Eurasia Specialized Veterinary Publication

International Journal of Veterinary Research and Allied Science

ISSN:3062-357X

2022, Volume 2, Issue 1, Page No: 122-132

Available online at: www.esvpub.com/

Analysis of the Occurrence of Cattle Respiratory Pathogens in Clinical Samples Submitted to UK Veterinary Laboratories: A Retrospective Study

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ABSTRACT

This study aimed to assess the prevalence and seasonal distribution of respiratory pathogens in cattle in the UK, using clinical samples submitted for laboratory PCR analysis. Data were retrospectively collected from a central laboratory in Scotland, comprising 407 pooled clinical samples provided by 95 veterinary practices across the UK between November 2020 and September 2022. Analyses included spatial mapping using choropleth techniques, chi-squared tests, and both Poisson and logistic regression models to explore associations. Analysis showed that 77.6% of samples contained multiple bacterial species, while 17.7% harbored more than one virus. The odds of infection with certain respiratory pathogens were significantly lower in the warmer months (March–August) compared with autumn and winter (September–February). Poisson regression indicated modest but significant univariable associations between weekly age and both total viral load (coefficient = –0.01, SE = 0.004, 95% CI = –0.02 to –0.003) and total pathogen count (coefficient = –0.005, SE = 0.002, 95% CI = –0.008 to –0.002). This study is inherently limited by its reliance on a single laboratory's submissions, introducing potential sampling bias, and by the retrospective nature of the analysis. Most bovine respiratory disease (BRD) cases involved multiple pathogens, with bovine coronavirus—typically not considered a major BRD contributor in the UK—being frequently detected.

Keywords: Bovine Respiratory Disease (BRD), Respiratory Pathogens PCR Diagnostics, Seasonal Distribution, Cattle

Received: 20 January 2022
Revised: 17 April 2022
Accepted: 18 April 2022

How to Cite This Article: O'Connor P, Doyle N. Analysis of the Occurrence of Cattle Respiratory Pathogens in Clinical Samples Submitted to UK Veterinary Laboratories: A Retrospective Study. Int J Vet Res Allied Sci. 2022;2(1):122-32. https://doi.org/10.51847/Noy6V0wU44

Introduction

Respiratory illness represents a major welfare issue and a leading cause of sickness and death in cattle [1–3], resulting in economic losses from treatment expenses and reduced growth, fertility, and milk output [4,5]. These diseases also have broader implications for human and environmental health, including the emergence of antimicrobial resistance [6,7]. Understanding the causative agents is therefore crucial.

BRD affects cattle across all ages [8], with newborn calves (<2 weeks) being particularly susceptible if they fail to ingest sufficient high-quality colostrum [3]. Respiratory infections also occur commonly in postweaning calves and adult cattle [9].

BRD can involve a wide range of viral agents such as infectious bovine rhinotracheitis (IBR, caused by BHV-1), bovine respiratory syncytial virus (BRSV), bovine parainfluenza virus 3 (BPI3), and bovine coronavirus (BCoV), as well as bacterial pathogens including Histophilus somni, Mannheimia haemolytica, Pasteurella multocida, and Mycoplasma species [10]. Some bacterial species (e.g., M. haemolytica, P. multocida, H. somni) are part of the normal respiratory microbiota [11] but can act as opportunistic pathogens under stress or viral co-infection [12]. While viruses are often considered the primary causative agents with bacteria as secondary invaders [13–15], co-

infections may interact synergistically, worsening disease severity [14]. Environmental and host factors, including immune status, also contribute to disease development [16,17]. Seasonality influences BRD incidence, with higher rates in colder months in the Northern Hemisphere due to indoor housing and limited ventilation [18–23].

