**Application**

Modelling the effect of length and intensity of commingling events on development of pneumonia and shedding of pneumonia-associated pathogens could aid in respiratory disease management and prevention.

**Introduction**

Bovine respiratory disease (BRD) is a leading cause of calf morbidity and mortality, costing the UK cattle industry approximately £50 million a year(Agriculture and Horticulture Development Board, 2024). Commingling events, such as mixing animals from different sources at auction markets or feedlots, are well-known to increase risk of disease transmission, and are a major risk factor for the development of BRD. In this study we aimed to model the relationship between commingling length and intensity, development of BRD, and shedding of bovine coronavirus (BCV).

**Materials and methods**

The study was conducted on weaned Angus x Holstein calves: 40 per cohort were obtained from five source farms (SF). In total, 80 calves (two cohorts) from seven SF were enrolled in the study, as due to practical constraints two SFs differed between cohorts. Animals were transported to the University of Liverpool research facility and quarantined for 12-14 days. Following quarantine calves were randomised within the strata of farm and sex, into one of four commingling groups: none (single-source pen), low-level (pen with 2 calves from one SF and 8 calves from a second SF), moderate (pen with 5 calves from one SF and 5 calves from a second SF), and intensive (pen with 2 calves from each of the 5 SFs). There was at least 3m air gap between pen groups. Serum, nasopharyngeal swabs and faecal samples were collected on source farms on the day of calf collection (arrival), the day of commingling (day 0), and then 3, 7 and 21-days post commingling. Swabs and faecal samples were subjected to quantitative PCR for BCV, targeting the conserved region of the membrane (M) protein. Serum samples were subjected to anti-BCV IgG ELISA at arrival, and then commingling days 0, 7 and 21. Furthermore, calves were scored daily for signs of clinical BRD using a modified Wisconsin system, and for subclinical BRD by thoracic ultrasonography on arrival and commingling days 0 and 21.

To assess the potential of airborne BCV transmission Sartorius MD8 Airport and SKC button air samplers were fitted in two selected pens on commingling days 7 and 21. Aerosol samples were collected from the button samplers by continuous filtration over gelatine membranes on sampling days (~ 8h) and from MD8 Airport samplers by 30-minute filtration both prior and during sampling events. RNA extracted from aerosol samples was subjected to quantitative PCR for BCV. Furthermore, biological material extracted from aerosol samples was subjected to serial passage in VERO cells as previously described (Gould *et al.* 2022).

Relationship between commingling length and intensity, development of BRD, and BCV shedding was modelled with linear mixed-effects models in R studio release 2024.09.0+375.

**Results**

Based on preliminary results from the first cohort, crude prevalence of nasopharyngeal BCV on arrival and on the day of commingling was 10 and 5%, respectively. After commingling, nasal BCV prevalence remained relatively low (<10%) until day 21, when 53% of samples were positive. Highest prevalence (100% on day 21) was observed in the pen with animals from a single source farm, with prevalence decreasing with commingling intensity: 12.5% in low and moderate commingling pens, and 7.5% in the intensive-commingling pen. Intensity of nasal BCV shedding was also relatively low until day 21, rising from an average of 104-5 to 107 viral copy number/µL in positive animals. Additionally, air swabs collected in the pens were positive for BCV RNA at low copy number (< 104 viral copy number/µL).

Crude prevalence of faecal BCV was 15 and 10% on arrival and commingling day respectively. Following a similar pattern to nasal BCV, faecal prevalence remained at <10% until 21 days post-commingling when it rose to 43%. There was a positive correlation between nasal and faecal BCV shedding, with the odds of calves shedding faecal BCV being 3.8 times greater if they were also shedding BCV from the nares at any experimental timepoint.

All calves were seropositive for BCV antibodies on arrival and their antibody levels remained high throughout the study. Both commingling time and intensity influenced BCV shedding, with commingling time of 21 days modelled to increase BCV shedding by 3.1 log viral copies/µL relative to the day of commingling (p=2.11e-10) and assignment to high-commingling pen to decrease it by 1.27 log viral copies/µL relative to the single source pen (p=0.0062). Neither clinical pneumonia signs, nor presence of lung lesions were found to be significantly associated with BCV shedding.

**Conclusions**

Nasal BCV shedding was significantly impacted by commingling length and intensity; however, we found no association between BCV shedding and development of clinical or subclinical pneumonia. At the time of submission, we have completed sample collection of two cohorts and data analysis of the first cohort. Data analysis of the second cohort, including additional environmental sampling, is ongoing. Results from these analyses should help to further disentangle the relationship between BCV and BRD.

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**References**

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