Influence of *Brucella* infection on circadian rhythm of haematological parameters in dairy cows

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SUMMARY

The circadian rhythms have evolved in domestic animals in more different breeding management, and reflect an adaptive mechanism to react in advance to the regular environmental changes. The aim of this research was to evaluate the circadian rhythm of haematological parameters in cows and to study if *Brucella abortus* infection in cows can alterate their rhythmicity. For this purpose, five healthy cows and five *Brucella* infected cows were enrolled. Blood samples were collected in spring (sunrise 06:30, sunset 19.00), every 4 h over a 24 h period, starting at 12:00 on day 1 and finishing at 12:00 on day 2, and a complete blood cell count with differential was performed. One-way ANOVA for repeated measures was applied to evaluate the effect of time on haematological parameters. Using cosinor rhythmometry, four rhythmic parameters were determined: mesor, amplitude, acrophase and robustness. One-way repeated measures Analysis of Variance were applied to evaluate a significant effect of time of day on each parameter. *P* values <0.05 were considered statistically significant. The application of one-way ANOVA showed a statistical significant effect of time of day on haematocrit (PCV) and haemoglobin (Hb) in healthy cows. In *Brucella*-infected cows, statistical significant changes of neutrophils, eosinophils and lymphocytes were observed. Using Cosinor method, the same parameters showed a daily rhythmicity.

Our results showed that a daily rhythmicity exists both for healthy and infected cows, but in different haematological parameters. In particular, hemoglobin, and hematocrit in and neutrophils, eosinophils and lymphocytes in. This study is a preliminary observation in changes of circadian clocks during pathological events in domestic animals. In particular, further investigations should be conducted to evaluate how the molecular clock components are influenced not only by an inflammatory response but contemporarily by a change in L/D cycles.

KEY WORDS

Circadian rhythms; cows; *Brucella* abortus infection; haematological parameters.

INTRODUCTION

The circadian rhythms have evolved in domestic animals in more different breeding management, and reflect an adaptive mechanism to react in advance to the regular environmental changes[1]. The ability to adoptively anticipate predictable changes in the environment, as conferred by the circadian system, is an integral component of homeostasis that interact with the immune system to guarantee survival[2]. The molecular components of the circadian system have been found to be essential for a diversity of basic and homeostatic systems ranging from the control of cell cycle or the regulation of cardiac and metabolic function[3]. The mammalian circadian timing system is composed of many individual clocks, and these are synchronized by a central pacemaker, which coordinates their physiology and behaviour[1]. Evidence suggests that peripheral immune activation may act as a timing signal for circadian clocks in peripheral tissues[4;5]. The synchronization of these cell-clocks results in the generation of rhythmic phenomena which involve the behaviour of both physiological activities and haematological and haematochemical parameters[6]. In peripheral blood, circadian rhythmicity is manifested in term of clock gene expression[7;8] and of circulating levels and function of different cell types[9;10]. Numerous studies have described the effects of photoperiod on the immune system[11]. In addition to signals emanating from the suprachiasmatic nuclei (SCN), clock gene expression in peripheral tissues can also be entrained by pro-inflammatory stimuli[12].

On the basis of recent advances made in large animal chronobiology and the increasing interest on the circadian time-specified treatment and its relevance in veterinary practice, the aim of this study was to investigate the daily rhythm of haematological parameters in healthy cows and in cows infected with *Brucella abortus*, a highly contagious, zoonotic pathogen with worldwide distribution.

MATERIALS AND METHODS

Before the start of the study, a routine screening for the detection of endemic bovine diseases (brucellosis, chlamydiosis, leptospirosis, Q fever, neosporosis, toxoplasmosis, theile-

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riosis, babesiosis, anaplasmosis, bovine viral diarrhoea infection, infectious bovine rhinothracheitis, virus respiratory syncytial bovine infection, bovine parainfluenza 3 and bovine herpesvirus 4 infection) was performed by Istituto Zooprofilattico della Sicilia "A. Mirri" (Italy). In particular, for the detection of antibodies anti-*Brucella abortus* the sera were tested with Rose Bengal (RB) and complement fixation (CFT) tests, the official tests used in the European Union countries[13]. Moreover, for measuring antigen/antibody interaction, the fluorescence polarisation assay (FPA) was performed as a control test. On the basis of screening results two farms, located in the same area (Lat 37° 55', Long 14° 21'), were selected. The animals of the first farm resulted negative for all the diseases tested, while the animals of the second farm were positive only for brucellosis.