Diagnosing BRD is challenging because of its complex aetiology. Diagnostic approaches include deep nasopharyngeal (NP) swabs, transtracheal washes, bronchoalveolar lavage (BAL), serology (single or paired), and postmortem examination. NP swabs provide early detection of upper respiratory pathogens and allow pooled sampling from multiple untreated, clinically affected calves to efficiently characterize herd-level pathogen profiles [24, 25]. BAL fluid helps determine the type and severity of pulmonary inflammation. Effective diagnostic strategies depend on careful selection of animals, disease prevalence, and the limitations of testing methods (sensitivity and specificity) [24, 26]. Serology informs on previous pathogen exposure at the group level. Timing is critical, as chronic infections may complicate detection of primary pathogens [22]. Examination of recent mortalities or euthanized animals provides additional insights. Real-time reverse transcriptase PCR (RT-PCR) allows rapid and cost-effective detection of pathogen genetic material in BAL, NP, and lung tissue samples [27]. This study aimed to evaluate the prevalence and seasonal trends of respiratory pathogens in UK cattle, hypothesizing that both animal age and season significantly influence pathogen frequency.

Materials and Methods

This investigation relied on retrospective data from a central Scottish laboratory, including 407 clinical samples from cases of bovine respiratory disease (BRD) collected on both dairy and beef farms by 95 veterinary practices across the UK between November 2020 and September 2022 **(Figure 1)**. The bulk of specimens consisted of nasal swabs (n = 373, 91.6%), while lung tissue obtained during postmortem examination accounted for 24 samples (5.9%), with the remainder unlabelled.

To detect pathogens, samples were analyzed for both bacterial (Mannheimia haemolytica, Pasteurella multocida, Histophilus somni, Mycoplasma bovis) and viral (bovine herpesvirus 1 [BHV-1], bovine parainfluenza virus 3 [BPI3], bovine coronavirus [BCoV], bovine respiratory syncytial virus [BRSV]) genetic material using RT-PCR. For efficiency, up to five swabs or tissue samples were combined to create a single pooled specimen at the laboratory. Nucleic acids (DNA and RNA) were extracted from these pooled specimens using the MagMAX Viral/Pathogen Nucleic Acid Isolation Kit (Thermo Fisher Scientific).

Detection was performed with the VetMAX Ruminant Respiratory Screening Kit, complemented by VetMAX IBR/BHV-1 reagents. Each assay in the VetMAX system is highly specific, designed to detect only its intended bacterial or viral target without cross-reactivity. The reported detection limit for these assays ranged from 10 to 40 nucleic acid copies per PCR reaction (see Supporting Information for the VetMAX validation report).

Figure 1. Distribution of 95 veterinary practices that submitted clinical bovine respiratory disease (BRD) samples to one diagnostic laboratory from November 2020 to September 2022

Data on the month of submission and the age of the animal were obtained from the submission forms. Information on whether the production type (beef or dairy) was not recorded.

Pathogen results were reported semi-quantitatively by the laboratory as '–' (negative), '+' (weak), '++' (moderate), or '+++' (strong) based on cycle threshold (Ct) values **(Table 1)**. The Ct value represents the number of amplification cycles required for the fluorescent signal to exceed background fluorescence; it is inversely related to the amount of target nucleic acid present in the sample:

- Ct ≤ 29: strong positive (+++), indicating high/abundant viral or bacterial nucleic acid
- Ct 30–37: moderate positive (++), indicating moderate amounts of target nucleic acid
- Ct 38–40: weak positive (+), indicating low/minimal amounts of target nucleic acid

Table 1. Interpretation of cycle threshold cycle (Ct) values for the VetMAX ruminant respiratory pathogen PCR panels (Applied Biosystems)

FAM Ct value (targets)	Level	Result	JOE Ct value (IPC)	Result
VetMAX Ruminant Respiratory Screening PCR				
≤20	+++	High quantity	≤45	IPC detected
>20 to ≤30	++	Intermediate quantity	>45	IPC not detected
>30 to ≤36	+	Low quantity		
>36 to ≤45	+/–	Investigate		
>45	–	Negative		
			JOE Ct value (Xeno DNA)	
VetMAX IBR/BHV-1 PCR				
≤25	+++	High quantity	<35	Xeno DNA detected
>25 to ≤30	++	Intermediate quantity	≥35	Xeno DNA not detected
>30 to ≤36	+	Low quantity		
>36 to ≤45	+/–	Investigate		
>45	–	Negative		

Notes

Borderline amplification results (for all targets and traces) were carefully evaluated to distinguish true positive amplification from non-specific effects. Only those showing a clear exponential amplification curve were classified as positive ('+'); non-exponential or non-specific amplification was recorded as negative ('–').

