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Comparative analysis of shed and external ambient temperature and humidity data on mastitis incidence in housed dairy cattle

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Heat stress affects animal health and welfare, significantly impacting dairy cattle production (Tao *et al.*, 2020). This study examines the relationship between shed and external environmental conditions, and its impact on mastitis occurrence. Shed temperature and humidity were measured hourly between June 2023 and October 2024 in an open-sided cattle shed, housing animals year round. External temperature was measured by a fixed and calibrated on-farm weather station, recording daily maximum, minimum, wet and dry bulb temperatures. Data regarding animal factors, including genetics, health and production were also recorded.

Maximum and minimum daily shed temperatures were compared and demonstrated significantly higher temperatures than ambient temperature (16.3 ± 5.5 vs. 14.5 ± 5.0 °C and 9.7 ± 4.5 vs. 7.4 ± 4.8 °C, respectively, $P < 0.01$). Association between shed and external maximum temperatures showed a strong positive correlation ($r = 0.94$, $P < 0.01$). The difference between maximum (ΔT_{max}) and minimum (ΔT_{min}) shed and external temperatures between seasons demonstrated that the highest ΔT_{max} (2.5 ± 1.6 °C higher in-shed) was observed during summer, followed by spring (1.9 ± 1.4 °C higher in-shed), winter (1.2 ± 3.0 °C, SD = 2.98), and autumn (1.0 ± 1.8 °C). ΔT_{min} showed less seasonal variation, with only summer (2.3 ± 1.5 °C higher in-shed) and winter (1.9 ± 2.5 °C,) being significantly different ($P < 0.05$). Temperature humidity index (THI, Mader *et al.*, 2006) peaked at 77.9, indicative of mild-moderate thermal stress risk. Comparison of average shed and external THI showed that in-shed THI (60.7 ± 7.95) was significantly higher ($P < 0.01$) than external (57.9 ± 8.29). Seasonal variation between internal and external measurements was not significant ($P > 0.05$). The percentage of the herd with mastitis increased with THI, up to THI 70 (Table 1). Though not significant when assessed with general linear model ($P = 0.76$), this indicates high THI negatively impacts animal health.

We found that in-shed temperatures and THI are higher than ambient temperatures but are strongly correlated. Importantly, in the absence of in-shed temperature measurements, this suggests free and publicly available data on external conditions can be used to indicate animal heat stress risk without the need for farm investment in additional sensors.

Table 1 Number of cows to experience mastitis in each THI range

THI Range	Number of Cows in Herd	Number of Cows to Experience Mastitis	Incidence %
<45	142	5	3.5
45-60	220	14	6.4
60-70	242	24	9.9
>70	234	12	5.1

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Tao S, Rivas RMO, Marins TN, Chen YC, Gao J and Bernard JK (2020). Impact of heat stress on lactational performance of dairy cows. *Theriogenology*, **150**, 437-444.

The impact of wearable technology for cows, on dairy farmers' work and wellbeing

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Dairy farming is among the most stressful occupations, with elevated rates of depression, anxiety, and suicide relative to the general population (Lunner Kolstrup et al., 2013; Wheeler and Lobley, 2023). There is extensive research on dairy farmers' experiences of job stressors, such as heavy workloads, physically demanding tasks, weather patterns, disease outbreaks, farm profitability, government policy, work and place isolation, and societal attitudes to farming (e.g. Wheeler and Lobley, 2021; Dolbec et al., 2024). With the increasing use of new technologies on dairy farms, especially in bigger farms, there are expectations on their potential to increase farm productivity and become a source of support for farmers by, for example, decreasing physical demands of tasks, reducing workloads, increasing work flexibility, etc. While much has been written about technologies productivity gains (e.g. Flett et al., 2004; Eastwood et al., 2021), there is very limited knowledge of technologies' social impact in terms of labour processes (e.g. on-call 24/7) and workers wellbeing (e.g. technostress).

