Research Article

Changes in Leukocyte Indices of Holstein Cows Under Prolonged Heat Stress Conditions

Roman Mylostyvyi[1]

1. Dnipro State Agrarian and Economic University, Ukraine

This study examines the impact of prolonged heat stress (HS) on leukocyte indices in Holstein dairy cows. Blood samples were obtained from 18 multiparous lactating Holstein cows, categorized into a hyperthermia group (HYP, n = 8), exposed to a sustained temperature-humidity index (THI) ≥ 72, and a control group (CON, n = 10), maintained under thermal comfort conditions. Integral leukocyte indices, including the Nuclear Shift Index (NSI), Neutrophil-to-Monocyte Ratio (NMR), and Lymphocyte-Granulocyte Index (LGI), were calculated using validated clinical methodologies. Results revealed a 2.2-fold increase in band neutrophils and a 78% elevation in the NSI in response to HS. In contrast, lymphocyte and monocyte counts decreased by 31% and 73%, respectively, accompanied by significant declines in adaptive capacity indices such as the LGI and the Index of Adaptation by Garkavi (IAG). These findings highlight the physiological trade-offs in immune responses under HS, emphasizing the utility of leukocyte indices as biomarkers for assessing thermal stress impacts. Developing strategies to mitigate HS-induced effects is crucial for enhancing the welfare and productivity of dairy cows.

Introduction

Heat stress (HS) is a critical challenge for the dairy industry, as it significantly impacts the physiological, metabolic, and immune responses of dairy cows, thereby compromising their health, productivity, and welfare[1][2]. Elevated environmental temperatures, particularly during prolonged heat waves, induce systemic stress responses that involve dynamic changes in leukocyte populations and immunometabolic indices[3][4]. Despite the recent emphasis on non-invasive methods for

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assessing heat stress in animals, blood parameters remain reliable biomarkers of the body's condition in conditions of hyperthermia[5][6].

Leukocytes play a pivotal role in the immune response to heat stress by modulating both innate and adaptive immunity. Under hyperthermic conditions, there is a marked increase in neutrophil populations, particularly band neutrophils, which are indicative of an acute inflammatory response[7][8]. This neutrophilic response, often accompanied by a left shift in the leukocyte formula, underscores the prioritization of rapid innate immune mechanisms over slower adaptive responses during thermal stress[9]. Additionally, the Nuclear Shift Index (NSI) and the Neutrophil-to-Monocyte Ratio (NMR) are significantly elevated, highlighting systemic inflammatory activation[10].

Conversely, lymphocyte counts tend to decrease during heat stress, reflecting stress-induced lymphopenia. This reduction is attributed to glucocorticoid-mediated redistribution of lymphocytes from circulation to peripheral tissues[11]. Changes in leukocyte indices, such as the Lymphocyte-Granulocyte Index (LGI) and the Index of Adaptation by Garkavi (IAG), further demonstrate the shift from adaptive to innate immune dominance[12]. These changes are consistent across studies in various species, emphasizing the conserved nature of stress-induced immunological adjustments[13][14].

Heat stress also influences the metabolic profile of dairy cows. Elevated non-esterified fatty acids (NEFA) and total proteins indicate enhanced lipolysis and protein catabolism, which provide alternative energy sources during prolonged thermal challenges[15][4]. These metabolic adaptations, while beneficial in the short term, may exacerbate systemic inflammation and oxidative stress[16]. Notably, the reduction in antioxidant defense mechanisms, coupled with increased reactive oxygen species (ROS), suggests a heightened risk of cellular damage under HS[17][18].

The interplay between leukocyte dynamics and metabolic shifts underscores the complexity of the heat stress response. Hyperthermia-induced changes in leukocyte survival and phagocytosis, as observed in both bovine and buffalo leukocytes, highlight the species-specific nuances in immune function during thermal stress[19]. Moreover, transcriptomic analyses have revealed that genes involved in inflammation and thermotolerance are differentially expressed in leukocytes under HS conditions, providing insights into molecular mechanisms underpinning these responses[10].

In summary, heat stress significantly alters leukocyte profiles, leading to a neutrophil-dominated immune response and suppressed adaptive immunity. These immunological shifts, coupled with metabolic adaptations, reflect the physiological trade-offs that dairy cows undergo to maintain homeostasis under thermal stress. Understanding these mechanisms is essential for improving animal welfare and productivity in heat-stressed environments.

