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RESEARCH ARTICLE

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Heart rate and heart rate variability in lambs, measured by biologgers, during transport and its relationship to basal cortisol concentration

Isabella Manenti[a], José A. Abecia[b], Irene Viola[a], Paola Toschi[a], Francisco Canto[c] and Silvia Miretti[a]

[a]Dipartimento di Scienze Veterinarie, University of Turin, Grugliasco, Italy; [b]Instituto de Investigación en Ciencias Ambientales de Aragón (IUCA), University of Zaragoza, Zaragoza, Spain; [c]Centro Regional de Investigación Remehue, Instituto de Investigaciones Agropecuarias, Osorno, Chile

ABSTRACT
In this study, we investigated the relationship between heart rate variability (HRV) and basal cortisol levels in lambs in the course of a short transport event. Fourteen lambs received a subcutaneously implanted biologger that was capable of recording body temperature (T) and HR at one-minute intervals. In addition, lambs wore collars that held triaxial accelerometers, which were used to record locomotor activity (LA) before and during transport (73 km). Saliva samples for cortisol measurements were collected 24 h and 4 h before transport and 1 h post-transport. Based on basal cortisol concentrations, lambs were assigned to one of two groups, low cortisol (LC) or high cortisol (HC). HR and HRV were measured at 1 h before, during, and 1 h after transport. Mean HR was similar at all three points, but HRV variables (SDNN and RMSSD) were higher ($p < 0.01$) during transport than they were 1 h before and 1 h after transport, particularly in the LC group. Accelerometry data revealed that the LC lambs displayed a lower ($p < 0.001$) LA than did the HC lambs before and during transport. In conclusion, lambs that had lower baseline cortisol levels were better adapted physiologically to the stress of transport, as reflected by their greater HRV response and calmer behaviour, than were those who had higher cortisol levels. Subcutaneous biologgers have been shown to be a powerful tool for detecting subtle physiological responses to stressors, even in situations where traditional markers such as HR or cortisol levels do not vary significantly.

HIGHLIGHTS
- Lambs with lower basal cortisol showed higher HRV and calmer behaviour during transport.
- Subcutaneous biologgers detected stress responses not seen in HR or cortisol levels alone.
- High cortisol lambs were more active and less physiologically adapted to transport stress.

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Lambs; biologgers; accelerometer; heart rate variability; locomotor activity

Introduction

From an animal welfare perspective, the transport of live animals, especially livestock such as lambs, is one of the most important stages in the production process. In transport, animals are subjected to various potentially stressful factors; e.g. handling, vehicle movement, a novel environment, or proximity to conspecifics, which can cause a variety of physiological and behavioural responses (Grandin 2014; European Commission 2023). Traditionally, the assessment of stress in ruminants during transport has been based on hormonal measurements such as blood or saliva cortisol concentrations, which have been used extensively to assess activation of the hypothalamic-pituitary-adrenal (HPA) axis (Palme 2019; European Commission 2023). Typically, concentrations of cortisol, a 'stress hormone', are quantified along with other data to assess an animal's welfare state and stress response (Boucher and Plusquellec 2019). Cortisol, which can be produced and secreted into the bloodstream, quickly, is deposited in various biological matrices, which allow a targeted analysis of short-term (acute) and prolonged (chronic) stress based on the matrix chosen (Palme 2012). Typically, however, that approach provides *ad hoc* information and does not accurately reflect the dynamics of the animal's emotional state over time.

CONTACT José A. Abecia alf@unizar.es Instituto de Investigación en Ciencias Ambientales de Aragón (IUCA), University of Zaragoza, Miguel Servet, 177. 50013 Zaragoza, Spain.

Continuous monitoring of physiological variables such as heart rate (HR) and, especially, heart rate variability (HRV) has become reliable measures for assessing stress response non-invasively and in real time (von Borell et al. 2007; Kovács et al. 2014). HRV, the degree of variation in the length of intervals between consecutive heartbeats, reflects the balance between the animal's sympathetic and parasympathetic systems. Typically, an increase in HRV is correlated with an increase in the flexibility of the autonomic nervous system and, therefore, with an increase in the capacity to adapt to adverse situations (Porges 2007).

