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Analysis of physiological variables of native sheep kept in a climatic chamber under different environmental conditions

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ABSTRACT: The aim of the study was to observe the physiological responses of non-acclimatized native sheep subjected to different environmental conditions in a climatic chamber. Eighteen male sheep, six of each breed, were distributed in an entirely randomized design and submitted to eight treatments (four air temperatures - T20, T25, T30 and T35ºC; and four air temperatures + environmental modification - T20+WA, T25+HF, T30+HF and T35+HF ºC), and 60 to 80% relative humidity. The physiological variables heart rate (HR) and respiratory rate (RR), and rectal temperature (RT), skin temperature (ST) and coat surface temperature (CT) were collected. In general, the physiological responses of the animals showed an interaction between them, where each one of these responses is dependent on each other, and there is susceptibility of these physiological responses to climate change. Understanding the functioning of the physiological mechanisms for heat dissipation, it is possible to adapt the animal management in order to increase production without increasing heat, which may cause stress to the animal.

Key words: adaptation; heat tolerance; homeokinesis

Análises das variáveis fisiológicas de ovinos nativos mantidos em câmara climática em diferentes condições ambientais

RESUMO: O objetivo do estudo foi observar as respostas fisiológicas de ovinos nativos não aclimatizados e submetidos a diferentes condições ambientais em câmara climática. Dezoito ovinos machos, seis de cada raça, foram distribuídos em um delineamento inteiramente casualizado e submetidos a oito tratamentos (quatro temperaturas do ar – T20, T25, T30 e T35ºC; e quatro temperaturas do ar + modificação do ambiente – T20+AM, T25+FC, T30+FC e T35+FC ºC), e 60 a 80 % de umidade relativa. Foram coletadas as variáveis fisiológicas frequência cardíaca (FC) e respiratória (FR), e temperaturas retal (TR), da pele (TP) e superficial do pelame (TS). De maneira geral, as respostas fisiológicas dos animais mostraram uma interação entre elas, onde cada uma dessas respostas é dependente uma das outras, e há susceptibilidade dessas respostas fisiológicas as mudanças climáticas. Entendendo o funcionamento dos mecanismos fisiológicos para a dissipação de calor, é possível adequar o manejo animal de modo a aumentar a produção sem que haja incremento calórico, podendo causar estresse no animal.

Palavras-chave: adaptação; tolerância ao calor; homeocinese

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Introduction

Thermal stress is a major concern in many production systems, not only in warm climates but also in temperate regions, due to increasing temperatures associated with climate change (Yamin et al., 2022; Carabaño et al., 2022). The detrimental effect of extreme temperatures on the energy expenditure of animals is unquestionable, with a consequent increase in production costs.

High temperatures promote several physiological and behavioral changes in small ruminants, including reduced dry matter intake, increased water intake, loss of electrolytes, changes in osmolarity and blood volume, altered microbial profile and rumen activity, and consequent loss of productivity (Ferreira et al., 2021; Sejian et al., 2021).

To ensure the success of the sustainable production system, it is essential to understand how the relationship between the animal and the environment occurs, as well as to know the adaptive mechanisms by which breeds exploit the environment to optimize productive responses according to their genetic potential and improve overall health and well-being (Giannetto et al., 2017; Santos et al., 2021).

Despite the good adaptability of many goat and sheep breeds in extreme environments, the basal energy and metabolism of these animals are influenced by various climatic elements. However, with the advent of global warming, many researchers are looking at how native breeds, which are considered adapted, maintain homeostasis in drylands (Titto et al., 2016; Leite et al., 2021). Raising these animals represents an important economic activity for farming systems in tropical developing countries (Pantoja et al., 2017; Sejian et al., 2021).

Animals respond differently to sudden temperature changes, altering various aspects of their physiology and behavior (Kahwage et al., 2018). These include changes in physiological parameters (Sejian et al., 2021). These parameters can vary within the same species due to factors such as diet, age, physiological state, breed, production level, management, and especially climatic stress (Thornton et al., 2021).

When the effects of thermal stress on these animals are recognized, it is possible to improve the management used, optimizing production with the least possible losses. This information can be used as a technical foundation for the sustainable exploitation of small ruminants, or even to guide a genetic improvement program to obtain breeds that are better adapted to extreme environmental conditions (Avendaño-Reyes et al., 2020; Lima et al., 2022).

The objective of this study was to observe the physiological responses of non-acclimatized native sheep submitted to different environmental conditions in a climatic chamber.