Abbreviations

BHV-1: bovine herpesvirus 1; IBR: infectious bovine rhinotracheitis; IPC: internal positive control.

Statistical analysis

Data were analysed in Stata (version 17) using the spatial analysis packages 'shp2dta' and 'spmap'. A shapefile was generated from the geographic coordinates of participating veterinary practices and converted into a Stata-compatible dataset. Base maps of UK administrative areas were obtained from the DIVA-GIS website. Choropleth maps were produced using point data tied to practice locations, with darker red shading indicating locations where multiple viral or multiple bacterial pathogens were detected in the same clinical sample.

Additional variables were derived, including totals for bacteria, viruses, and overall pathogens to reflect co-infections. Animal age was standardised to weeks; animals over 100 weeks were grouped into a single "adult" category. Because age data were available for only a subset of samples (n = 213), a separate dataset was created for age-related analyses. Binary (positive/negative) variables were created for each pathogen. Seasons were defined as winter (December–February), spring (March–May), summer (June–August), and autumn (September–November), and further collapsed into a binary "cold" (autumn + winter) vs. "warm" (spring + summer) variable. A geographic variable grouped practices into Scotland, Wales, Northern Ireland, and English regions (East Midlands, West Midlands, East, North East, North West, South East, South West).

Associations between individual bacterial and viral pathogens were tested using chi-squared tests, with Bonferroni correction applied for multiple comparisons (significance threshold adjusted to $p = 0.007$). Where cell counts were <5 and chi-squared assumptions were violated, Fisher's exact test was used instead. Univariable logistic regression models were built for each pathogen (positive vs. negative) with season and age (in weeks) as predictors. Potential confounding was assessed by a ≥20 % change in coefficients, and interaction terms between age and season were

explored. Poisson regression models were also fitted using season and age to predict counts of total bacteria, total viruses, and total pathogens. Goodness-of-fit tests for the Poisson models were non-significant ($p > 0.05$), indicating acceptable model fit.

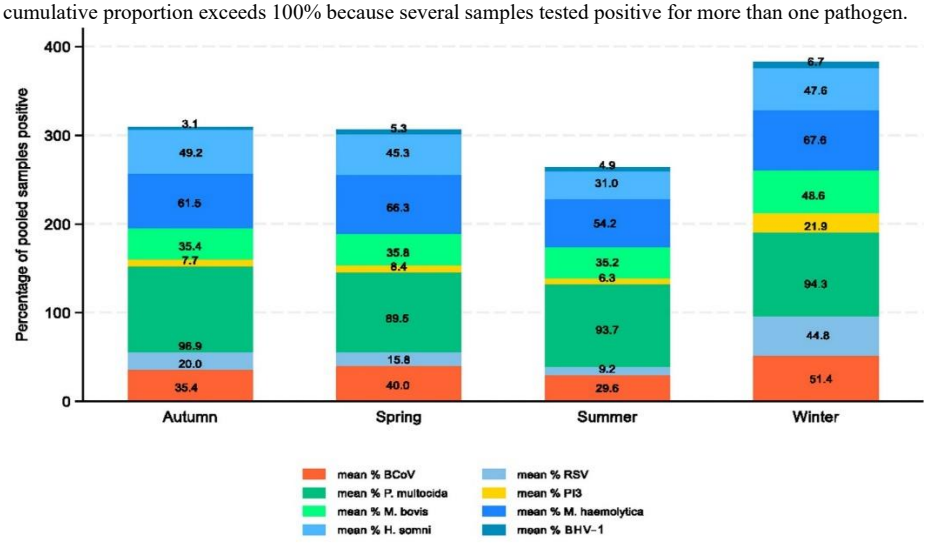
Results

Table 2 presents the frequency of positive results for each pathogen, taking into account the strength of positivity recorded. **Figure 2** displays the proportion of samples testing positive for each pathogen by season (using total submissions as the denominator). The highest proportions of positive samples occurred in winter, with particularly elevated detection rates for bovine coronavirus (BCoV), bovine respiratory syncytial virus (BRSV), bovine parainfluenza virus 3 (BPI3), and Mycoplasma bovis.

