Summary of PhD thesis

Investigation of environmental diagnostic and herd health monitoring options for the prevention of multifactorial respiratory diseases in calf herds

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Budapest, 2024

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1. Background and objectives of the doctoral thesis

1.1. Background

The bovine respiratory disease complex (BRDC) is one of the main causes of losses in the dairy industry. As a multifactorial disease, viral and bacterial infectious agents, individual resistance, microclimate, husbandry and management factors are all decisive factors in its development. The latter factors play a role in the survival and spread of pathogens and, in case of increased stress, weaken the overall resistance of calves and the efficiency of respiratory defense mechanisms. The main risk factors include group housing and large group sizes, poor litter, direct contact with older animals, adverse weather conditions (heat, cold, snow, frost) and inadequate air quality such as high humidity, increased concentrations of particulate matter and harmful gases. Herd size can also have some impact if farmers are less conscious and spend less time observing animals in larger herds.

Aerosols in livestock buildings can also play an important role in BRDC as they consist of feed, litter, organic matter from animals (e.g. epithelial cells, hair, urine, faeces), microorganisms and toxins. Due to their size, PM10 and PM2.5 particles are dominant as they irritate the conjunctiva and respiratory tract if inhaled. In addition, PM2.5 particles accumulate in the lung parenchyma, thereby leading to serious respiratory and systemic diseases. High temperature, low humidity, air movement (especially drafts) and increased activity of animals promote drying out of litter and dust formation, as well as the particles become airborne. Bioaerosols in the livestock farm environment can serve as vectors for microorganisms, and although most of them are non-pathogenic, they can burden the defense mechanisms of the respiratory system. Therefore, the aerial germ load (CFU/m^3) can also be used as an air hygiene marker. Among the gaseous pollutants in the barn air, ammonia is of the greatest importance, as it directly damages the respiratory epithelium, and

tissue changes in the lungs can be detected by ultrasound examination even at 4 ppm concentration.

Nowadays, a wide range of clinical diagnostic methods and tools are available in veterinary clinics, but our possibilities are limited in farm conditions, especially in terms of early detection of diseases. Detailed clinical examinations of large numbers of animals are limited due to lack of time, manpower and work organisation. Even in the case of individual tests, lack of appropriate conditions such as examination room or restraining equipments (e.g. examination stand or headlock) can be a common problem, but it can also be a problem to quickly send the collected samples to the laboratory. On the other hand, timely clinical and laboratory diagnostic tests are essential for the treatment of diseases.

1.2. Objectives

In our research, we examined whether continuous, real-time measurements are suitable for monitoring the long-term dynamics and daily patterns of critical microclimatic factors, as well as

the relationships of individual parameters, and thus serve as a basis for the development of a precision livestock farming (PLF) system that can be used to predict BRDC.

Our main hypothesis was that with strict control of the environment and the use of certain clinical and laboratory diagnostic tests, the detection of disease symptoms and the duration of any necessary interventions could be shortened, animals are exposed to adverse effects for less time, thus the number of diseases (primarily respiratory, BRDC) decreases.

Our main objectives were:

1. Examining the applicability of environmental diagnostic PLF technologies in different calf rearing environments.
2. Monitoring critical environmental factors and detecting critical values related to BRDC.
3. Search for specific patterns of these factors and examining the related technological processes.

4. Comparison of different calf rearing buildings in terms of environmental factors.
5. Monitoring of a group of calves reared in different calf rearing buildings during the calf rearing period: perform clinical and laboratory diagnostic tests that can be organised and carried out under farm conditions to investigate applicability in herd health diagnostic.
6. Based on clinical and laboratory diagnostic results, comparison of calf rearing technologies of different buildings.
7. Compare environmental- and herd health diagnostic results and make recommendations for the development of monitoring systems for prevention and timely detection of BRDC.

