

Vaginally use of iButton® loggers to measure body temperature in grazing red deer (*Cervus elaphus*)

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Abstract. When collecting accurate physiological data, it is important to eliminate human interference in situations when it is necessary to record continuously over several days. Non-invasive methods for measuring body temperature can be useful for monitoring health status and assessing environmental and climatic effects. The aim of this study was to describe a) two methods for automatically recording of body temperature, by means of a vaginal device in grazing red deer (*Cervus elaphus*) and b) the daily body temperature pattern. All animals were sedated with xylazine, and had automatic thermal recording devices (iButton®, Thermochron™, Maxim Integrated, San Jose, CA, USA) inserted into a vaginal sponge (10 animals, 10 days) or CIDR-G (13 animals, 10 days). Devices were programmed to record temperature every 10 minutes. All devices except one corresponding to a sponge were successfully recovered. The devices recorded a rhythmic pattern of body temperature, with the highest value registered during the afternoon and the lowest during the morning. No major inconveniences were observed, suggesting that the iButton® device can be successfully used in cases where records of continuous body temperature recording are required for up to 10 days while avoiding any notable interference.

Keywords: physiological traits; heat stress; circadian rhythm; daily pattern.

Uso intravaginal de registradores iButton para medir la temperatura corporal en ciervos rojos (*Cervus elaphus*) a pastoreo

Resumen. Para recopilar datos fisiológicos precisos, es importante eliminar la interferencia humana y tener un registro continuo durante varios días de ser necesario. Los métodos no invasivos para medir la temperatura corporal pueden ser útiles para monitorear el estado de salud e investigar los efectos ambientales y climáticos. El objetivo de este estudio fue describir a) dos métodos para el registro automático de la temperatura corporal mediante un dispositivo vaginal en ciervo rojo en pastoreo (*Cervus elaphus*) y b) el patrón diario de temperatura corporal. Todos los animales fueron sedados con xilazina y se colocaron dispositivos automáticos de registro térmico (iButton®, Thermochron™, Maxim Integrated, San Jose, CA, USA) adheridos a una esponja vaginal (10 animales, 10 días) o CIDR-G (13 animales, 10 días). Los dispositivos se programaron para registrar cada 10 minutos. Todos los dispositivos se recuperaron con éxito con excepción de uno de los animales con esponja. Los dispositivos registraron un patrón rítmico de la temperatura corporal, con el valor más alto durante la tarde y el más bajo durante la mañana. No se observaron mayores inconvenientes, lo que sugiere que el dispositivo iButton® puede ser utilizado con éxito en los casos en que se requiera un registro continuo de la temperatura corporal hasta por 10 días sin mayores interferencias.

Palabras clave: datos fisiológicos; estrés térmico, ritmo circadiano; patrón diario.

Uso intravaginal de registradores iButton para medir a temperatura corporal em veado-vermelho (*Cervus elaphus*) em pastejo

Resumo. Para coletar dados fisiológicos precisos, é importante eliminar a interferência humana para obter registros contínuos por vários dias. Métodos não invasivos para medir a temperatura corporal podem ser úteis para monitorar o estado de saúde e investigar os efeitos ambientais e climáticos. O objetivo deste estudo foi descrever a) dois métodos de registro automático da temperatura corporal usando um dispositivo vaginal em veado-vermelho (*Cervus elaphus*); e b) o padrão diário de temperatura corporal por 10 dias. Os dispositivos foram programados para gravar a cada 10 minutos. Todos os dispositivos foram recuperados com sucesso, com exceção de um dos animais com esponja. Os aparelhos registraram um padrão rítmico de temperatura corporal, com maior valor à tarde e

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menor pela manhã. Não foram observadas grandes desvantagens, sugerindo que o dispositivo iButton pode ser usado com sucesso e sem grandes interferências nos casos em que é necessário o registro contínuo da temperatura corporal por até 10 dias.

Palavras-chave: dados fisiológicos; estresse calórico, ritmo circadiano; padrão diário.

Introduction

Body temperature is one of the most relevant physiological traits of animals (Godyń *et al.*, 2019). Temperature values may indicate an animal's metabolic state (Burfeind *et al.*, 2014), presence of infections (Lee *et al.*, 2015; Godyń *et al.*, 2019), or the exposure to a challenging thermal environment (Kinahan *et al.*, 2007; De *et al.*, 2017). Rectal temperature is the most reliable indicator of core body temperature. However, the handling practices required to repetitively record rectal temperature may cause serious problems, such as stress-induced hyperthermia and possible animal welfare affectations (Hilmer *et al.*, 2010; Chapon *et al.*, 2012).