Our research, funded by the HDRF, will address this knowledge gap by exploring the impact of wearable technology for cows (biosensor devices that monitor a range of cows' biometrics) on dairy farmers' work and wellbeing. To achieve this, qualitative interviews with dairy farmers who have adopted wearables for cows, currently or in the past, will be conducted to understand how the technology has influenced their work (i.e. labour processes) and wellbeing (i.e. job satisfaction). Additional interviews with those that have not adopted wearables, whether they are thinking of adopting them or not in the future, will explore their perceptions and experiences. Interviews with around 25 farms, will take place primarily in Scotland, with a small number of interviews in Ireland and southwest England to provide a wider geographical perspective. Outputs are expected to benefit farmers and the wider industry contributing to knowledge on this issue and producing evidence-based recommendations that will be of interest to a range of agricultural, technology, policy, and academic audiences.

Dolbec D, Dubreuil P and Larouche L (2024) Farmers' Mental Health: The Mediating Role of Chronic Fatigue *Journal of Agromedicine* **29**, 307-320.

Eastwood CR, Edwards J P and Turner J A (2021) Review: Anticipating alternative trajectories for responsible Agriculture 4.0 innovation in livestock systems *Animal* **15**, 100296.

Flett R, Alpass F, Humphries S, Massey C, Morriss S and Long N (2004) The technology acceptance model and use of technology in New Zealand dairy farming *Agricultural Systems* **80**, 199–211.

Lunner Kolstrup C, Kallioniemi M, Lundqvist P, Kymäläinen HR, Stallones L and Brumby S (2013) International perspectives on psychosocial working conditions, mental health, and stress of dairy farm operators *Journal of Agromedicine*, **18**, 244–255.

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Evaluation of *C. burnetii* detection by qPCR in different sample types collected from Scottish dairy cattle.

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Coxiella burnetii is a gram-negative intracellular zoonotic bacterium that infects cattle, sheep, and goats, and has also been detected in wildlife and humans worldwide. Cattle and small ruminants are thought to be the main sources of infection for humans. In the UK, Q fever is a mandatory notifiable disease under the Health Protection Regs 2010 in humans and since 2021, the detection of the organism by a laboratory test has been a reportable disease in the EU and Great Britain (GB) for animals. Although studies indicate widespread infection, the epidemiology of infection in UK dairy cattle remains poorly understood.

The objective of the study was to compare the ability to detect *C. burnetii* by IS1111 qPCR in different sample types collected from dairy cattle. A total of 100 dairy cows from a herd with high prevalence of *Coxiella burnetii* infection and evidence of increased endometritis and reproductive disease problems were sampled 1-7 days post-partum. Vaginal swabs, urine and milk were collected for each animal and IS1111 qPCR analysis was conducted on extracted DNA from all sample types to evaluate bacterial shedding by different routes.

Coxiella burnetii was detected by qPCR in 84 of 98 (85%) vaginal swab samples, 23 of 100 (23%) urine samples and 18 of 94 (19%) milk samples. The agreement between IS1111 qPCR test results for the different sample types, measured with Cohen's kappa (k), was poor between vaginal swabs and both urine (k=0.03) and milk (k=0.05), There was slight agreement between urine and milk samples (k=0.19).

The study findings indicate very high prevalence of infection in this herd, with 87 of 100 (87%) animals positive in one or more sample type. Assuming 'true' infection positivity in most if not all samples, testing of vaginal swabs is most sensitive for the detection of *C. burnetii* by qPCR. These findings agree with the wider literature indicating high sensitivity of *C. burnetii* detection in vaginal swabs from post-parturient animals and clear variation in *C. burnetii* shedding by different routes. The high sensitivity for *C. burnetii* detection and the ease of collection of vaginal swab samples highlight the value of this sample type for surveillance and monitoring of *C. burnetii* in ruminants.

Integration of multiple data streams to enable evidence based veterinary advice.