Table 2. Frequency and proportion of respiratory pathogens identified in 407 samples from 95 UK veterinary practices between November 2020 and September 2022

PCR Result	Bovine Coronavirus, % (n)	Bovine Herpesvirus 1, % (n)	Histophilus somni, % (n)	Mannheimia haemolytica, % (n)	Mycoplasma bovis, % (n)	Parainfluenza Virus 3, % (n)	Pasteurella multocida, % (n)	Respiratory Syncytial Virus, % (n)
Negative (–)	61.4 (250)	94.8 (386)	58.7 (239)	38.3 (156)	60.9 (248)	88.5 (360)	6.6 (27)	78.4 (319)
Low positive (+)	15.7 (64)	3.9 (16)	17.9 (73)	27.0 (110)	14.0 (57)	8.6 (35)	32.2 (131)	11.3 (46)
Moderate positive (++)	21.6 (88)	0 (0)	21.4 (87)	33.9 (138)	20.4 (83)	2.5 (10)	43.0 (175)	8.6 (35)
High positive (+++)	1.2 (5)	1.2 (5)	2.0 (8)	0.7 (3)	4.7 (19)	0.5 (2)	18.2 (74)	1.7 (7)
Overall positive (%)	38.6	5.2	41.3	61.7	39.1	11.5	93.4	21.6

• Note: The total cumulative proportion exceeds 100% because several samples tested positive for more than one pathogen.



Footnote: percentage figures on the y axis exceed 100% since many samples were pooled and there were multiple pathogens isolated from each sample

BCoV= bovine coronavirus; P. multocida= Pasteurella multocida; M.bovis=Mycoplasma bovis; H.somni= Histophilus somni; RSV=respiratory syncytial virus; PI3= parainfluenza virus 3; BHV-1 bovine herpesvirus 1

Figure 2. Proportion of pooled samples testing positive for specific respiratory disease pathogens across seasons

As indicated in **Table 3**, 316 out of 407 samples (77.6%) contained more than one bacterial species, while 72 out of 407 samples (17.7%) contained multiple viruses. **Figures 3** and **4** depict the total bacteria and viruses detected

in the clinical samples using choropleth point mapping, where larger and darker points correspond to higher counts.

Table 3. Count of bacterial and viral pathogens detected via PCR in 407 samples submitted by 95 UK veterinary practices

Number of pathogens identified	Number of viral samples, % (*n*)	Number of bacterial samples, % (*n*)
0	43.5 (177)	3.4 (14)
1	38.8 (158)	18.9 (77)
2	15.2 (62)	32.2 (131)
3	2.2 (9)	29.7 (121)
4	0.3 (1)	15.7 (64)

Note: The bacteria isolated included Mannheimia haemolytica, Pasteurella multocida, Histophilus somni, and Mycoplasma bovis. The viruses detected were bovine herpesvirus 1 (BHV-1), bovine parainfluenza virus 3 (BPIV-3), bovine respiratory syncytial virus (BRSV), and bovine coronavirus.