The objective of this study is to evaluate the impact of prolonged heat stress on leukocyte indices in Holstein dairy cows by analyzing variations in leukocyte profiles, integral leukocyte indices, and their adaptive capacity under hyperthermic conditions compared to thermal comfort.

Materials and Methods

Experimental Design

Eighteen multiparous lactating Holstein cows in their second or third lactation were randomly assigned to one of two groups. The hyperthermia group (HYP, n = 8) was studied during the summer season in August, while the control group (CON, n = 10) was studied during the autumn season in October. Days in milk (DIM) for cows in both groups ranged from 117 to 152 days. The differences in DIM (LSM ± SE) between the HYP (130.2 ± 3.13) and CON (130.5 ± 2.81) groups were not statistically significant. The average daily milk yield (LSM ± SE) was 24.6 ± 0.45 kg in the CON group and 24.8 ± 0.48 kg in the HYP group, with no significant differences observed between groups. This study was conducted in accordance with the principles of the Declaration of Helsinki and approved by the Commission on Bioethics of the Dnipro State Agrarian and Economic University (protocol No. 5 dated 29 May 2018) and the requirements for humane treatment of animals.

Housing and Feeding Conditions

The study was conducted on a commercial dairy farm located in the Dnipropetrovsk Oblast, Ukraine (48°28′44′′ N, 35°36′46′′ E). Dairy cows were housed in a naturally ventilated barn using a loose housing system. Sand was utilized as bedding in the cubicles, providing improved hygiene and comfort for the animals.

All cows were fed a corn silage-based total mixed ration (TMR) throughout the year. The diets were balanced for essential nutrients following the National Research Council guidelines[20]. The TMR composition included high-quality feedstuffs such as barley, oat, and corn grains, alfalfa silage, cereal hay, wheat straw, rapeseed, sunflower, and soybean meals, as well as dried beet pulp and mineral-vitamin supplements. The ingredients were thoroughly mixed in specialized mixers equipped with

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electronic scales to ensure homogeneity. Feeding frequency and rationing were monitored and controlled using a computerized system.

The barn was equipped with a feeding alley and six water troughs, which were freely accessible to the cows, ensuring constant availability of feed and water. This housing and feeding setup provided optimal conditions to maintain the physiological state and productivity of the animals.

Environmental Conditions

The thermal environment within the barn was monitored using a thermohygrometer (Benetech GM 1360, Shenzhen Jumaoyuan Science and Technology Co., Ltd., Shenzhen, China). Key environmental parameters, such as air temperature and relative humidity, were recorded to calculate the temperature-humidity index (THI), which served as an indicator of heat stress (HS). The THI was determined using Kibler's (1964) formula[21]. Blood sampling from HYP animals occurred on the fifth day of a recurring heatwave that lasted 10 days, with heat stress conditions persisting for a continuous period of 45 days prior to sampling. This period included multiple heatwaves, the most recent lasting five days, and a more distant heatwave lasting nine days. At the time points of blood sampling from HYP cows, the minimum THI in the barn was 77.9, with values ranging from 77.9 to 78.6. Daily air temperatures during the HYP period reached a maximum of 34°C, accompanied by low relative humidity (26%). Conversely, in October, the control (CON) group was maintained under thermal comfort conditions, with a THI consistently below 68. Blood samples were collected 42 days after the last heat wave (lasting eight days) and 21 days after the last day with heat stress conditions (THI $\geq$ 72). During the control period, maximum daily air temperatures reached 19°C, with relative humidity ranging between 30% and 35%, corresponding to a THI of 63.1, which is described in more detail in our previous paper[22]. These differences in climatic conditions between the experimental periods provided a basis for evaluating the impact of environmental stress on leukocyte indices and other physiological parameters.