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The continuing development of appropriate technology is essential for UK agriculture as it strives to address the need for increased efficiency, productivity, and sustainability in farming practices. For the dairy sector these include the optimisation of production systems, extending the productive life of the breeding herd, enhanced disease and parasite control and overall improved system resilience. In order to address these priorities, it is essential that there is improved and more automated data collection and that this data is then aggregated to enable analysis and provide actionable insights. For veterinary surgeons working with dairy clients the data on farm performance and animal health can take multiple different forms ranging from notes made in the farm diary to alerts from the most recent advances in wearable technology and data from automatic milking systems. In order to make best use of this data and the insights it offers it is important that it can be integrated, analysed and reported back to the vet and farmer in a timely and readily interpreted manner. Over the last five years VetPartners has developed its own data solution Digifarm which enables data from multiple different sources to be brought together in order to provide our clients with a holistic view of herd performance and identify the key opportunities for improvement and optimise routine health monitoring.

Now with over 1000 herds registered on the system Digifarm draws on multiple data sources from both the farm and the veterinary practices and integrates this to provide a series of targeted reports that examine the key drivers of health and performance on those farms. Reports such as the dairy health tracker, udder health insight and fertility insight along with the infectious disease check help facilitate discussions between the vet and the farmer on the farms current performance but also make use of anonymous benchmarking across the herds to provide farmers feedback on how they are performing relative to other farms on the system.

Understanding the faecal and oral microbiomes of cattle to improve health and productivity.

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Shotgun metagenomic sequencing has revolutionised our ability to characterise the bovine gut and oral microbiomes. This sequencing technique generates large, multi-dimensional datasets with isolate-level taxonomic resolution and provides insights into the functionality of these microorganisms. Ruminants, including dairy cows, rely on their gut microbiota to provide them with energy and essential nutrients. It is therefore important to understand how these microbial communities may change during the transition and lactation periods when the cow's metabolic demands are at their highest.

The studies presented here examined oral and faecal microbiomes, the latter serving as a non-invasive proxy of the gut microbiome. Samples were collected from 150 Holstein cows and heifers at four timepoints from 30 days prepartum to 250 days in milk (DIM). Oral swabs, rectal swabs and corresponding voided faecal samples from ten individuals were used in an initial pilot study to assess the suitability of the sample processing and analysis pipeline as well as the impact of read depth on the perceived taxonomic composition. Variations in microbiome composition between individual animals and sample type were also explored.

Shotgun sequencing produced 144 gigabytes of data which comprised 2.8 billion raw reads. The sample read depths ranged from 47.5M to 74.9M base pairs (bp) after removing low quality and adaptor sequences. Subsampling trials confirmed that read depth was proportional to the number of bacterial reads and inversely proportional to the number of unmapped reads. Clustering similar samples together in a single run also improved the recovery of bacterial reads. Voided faecal samples and rectal swabs did not differ significantly in their read depths ($P = 0.85$, paired t-test), however there was a statistically significant difference in the proportions of bacteria between the two sample types ($P = 0.003$, paired t-test).

These results demonstrate that the sample processing pipeline employed is compatible with both oral and faecal samples. They also indicate that cautious interpretation is needed when analysing samples with different read depths and methods of collection. Future studies will focus on whether gut and oral microbiome signatures can be used to predict health status and production capabilities of dairy cows.

Genetic merit for digital dermatitis resistance is associated with disease incidence and granulocytes migration in dairy heifers.

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Digital dermatitis (DD) is a poly-bacterial skin disease associated with strawberry-like granulomatous formation and lameness, which is endemic to most UK dairy farms. Research indicates that resistance to DD is heritable, and selective breeding could reduce disease incidence. This study aimed to investigate the association between the DD index, published by the UK Agriculture and Horticulture Development Board, and actual incidence of DD in a population of maiden heifers. As neutrophils play an important role in the development of clinical DD, migration of cells from heifers with differing genetic merit for DD resistance was also investigated to potentially link genotype to phenotype. A total of 320 heifers were examined over a four-month period and their clinical phenotypes classified. Associations between the DD index and DD incidence were investigated using logistic regression models. Animals were grouped into terciles based on their estimated breeding values for the DD Index (high, medium, or low genetic merit). Cells from 3 heifers each with either high genetic merit that were healthy (HGH), low genetic merit that were infected and then healed (LGH), and low genetic merit that were chronically infected (LGC) were used for migration assays. Primary fibroblasts cells were generated from a foot skin biopsy and used with homologues granulocytes in a transwell migration assay using fibroblasts infected with *Treponema phagedenis* or various controls. For every 1 SD increase in the DD Index the OR for any, active, chronic and M2 DD lesion presence were 0.66 (95%CI 0.53-0.82), 0.58 (95%CI 0.45-0.73), 0.63 (95%CI 0.51-0.79) and 0.6 (95%CI 0.42-0.84) respectively. Lower numbers of granulocytes migrated when derived from HGH heifers compared to those generated from LGH and LGC, suggesting that genetic factors associated with high merit may influence immune cell function, potentially leading to a more balanced immune response or altered regulation of cell migration. These preliminary findings provide insight into the immune mechanisms associated with the host genetics of resistance to DD.