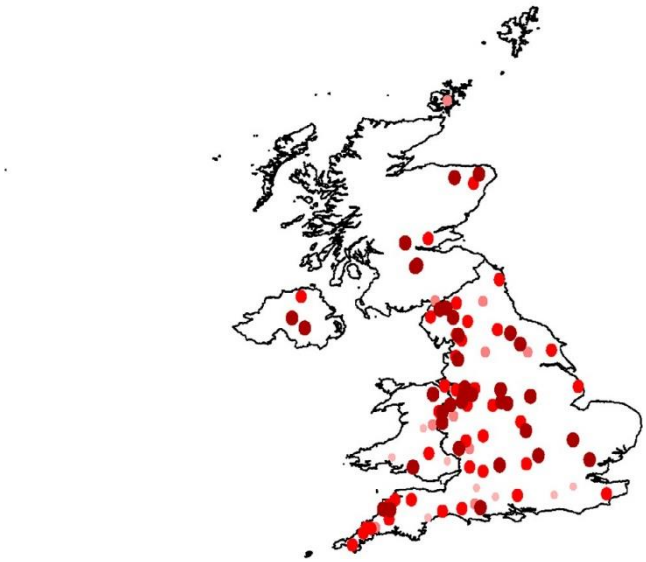


Figure 3. Choropleth point map illustrating the geographic distribution of total bacteria detected in 407 clinical samples from cases of bovine respiratory disease. These samples were submitted by 95 veterinary practices across the UK from November 2020 to September 2022. Darker shades represent practices with a higher proportion of bacterial detections relative to the total number of submissions

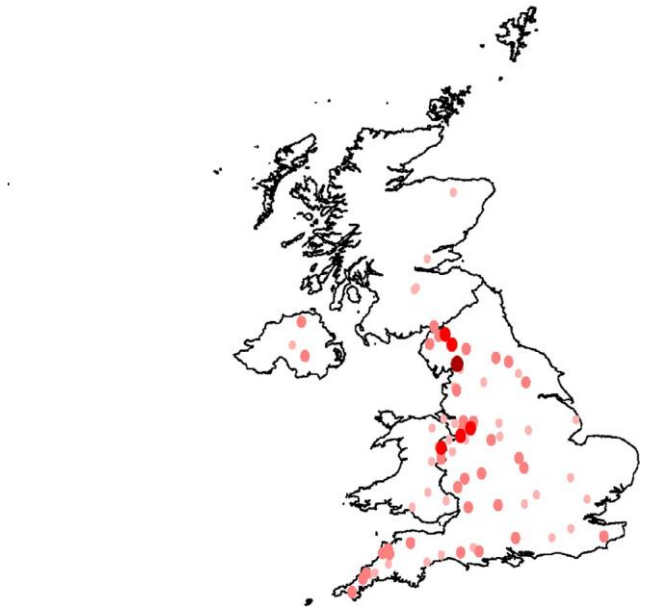


Figure 4. Choropleth point map displaying the geographic distribution of total viruses detected in 407

clinical bovine respiratory disease samples submitted by 95 UK veterinary practices between November 2020 and September 2022. Darker colours denote a higher relative frequency of viral detections per practice submission

Significant pathogen associations (Bonferroni-adjusted, $p < 0.007$) included:

- Mycoplasma bovis with both Pasteurella multocida (Fisher's exact test, $p < 0.01$) and Histophilus somni (χ^2 test, $p < 0.01$).
- Bovine coronavirus (BCoV) with both Mannheimia haemolytica (χ^2 test, $p < 0.01$) and bovine parainfluenza virus 3 (BPI3; χ^2 test, $p < 0.01$).
- Mannheimia haemolytica with both Pasteurella multocida (χ^2 test, $p < 0.01$) and Mycoplasma bovis (χ^2 test, $p < 0.01$).

These analyses treated pathogens individually in a binary framework and did not account for co-infections. Full results of the chi-squared analyses (with Fisher's exact test used when any category contained ≤5 samples) are presented in **Table 4**.

Table 4. Pairwise associations between respiratory pathogens detected in 407 clinical bovine respiratory disease samples (chi-squared tests with Fisher's exact test applied when expected cell counts ≤5). P-values are shown; statistically significant associations after Bonferroni correction ($p \leq 0.007$) are highlighted in bold

	BPI3	P. multocida	BCoV	BRSV	H. somni	BHV-1	M. bovis	M. haemolytica
BPI3	–	0.21	**<0.01**	0.38	0.29	0.58	0.25	0.47
P. multocida	0.21	–	0.07	0.27	0.09	0.04	**<0.01**	**<0.01**
BRSV	0.38	0.27	0.61	–	0.72	0.51	0.15	0.95
BCoV	**<0.01**	0.07	–	0.61	0.32	0.96	0.01	**<0.01**
H. somni	0.29	0.09	0.32	0.72	–	0.90	**<0.01**	0.04
BHV-1	0.58	0.04	0.96	0.51	0.90	–	0.08	0.63
M. haemolytica	0.47	**<0.01**	**<0.01**	0.95	0.04	0.63	**<0.01**	–
M. bovis	0.25	**<0.01**	0.01	0.15	**<0.01**	0.08	–	**<0.01**