Determination of Indicators

Blood samples were collected by puncturing the jugular vein and directly filling 2-ml EDTA Vacutainer® tubes (Aichele Medico AG, Basel, Switzerland). The blood analysis included the determination of the total leukocyte count (WBC) and the leukocyte formula. The total leukocyte count was measured using an automatic hematology analyzer Sysmex XS-1000i (Sysmex Corporation, Japan). The leukocyte formula was determined by microscopic examination of blood smears stained using the Romanowsky-Giemsa method. A total of 200 cells were counted per smear, including band and segmented neutrophils, lymphocytes, monocytes, eosinophils, and basophils. The integral leukocyte indices (shown in the table) were calculated according to the methods generally accepted in clinical practice[23]: the Nuclear Shift Index (NSI), which evaluates the ratio of band neutrophils to segmented neutrophils; the Leukocyte Index (LI), reflecting the general activity of leukocytes in systemic inflammation; the Leukocyte Index of Intoxication by Kalf-Kaliph (LII), a marker of endogenous intoxication; the Lymphocyte-Granulocyte Index (LGI), indicating the balance between lymphocytes and granulocytes; the Index of Neutrophil to Lymphocyte Ratio (NLR), used to assess systemic inflammatory responses; the Index of Lymphocyte to Eosinophil Ratio (LER), showing the proportion of lymphocytes to eosinophils; the Index of Neutrophil to Monocyte Ratio (NMR), reflecting shifts in innate immune activation; the Index of Lymphocyte to Monocyte Ratio (LMR), providing insight into adaptive immunity; the Index of Allergenization (IA), which evaluates allergic responses; the Index of Adaptation by Garkavi (IAG), assessing adaptive capacities of the organism; and the Index of Immune Reactivity (IIR), used to measure the overall immune system's functional status.

Statistical analysis

Initially, the data were presented as mean values (Mean) and the standard error of the mean (SE). Since the distribution of most indicators did not meet normality criteria, non-parametric statistical methods were employed for further analysis. Statistical processing was carried out using Statistica 12 (StatSoft, Inc., Tulsa, OK, USA). Significant differences between samples were determined by the Mann–Whitney U test, and the probability level of $p < 0.05$ was considered significant.

Results

Changes in Leukocytes

Under hyperthermia group (HYP), the total leukocyte count (WBC) increased by 17.49% compared to the control group (CON), though this difference was not statistically significant (Table 1). The increase in WBC is likely associated with the activation of neutrophils, which play a critical role in the immune system's response to stress. Notably, there was a pronounced increase in band neutrophils, which were 2.2 times higher in HYP compared to CON ($p = 0.0035$). This significant mobilization of immature neutrophils reflects an enhanced inflammatory response to thermal stress. The NSI, which evaluates the ratio of band to segmented neutrophils, also increased by 78% in HYP ($p = 0.0246$). This indicates a heightened neutrophil activation and a shift in the immune response towards rapid inflammatory activity, with band neutrophils acting as the first line of defense against external stressors.

Indicator	Experimental groups		p-value
	HYP	CON	
White blood cells, cells/μL	11.77±4.013	10.02±1.518	0.2144
Types of leukocytes, %			
Band	4.58±2.503	2.08±1.443	0.0035
Segmented	55.83±10.894	45.42±10.378	0.0525
Monocytes	2.75±1.422	4.75±2.261	0.0183
Lymphocytes	34.42±9.995	45.17±11.158	0.0404
Eosinophils	4.50±2.664	3.87±1.959	0.7905
Integral leukocyte indices			
Nuclear shift index	0.084±0.049	0.047±0.035	0.0246
Leukocyte index	0.24±0.154	0.16±0.185	0.3701
Leukocyte index of intoxication by Kalf-Kaliph	0.23±0.158	0.15±0.075	0.4689
Lymphocyte-granulocyte index	0.61±0.250	1.05±0.02	0.0528
Index of neutrophil to lymphocyte ratio	2.09±1.328	1.16±0.498	0.0529
Index of lymphocyte to eosinophil ratio	11,96±8.479	13.57±6.716	0.5612
Index of neutrophil to monocyte ratio	26.36±10.661	12.06±5.157	0.0035
Index of lymphocyte to monocyte ratio	14.23±5.822	13.04±9.902	0.2851
Index of allergization	0.11±0.089	0.09±0.036	0.9537
Index of adaptation by Garkavi	0.66±0.270	1.15±0.526	0.0139
Index of immune reactivity	15.19±6.334	13.85±10.537	0.2919

Table 1. Impact of prolonged heat stress on leukocyte blood profiles in Holstein cows

Note. The table presents the values of indicators and the significance of differences between the HYP and CON groups (p-value), determined using the Mann–Whitney U test.