Technologies such as biologgers can provide accurate continuous recordings of variables such as HR, body temperature (T), and locomotor activity (LA), even during transport, without interfering with the animal's natural behaviour. Those types of recordings offer the possibility of detecting differences among individuals, which is important because not all individuals respond the same way to the same stressful stimulus (Forkman et al. 2007; Van Reenen et al. 2005a, 2005b).

The objective of this study was to document HR and its variability in lambs during short-term transport based on data collected by biologgers and to assess whether these physiological responses are correlated with basal salivary cortisol levels. In doing so, we sought to increase our understanding of the stress response in sheep and provide useful information for the design of management strategies that consider both the environment and individual differences among animals.

Material and methods

Ethical statement

The study was conducted at the experimental farm of the University of Zaragoza, Spain (41° 63 N), following procedures approved by the Ethics Committee for Animal Experiments at the University of Zaragoza (PI29/21). The care and use of animals followed the Spanish Policy for Animal Protection (RD 53/2013), which meets the European Union Directive 2010/63 on the protection of animals used for experimental and other scientific purposes.

Animals, transport, and sensors

To measure changes in body T, HR, HRV, and LA during a transport, 14 lambs (8 males, 6 females) (mean age (± S.D.) = 94 ± 2 d; live weight = 24 ± 2 kg) received a subcutaneous biologger implant (8.3 × 25.4 mm; 3.3 g; DST micro-HRT, Star Oddi, Iceland) (Figure 1), which recorded body T and HR at 1–min intervals. To minimise data storage needs, HRV variables were calculated based on raw ECG data that was recorded every 5 min between 0900 h and 1700 h. The biologgers were sterilised by immersion in a 0.55% ortho-phthalaldehyde solution (CIDEX-OPA, Johnson & Johnson, New Jersey, USA) for 24 h. For the surgical procedure, lambs were placed supine on a stretcher. The site of the incision was anointed by a povidone-iodine soap solution (Betadine Scrub 7.5%, Alcon Laboratories, Inc., Fort Worth, TX), and a local anaesthetic was administered s.c. (1 ml of lidocaine hydrochloride, Anesvet, Ovejero, León, Spain). The biologger was implanted under the skin on the left side of the thorax, and the sensor axis was aligning with the cardiac axis (Figure 2) (Abecia et al. 2022). An incision was made to create a subcutaneous pocket in which the device was housed. The incision was closed by 2 or 3 stitches, and the incision was treated with aluminium spray (Aluspray, Vetoquinol, Madrid, Spain). At the end of the experiment, a similar surgical procedure was performed to remove the biologger (Abecia et al. 2022).

Figure 1. Subcutaneous biologgers (8.3 x 25.4 mm; 3.3 g; DST micro-HRT, Star Oddi, Iceland) and triaxial accelerometers (46 × 33 × 15 mm, 19 g; ActiGraph wGT3X-BT, actigraph, Pensacola, FL, USA) that were used to record body temperature, heart rate,and heart rate variability, and locomotor activity, respectively, in lambs.

Figure 2. Surgical procedures to insert the subcutaneous biologgers (left), and the lambs carrying the collars with the accelerometers, in the lorry (right).

To calculate HRV, the Star-Oddi HRT Analyser software was used, which provides HR (bpm) and HRV based on the variables SDNN (standard deviation of normal-to-normal R-R intervals) and the RMSSD (root mean square of consecutive deviations between normal heartbeats) (ms).

$$\mathrm{SDNN} = \sqrt{\frac{1}{N-1}\sum_{i=1}^{N}\left(RR_i - \overline{RR}\right)^2}$$

$$\overline{RR} = \text{mean of RR intervals}$$

$$\mathrm{RMSSD} = \sqrt{\frac{1}{N-1}\sum_{i=1}^{N-1}\left(RR_{i+1} - RR_i\right)^2}$$

The animals wore a collar that carried a triaxial accelerometer ($46 \times 33 \times 15$ mm, 19 g; ActiGraph wGT3X-BT, Actigraph, Pensacola, FL, USA), which recorded LA (Figure 1 and 2). Those data were downloaded through the ActiLife software (ActiGraph, LLC, Pensacola, FL, USA), which generates three columns of data; i.e. activity in the x-, y-, and z-axes. The activity counts for the three axes were used to create minute-by-minute activity (counts per 1 min intervals) records; i.e. VM, which is the magnitude of the vector that is formed from the combination of the recorded accelerations from the three axes on any device.

