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Cardiac autonomic activity has a circadian rhythm in summer but not in winter in non-lactating pregnant dairy cows

Levente Kovács [a,b,*], Fruzsina Luca Kézér [a,b], Ferenc Ruff [c], Ottó Szenci [a,d]

[a] MTA–SZIE Large Animal Clinical Research Group, Üllő-Dóra Major H-2225, Hungary
[b] Institute of Animal Husbandry, Faculty of Agricultural and Environmental Science, Szent István University, Páter Károly utca 1, Gödöllő H-2100, Hungary
[c] Department of Methodology, Hungarian Central Statistical Office, Keleti Károly utca 5-7, Budapest H-1024, Hungary
[d] Szent István University, Faculty of Veterinary Science, Department and Clinic for Production Animals, Üllő-Dóra Major H-2225, Hungary

HIGHLIGHTS

- A circadian profile was observed for every parameter in summer but not in winter.
- Sympathovagal balance shifted towards sympathetic dominance during daytime.
- Nonlinear HRV showed a chaotic behavior of cardiac function during the afternoon.
- Season had an expressed effect on cardiac activity both for daytime and nighttime.
- Results suggest an impaired cardiac autonomic function during daytime in summer.

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ABSTRACT

This investigation was conducted to examine circadian and seasonal rhythms of heart rate and heart rate variability (HRV) by means of hour-by-hour recordings over 24 h in a large population of non-lactating Holstein–Friesian pregnant cows [$N = 56$, summer (June–July); $N = 61$, winter (November–December)]. Data were collected during a 5-day period from each animal. Besides parameters of cardiac autonomic function [the high-frequency (HF) component of HRV and the ratio between the low-frequency (LF) and the HF components (LF/HF ratio)], the RR triangular index and L_{max} were calculated. A clear circadian profile was observed for every parameter in summer. Heart rate elevated gradually with the course of the day from 7:00 to 17:00 o'clock and then slightly decreased from 18:00 to 6:00. Sympathovagal balance shifted towards sympathetic dominance during the daytime (increased LF/HF ratio), whereas parasympathetic activity was predominant during the night (increased HF). L_{max} reflected a chaotic behavior of heart rate fluctuations during the afternoon in summer. Decreased values of RR triangular index indicated a sensitive period for cows between 14:00 and 16:00 o'clock in summer. During winter, except for the RR triangular (RRtri) index reflecting a high overall variability in R–R intervals between 12:00 and 23:00 o'clock, heart rate and HRV showed no periodicity over the 24-h period. The results suggest an impaired cardiac autonomic function during daytime in summer. HF, L_{max} and RRtri index showed seasonal differences for both daytime and nighttime. Heart rate was higher in summer than in winter during the daytime, whereas the LF/HF ratio was higher in winter during the nighttime. Circadian and seasonal rhythms of cardiovascular function are presumably related to the differing temperature, and animal activity associated with summer and winter. As all of the investigated parameters are commonly used in bovine HRV research, these findings have practical implications for behavioral, physiological and welfare studies on dairy cattle.

1. Introduction

A number of biological variables that are influenced by external stimuli and internal homoeostatic control mechanisms including behavior, physiological functions and several biochemical factors, may show fluctuations in the short, medium, and long term [1]. The most common variation is that of a 24-h rhythm, referred to as circadian, which is determined by sunrise and sunset [2]. In mammals, circadian rhythms are the result of both external stimuli and endogenously controlled self-contained homeostatic control mechanisms, i.e. the so-called circadian clocks [3,4].

The cardiovascular system is one of the series of biological systems having rhythmic variations. Cardiovascular function has been described by heart rate variability (*HRV*), i.e., the short-term fluctuations in the successive R–R intervals of the ECG [5–7]. This beat-to-beat variation

* Corresponding author.
E-mail address: Kovacs.Levente@mkk.szie.hu (L. Kovács).

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results from the cyclic interplay of the sympathetic and vagal branches of the autonomic nervous system (*ANS*) [8–10] and other local or systemic control and feedback mechanisms [11,12]. Frequency-domain parameters of HRV enable a detailed assessment of ANS activity in several species [13–16], and it is possible to measure the balance between the two branches [17,18]. Spectral parameters of HRV, i.e. the high-frequency (*HF*) component as an indicator of vagal regulation and the ratio between the low-frequency (*LF*) and the HF band (*LF*/*HF*) are appropriate indices reflecting stress in dairy cattle [19]. For studying the autonomic modulation of cardiac activity, monitoring the rhythmic patterns in heart rate and HRV is of scientific interest in humans [20–22] and in laboratory species in both health and disease [23–26]. The observations made in rodents and humans indicate that cardiac autonomic function is synchronized with the time of day.

The circadian variation of heart rate and arterial blood pressure in farm animal species is well known [27]; however, rhythmic fluctuations in HRV are poorly studied in domestic animals. The few existing studies on the diurnal patterns of HRV are restricted to horses [28] and focus only on differences between the day/night cycle of spectral parameters. To the best of our knowledge, no data are available on hour-by-hour fluctuations in heart rate and HRV in dairy cattle. Since baseline and response values of HRV parameters were established mainly during different periods of the day, without taking into consideration the circadian rhythms of cardiac activity, the interpretation and comparison of results obtained in stress and behavioral studies are sometimes difficult.