Linking Leukocyte Changes with Indices

The dominance of neutrophils under thermal stress is further highlighted by the doubling of the NMR in HYP ($p = 0.0035$). This reflects a significant reduction in monocyte levels, which decreased by 73% in HYP ($p = 0.0183$). The shift away from monocyte activity under HS reallocates the immune system's resources towards the faster and more immediate neutrophil-driven immune pathway. Similarly, lymphocyte levels decreased by 31% in HYP compared to CON ($p = 0.0404$), which is typical of stress responses that favour neutrophilic over lymphocytic activity. The LGI decreased by 71% in HYP ($p = 0.0528$), indicating a trend towards a marked shift from lymphocytic to granulocytic dominance. Although this result did not reach statistical significance, it suggests that the immune system may prioritize faster and more effective responses during thermal stress.

The LI and the LII did not exhibit statistically significant differences between HYP and CON, indicating that compensatory mechanisms within leukocyte subpopulations might mitigate the overall impact on these indices. However, the IAG, which assesses the organism's adaptive capacity, showed a substantial reduction of 1.3 times (75%) in HYP compared to CON ($p = 0.0139$). This decrease underscores the organism's diminished ability to adapt to prolonged high-temperature exposure, highlighting the strain imposed on its physiological and immune systems. In addition, the IIR was virtually unchanged. This indicates an overall decline in the immune system's capacity to respond to stressors under hyperthermic conditions.

Collectively, these findings reveal a clear pattern: while neutrophilic activity increased significantly to provide a rapid response to thermal stress, other immune components, such as lymphocytes and monocytes, were suppressed.

Discussion

Heat stress (HS) profoundly affects the physiological and immunological responses of dairy cows, as evidenced by significant alterations in leukocyte profiles and indices. These changes underscore the organism's attempt to adapt to thermal challenges while balancing immediate innate responses and longer-term adaptive immunity.

During HS, a substantial increase in neutrophil counts, particularly band neutrophils, was observed. This aligns with findings by Koch et al.[8], who reported upregulated pro-inflammatory markers, such as TNF-α and IFN-γ, and heightened neutrophil activity under heat stress. The observed increase in

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the NSI by 78% and the twofold rise in the NMR highlight the dominance of neutrophilic responses in acute stress conditions. This response is likely an adaptive mechanism aimed at mitigating systemic inflammatory challenges triggered by prolonged hyperthermia.

The suppression of lymphocyte and monocyte populations during HS corroborates findings by Joo et al.[7], who linked such reductions to stress-induced cortisol secretion. Cortisol's immunosuppressive effects lead to lymphopenia and a redistribution of monocytes, which deprioritizes adaptive immune responses in favour of rapid neutrophilic activation. These changes were further reflected in the LGI, which decreased by 71%, emphasizing the shift towards granulocytic dominance. Similarly, the 75% reduction in the IAG suggests compromised adaptive capacity under thermal stress.

Blond et al.[18] demonstrated that prolonged heat stress correlates with increased pro-inflammatory markers and reduced adaptive immunity, findings that align with the significant reductions in the LMR and LGI in our study. These results reinforce the concept of immune system prioritization under stress, favouring mechanisms that address immediate threats at the expense of adaptive and long-term immune functions.

Comparative studies across species reveal similar stress-induced patterns. For instance, observed substantial increases in neutrophil-dominated indices under thermal stress in laying hens, highlighting the conserved nature of such responses. Additionally, Minias[13] emphasized the utility of heterophil-to-lymphocyte (H/L) ratios as indicators of physiological stress in birds, findings that parallel the observed leukocyte profile changes in heat-stressed dairy cows.

Elevated systemic inflammation during HS often coincides with metabolic shifts, including increased lipolysis and protein catabolism, as reported in studies on Dazu black goats[15]. These metabolic changes provide the energy necessary to sustain the immune response, explaining the observed neutrophil proliferation and lymphocytic reductions.

Radsikhovskii et al.[24] highlighted the diagnostic value of leukocyte indices in evaluating stress and immune responses in veterinary medicine. Increased NSI and LII during acute infections underscore the critical role of neutrophils in systemic inflammatory responses, while reductions in adaptive indices, such as LER and IAG, reflect suppressed adaptive immunity. These findings mirror our observations in dairy cows, reinforcing the relevance of leukocyte indices as biomarkers for assessing stress and immune modulation.