Five adult Italian Brown cows for each farm, respectively Group A and B, were enrolled for our research. The animals of Group A were clinically healthy, as determined by history and clinical examination.

All cows were subjected to the same type of management. Blood samples (10 ml) were collected in spring (sunrise 06:30, sunset 19.00), every 4 h over a 24 h period, starting at 12:00 on day 1 and finishing at 12:00 on day 2.

On each animal, in order to allow multiple blood sampling, an intrajugular catheter (Surflo, Terumo Corporation, Japan) was placed on the day prior to the beginning of the study. For the blood collection, two syringes were used: the first one was used to withdraw no less than twice the internal volume of the catheter and the second one was used to withdraw the sample, collected in a tube with K_3 EDTA as anticoagulant. After each blood sampling the catheter was flushed with heparinised solution to maintain its patency.

On the whole blood, a complete blood cell count was performed by means of an automated analyzer for haematology (He Co Vet C, SEAC, Florence). Differential leukocyte count was performed microscopically on Giemsa stained blood smears.

One-way repeated measures Analysis of Variance were applied to evaluate a significant effect of time of day on each parameter. *P* values <0.05 were considered statistically significant. Data were analyzed using the software STATISTICA 7.5 (StatSoft Inc., USA).

Using cosinor rhythmometry, four rhythmic parameters were determined: mesor (mean level), amplitude (half the range of oscillation) acrophase (Φ, time of peak) and robustness. Rhythm robustness was computed as the quotient of the variance associated with sinusoidal rhythmicity and the total variance of the time series[14] Robustness greater than 65% is above the noise level and indicates statistically significant rhythmicity.

RESULTS

The application of one-way ANOVA showed a statistical significant effect of time of day on haematocrit (PCV) and haemoglobin (Hb) in Group A. In Group B, statistical significant changes of neutrophils, eosinophils and lymphocytes were observed (Table 1). Using Cosinor method, the same parameters showed a daily rhythmicity (Table 1 and Figure 1).

DISCUSSION

Our results showed that in healthy cows (Group A) a daily rhythmicity was observed in Hb and PCV, as previously reported[15]. The rhythm showed a diurnal acrophase, observed in the late morning, and a good percentage of robustness, 84 and 70,90% for Hb and PCV respectively. In cows with *Brucella* infection, the daily rhythmicity of Hb and PCV was loss and a daily rhythm of lymphocytes, neutrophils and eosinophils was observed. Some studies demonstrated that mediators of the immune response can influence clock gene expression[3]. Moreover, whereas clinical signs of inflammatory disease display circadian variations, the haematopoietic lineage per se, seems to be regulated in a circadian fashion[16]. The circadian rhythms of the haemato-immune system seem to be synchronized by two clocks, the first one, endogenous, is based on clock gene activity in the SCN. The second one, exogenous, is based on immune stimuli[17;16]. Neutrophils and eosinophils showed nocturnal acrophases and this could be due to the entrainment of endogenous clock that facilitates an alteration in the immune system, which counter external attacks in day time and induces repair and development by night[16;18]. Lymphocytes count showed acrophase in late afternoon. Respect to the other WBC, their rhythms is not driven by the endogenous pacemaker, but it is influenced by cortisol daily rhythm[19].

Blood glucocorticoid concentrations, which increase following inflammation, are believed to regulate 24h patterns of white blood cells clock gene expression and functionality in both rodents and humans[19]. In particular, cortisol has an important immunosuppressive activity and its alternations could be considered the grand of the circadian rhythm in the circulating lymphocyte count[20]. The most important virulence determinant of *B. abortus* is the lipopolysaccharide component of the cell wall of the bacterium, and several monoclonal antibodies, directed against the O-chain of lipopolysaccharide, were shown to protect against infection[21]. We hypothesize that the infection could interfere, not only at level of peripheral circadian clocks, but also in the SCN, and, nevertheless, such interference occurs in the endogenous mechanism, since that the L/D cycles were not altered in our experiment[22].

Table 1 - Statistical analyses (ANOVA and Cosinor) of the significant haematological parameters in both groups (A and B).