Materials and Methods

The procedures performed in this study were approved by the Research Ethics Committee (Comitê de Ética em Pesquisa - CEP) of the Universidade Federal de Campina Grande, municipality of Campina Grande, PB, Brazil, Protocol CEP No. 097.2019.

Location

The present study was conducted at the Laboratory of Rural Constructions and Ambience (Laboratório de Construções Rurais e Ambiência - LaCRA) (7° 13′ 51″ S, 35° 52′ 54″ W), of the Universidade Federal de Campina Grande, municipality of Campina Grande, PB, Brazil, between the months of May and June 2021.

Animals and accommodation

Eighteen uncastrated male sheep of the Soinga (SOI), Morada Nova (MN), and Santa Inês (STI) breeds were used, six animals of each, with an average age of 4.0 ± 0.5 months and an average weight of 15.00 ± 3.60 kg, kept inside a climate chamber. The animals were dewormed at the beginning of the experiment, kept in collective stalls with dimensions of 1.60 × 2.85 m in length and width (4.56 m^2 of area), provided with feeders and drinkers, with a floor covered with wood shavings, where each stall housed six animals of the same ecotype. The animals were distributed in an entirely randomized design, with an 8 × 3 factorial scheme, with eight treatments (four air temperatures and four air temperatures + environment modification), 3 breeds (Soinga, Morada Nova, and Santa Inês), and 6 repetitions (animals of each breed).

Temperatures

The proposed air temperatures were determined based on the thermal comfort zone (TCZ) for sheep that is between 20 and 30 °C, with relative humidity at 60% (Baêta & Souza, 2010; Eustáquio Filho et al., 2011), proposing four controlled average temperatures added of environmental modifications, obtaining eight thermal conditions: T20 (20 °C, threshold temperature between thermal comfort zone and thermal stress by cold), T25 (25 °C, thermal comfort zone), T30 (30 °C, threshold temperature between thermal comfort zone and thermal stress by heat), and T35 (35 °C, above the TCZ). An environmental modification was added to each temperature, where at T20 the animals were wetted in order to further reduce the temperature of the medium (T20 + WA); at temperatures T25, T30, and T35 an extra heat source was added, simulating the heat produced by absorbing solar radiation (T25 + HF; T30 + HF, and T35 + HF).

To wet the animals, the water was deposited in a container with a spray bottle and volumetric identification (1 L) to ensure that the amount of water was the same for the three groups and, every 30 minutes, the animals were wetted again, preventing them from drying out beforehand of the period of experimentation of the temperature in question, being the average of water used of 12 L applied in each group per day, quantifying 2 L/animal. As heat sources, a 250W LED infrared lamp was added to each stall. In all environments the average wind speed averaged 0.5 m s^{-1}.

Experimental procedures

For each thermal condition, no adaptation period of the animals to a controlled environment was adopted, with data collection being performed over a period of three days for each treatment. In the interval between treatments, the 18 animals were exposed to ambient air temperature and relative air humidity (with the chamber open) for the restoration of their physiological functions, for two days. Thus, taking into consideration the days of treatments and the days of restoration of physiological functions, the experiment had a total duration of 38 days.

At each study stage in the climatic chamber, the animals were subjected to a 6/18 h cycle (experimental air temperature/ambient air temperature). The chamber was always turned on at 7 a.m., and the first hour was used to stabilize the temperature and relative air humidity inside the chamber. After stabilization, the experimental period began at 8:00 a.m. and lasted until 2:00 p.m.

The feed was supplied to the animals in two schedules, 7:00 a.m. and 6:30 p.m., avoiding the influence of caloric increment in the collections, water was supplied ad libitum, and the feed offered to the animals was composed of Tifton hay (*Cynodon dactylon* (L) Pers), which constituted 39.94% of the total volume of the ration, ground corn (43.41%), soybean meal (11.15%), urea (0.89%), calcitic limestone (0.89%), and vegetable oil (3.59%), according to the composition indicated by the NCR (2007).

Environmental variables

The air temperature (AT, °C) and relative air humidity (RH, %) were controlled and monitored through a microcomputer with the aid of free software SITRAD® connected to a controller type MT-530 PLUS from Full Gauge Controls®. The controller received the average temperature and relative air humidity data from the sensors, thermistor, and humidiostat, respectively, every 15 minutes, checking and controlling these variables so that they always remained in the desired control range (Setpoint).