Rectal and vaginal temperature have shown to be nearly identical, and their agreement indicates that they are interchangeable (Suthar *et al.*, 2013; Lees *et al.*, 2018); differences between them are insignificant and both values are considered reliable indicators of physiological information (Vickers *et al.*, 2010; Burdick *et al.*, 2012; Maeder *et al.*, 2012; Suthar *et al.*, 2013). Recording body temperature over a prolonged period requires the use of an automated device that once implanted, continues to record without interrupted. A variety of devices fulfill these requirements (Hilmer *et al.*, 2010; Vickers *et al.*, 2010; Burdick *et al.*, 2012; Maeder *et al.*, 2012). Automatic devices for body temperature assessment now provide a valuable tool for researching almost any kind of animal and its habitat (Hartman and Oring, 2006; Hilmer *et al.*, 2010; Signer *et al.*, 2010; Chapon *et al.*, 2012; Roznik and Alford, 2012); however specific techniques for their use must be evaluated.

The iButton® device (Thermochron™, Maxim Integrated, San Jose, CA, USA) is one of the most popular temperature dataloggers, it records and stores data points into an internal memory (Davidson *et al.*, 2003). For continuous body temperature recording, iButton® devices can either be ingested (Kinahan *et al.*,

2007), surgically implanted (Taylor *et al.*, 2004; Hilmer *et al.*, 2010; Lee *et al.*, 2015) or attached to the animal's body surface (Grayson and Dorcas, 2004; Munn *et al.*, 2009; Abecia *et al.*, 2019). In cows, it has been used subcutaneously (Lee *et al.*, 2015) or attached to an intravaginal-intrarectal implant (Lea *et al.*, 2008; Polsky *et al.*, 2017; Lees *et al.*, 2018).

The pattern of daily body temperature is usually defined as a circadian rhythm that extends over an approximate 24 h period. Mean value of body temperature (mesor), together with amplitude of the oscillation and shape (waveform) of the rhythm are also important factors; these parameters may have a strong physiological significance. The rhythm reflects a constant conflict between homeostasis and circadian rhythmicity for control of the animal's core temperature, it is the result of an interplay of mechanisms of heat production and heat loss, controlled by the circadian system (Refinetti, 1992, 2010).

Red deer (*Cervus elaphus*) is a wild ruminant that has been introduced into different regions of the globe. In many of these regions, the species is exposed to thermal conditions that impose a challenge and require monitoring (Mattiello, 2009; Alvarez Ramírez *et al.*, 2021). In these cases, it is necessary to study body temperature without disturbing the animal. In species of this kind, it is not possible to record body temperature using traditional methods, nor has an easy, automatic method that avoids disturbing the red deer been described. The objective of this document was to describe a) the use of the iButton® device to record vaginal temperature in red deer, coupled either to a CIDR or to a polyurethane sponge, and b) assess daily vaginal temperature pattern and its relationship with particular environmental factors.

Materials and Methods

The study was undertaken with the approval from the Animal Care and Use Committee of the Faculty of Veterinary Medicine, National Autonomous University of Mexico (protocol #028).

Location and animals

The study was carried out at a research station located north of Mexico City (Alvarez Ramírez *et al.*, 2021) among a captive population of non-cyclic and non-pregnant adult red deer (1.2 - 2.3 years of age). According to the



farm's management program, animals were maintained in rotational grazing conditions, in 5000 m² alfalfa paddocks (*Medicago sativa*), where no artificial or natural shade was available. Water was permanently provided using plastic 200 L basins.

Part A) iButton® devices in polyurethane sponges

During the month of December, a total of 10 red deer females were vaginally implanted with a polyurethane

sponge containing a data logger (iButton®, models DS-1921G and DS-1921H; Thermochron™, Maxim Integrated, San Jose, CA, USA). Sponges were elaborated using hormone-free commercial sterilized polyurethane. A horizontal line was cut in the middle of the sponge (figure 1A) to enable total introduction of the iButton® (figure 1B), and subsequently this slit was closed with simple stitches, using sterile non-absorbable thread (figure 1C).

