

Bacterial colonisation dynamics and development of the bovine respiratory microbiome

Amy Thomas, Mark Eisler, Hannah Fleming and Michael Lee

Bovine respiratory disease (BRD) is a multifactorial disorder and one of the most important disease syndromes affecting dairy replacement and beef rearing systems. BRD can affect animals of any age but is most commonly associated with calves aged under 6 months, typically shortly after housing or transportation. In the UK a complex of viral and bacterial pathogens is identified in clinical cases. Bacterial involvement in the pathogenesis of BRD is thought to occur following stress or infection with respiratory viruses, allowing proliferation of what are normally nasopharyngeal commensals (most commonly members of the Pasteurellaceae: *Pasteurella multocida*, *Mannheimia haemolytica* and *Histophilus somni*). Proliferation often occurs initially in the tonsillar crypts and nasal/sinus mucin, then bacteria penetrate deeper into the respiratory tract and cause pneumonia.

Previous research has focused on animals of various ages, usually between 1 – 4 months of age, and methods have been predominately directed at culturing members of the Pasteurellaceae, sometimes supplemented with PCR to aid in identification. To our knowledge, only one longitudinal study has investigated the neonatal upper respiratory tract (URT) microbiome of healthy calves. However, that study focused exclusively on Holstein dairy calves using high-throughput 16s rRNA sequencing to look at relative abundances of bacterial genera, and did not attempt absolute quantification at the species level.



For the first time we describe colonisation dynamics for Pasteurellaceae in URT of healthy neonatal beef suckler calves in a long-term, longitudinal study using both molecular and culture-based methods. We collected short nasopharyngeal swabs from thirty housed calves (Stabiliser or crossbred from Hereford-Friesian dams by either Charolais or Limousin sires) within their first week of life and at approximately monthly intervals thereafter until 10 months of age. Using published PCR gene targets and 16s rRNA sequences we have developed three real-time PCR (qPCR) assays, allowing both detection and quantification of *P. multocida*, *M. haemolytica*. Our preliminary findings using qPCR reveal marked differences in colonisation patterns of commensal Pasteurellaceae. Within the first week of life 27% of calves were colonised with *H. somni*, with carriage rates subsequently maintained at similar rates up to 10 months of age. In contrast, colonisation with *P. multocida* was not detected until 3 months of age (3% prevalence), with carriage rate increasing with age thereafter.

Threshold cycle (Ct) values indicated that the density of carriage for *H. somni* was low in comparison to that for *P. multocida* (*H. somni* mean Ct = 33 at 3 months, 32 at 10 months; *P. multocida* mean Ct = 25 at 3 months, 27 at 10 months). All Ct values will be converted to CFU/mL (equivalent to gene copies/mL) for direct comparison of bacterial density between species.

Our results suggest that bacterial colonisation with age is dynamic. However, it is not yet clear how these colonisation dynamics affect or are affected by host mucosal immune responses, although early life colonisation could be an important factor in determining later respiratory health. The trends in bacterial colonisation we describe in cattle appear to be analogous to those seen in the nasopharynx of children, where *Staphylococcus* is frequent in the early days of life, but declines between 1 & 4 years of age when species such as *Streptococcus pneumoniae*, *Haemophilus influenzae* and *Moraxella catarrhalis* predominate. Emerging epidemiological data in humans suggests that bacterial colonisation of the URT in early life may modulate the risk of developing respiratory dysfunction later in life, and may also impact on mucosal immune development. Further work will focus on defining prevalence and density of *M. haemolytica* and a subset of samples will be cultured to validate qPCR results. Colonisation rates and densities for all three species of Pasteurellaceae will be investigated over time using mixed effect repeated measures models, taking into consideration covariates such as gender and breed.