2. Methods

2.1. Investigation of environmental monitoring options

We examined microclimatic and air quality parameters under different calf rearing conditions between July 2019 and February 2020 in a large-scale dairy farm in Central Hungary. Our studies were carried out paralell or partially paralell during the given period with mounted monitoring devices suitable for real-time measurements (U1, U2 and U3) in individual calf hutches (ICH, U2), group calf pens (7GCP, U2), experimental group calf hutches (GCH, U1) and in a conventional group calf barn (CGB, U3).

All mounted devices were capable of measuring temperature (T), relative humidity (RH), wind speed (WS) and PM2.5 and PM10 particulate matter concentrations, while the device installed in the CGB (U3) unit also measured carbon monoxide (CO), carbon dioxide (CO2), hydrogen sulfide (H2S), ammonia (NH3) and methane (CH4) concentrations.

During the winter months, point measurements were regularly carried out with a handheld particle counter to examine T, RH and particulate matter [PM2.5 and PM10 concentrations in μg/m^3 and 0.3 – 10.0 μm particle number (pcs/m^3) according to size] and an impaction air sampler to measure the aerial germ load of the air (CFU/m^3).

We prepared descriptive statistics about our observations by locations and microclimatic parameters. During the inductive statistical analyses, the correlations of microclimatic parameters were examined by Pearsson correlation, and a linear model was prepared to compare the individual calf rearing units. To compare the values measured with the handheld particle counter and the aerial germ load of the air between the individual rearing units, an ANOVA test was performed.

2.2. Investigation of herd health monitoring options

Clinical and laboratory examinations were conducted between September 2019 and February 2020, in wich 28 Holstein-Friesian female calves born in September were included according to their order of birth. We wanted to explore the biological responses of calves to environmental changes through scheduled examinations, which were carried out a total of 7 times according to the milestones of the husbandry technology, from 24-72 hours to 150 days of age, and the health status of the calves was checked three times a week. The entire series of examinations took a total of about half a year, thus covering the period of calf rearing in the autumn and winter seasons.

All animals were kept in the same housing technology system until weaning (until 5 weeks of age in outdoor ICHs, between 5 and 12 weeks of age in outdoor 7GCPs), while after weaning, two groups of calves (12-17 weeks old) were formed to investigate

the impact of two different housing conditions on calf health: 16 calves were placed in CGB (CON = control group) and 12 calves in GCHs (EXP = experimental group). Finally, to further investigate the effects of different husbandry techniques on later life, individuals from both groups were housed in the CGB building (17-22 weeks old).

The clinical examinations of calves were based on regular rectal body temperature measurement. In addition, we looked for the presence of characteristic symptoms of BRDC and other calf diseases, which were recorded in a binary system (present/absent). During scheduled examination sessions, the body weight of the calves was measured. Activity (lying time) was also investigated at the time of weaning and during the first days spent in CGB (CON) and GCH (EXP) enclosures.

Passive transfer of maternal antibodies was examined from serum samples by digital Brix refractometer at 24-72 hours of age. Haematological examinations (quantitative and qualitative blood

count) were performed to assess inflammatory processes and possible stress effects. Virus neutralization (VN) serological tests were performed during scheduled sampling to check maternal protection and detect viral infections contributing to respiratory diseases (BHV-1, PI-3, BRSV and BVDV). In order to identify bacterial pathogens causing BRDC, nasal swab samples were collected for microbiological culture and PCR during specified scheduled sampling and in case of clinical symptoms, while faecal samples were collected to assess protozoal infection.

Body weight (t-test) and activity data (t-test and paired t-test) were compared in CON and EXP groups. We prepared descriptive statistics about clinical and laboratory diagnostic results. Based on the Brix and VN titre values, the characteristics of colostrum supply and maternal protection (Spearman correlation, two-sample t-test) were analysed.

Based on the clinical examination results, disease categories were determined, their frequency

was compared by chi-squared test (fit test) in CON and EXP groups. The number and proportion of calves belonging to each disease category were determined in different calf rearing periods, then the severity of diseases in CGB and GCHs was compared using a nonparametric Kruskal-Wallis test.

Herd-specific hematological reference ranges were calculated from blood count data of healthy calves (t-distribution, with a 95 % confidence interval).