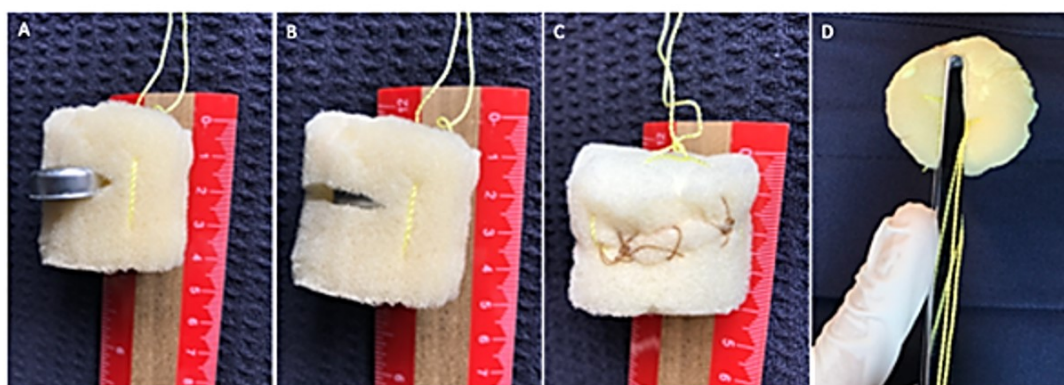


Figure 1. Preparation of polyurethane sponges for insertion of the iButton® device.

Part B) iButton® devices in a CIDR-G

During the month of February, a total of 13 red deer females received hormone-free recycled sterilized CIDR-G devices with an attached iButton® data logger. The middle of the CIDR (figure 2A) was slit open 16

mm. A datalogger was inserted into the slit (figure 2B) and then this portion of the CIDR and the iButton® were covered using Thermofilm tape (Steren® Mexico; figure 2C). Hot air from a hair dryer was applied to cause shrinkage to the Thermofilm, thus firmly attaching the iButton® to the CIDR (figure 2D).

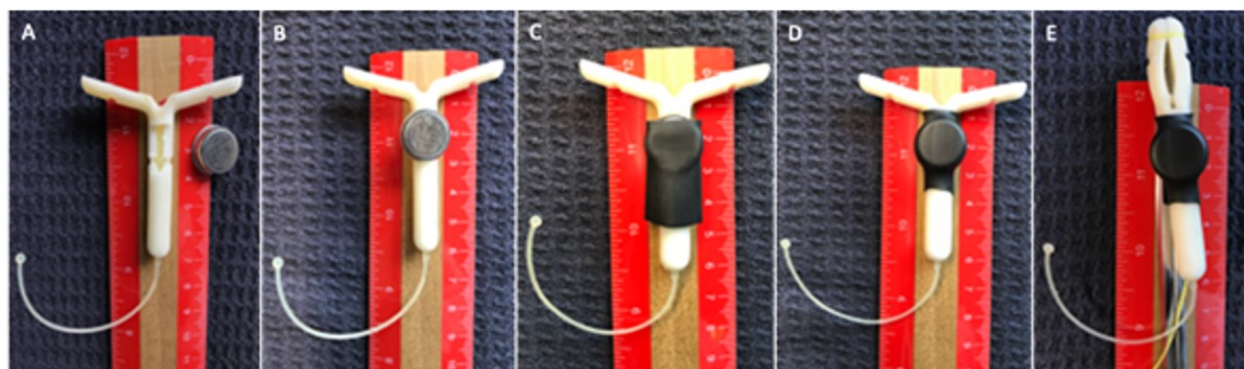


Figure 2. Procedure to adhere an iButton® device into a CIDR-G.

All dataloggers were factory-calibrated and previously programmed to record temperature every 10 minutes. iButtons were not waxed. Sponges and CIDRs were sterilized by autoclaving, prior to their preparation. Once prepared, sponges and CIDRs were smeared with disinfectant-lubricant cream and introduced into the vagina using straight forceps (figures 1D, 2E). The devices were withdrawn after 10 days of effective recording. For the insertion and removal of the devices, all animals were previously sedated with an i.m. injection of xylazine (0.7 mg/kg) (Walsh and Wilson, 2002) within the research station's management pen.

Environmental factors

Solar radiation (SR), environmental temperature (ET), sunrise and sunset time were recorded from an online meteorological station, located at 3 km from the experimental site (www.wunderground.com). During December, average ET was 10.9 ± 0.12 °C with a minimum of 1.3 °C and a maximum of 21.3 °C; during February, average ET was 18.2 ± 0.14 °C (\pm SE) with a minimum of 6.6 °C and a maximum of 27.4 °C. Recorded SR was 329 ± 9.4 w/m² and 492 ± 8.6 w/m² during December and February respectively (mean \pm SE). Average sunrise and sunset time during December were 07:18 h (07:17-07:20 h) and 18:14 h (18:11-18:17 h) respectively.