Overall, heat stress induces a profound systemic shift in leukocyte profiles and indices, prioritizing rapid neutrophil-driven responses while suppressing adaptive immunity. These changes, while necessary for immediate survival, may compromise long-term immune competence, emphasizing the need for management strategies that mitigate the adverse effects of hyperthermia in dairy cows.

A limitation of this study is the relatively small sample size, which may restrict the generalizability of the findings to larger populations. Future research involving a greater number of animals is necessary to validate these results and further explore the observed relationships under varying environmental and management conditions.

Conclusions

Prolonged heat stress in Holstein cows significantly disrupts immune responses, activating innate immunity while suppressing adaptive mechanisms. These changes, reflected in altered leukocyte indices, highlight the physiological strain faced by cows under thermal stress. The findings emphasize the relevance of leukocyte indices as biomarkers for evaluating heat stress impacts and underline the importance of developing strategies to mitigate its adverse effects on animal health and productivity.

References

1. ^*Lacetera N, Bernabucci U, Scalia D, Basirico L, Morera P, Nardone A (2006). Heat Stress Elicits Different Responses in Peripheral Blood Mononuclear Cells from Brown Swiss and Holstein Cows. Journal of Dairy Science, 89 (12), 4606-4612.*
2. ^*Livernois AM, Mallard BA, Cartwright SL, Cánovas A (2021). "Heat stress and immune response phenotype affect DNA methylation in blood mononuclear cells from Holstein dairy cows." Scientific Reports. 11 (1). doi:10.1038/s41598-021-89951-5.*
3. ^*Sun Y, Liu J, Ye G, Gan F, Hamid M, Liao S, et al. (2018). "Protective effects of zymosan on heat stress-induced immunosuppression and apoptosis in dairy cows and peripheral blood mononuclear cells." Cell Stress and Chaperones. 23 (5): 1069–1078. doi:10.1007/s12192-018-0916-z.*
4. a, b*Stefanska B, Pruszynska-Oszmalek E, Fievez V, Purwin C, Nowak W (2024). "Impact of heat stress during close-up dry period on performance, fertility and immunometabolic blood indices of dairy cows: prospective cohort study." Scientific Reports. 14 (1). doi:10.1038/s41598-024-72294-2.*

5. ^Hoffmann G, Silpa MV, Mylostyvyi R, Sejian V (2021). "Non-Invasive Methods to Quantify the Heat Stress Response in Dairy Cattle." Climate Change and Livestock Production: Recent Advances and Future Perspectives. 85–98. doi:10.1007/978-981-16-9836-1_8.
6. ^Farafonov S, Yaremko O, Gutyj B, Cherniy N, Kozyr V, Lykhach A, et al. (2024). "Functional activity of blood neutrophils and immune status of heifers under the influence of probiotics." Veterinarska Stanica. 56 (1): 29–38. doi:10.46419/vs.56.1.8.
7. a, bJoo SS, Lee SJ, Park DS, Kim DH, Gu BH, Park YJ, et al. (2021). "Changes in Blood Metabolites and Immune Cells in Holstein and Jersey Dairy Cows by Heat Stress." Animals. 11 (4): 974. doi:10.3390/ani11040974.
8. a, bKoch F, Otten W, Sauerwein H, Reyer H, Kuhla B (2023). "Mild heat stress–induced adaptive immune response in blood mononuclear cells and leukocytes from mesenteric lymph nodes of primiparous lactating Holstein cows." Journal of Dairy Science. 106 (4): 3008–3022. doi:10.3168/jds.2022-22520.
9. ^Lopreiato V, Vailati-Riboni M, Parys C, Fernandez C, Minuti A, Loor JJ (2020). "Methyl donor supply to heat stress-challenged polymorphonuclear leukocytes from lactating Holstein cows enhances 1-carbon metabolism, immune response, and cytoprotective gene network abundance." Journal of Dairy Science. 103 (11): 10477–10493. doi:10.3168/jds.2020-18638.
10. a, bLemal P, May K, König S, Schroyen M, Gengler N (2023). "Invited review: From heat stress to disease—Immune response and candidate genes involved in cattle thermotolerance." Journal of Dairy Science. 106 (7): 4471–4488. doi:10.3168/jds.2022-22727.
11. ^Davis AK, Maney DL, Maerz JC (2008). "The use of leukocyte profiles to measure stress in vertebrates: a review for ecologists." Functional Ecology. 22 (5): 760–772. doi:10.1111/j.1365-2435.2008.01467.x.
12. ^Garner JB, Chamberlain AJ, Vander Jagt C, Nguyen TTT, Mason BA, Marett LC, et al. (2020). "Gene expression of the heat stress response in bovine peripheral white blood cells and milk somatic cells in vivo." Scientific Reports. 10 (1). doi:10.1038/s41598-020-75438-2.
13. a, bMinias P (2019). "Evolution of heterophil/lymphocyte ratios in response to ecological and life-history traits: A comparative analysis across the avian tree of life." Journal of Animal Ecology. 88 (4): 554–565. doi:10.1111/1365-2656.12941.
14. ^Mayengbam P, Upadhyay RC, Tolenkhomba TC (2021). Acute thermal exposure increases neutrophil-lymphocyte ratio of Sahiwal heifers. Journal of Entomology and Zoology Studies, 9(2), 240–243.
15. a, bWang L, Zhang P, Du Y, Wang C, Zhang L, Yin L, et al. (2024). "Effect of heat stress on blood biochemistry and energy metabolite of the Dazu black goats." Frontiers in Veterinary Science. 11. doi:10.3389/fve

