or recurrent BRD event. The coefficient estimates for serum VAS, SAA and Hp were not statistically significant. The estimate for the follow-up period after the day of the mild BRD event was 0.080 (P = 0.005), with a standard error of 0.028 and an OR of 2.23 for each 10-day increase in follow-up period. This association indicates that with a longer follow-up period after the day of the BRD event, the odds of having recurrent BRD events significantly increases (P = 0.005). No significant associations were found for VAS, SAA and Hp and 2-way interactions between them in the odds of having recurrent events of BRD in this model.

Discussion

This study showed that neither serum concentrations of SAA or Hp at the day of detection of a mild BRD event were not significantly associated with experiencing recurrent BRD events. However, the length of follow-up period after the BRD event was statistically significant associated with the calf having recurrent events of BRD. As mentioned in the introduction, it was hypothesized that calves with several BRD events might have higher amounts of injured lung

tissue, which causes higher serum concentrations of SAA and Hp and make the calves more susceptible to new infections. Other studies found that viral pathogens cause milder clinical signs of BRD and have not been found associated with increased serum concentrations of SAA, Hp and other APPs (Gånheim et al., 2003; Nikunen et al., 2007). On the other hand, Orro et al. (2011) examined calves weekly and found that SAA and Hp increased within the first week post-infection with bovine respiratory syncytial virus up to 100 mg/L and 500 mg/L, respectively. When the same calves had a secondary bacterial infection, SAA and Hp increased even more, and the clinical signs also became more pronounced. Overall, this study found almost equal mean values for both SAA and Hp on the day of detection of BRD in both calves having one event and recurrent events. This is probably explained by the fact that the calves investigated in our study had mild clinical signs of disease and were detected very early in the BRD disease course by using a VAS as the clinical detection tool (Møller et al., 2024). This may indicate that the included calves, which had mild clinical signs detected in relation to the BRD event, might have less useful information from the SAA and Hp response in order to analyze for prognostic value because the concentrations were almost the same in the two groups of calves.

According to Jacobsen et al., (2004), serum SAA should increase rapidly around the time of disease and rapidly decrease after a certain treatment where initiated whereas Serum Hp will have a slower increase and peak later than SAA around 72 hours after the start of the disease course. A reference interval has not been established for the used SAA ELISA kit, however, preliminary results suggest that the cut-off between healthy and diseased individuals could be somewhere between 20 and 30 mg/L (Personal communication, Lise N. Nielsen, 12 August 2023), which supports the results in this study because the mean values of SAA and Hp lies within this concentration.

The model suggested that with increased length of follow-up period after the BRD event, the odds of having recurrent BRD events increased (OR = 2.23). This indicates that disease at a younger age and thereby a longer time in risk of having new infections, is important for the later risk of disease in the individual calf, which supports our results. The age of the calf at detection of the first BRD event and the follow-up time, were highly correlated (results not shown) and these two predictors could therefore not be included in the same statistical model. An optimization of the study protocol could include a fixed follow-up time after the BRD event, meaning that the calves had equal time to develop recurrent BRD events. This approach would make it possible to include the individual calves age as a predictor in the statistical model, which might have been informative.

When investigating SAA as a prognostic biomarker it must be taken into account that Orro et al. (2008) found that newborn calves had a naturally high level of SAA up to approximately 112 mg/L in the first three weeks after birth and thereafter stabilized on a certain level below 30 mg/L. This might not have had any influence on our results as the mean follow-up time of the calves at the on-set of the first mild BRD event and thereby related to the calves age was 25 days (one event) and 33 days (recurrent events), and the SAA concentration should have returned to normal levels by then when adding the age of the calves at enrolment which was around 17–24 days of age.

In general, biomarkers in serum should be further investigated to conclude more specifically on the responses under commercial settings in a herd, where other diseases and events, such as disbudding, movement etc. are difficult to control. A limitation of this study was the lack of follow-up detection by e.g. thoracic ultrasound or pathogen-detection. It could have aided in the certainty and quantification of the BRD detection, where a gold standard is missing.

Conclusions

There was not any statistically significant associations between calves having recurrent events of BRD and VAS and the serum concentrations of SAA and Hp. The calves in this study were detected early in

Table 1
Results of the logistic regression model including the intercept, Follow-up time in days, Visual Analogue Scale Score (VAS) in cm, Serum Amyloid A (SAA) in mg/L and Haptoglobin (Hp) in mg/L concentration estimate, standard error (SE), Odds ratio (OR), 95 % confidence interval (95 % CI) and level of significance of estimate Pr(>|z|) for calves having either one mild BRD event or recurrent BRD events based on the variables measured on the day of the clinically-detected BRD event (Day 0).

Parameter	Estimate	SE	OR	95 % CI	Pr (> z)
Intercept	-3.40	2.57			0.185
Follow-up time	0.080	0.028	2.23 ^a	[1.28;3.86]	0.005*
VAS	0.385	0.84	1.21 ^b	[0.53;2.75]	0.648
SAA	-0.008	0.023	0.96 ^c	[0.76;1.20]	0.719
Haptoglobin	0.0016	0.0012	1.08 ^d	[0.96;1.22]	0.168

^a 10-day unit change in follow-up time
^b 0.5 cm unit change in VAS score
^c 5 mg/L unit change in SAA concentration
^d 50 mg/L unit change in Hp concentration

their BRD course and the sample size were relatively limited, which might explain the lack of association between recurrent BRD and VAS, SAA and Hp. The use of VAS, SAA and Hp as tools to predict clinical outcomes of BRD in calves, needs further investigation.

CRediT authorship contribution statement

L.R. Nielsen: Writing – review & editing, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **M.A. Krogh:** Writing – review & editing, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Formal analysis, Conceptualization. **N. Capion:** Writing – review & editing, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **M.B. Petersen:** Writing – review & editing, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Formal analysis, Conceptualization. **H.H. Møller:** Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of Competing Interest

None of the authors has any financial or personal relationships that could inappropriately influence or bias the content of the paper.

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