	Parameters	MESOR	Amplitude	Acrophase (hours)	Robustness (%)	$F_{(2,4)}$ (ANOVA)	P< (Cosinor)
Group A	PCV (%)	34.09	3.14	10:56	70.90	9.24	0.01
	Hb (g/dL)	14.22	1.46	12.08	84.10	18.11	0.01
Group B	Neutrophils (K/ul)	0.70	0.32	05:23	69.5	10.99	0.01
	Lymphocytes (K/ul)	5.93	0.59	17:37	86.8	28.55	0.001
	Eosinophils (K/ul)	0.87	0.07	23:06	88.0	24.99	0.001

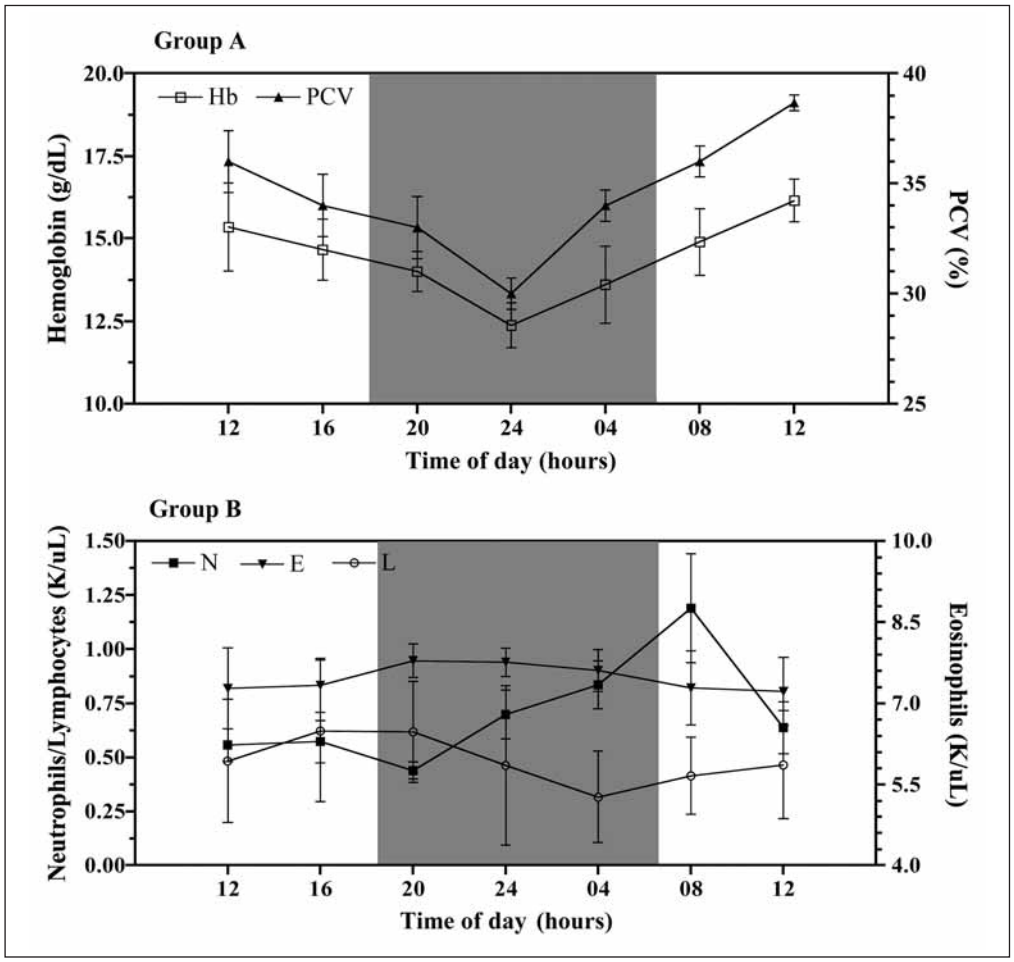

Figure 1
Patterns (Mean ± SD) of the haematological parameters analyzed in two groups of dairy cows (Group A healthy; Group B, infected by *B. abortus*), which showed a daily rhythmicity.

CONCLUSIONS

This study is a preliminary observation in changes of circadian clocks during pathological events in domestic animals. In particular, further investigations should be conducted to evaluate how the molecular clock components are influenced not only by an inflammatory response but contemporarily by a change in L/D cycles. In fact, sometimes the same pathology can alterate the sleep/wake cycles and, of course, all the rhythms of parameters which depends by these.

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