During February, sunrise occurred at 07:05 h (07:09-07:02 h) and sunset at 18:44 h (18:42-18:46 h).

Data analysis

Data was analyzed using descriptive statistics, cosinor analysis (Molcan, 2019; <https://cosinor.online/>

app/cosinor.php) to explore rhythmicity; Pearson, linear regression, and the Bland-Altman plot (Bland and Altman, 1986; Doğan, 2018) were also used to evaluate correlations and agreement between methods of recording.

Results and Discussion

Little or no reaction to the introduction of the vaginal devices were observed. Following recovery from sedation, some animals were seen to urinate, but then returned to grazing without any other perceived discomfort.

All vaginal devices except one, inserted into a sponge were recovered. On removal, mucous and purulent secretion was observed around the sponges, a common finding when using these devices (Fleisch *et al.*, 2012), but no other complications emerged. No blood was detected on any of the devices. No or little vaginal

discharge was observed on CIDRs at withdrawal. Removal of CIDRs appeared to be slightly easier, probably due to the material from which they are made and to their diminished adhesion to vaginal mucosa, compared to sponges (Fleisch *et al.*, 2012).

One iButton® from the sponge group ceased recording on the last day of the study. Mean body temperature obtained during the 10 days of recording, relating to each vaginal device is presented in figure 3.

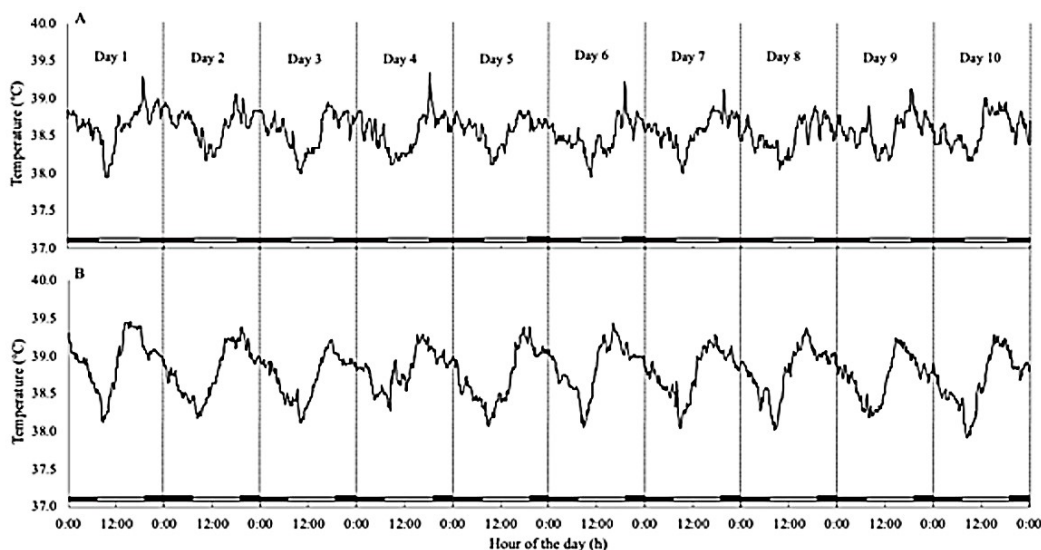


Figure 3. Mean daily pattern of body temperature during ten consecutive days of recording, using the iButton® device embedded in vaginal polyurethane sponges (A) and CIDR-G vaginal inserts (B). The black and white horizontal bars represent the dark and light phases of the prevailing sunrise-sunset cycle.

In both cases, body temperature showed a rhythmic pattern over a period of 24 h ($p < 0.05$), with a mesor of 38.5 and 38.7 °C, an amplitude of 0.22 and 0.38 °C; acrophase occurred at 20 and 19 h, and bathyphase at 8.5 and 7 h of the day for sponge and CIDR devices, respectively (figure 4). Recorded means for vaginal temperatures in both cases (mesor) resemble rumen temperature among red deer living in semi-natural conditions (Turbill *et al.*, 2011) and also body temperature of other domestic ruminants (Jessen and Kuhnen, 1996; Piccione *et al.*, 2003). During both recording periods, the correlation between mean body temperature and ET was positive. A high correlation

was found in February ($r = 0.72$, $p < 0.00001$), when ET was also higher, while the correlation during December was weak and non-significant ($r = 0.14$, $p = 08$).