According to recent research on humans, geometric [29] and nonlinear [30,31] measures HRV are suitable for chronic stress assessment. The major advantage of geometric methods lies in their relative insensitivity to the analytical quality of the series of R–R intervals [32]. Nonlinear techniques are derived from the chaos theory and the nonlinear system theory [33] and are designed to assess the quality of the R–R time series [34]. Some of these procedures are extremely robust against nonstationary data, which are widespread in physiological time series [6]. Although non-linear and geometric indices are promising tools for the assessment of chronic physiological conditions in cattle [19], up to now only a few studies on the welfare of dairy cattle have used non-traditional components of HRV [35–37].

In their review article, von Borell et al. [18] summarized the unresolved problems in physiological studies on HRV in farm animals. They suggested determining the diurnal variation of cardiac activity and the effects of season on HRV. From that fundamental work, several questions have arisen with regard to these rhythms and their possible influence on cardiovascular function. As extensive research focusing on complex behavioral concerns of HRV in dairy cattle has been started [38,39], it would be urgent to develop and establish standards for HRV analysis. Recent studies on behavioral physiology have performed behavioral tests at different times of the day; however, basal HRV was recorded mostly once a day.

The aim of our study was to examine the possible circadian profile and seasonal differences of heart rate and HRV in dairy cows. Besides the heart rate and the ANS-related frequency domain parameters of HRV, nonlinear and geometric measures were also investigated. We used hour-by-hour analysis for heart rate and HRV parameters, enabling to investigate the 24-h profile in more detail, instead of monitoring only day–night variations.

2. Materials and methods

2.1. Animals and housing

The experiment was carried out at the Prograg Agrárcentrum Ltd. in Ráckeresztúr, Lászlópuszta, Hungary (N47°18′191″ E18°48′336″), which has a herd of 900 Holstein–Friesian cattle. Our study was conducted as part of a larger research project on behavioral and physiological aspects of the transition period in dairy cows. The farm was visited for a 40-day period between June 21 and July 30 [temperature; average/min/max (°C): 21.3/15.6/34.8] and for a 43-day period between November 10 and December 12 [temperature; average/min/max (°C): 3.5/−4.7/8.2] in 2013. One hundred and fifty non-lactating multiparous cows were selected at random from the herd for this study, between 250 and 260 days of gestation. All selected cows were inspected physically before heart rate recording. Twenty-two animals with health problems were not included, in order to help exclude possible causes of stress caused by pathological conditions. Three of the cows were excluded due to technical problems during the measurement (dried-out electrodes, disturbance caused by group mates during data recording). Temperamental animals ($N = 8$) were also excluded from the experiment. Finally, a total of 117 clinically healthy (without any pharmacological treatment, without visible lesions), non-lactating multiparous pregnant cows ($N = 56$, summer; $N = 61$, winter) were included in the study (means ± SD; age = 6.4 ± 1.2 years; parity = 3.6 ± 0.7; BCS = 2.9 ± 0.3).

From approximately 4 weeks before calving, cows were kept in a 40 m × 8 m group pen bedded with deep straw and including 50–60 animals. TMR was provided twice a day at 5:00 and 16:00 o'clock, and animals had free access to water.

2.2. Measurement preparation and R–R data collection

Twenty-four hour R–R interval recordings were obtained using a mobile recording system, which contained a Polar Equine T56H transmitter with two integrated electrodes with a compatible Polar H7 heart rate sensor and a Polar RS800 CX heart rate receiver (Polar Electro Oy, Kempele, Finland). Electrode sites were covered with ultrasound transmission gel (Aquaultra Blue, MedGel Medical, Barcelona, Spain). The electrodes were positioned and fitted to cows as advised by von Borell et al. [18] and the electrode belt was protected against external impacts by a leather girth. For ease of later visual identification, cows were marked on their hind legs and backs at the time of fixing the devices.

After fixing the heart rate monitors, 10 cows were moved from the group pen into a 15 m × 10 m separated experimental area, which had a 3.5 m high solid wall made of wood, facing north. As the experimental area was covered with wooden shade structures, protection from direct solar radiation was provided for the animals at all times. The cows always had visual and auditory contact with animals staying outside the experimental area. Experimental groups were formed 24 h before the start of observations, and heart rate monitors were fixed on the animals at that time to reduce the effects due to adaptation to wearing the devices.

Because of the limited storage capacity of the heart rate receivers for about 25,000 R–R intervals, data were downloaded every 48 h. This procedure lasted approximately 10 min/animal and was performed regularly when animals were standing at the feed bunks. During all times of data downloading the electrodes were covered with extra gel. Except for data downloading and preparation of the electrodes, any practice that could potentially disturb the animals was avoided during the measurement period. R–R data were recorded continuously for 5 days from each animal.

2.3. Behavioral observations

Since HRV differs between standing and lying posture in cattle [36,38], disturbances of the measurements due to physical activity of the cows were excluded by using for later analysis only R–R data recorded during undisturbed lying periods [40]. Criteria of lying were the following: 1) the cow is lying comfortable without any disturbance from her herd mates; 2) the cow finished feeding or walking until 10 min before the start of data recording. During the last 2 min before data recording and throughout the period of interest any kind of disturbances (presence of stockmen, sudden noise) were recorded.