Digestive health and production consequences of incorporating a rumen buffer in beef fattening diets at moderate and high inclusion rates.

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Beef fattening cattle are fed high-starch diets which provide sufficient energy to optimize animal performance thereby shortening time to slaughter. Transitioning abruptly to high concentrate diets can reduce ruminal pH leading to metabolic disorders such as acute- and subacute-ruminal acidosis (ARA and SARA, respectively). Including pH-buffers in high-concentrate diets can mitigate this reduction, stabilizing ruminal pH, thereby diminishing SARA associated consequences. This study aimed to assess the effect of including a commercially available rumen buffer (Alkacid, HARBRO) at currently advised (30 g/head/day) and high (100 g/head/day) inclusion rates to a barley-based fattening diet on animal production (daily liveweight gain (DLWG), proportion within DLWG target and starch utilization) and digestive health (fecal pH and lipopolysaccharide (LPS)). Cattle were fattened in four pens across two experimental periods (2022 and 2024) on a commercial fattening unit (Aberdeenshire, Scotland). Fecal samples were collected from observed defecations from 5 animals on 3 days; start of transition (day-1), end of transition (day-21), post-transition (day-42) for fecal analysis. Weight data was collected from individual animals at approximately three-week intervals until slaughter. Comparisons between treatment groups were performed using linear mixed effect models which included pen in experimental period as random variable for all variables except LPS (t-test) as only a single repeat of this outcome variable was performed. Fecal pH and starch did not differ between treatment groups on day 1 and day 3 although a tendency towards significant was observed on day 2. Fecal LPS differed between treatment groups on day 2 only. Mean DLWG and proportion of animals within DLWG targets did not differ between treatment groups except that fewer animals in the T group were below DLWG target between day-2 and day-3. This study suggests but does not provide conclusive evidence that the addition of a rumen buffer to a beef fattening diet at a high inclusion rate results in positive production and digestive health consequences.

Enhancing IgG goat colostrum determination using colour-based techniques and data science.

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Newborn goats require adequate immunoglobulin G (IgG) concentration for immunity, primarily obtained through colostrum ingestion due to minimal antibody transfer during gestation. Standard techniques for measuring IgG, like ELISA or RID, are reliable but often unaffordable for small-scale farmers. This study explores a practical, cost-effective alternative, combining colour analysis with machine learning models to estimate IgG concentration in goat colostrum. By focusing on the relationship between colostrum colour and IgG concentration, this research introduces a predictive model feasible for farm use, enhancing decision-making processes in colostrum quality assessment. The dataset comprised colostrum samples from Majorera goats, which were analysed for colour attributes (using the CIE LCh colour space) and IgG concentration. Two regression models, a decision tree and a neural network, were trained on these data to evaluate their predictive capabilities. The decision tree model demonstrated higher performance, achieving an accuracy of 98.16% and a sensitivity of 100% in distinguishing high from low IgG concentrations based on a 20 mg/mL threshold. This performance exceeded that of a previous study using multiple linear regression, indicating the enhanced predictive power of these machine learning approaches for biological data. Both models provided results closely matching ELISA-derived IgG concentrations, validating their effectiveness as accessible, on-farm tools for IgG assessment.