Daily rhythmicity of body temperature has been documented among a wide variety of species; its specific pattern can be affected by environmental cycles by means of synchronizing the endogenous clock and thus changing its period. Other environmental variations may also modify the circadian rhythm by disturbing its wave form and altering its mesor and amplitude. The light-dark cycle, ET and food availability have been shown to represent some of the

most important factors entraining and affecting daily rhythm of body temperature and its wave form (Rensing and Ruoff, 2002; Mendoza, 2007; Refinetti, 2010). For example, in goats exposed to different ET, circadian rhythm of body temperature is preserved but its amplitude and mean value are significantly affected (Piccione *et al.*, 2005). Furthermore, the diurnal phase is clearly associated with an increment of body temperature, whereas during the night a clear drop has been documented in goats (Ayo *et al.*, 1999; Piccione *et al.*, 2005) and donkeys (Zakari *et al.*, 2018), as in our case. Other factors as locomotor activity, body size and reproductive state can also affect the appearance of the

pattern; however, although changes in these environmental and biological factors can affect body temperature, its circadian rhythm is not a consequence of them (Refinetti and Menaker, 1992). Although our study was not designed to determine the specific influence of those factors on deer body temperature, it shows a clear rhythmic pattern with the lowest and highest values at the beginning and the end of the light phase of the day, respectively (figures 3 and 4). To our knowledge, this is the first report to characterize a circadian rhythmic pattern of body temperature in red deer.

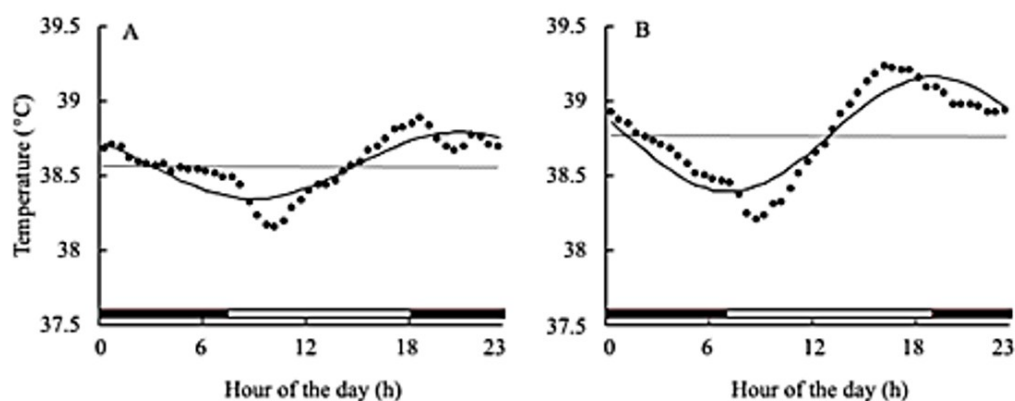


Figure 4. Mean pattern of daily oscillation (real data, •), cosinor curve (-) and mesor (--) of red deer vaginal temperature recorded with iButtons attached to a sponge (A) or to a CIDR-G (B). The black and white horizontal bars represent the dark and light phases of the prevailing sunrise-sunset cycle.

Pearson correlation showed a strong association between the values recorded, using both methods ($r = 0.83$, $p < 0.05$). The regression line indicated a good agreement between both methods ($r^2 = 0.68$; $y = 1.3665x - 13.921$), although the data did not show a perfect dispersion from the line; instead revealing considerable discrepancy, as the use of sponges seemed to show lower estimates than those obtained with CIDR. However, caution should be exercised when making this interpretation, as information was obtained during different periods and in different animals, possibly influencing the values.

The Bland-Altman plot showed the mean bias \pm SD between sponge and CIDR measurements as -0.21 ± 0.17 °C, and the limits of agreement were -0.56 and 0.13 . This means that when the sponge was used, iButton® recordings were 0.21 °C lower on average than those for CIDR (figure 5). As this study did not intend to validate the methods used, no objective recommendation can be given regarding which method is better for reflecting physiological data.