Note: Associations reaching statistical significance after Bonferroni correction for multiple comparisons (adjusted threshold $p \leq 0.007$) are shown in bold. Each pathogen was analysed on a simple present/absent basis. Where any contingency table cell contained five or fewer samples, Fisher's exact test was substituted for the chi-squared test, and the corresponding p-values are displayed in italics.

Abbreviations

BCoV= bovine coronavirus
BHV-1= bovine herpesvirus 1 (infectious bovine rhinotracheitis virus)
BPI3= bovine parainfluenza virus type 3
BRSV= bovine respiratory syncytial virus

Compared to the colder period (September–February), the warmer months (March–August) were linked to markedly reduced odds of detecting certain BRD pathogens (BRSV, BPI3, BCoV, and H. somni), whereas no such seasonal effect was observed for BHV-1, M. haemolytica, M. bovis, or P. multocida **(Table 5)**. Adding submission year to the models did not change these findings, confirming a consistent seasonal pattern in pathogen occurrence. Geographic location showed no significant association with the detection of total bacteria, total viruses, or any individual pathogen in the logistic regression analyses.

Table 5. Univariable logistic regression analysis examining the association between season (cold months September–February as reference; warm months March–August) and detection of selected respiratory pathogens in 407 clinical BRD samples from 95 UK veterinary practices. Statistically significant associations ($p < 0.05$) are shown in bold

Pathogen	Odds ratio (warm vs. cold)	SE	p-value	95% CI
Bovine parainfluenza virus 3 (BPI3)	**0.39**	0.13	**<0.01**	**0.21 – 0.74**
Pasteurella multocida	0.57	0.25	0.19	0.24 – 1.33
Bovine respiratory syncytial virus (BRSV)	**0.25**	0.06	**<0.01**	**0.15 – 0.41**
Bovine coronavirus (BCoV)	**0.62**	0.13	**0.02**	**0.41 – 0.92**
Histophilus somni	**0.62**	0.13	**0.02**	**0.42 – 0.93**
Bovine herpesvirus 1 (BHV-1)	0.95	0.43	0.92	0.39 – 2.32
Mannheimia haemolytica	0.77	0.16	0.20	0.51 – 1.15
Mycoplasma bovis	0.71	0.15	0.10	0.48 – 1.07

Note: The reference season for all models is the colder period (September–February). Statistically significant associations ($p < 0.05$) are shown in bold.

Abbreviations
CI= confidence interval
SE= standard error

Age data were available for only 213 of the 407 samples (52.3%), which restricted the reliability of age-related analyses. Moreover, the age distribution was heavily skewed toward younger animals, with 59.2% (126/213) being ≤12 weeks old.

In univariable logistic regression, each additional week of age was associated with reduced odds of detecting Mannheimia haemolytica (OR = 0.98, $p < 0.01$) and bovine coronavirus (OR = 0.94, $p < 0.01$; **Table 6**).

Univariable Poisson regression revealed modest but statistically significant negative relationships between increasing age (per week) and both the total number of viruses detected (coefficient = −0.01, SE = 0.004, 95 percent CI −0.02 to −0.003) and the total number of pathogens overall (coefficient = −0.005, SE = 0.002, 95 percent CI −0.008 to −0.002). Multivariable Poisson models for counts of total viruses, total bacteria, and total pathogens are presented in **Table 7**.