These findings support the potential of machine learning to advance colostrum quality evaluation, offering small-scale farmers a cost-effective and reliable method for IgG prediction. By integrating data science techniques with a colour-based approach, this methodology not only aids in improving animal welfare by ensuring timely IgG monitoring but also fosters sustainable livestock management through reduced dependency on laboratory testing.

Field bean (*Vicia faba* L.) to spring barley (*Hordeum vulgare* L.) production in Scotland: a life cycle assessment.

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The urgent need to reduce greenhouse gas emissions has increased interest in legume-based cropping systems. Among the various crop-legume species available, the cool-season species *Vicia faba* L. (cv. minor; field beans) has attracted significant interest. Assessment of the environmental impact reduction potential of field beans in a crop-sequence (rotation) is understudied. Data from a long term (2009-2023) field bean experimental platform (the Centre for Sustainable Cropping, CSC; James Hutton Institute, Scotland, UK) were analysed using attributional life cycle assessment (aLCA). This focused on elaborating the impacts of a spring field bean to spring barley (*Hordeum vulgare* L.) cropping sequence. Field beans were shown to decrease the environmental impact of spring barley cropping in four of the five two-year crop-sequence periods. Of the 6 impact categories reported here (namely acidification, climate change (GWP100), eutrophication (freshwater), ozone depletion, resource use (fossils), water use), field beans reduced impacts by an average of 25% across the sequences. Additionally, spring barley yields increased by an average of 1.2 t/ha following field beans, except in drought conditions. A combined index analysis revealed that a combination of humidity, maximum temperature, and previous field bean yields were the strongest determinants of spring barley production. The integration of field beans to barley-dominated rotations may be of interest to major industries seeking to improve value chain sustainability, through further investigation of field bean performance in drought conditions is required.

Investigation of livestock transport trailers as potential fomites for antibiotic-resistant *Escherichia coli*.

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Dairy cattle and calves are regularly transported to auction markets, calf rearers, and slaughterhouses by livestock transport vehicles from 12,000 dairy farms in the United Kingdom. UK Government guidelines require transport vehicles to be cleaned and disinfected within 24 hours of use or before re-use within that period. It is feasible, however, that if cleaning fails to eradicate bacteria then transport vehicles can act as a fomite in the spread of antibiotic-resistant (AMR) pathogens. The purpose of this pilot study was to assess the contamination levels of trailers used for calf transport by *Escherichia coli*, a critically important foodborne pathogen, before and after the application of disinfection with FAM30.

Calves were transported for 40-60 minutes from the dairy farm (n = 7) to the University of Liverpool. Within 20 minutes of unloading the trailer was cleaned with a pressure washer, and disinfected. Swab samples from five sites within the trailer were collected from a total of nine trailer-loads before and 30 minutes after application of the disinfectant. Two university-owned trailers (2.4x1.5m and 3.7x1.8m) were used. A bacterial count for *E. coli* was performed through growth on selective agar for each site and trailer-load. Species identification was confirmed by MALDI-TOF for a subset of twenty isolates selected for antibiotic susceptibility screening, and broth microdilution minimum inhibitory concentration assay was performed for a panel of both veterinary and human antibiotics. *E. coli* were recovered from all trailer-loads, both before and after disinfection. The sites with highest level of contamination were underneath side rails and the trailer ramp. However, bacterial load was reduced for several sampled sites following disinfection. Furthermore, high levels of resistance to several antibiotic classes, namely β -lactams (21%), tetracyclines (42%) and sulphonamides (89%) was observed. Twenty percent of screened isolates were also classified as multidrug-resistant (i.e. resistant to at least one antibiotic from three or more antibiotic classes).

This study demonstrated that livestock trailers can harbour zoonotic pathogens with AMR properties. Disinfection is an important step in reducing, but not eliminating, bacterial populations in livestock trailers.

Unlocking dairy potential: Insights into drinking water minerals composition effects on lactating cow performance.