Data on air temperature, relative air humidity, black globe temperature (BGT, °C), dew point temperature (DPT, °C) were stored in a HOBO U12-012 ONSET Comp® datalogger, with an external and an internal channel coupled to a black globe placed at a height similar to that of the animals (1 m from the ground), in each stall. Data were taken and stored daily every 30 minutes throughout the experimental period.

The temperature and humidity index (THI) and the black globe temperature and humidity index (BGTHI) were used to evaluate the level of heat stress induced by the environment to the animals and calculated using the equation reported by Ravagnolo et al. (2000) and Buffington et al. (1981), according to Equations 1 and 2, respectively:

$$\mathrm{THI} = (1.8\mathrm{AT} + 32) - [(0.55 - 0.0055\mathrm{RH}) \times (1.8\mathrm{AT} - 26)] \quad (1)$$

where: THI - temperature and humidity index; AT - air temperature (°C); and, RH - relative air humidity (%).

$$\mathrm{BGTHI} = \mathrm{BGT} + 0.36\mathrm{DPT} + 41.5 \quad (2)$$

where: BGTHI - black globe temperature and humidity index; BGT - black globe temperature (°C); and, DPT - dew point temperature (°C).

The comfort/thermal stress ranges experienced were classified according to Silanikove & Koluman (2015) who defined THI ranges classified as 74 or less (comfortable), 75-79 (moderate stress), 80-85 (stressful), 86-88 (very stressful), and 88 or more (extreme suffering). BGTHI values of up to 74 indicate comfortable conditions, 74 to 78 indicate alert conditions, 79 to 84 indicate dangerous conditions, and 84 and above indicate emergency conditions (Buffington et al., 1981).

Physiological variables

On each treatment day, three measurements of physiological variables were taken at 10:00 a.m., 12:00 a.m., and 1:00 p.m., when the animals were already under the influence of the experimental temperature.

The surface coat temperature (CT, °C) was obtained as the arithmetic mean of the temperatures of the cervical (CTce, °C), thoracic (CTth, °C), and gluteal (CTgl, °C) regions of the animals using an infrared thermometer (ST-900 Incoterm, Porto Alegre, RS, Brazil). Skin temperature (ST, °C) was also obtained by the arithmetic mean of the temperatures of the cervical (STce, °C), thoracic (STth, °C), and gluteal (STgl, °C) regions of the animals in trichotomized areas, with the aid of an infrared thermometer (ST-900 Incoterm, Porto Alegre, RS, Brazil). Rectal temperature (RT, °C) was recorded using a clinical thermometer (Instrutherm, São Paulo, SP, Brazil) inserted into the rectum with minimal disturbance of the animal, remaining inserted until the reading stabilized.

Respiration rate (RR) was measured by visually observing rib movement for 15 seconds at 1 m distance, extrapolating to one minute, and these data were expressed as movements per minute (mov min^{-1}). Heart rate (HR) was measured by counting the heartbeats with a flexible stethoscope over a period of 15 seconds, and extrapolated to one minute, these data were expressed as beats per minute (beats min^{-1}).

Statistical analysis

Data were analyzed by analysis of variance (ANOVA) and F test using the ExpDes.pt package of the R statistical software version 3.4.1. The probability value denoting statistical significance was stated at $p < 0.05$. Paired comparisons were performed by Tukey test.

The data were subjected to the exploratory Principal Component Analysis (PCA) procedure. The choice of Principal Components (PCs) was based on eigenvalues greater than one ($\lambda > 1.0$) according to Kaiser (1958) criterion, and explaining a percentage greater than 10% of the total variance (Hair Jr. et al., 2009).

Results and Discussion

There was a gradual increase in AT and BGT. The BGT (Table 1) showed values above the recommended for the

Table 1. Mean values of air temperature (AT, °C), black globe temperature (BGT, °C), relative air humidity (RH, %), BGTHI, and THI, recorded in the treatments.

Treatment	AT	BGT	RH	BGTHI	THI
T20+AM	20.96	21.06	80.22	68.87	68.29
T20	21.22	21.64	79.44	69.40	68.71
T25	24.47	24.57	82.12	73.68	74.23
T25+FC	25.08	27.92	75.11	76.74	74.46
T30	29.50	29.61	80.08	80.34	82.01
T30+FC	29.06	32.69	72.90	82.78	80.36
T35	31.41	31.37	75.18	82.41	84.19
T35+FC	32.80	35.69	74.91	87.36	86.52

species (Eustaquio Filho et al., 2011), which shows the high absorption of radiant heat energy from the medium. The RH, on the other hand, remained high in all treatments, a fact that can be explained by the lack of air circulation inside the climatic chamber, which has wind speed values below 1 m s^{-1} (Miranda et al., 2018).