The animals were transported in the central part of the lower floor of a two-axle truck to and from the experimental farm at the University of Zaragoza, for a total distance of 73 km at an average speed of 68 km/h. The entire event lasted 2.5 h (including loading and unloading). The truck bed ($5 \times 2.25 \times 2.20$ m) provided 0.32 m^2 per lamb (Consortium of the Animal Transport Guides Project (2017-rev1) 2018). The livestock lorry (IVECO®, Turin, Italy; model ML80E14, 8000 kg capacity) was designed for sheep transport, with two axles and two floors. The vehicle had a hydraulic elevator for loading and unloading animals.

Animals were housed in a 25-m^2 paddock and were fed a 14.8% crude protein concentrate (Cadecor-2, Agroveco, Zaragoza, Spain), and barley straw and water available ad libitum.

Estimation of salivary cortisol

To measure cortisol concentrations, saliva samples were taken 24 h and 4 h before transport, and 1 h after transport. For the purposes of the analysis, lambs were divided into two groups ($n = 7$ each) based on their mean basal cortisol concentrations (i.e. above the mean = high cortisol (HC) group, or below the mean = low cortisol group (LC)), which were based on the two samples that were collected before transport. Saliva samples were obtained by a polyethylene swab (Salivette®, Sarstedt AG & Co., Nümbrecht, Germany), which was kept in the lamb's mouth by a clamp for 3 min to allow for chewing. Saliva was extracted from the swab by centrifugation at 3,500 rpm for 10 min at 5 °C and stored at −20 °C (Manenti et al. 2025).

To measure the cortisol concentrations in saliva, an ELISA immunoassay was performed in duplicate by a commercial DRG Salivary Cortisol ELISA Kit (DRG Instruments GmbH®, Germany), following the manufacturer's protocol. The sensitivity range of the assay was between 0.09 and 30 ng/mL. According to the manufacturer, the kit produces the following cross-reactivities: 23.40% with progesterone, 11.39% with DHEA, 1.97% with androstenedione, and 1.47% with oestriol. Results are expressed as the concentration of cortisol in saliva (ng/mL).

Statistical analyses

The transport protocol involved three phases (1 h before transport, transport, and 1 h after transport), and mean (±S.E.) T, HR, HRV, and LA in each phase were calculated for the two groups. The assumption of normality was verified by Q-Q plots and supported by the results of the Kolmogorov–Smirnov test ($p > 0.05$). The statistical evaluation of the effects of time related to transport (before, during, after) and basal cortisol (LC vs. HC) on the physiological variables was performed based on a multifactorial model, which included these variables as fixed effects and used the Least Squares Method *via* the GLM procedure in SPSS v.29. The sex of the lambs was not considered in the final model, as preliminary analyses indicated it did not have a statistically significant influence on the outcomes measured. Significant differences within the fixed effects were tested by analysis of variance. The general structure of the model is as follows: $y = xb + e$, where 'y' is an Nx1 vector of observations, 'b' represents the fixed effects within the association matrix 'x', and 'e' is the vector of residual effects.

Table 1. Mean (± S.E.) body temperature (T, °C), heart rate (HR, bpm), and HR variability (standard deviation of normal to normal R-R intervals, SDNN, ms; and the root mean square of consecutive deviations between normal heartbeats, RMSSD, ms) in lambs before, during, and after transport (a,b indicates differences among moments, $p < 0.05$).

	Before	During	After
T	38.8 ± 0.1	38.9 ± 0.1	38.9 ± 0.1
HR	124.3 ± 3.1	119.7 ± 4.3	123.5 ± 4.4
SDNN	17.0 ± 4.5^{a}	40.4 ± 8.1^{b}	21.1 ± 7.2^{a}
RMSSD	20.3 ± 5.1^{a}	50.2 ± 11.0^{b}	26.4 ± 9.2^{a}

Results

Mean basal salivary cortisol concentrations of the lambs in the LC and HC groups were 0.460 ± 0.002 and 1.176 ± 0.015 ng/ml, respectively ($p < 0.001$). Mean T and HR did not differ significantly between 1 h before, during, and 1 h after transport (Table 1). HRV was significantly ($p < 0.001$) higher during transport than it was either 1 h before or 1 h after. The increase in HRV during transport was significantly ($p < 0.01$) higher in LC than it was in HC lambs (Table 2). Specifically, SDNN and RMSSD were significantly ($p < 0.01$) higher in LC than they were in the HC lambs during transport. Both groups exhibited a significant ($p < 0.001$) increase during transport, to reach similar values after transport (Table 2). Lambs in the two groups did not differ significantly in body T at the three stages of the experiment. The HR of HC lambs was significantly ($p < 0.05$) lower during transport than it was either 1 h before and or 1 h after.