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The aim of this study was to measure the effect of drinking water salinity and hardness (calcium and magnesium) on lactating cow performance. Genetic selections, optimizing feed, inseminations, and housing conditions are among the major strategies aimed at improving production and profit in the dairy industry, with a great number of studies focusing on those topics. Absent from the list is a strategy that explores the ability to control the mineral composition of drinking water. The common practice is to ensure unlimited access to fresh water, and as long as the water is not too loaded with dissolved solids (below 1000 ppm), it is considered safe to drink. However, water is one of the most consumed nutrients required for life, and lactating cows consume it in large volumes, diverting it to milk, urine, feces, and evaporation.

After constructing experimental systems capable of controlling and measuring the drinking of individual cows and executing two independent trials (four by four Latin square), we found that lactating cows (Israeli Holstein) drank approximately 142 liters in an average of eight drinking events per day, ranging from a few liters to up to 83 liters in a single event. Cows consumed from 120 to 160 liters per day, with intake positively related to feed intake (R-squared equals 0.66). Results of the first trial, conducted in summer 2022, examined the effect of drinking water salinity by adding sodium chloride to create water with electrical conductivities of 400, 600, 800, and 1000 μS per cm. This analysis revealed an increased milk yield of up to two kg per day with higher water salinity. In our second trial, conducted in summer 2023, we found that increasing drinking water hardness to levels of 80, 160, 240, or 360 ppm (calcium carbonate equivalent) by adding 46, 92, or 138 mg per liter of calcium chloride and magnesium sulfate (with a calcium-to-magnesium ratio of two to one) raised milk yield by up to 2.2 kg per day. These findings suggest the potential of utilizing drinking water to enhance dairy production, warranting further research in this area.

The effect of neonatal calf diarrhoea with and without dehydration and calf characteristics on saliva pH and conductivity.

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Neonatal calf diarrhoea (NCD) affects an average of 48% of calves in the first 9 weeks of life (1). This study aimed to assess the relationship between saliva parameters and naturally occurring dehydration in calves with NCD. One hundred and forty-one group housed dairy-bred calves had access to seven litres of milk replacer daily using an automatic milk feeder and ad libitum access to water, concentrate and straw. Calves left the trial at 26 days of age or after an episode of NCD. Daily health scoring including tail, perineum and hindleg cleanliness (CLEAN, 0-3), skin tent elasticity and capillary refill time, Wisconsin calf health score (HEALTH) (2) and faeces score (0-3) where possible. NCD was classified as a faeces score of ≥ 2 or a CLEAN score of ≥ 2 . Dehydration was classified by a return of the skin tent of >3 seconds. Saliva was collected on the day of recruitment and approximately weekly thereafter using the Salivette saliva collection system and saliva pH and conductivity measured using portable meters. Non-diseased samples that were taken within two days of the development of NCD or where HEALTH was intermediate or diseased (≥ 4) were removed. Samples were classified as being 'NCD without dehydration' (NCD-H), 'NCD with dehydration' (NCD-D) or 'healthy'. General linear mixed models tested the fixed effect: disease status, sex, sire breed type, age, the interaction between disease and age, age at inclusion into the group pen, and date. Saliva pH was lower in NCD-H ($p < 0.01$) and NCD-D ($p < 0.01$) calves compared to healthy calves (Figure 1). However, NCD-D calves were not different from NCD-H calves ($p > 0.05$, Figure 1). Saliva conductivity had no relationship with NCD or dehydration. In conclusion, saliva pH shows some promise as a pen side diagnostic for NCD with and without dehydration.