3. New scientific results of the thesis

3.1. Environmental monitoring

1. In common technological systems of calf husbandry, precision, real-time monitoring of microclimatic factors can be used to identify farm-specific patterns, long-term and intraday periods exceeding limit values and daily fluctuations of individual parameters.

2. In outdoor small-group calf rearing units in autumn and winter, the frequency of risky periods from BRDC point of view depend: in case of > 80 % relative humidity on the month, in case of >0,2 m/s and >0,6 m/s air movement on the location, while in case of >45 µg/m3 PM10 and >15 µg/m3 PM2.5 particulate matter concentrations as well as in case of >85% relative humidity on both month and location.

3. The uncovered and unshaded small group calf rearing unit is significantly draughtier and has a significantly lower proportion of periods of high humidity compared to the covered and shaded outdoor small group unit.

4. In different calf rearing units, weak negative correlations were observed between relative humidity and air movement, particulate matter and temperature, as well as particulate matter and air movement. Particulate matter concentration has a weak positive correlation with relative humidity.

3.2. Herd health monitoring

5. In the approximately 1.5-2 month period following weaning, the different husbandry did not significantly affect body weight gain, but the number of cases associated with more severe febrile conditions (>39.5 °C) were significantly higher in conventional housing compared to small group housing.

6. A moderate positive correlation was found between serum Brix values measured after colostrum intake and initial PI-3V and BRSV antibody titre values.

7. Haematological reference ranges were established for calves without clinical signs from neonatal to 5 months of age in 6 age groups.

4. Publications related to the thesis

4.1. Papers published in peer-reviewed scientific journals

1. Sáfár, J., Hejel, P., Vass-Bognár, B., Kiss, L., Könyves, L., 2024.: Long-term monitoring of environmental risk factors of bovine respiratory disease complex in different dairy calf rearing conditions. **Accepted for publication in Acta Veterinaria Brno.**
2. Sáfár, J., Hejel, P., Vass-Bognár, B., Kiss, L., Könyves, L., 2024.: A szarvasmarhák légzőszervi betegség komplexe (BRDC) klinikai- és labordiagnosztikai vonatkozásai a telepi állatorvosi gyakorlatban - Irodalmi összefoglaló. **Magyar Állatorvosok Lapja** 146, 215-230.
3. Sáfár, J., Hejel, P., Vass-Bognár, B., Kiss, L., Seregi, B., Könyves, L., 2023.: The impact of environmental factors on bovine respiratory

disease complex in dairy calves - a review. **Acta Vet. Brno** 92, 213–231.

4.2. Publication of results at scientific conferences

1. Sáfár, J., Hejel, P., Vass-Bognár, B., Kiss, L., Brydl E., Könyves, L..: Long-term monitoring of environmental risk factors of bovine respiratory disease complex in different dairy calf rearing conditions. **XXIII: Middle European Buiatric Congress, 2024. 04. 23-27., Brno.** Proceedings Book, University of Veterinary Sciences Brno, p. 77., 2024.
2. Sáfár J., Hejel P., Bognár B., Kiss L., Seregi B., Könyves L.: Hagyományos és kiscsoportos borjúnevelő egységek klimatikus jellemzőinek leíró elemzése egy Holstein-fríz állományban. **A Magyar Buiatrikus Társaság 30. Jubileumi Nemzetközi Kongresszusa 2022. 03. 20-23., Eger**. Proceedings, Magyar Buiatrikus Társaság pp. 57-64, 2022.

3. Sáfár J., Hejel P., Bognár B., Kiss L., Könyves L., 2022: Hagyományos és kiscsoportos borjúnevelő egységek klimatikus jellemzőinek leíró elemzése egy holstein-fríz állományban. **MTA Akadémiai Beszámolók, Budapest**, **2022**, 2021. évi 48. füzet
4. Sáfár J., Hejel P., Kiss L., Könyves L., 2021: Holstein-fríz borjak egyes légzőszervi vírusokkal szembeni maternális védelemének időbeli változása. **MTA Akadémiai Beszámolók, Budapest, 2021**, 2020. évi 47. füzet