LA in lambs in the LC group was significantly ($p < 0.001$) lower than it was in the HC group, both before (LC: 71 ± 3; HC: 101 ± 3 counts/min) and during transport (LC: 119 ± 3; HC: 137 ± 3 counts/min). In both groups, LA was significantly ($p < 0.001$) lower before transport than it was during transport. Salivary cortisol concentrations after transport did not differ significantly between groups (LC: 1.382 ± 0.003, HC:1.018 ± 0.012 ng/ml); however, in the LC group, only, cortisol concentrations during transport were significantly ($p < 0.001$) higher than were their mean basal cortisol levels.

Table 2. Mean (± S.E.) body temperature (T, °C), heart rate (HR, bpm), and HR variability (standard deviation of normal to normal R-R intervals, SDNN, ms; and the root mean square of consecutive deviations between normal heartbeats, RMSSD, ms) of lambs that had either a low or a high basal salivary cortisol level, before, during and after a transport (a,b differences within groups among moments; x,y differences within moments between groups, $p < 0.05$).

	Before transport		During transport		After transport	
	Low cortisol	High cortisol	Low cortisol	High cortisol	Low cortisol	High cortisol
T	38.6 ± 0.1	38.9 ± 0.1	38.8 ± 0.1	38.9 ± 0.1	38.8 ± 0.1	39.0 ± 0.1
HR	120.5 ± 3.3	128.0 ± 6.2^{a}	114.3 ± 4.2	123.5 ± 2.7^{b}	120.6 ± 4.4	125.0 ± 4.1^{a}
SDNN	20.3 ± 6.2^{a}	14.1 ± 4.4^{a}	56.4 ± 13.9^{bx}	22.8 ± 4.0^{by}	31.3 ± 11.8^{a}	11.9 ± 2.3^{a}
RMSSD	23.7 ± 6.8^{a}	17.1 ± 6.4^{a}	71.9 ± 18.2^{bx}	27.3 ± 6.4^{by}	39.3 ± 15.9^{a}	14.2 ± 3.5^{a}

Discussion

Animal transport is one of the most stressful stages in the production chain, and understanding how animals respond physiologically to transport is essential to implementing practices that prioritise their well-being. In this study, we quantified cardiac response (HR) and its variability (HRV) in lambs during transport, and assessed their relationship with basal levels of salivary cortisol, a hormone widely recognised as a stress marker (Palme 2019).

Although lamb body T and HR did not differ significantly among the three phases of transport phases, measures of HRV (SDNN and RMSSD) were significantly higher during transport than they were before or after transport, especially in lambs that had lowest basal cortisol concentrations. That difference suggests that LC lambs were better prepared for the challenge of transport than were HC lambs because, typically, high HRV reflects high vagal tone and autonomic flexibility (von Borell et al. 2007; Kovács et al. 2014). The low HRV during transport in HC lambs suggest that they were less prepared to face a stressful situation such as transportation, also associated with a higher level of LA outside and inside the truck. The increase in HRV in lambs in the LC group during stressful conditions suggests that they had a more proactive autonomic response than did HC lambs, which might have mitigating the impacts of acute stress that is associated with transport. Similarly, studies of other species have shown that the animals having the least reactive hormonal profiles tend to exhibit the most stable physiological responses to environmental stimuli (Rousing et al. 2001;Napolitano et al. 2006). Although lambs in the LC group had significantly elevated cortisol levels after transport, this response might reflect normal physiological activation and not necessarily a distress reaction. No subsequent differences in cortisol levels were observed between the two groups, which suggests that recovery was efficient in both groups. Studies have shown that HRV is a marker of stress resilience because low HRV has been correlated with poor adaptability to stressors, and an increase indicates effective physiological regulation (Pulopulos et al. 2018; Mazgelytė et al. 2021).