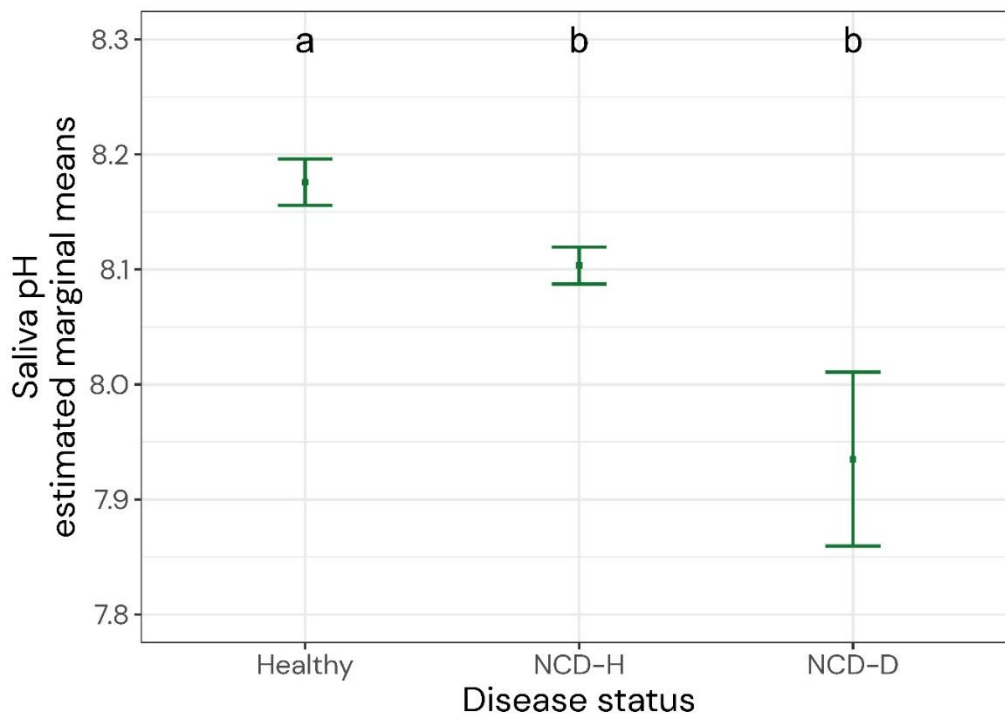


Figure 1: The effect of neonatal calf diarrhoea and dehydration on saliva pH. Differing letters indicates significance

(1) Johnson et al, 2017, Vet Rec Open, 4, e000226

(2) McGuirk 2008, Vet Clin North Am Food Anim Pract, 24, 139-53

Sustainable worm control with 'PASTURE' (parasite amplicon sequencing to understand resistance emergence).

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The cost of helminth infections in European dairy cattle was recently estimated at €941 million per annum, due to reduced productivity and the cost of anthelmintic treatment. These impacts of helminth infections are amplified by the global emergence of anthelmintic resistance to all three broad-spectrum anthelmintic classes licensed for use in cattle against gastrointestinal nematodes (GIN).

Diagnosis of resistance currently relies on the faecal egg count reduction test (FECRT); however, this is an indirect measure of parasite survival and can only detect late-stage resistance. Genetic markers are highly sensitive and can be used to assess resistance status without animal handling or drug treatment. Currently, genetic markers are only available for benzimidazole and levamisole resistance, and the genetic variants conferring macrocyclic lactone resistance are unknown. We developed a next-generation sequencing panel designed to amplify ten validated or putative resistance loci in *Ostertagia ostertagi* and published primers for surveillance of GIN community composition. This panel was used to generate PCR amplicons from pooled populations of ~1,000 larvae from eight Scottish dairy farms.

In total, 13 GIN species were identified, the most prevalent being *Cooperia oncophora* and *O. ostertagi*, present on all farms. Benzimidazole resistance was only detected on one farm; the F200Y variant was observed in 2% of reads before treatment and at 60.5% post-benzimidazole treatment. For levamisole, the resistance marker S168T in the *acr-8* gene was identified on two farms. This variant was present at a very low frequency and neither farm had reported levamisole use. These preliminary results suggest that neither levamisole nor benzimidazole resistance is currently widespread in Scottish dairy calves; this may reflect the greater reliance on macrocyclic lactones. However, the detection of benzimidazole resistance mutations at a high frequency on one farm should be a clear reminder that resistance variants are rapidly selected by drug treatment. This is the first report of S168T in UK cattle, and while the variant is currently at an extremely low frequency if the situation in cattle mirrors that of sheep, S168T will continue to circulate after levamisole treatment.