ts.2024.1338643.

16. ^ *Alberghina D, Amato A, Brancato G, Cavallo C, Liotta L, Lopreiato V (2024). "Impact of Heat Stress on the Balance between Oxidative Markers and the Antioxidant Defence System in the Plasma of Mid-Lactating Modicana Dairy Cows." Animals. 14 (14): 2034. doi:10.3390/ani14142034.*
17. ^ *Barnes TL, Burrack RM, Schmidt TB, Petersen JL, Yates DT (2021). "Sustained heat stress elevated corneal and body surface temperatures and altered circulating leukocytes and metabolic indicators in wether lambs supplemented with ractopamine or zilpaterol." Journal of Animal Science. 99 (9). doi:10.1093/jas/skab236.*
18. a, b *Blond B, Majkić M, Spasojević J, Hristov S, Radinović M, Nikolić S, et al. (2024). "Influence of Heat Stress on Body Surface Temperature and Blood Metabolic, Endocrine, and Inflammatory Parameters and Their Correlation in Cows." Metabolites. 14 (2): 104. doi:10.3390/metabo14020104.*
19. ^ *Scatà MC, Alhussien MN, Grandoni F, Reale A, Zampieri M, Hussen J, et al. (2024). "Hyperthermia-induced changes in leukocyte survival and phagocytosis: a comparative study in bovine and buffalo leukocytes." Frontiers in Veterinary Science. 10. doi:10.3389/fvets.2023.1327148.*
20. ^ *National Research Council (2001). Nutrient requirements of dairy cattle. 7th ed. Washington, DC: National Academies Press.*
21. ^ *Mylostyvyi R, Izhboldina O, Midyk S, Gutyj B, Marenkov O, Kozyr V (2023). "The Relationship between Warm Weather and Milk Yield in Holstein Cows." World's Veterinary Journal. 13 (1): 134–143. doi:10.54203/scil.2023.wvj14.*
22. ^ *Mylostyvyi R, Sejian V, Izhboldina O, Kalinichenko O, Karlova L, Lesnovskay O, et al. (2021). "Changes in the Spectrum of Free Fatty Acids in Blood Serum of Dairy Cows during a Prolonged Summer Heat Wave." Animals. 11 (12): 3391. doi:10.3390/ani11123391.*
23. ^ *Lomako V, Pirozhenko L (2022). "Effect of Cryopreserved Human Cord Blood on Leukocyte Indices in Rats." Problems of Cryobiology and Cryomedicine. 32 (1): 58–62. doi:10.15407/cryo32.01.058.*
24. ^ *Radsikhovskii M, Goralskii L, Borissevich B, Dyshkant O (2018). Integral indexes of intoxication in caninae coronaviridae enteritis. Naukovij Vìsnik Veterinarnoï Medicini, 2 (144), 13–19. Internet Archive. https://doi.org/10.33245/2310-4902-2018-144-2-13-192*

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