Although transport did not have a significant effect on lamb body T in our study, other have shown that typically, transported sheep experience an increase in body T because of stress, which can persist for several hours post-transport (Pascual-Alonso et al. 2017). In that study, the internal T in transported ewes increased steadily before it stabilised near the end of the journey and unloading. Subsequently, and up to 24 h after transport, the internal T of ewes returned to pre-transport values (Pascual-Alonso et al. 2017). Elsewhere, we have shown that an elevated HR was correlated with a reduction in subcutaneous T during the unloading procedure in transported lambs (Manenti et al. 2025). Note that, in our study, loading or unloading procedures, were not separated, because of the memory capacity of the loggers to record raw ECG data.

Studies have shown that maternal factors can influence cortisol levels in lambs. Shasa et al. (2015) reported that lambs born to obese mothers exhibited elevated cortisol levels at birth, which might have predisposed them to low HRV in postnatal life. Prenatal exposure to high cortisol levels might have lasting effects on the autonomic regulation of heart function in offspring. Other studies have shown that chronic stress leads to persistently elevated cortisol levels, which are associated with a reduction in HRV. For example, Altın et al. (2016) showed that environmental stressors such as extreme temperature can elevate cortisol levels significantly, which has a negative impact on HRV. In addition, the work of Caroprese et al. (2010) showed that stress-induced increases in cortisol levels can have an adverse effect on the behaviour and physiology, including HRV, of sheep.

In our study, the relatively low LA among lambs in the LC group before and during transport might have been related to their emotional stability and docile temperament. Studies have shown that levels of motor activity can differ based on the animal's personality, which can influence their perception of and ability to cope with potentially stressful environments (Van Reenen et al. 2005a, 2005b; Forkman et al. 2007). In our study, that might have been because those lambs were less reactive, which require the least physiological mobilisation to adapt to the new environment. In contrast, lambs that had the highest basal cortisol levels had the lowest HRV and the highest LA during the transport, which might reflect an increase in the activation of the sympathetic nervous system, a common response to acute stress (Porges 2007). In our study, lambs that had low basal cortisol levels exhibited a greater increase in HRV during transport and a lower LA pre-transport than did lambs in the high cortisol group, which suggests a more composed behavioural state in those lambs amidst the perceived stressor (von Borell et al. 2007).

In both groups, cortisol levels did not increase after transport, which suggest that, although the LC lambs were the most reactive, they managed to adequately modulate their endocrine response. Transport stress can influence the activity of transported lambs; e.g. a reduction in time spent resting immediately after transport (Pascual-Alonso et al. 2017).

The use of subcutaneous biologgers for monitoring physiological variables in animals is a significant advancement in the assessment of stress responses in natural environments. Studies have demonstrated the effectiveness of biologgers in providing continuous and unobtrusive measurements, which are essential for obtaining reliable data on variables such as HR and T (Palacios et al. 2021; Abecia et al. 2022, 2025). We have demonstrated that monitoring HRV *via* biologgers can yield insights into the animal's stress physiological state without the confounding effects that often accompany the more invasive techniques. Those types of approaches, which integrate physiological monitoring technologies such as biologgers and accelerometers, allow for a more detailed account of the nuances of individual responses to stress.

Conclusion

In conclusion, the results of our study have demonstrated the importance of considering not only the group average but also individual differences in physiological and behavioural biomarkers during transport. Lambs that had the lowest basal cortisol levels appeared to have the greatest adaptive capacity, as expressed by a higher HRV and lower LA. These findings highlight the fact that HRV, but not HR, responded significantly to the stressor, reinforcing the view that HRV is a more sensitive and nuanced marker of autonomic regulation under stress conditions. That information will be helpful in moving towards more individualised management, which considers the environment and the characteristics of each animal.

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Disclosure statement

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ORCID

Isabella Manenti http://orcid.org/0000-0002-7110-0960
José A. Abecia http://orcid.org/0000-0003-2827-3054
Irene Viola http://orcid.org/0000-0002-4268-5613
Paola Toschi http://orcid.org/0000-0003-0963-7254
Francisco Canto http://orcid.org/0000-0001-5124-1929
Silvia Miretti http://orcid.org/0000-0002-6693-2527

Data availability statement

Data are available from the corresponding author upon reasonable request.

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