Table 6. Univariable logistic regression analysis displaying significant associations between selected respiratory pathogens (as the outcome) and age (in weeks) for a subset of 213 clinical samples obtained from 95 UK veterinary practices

Pathogen	95% CI	Odds ratio	*p*-Value	SE
Bovine parainfluenza virus 3	0.96–1.02	0.99	0.42	0.02
Pasteurella multocida	0.98–1.02	1.0	0.88	0.01
Bovine respiratory syncytial virus	0.99–1.01	1.0	0.83	0.01
Bovine coronavirus	0.91–0.97	0.94	**<0.01**	0.02
Histophilus somni	0.98–1.00	0.99	0.38	0.01
Bovine herpesvirus 1	0.99–1.03	1.01	0.14	0.01
Mannheimia haemolytica	0.97–0.99	0.98	**<0.01**	0.01
Mycoplasma bovis	0.98–1.01	0.99	0.24	0.01

Note: Statistically significant p-values are highlighted in bold.
Abbreviations: CI= confidence interval; SE= standard error.

Table 7. Multivariable Poisson regression analysis illustrating the association of season and age (in weeks) with the total counts of viruses, bacteria, and overall pathogens (both viruses and bacteria) detected in 407 clinical samples submitted by 95 UK veterinary practices

Outcome	95% CI	Variable	*p*-Value	Coefficient	SE
Total viruses	–0.89 to –0.24	Season	<0.01	–0.56	0.16
	–0.02 to –0.003	Age (weeks)	<0.01	–0.01	0.005
Total bacteria	–0.007 to 0.000	Age (weeks)	0.06	–0.004	0.002
Total pathogens	–0.32 to –0.02	Season	0.03	–0.17	0.08
	–0.008 to –0.002	Age (weeks)	<0.01	–0.005	0.002

Note: Seasons were classified with cold (September–February) as the reference category and warm (March–August) as the comparison.
Abbreviations: CI= confidence interval; SE= standard error.

Discussion

As anticipated, the vast majority of clinical BRD cases (84.8%) involved mixed infections. Because the analysis relied on pooled samples, it was not possible to determine whether every animal in the pool was co-infected or whether individual animals carried different pathogen combinations.

Although Mycoplasma bovis is increasingly recognised globally as a primary respiratory pathogen in cattle [23], it was most commonly detected alongside other agents in this study, suggesting that co-infections predominate. The present results do not allow us to designate any pathogen as strictly primary, opportunistic or secondary, although bacteria are frequently implicated in secondary roles [28]. Bacterial pathogens were identified more often than viruses and were more likely to appear as multi-species complexes within the same sample. This pattern could reflect the presence of commensal bacteria in healthy cattle (e.g. Mannheimia haemolytica and Pasteurella multocida in tonsillar crypts), or it may indicate that samples were collected during the post-acute phase of outbreaks, after peak viral shedding had subsided. Such timing would support the widely held view that viruses

often act as primary initiators of BRD [13-15]. Despite the heightened sensitivity of PCR for viral nucleic acid, secondary bacterial invaders become more prominent as disease progresses. An Irish retrospective study similarly reported higher viral detection rates in pooled samples than in individual nasal swabs [14], which may explain the relatively high viral diversity observed here. A limitation of PCR-based diagnostics is the inability to perform culture and antimicrobial susceptibility testing, with potential consequences for treatment choice and the monitoring of resistance. Future PCR platforms could incorporate simultaneous detection of resistance genes.

Other UK surveillance initiatives for respiratory pathogens are underway [29]; however, methodological biases preclude direct prevalence estimates or cross-country comparisons. In a Slovenian study using comparable methodology, at least two pathogens were recovered from ~35% of clinical BRD samples and three or more from ~14%, with BRSV being the most common virus (41%), followed by bovine coronavirus (BCoV; 12%) and bovine parainfluenza virus type 3 (BPI3; 3%) [30]. In Poland, the same commercial kits applied to deep nasal swabs and tracheal washes identified P. multocida most frequently (18% of positives), while dual-pathogen samples were common (29.7%) and bacteria overall were detected in 57% of cases [31] – patterns broadly similar to the high BRSV prevalence seen in the current UK dataset.