Human presence and direct management can affect an animal's behavioural and physiological traits

(Burdick *et al.*, 2012); which might be more significant in less domesticated animals, such as red deer. Thus, using devices to automatically record animal responses, with minimal human interference, may represent an advantage (Reuter *et al.*, 2010; Angle and Gillette, 2011; Maeder *et al.*, 2012). Using iButtons in vaginal sponges or CIDRs in other animal species like cows, sheep or goats seems totally possible. In cows, successful experiences using the CIDR implant have already been reported (Suthar *et al.*, 2013; Polsky *et al.*, 2017).

Although both vaginal devices performed satisfactorily, the CIDR always proved easier to withdraw. In some cases, the sponges seemed to adhere to the vaginal walls, and in one case a speculum had to be used to remove it completely, a difficulty which was not observed with the CIDRs. This represents a limitation to the use of the iButton® in sponges and CIDRs over longer periods; for reproductive purposes, these vaginal implants have been used for up to 14 days; although in isolated cases, the CIDR was used over a longer period (20 and 45 days), with no reports of negative consequences (Wheaton *et al.*, 1993). However, we do not recommend

the use of either vaginal device for more than 14 consecutive days, as the risk of vaginitis and adhesions increases significantly (Martinez-Ros *et al.*, 2018). When

longer logging periods are required, dataloggers should be implanted using a different technique.

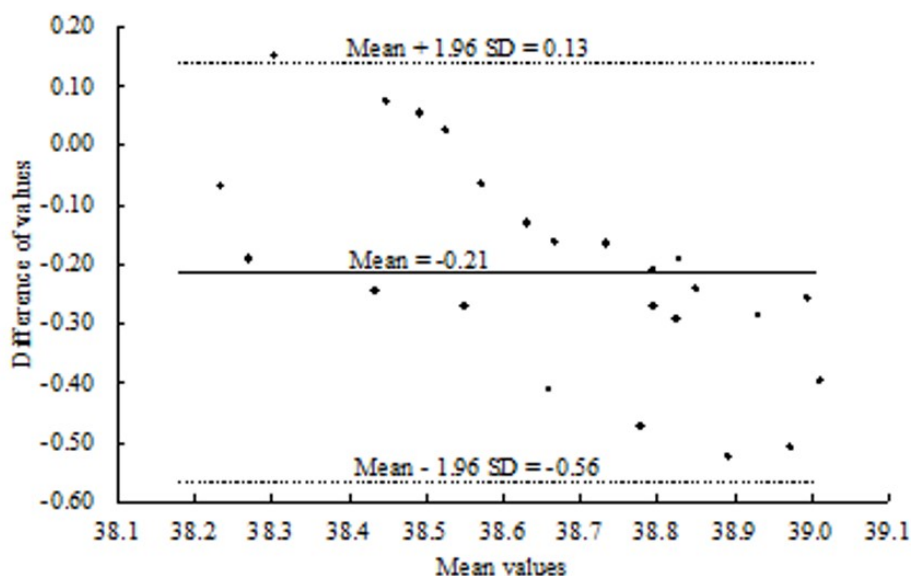


Figure 5. Bland-Altman plot for the agreement of temperature recorded using sponges or CIDR-G vaginal implants.

The iButton® is a datalogger that records date, time and temperature and stores the information in a single chip. This information can be recovered using OneWireViewer® software (Maxim Integrated™, San Jose, CA, USA) and a USB-port adapter. This device can be used in field studies and represents one of several methods for continually recording animal traits (Davidson *et al.*, 2003). These loggers have been used to record body temperature in a variety of ways and

among a number of animal species (intraperitoneal, shrew: Mzilikazi *et al.*, 2002; carapace, turtle: Grayson and Dorcas, 2004; rectal, cattle: Lea *et al.*, 2008; intraperitoneal, feral cat: Hilmer *et al.*, 2010; subcutaneous, calves: Lee *et al.*, 2015; external, sheep: Abecia *et al.*, 2019). To our knowledge, this is the first demonstration that the iButton® can be used vaginally in red deer to successfully measure body temperature for up to 10 continuous days.

Conclusion

In red deer, the iButton® device was successfully used in vaginal implants to continuously record body temperature for a period of up to 10 days. If validated, this procedure may become a valuable research and clinical follow-up tool in various areas of study

focusing on animal populations, where interference should be avoided. Red deer's body temperature showed a rhythmic pattern during the day, with the highest values during the afternoon and the lowest during the morning.

Conflict of interests: None

Ethics statement: The Internal Animal Care and Use Ethics Committee of the Faculty of Veterinary Medicine of the National Autonomous University of Mexico approved the experimental protocol (CICUA #028).

Author contributions: All authors contributed equally.

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