Commercial multiplex PCR kits typically do not disclose primer/probe sequences, target genes or full in-house validation data, preventing independent assessment of specificity and sensitivity [12]. Nevertheless, recent independent validation of analogous kits has demonstrated specificity of 100% and sensitivity of 95–100% [32].

The frequent detection of BCoV in this UK cohort is noteworthy. The biology of BCoV infection – limited duration of immunity after natural exposure, persistent subclinical carriage, initial respiratory replication followed by enteric amplification and prolonged faecal shedding [33] – favours extended detectability compared with many other viruses. Our findings confirm that BCoV is a common finding in British cattle, consistent with reports worldwide [34-36], and underline the need for further targeted research.

Increasing age (weeks) was associated with fewer viral species and fewer total pathogens overall, but not with fewer bacterial species. This may suggest that protective immunity against respiratory viruses develops more rapidly than against bacteria and/or that younger calves are particularly prone to complex bacterial co-infections. However, the absence of data on prior BRD episodes or vaccination history renders these interpretations speculative. Both viral and bacterial BRD pathogens employ sophisticated immune-evasion tactics, including suppression of immune-cell proliferation and interference with phagocytosis and intracellular killing [37]. Vaccine-induced immunity typically lasts ~6 months for BPI3 and BHV-1 and up to 12 months for BRSV [38], although live BHV-1 vaccines can extend protection to 12 months [39, 40]. M. haemolytica and P. multocida are normal tonsillar commensals whose virulence is heavily context-dependent; immunity against bacterial components of BRD is therefore less predictable and influenced by host factors and pathogen evasion strategies [41, 42].

Season exerted a significant influence on pathogen profiles, most likely reflecting weather-related variables that differ by region. Warmer months were associated with significantly lower total pathogen counts, aligning with observations in feedlot cattle in France [43] and North America [44]. Specifically, the odds of detecting BRSV, BPI3, Histophilus somni and BCoV were markedly reduced during warmer periods.

Veterinarians managing BRD outbreaks should recognise the inherently multivalent and multipathogenic nature of the syndrome, as demonstrated here. Preventive strategies must therefore be comprehensive rather than pathogen-specific, incorporating optimal colostrum management, high standards of housing, ventilation and environmental hygiene, and multivalent vaccination programmes that acknowledge the synergistic interactions among the many agents capable of contributing to clinical disease.

Study Limitations

This study is based on a inherently biased dataset, as it only includes clinical samples submitted to a single UK laboratory. Because the dataset is organized by submitting veterinary practice, details such as the number of outbreaks sampled or the number of farms involved are not provided. The analysis was conducted retrospectively without prior sample size calculation; however, the data still cover much of the UK and offer insight into the relative frequencies of respiratory disease isolates in the sampled clinical cases.

Mixed flora detected could reflect sample pooling at the laboratory rather than true co-infection in individual animals. Although bovine viral diarrhoea virus is often cited in the literature on the BRD complex due to its immunosuppressive effects, it was not included in this dataset. The analysis was further limited by incomplete

metadata; for example, only 213 of the 407 samples had recorded animal ages. More complete submission forms from veterinary clinicians would improve the value of retrospective surveillance studies. Additionally, nasal swabs may have captured upper respiratory tract commensals rather than true pathogens and may not adequately reflect lower respiratory tract infections.

Sample submissions were uneven over the study period, with more samples submitted between May and September 2022, potentially leading to overestimation of certain risk factors, such as seasonal effects. The generalizability of the total numbers of viruses and bacteria detected is also constrained by the lack of information on farm management, housing, and stocking densities.

Conclusion

Data from this single UK laboratory indicate that most clinical BRD samples in this dataset contained multiple pathogens. Bovine coronavirus (BCoV), although not traditionally considered a major contributor to the BRD complex in the UK, was frequently detected and aligns with global evidence of its clinical significance. Warmer seasons and older age in weeks were significantly associated with a lower total number of pathogens identified in clinical BRD samples.

Acknowledgments: The authors would like to thank Alice Cornish for her initial analysis of this dataset.

Conflict of Interest: None

Financial Support: None

Ethics Statement: The analysis was conducted with ethical approval from the University of Glasgow (EA46/23).

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