The THI in the treatments T20+WA, T20, T25, and T25+HF, recorded values close to or equal to 74, an environment classified as comfortable (Silanikove & Kolumam, 2015), suffering a gradual increase according to the increase in AT, reaching values above 80 in the treatments with higher temperatures T30, T30+HF, T35, and T35+HF, which characterizes these environments as stressful (T30, T30+HF, T35) and extreme suffering (T35+HF) for the animals.

The values recorded for BGTHI, presented a gradual increase as a function of the increase in AT, in front of the treatments, the BGTHI values also increased, reaching a very high value in T35+HF, characterizing an emergency situation. In the treatments T20+WA, T20, and T25, recorded values close to 74, environment classified as comfortable (Buffingtom et al., 1981).

The Principal Component Analysis (PCA) extracted two components in the order of their importance to explain 94.1% of the total variance observed (Table 2). The first plot represents principal component 1 (PC1), with 81.9 % of the total observed variance, composed of the variables HR, RR, and RT. The second plot represents principal component 2 (PC2), with 12.2 % of the total variance observed and formed by the variables ST, STce, STth, STgl, CT, CTce, CTth, and CTgl. Considering the variables of highest importance within each principal component, HF (0.711), RR (0.520), and RT (0.299) represent PC1, while the variables ST (0.323), STce (0.304), STth (0.323), STgl (0.322), CT (0.328), CTce (0.326), CTth (0.315), and CTgl (0.327) represent PC2 (Table 2).

With these results it was possible to generate the two-way graph of the factorial plane, defined by the coordinates of two principal components (Figure 1). In this sense, the primary heat stress detection variables (HR, RR, and RT) (Maia et al., 2015), are associated to the right of the horizontal (X) axis of PC1, while variables related to animals skin and coat temperature (ST, STce, STth, STgl, CT, CTce, CTth, and CTgl) are associated with the vertical (Y) axis of PC2.

The correct functioning of the physiological pathways involved in the allocation of energy resources during growth

Table 2. Correlation between original variables and principal components, eigenvalues, explained and accumulated variance of the first two principal components (PCs 1 and 2) among different environmental conditions under the physiological responses of native sheep.

Variables evaluated	Principal components	
	PC1	PC2
HR - heart rate	0.711	0.167
RR - respiratory rate	0.520	0.239
RT - rectal temperature	0.299	0.280
STce - skin temperature of the cervical region	-0.114	0.326
CTce - coat surface temperature of the cervical region	-0.186	0.304
STth - skin temperature of the thoracic region	-0.057	0.315
CTth - coat surface temperature of the thoracic region	-0.168	0.323
STgl - skin temperature of the gluteal region	-0.085	0.327
CTgl - coat surface temperature of the gluteal region	-0.151	0.322
ST - skin temperature	-0.086	0.328
CT - coat surface temperature	-0.172	0.323
λ - Eigenvalues	9.008	1.344
s^2 (%) - Explained variance	81.888	12.220
s^2 (%) - Accumulated variance	81.888	94.109

and development, productive life and reproductive periods are essential for a production animal to remain healthy and fertile (McManus et al., 2020). Resources must be prioritized between maintenance, growth, production, and reproduction during the animals life, depending on environmental and climatic conditions.

Being under caloric stress, food intake, digestibility, and absorption decrease (Sejian et al., 2021), reducing the energy reserves available for animal maintenance, production, and reproduction. This also reduces endogenous heat production and increases heat dissipation (Collier et al., 2018) through increased perspiration, respiration, and heart rate.

Small ruminants, such as sheep, are important in this scenario, especially for smallholder farmers in arid areas (McManus et al., 2020), accounting for 56% of the global domestic ruminant population. Therefore, understanding the effects of heat stress on the animal body can help understand the processes that lead to success in production settings.

The PCA procedure and the two-way graph are focused on the daily evaluation curves (Table 2 and Figure 1). In PC1 are the variables HR, RR, and RT. The grouping of these variables suggests the existence of a similarity relationship, and may reflect the considerable effects of the heat stress conditions experienced by both animals affect their physiological responses. For better understanding, the higher the rectal temperature of the animals, the higher will be the values of respiratory and heart rate, with direct effects and in the same proportion on the indications of thermal comfort. Because the increase in internal body temperature (RT),

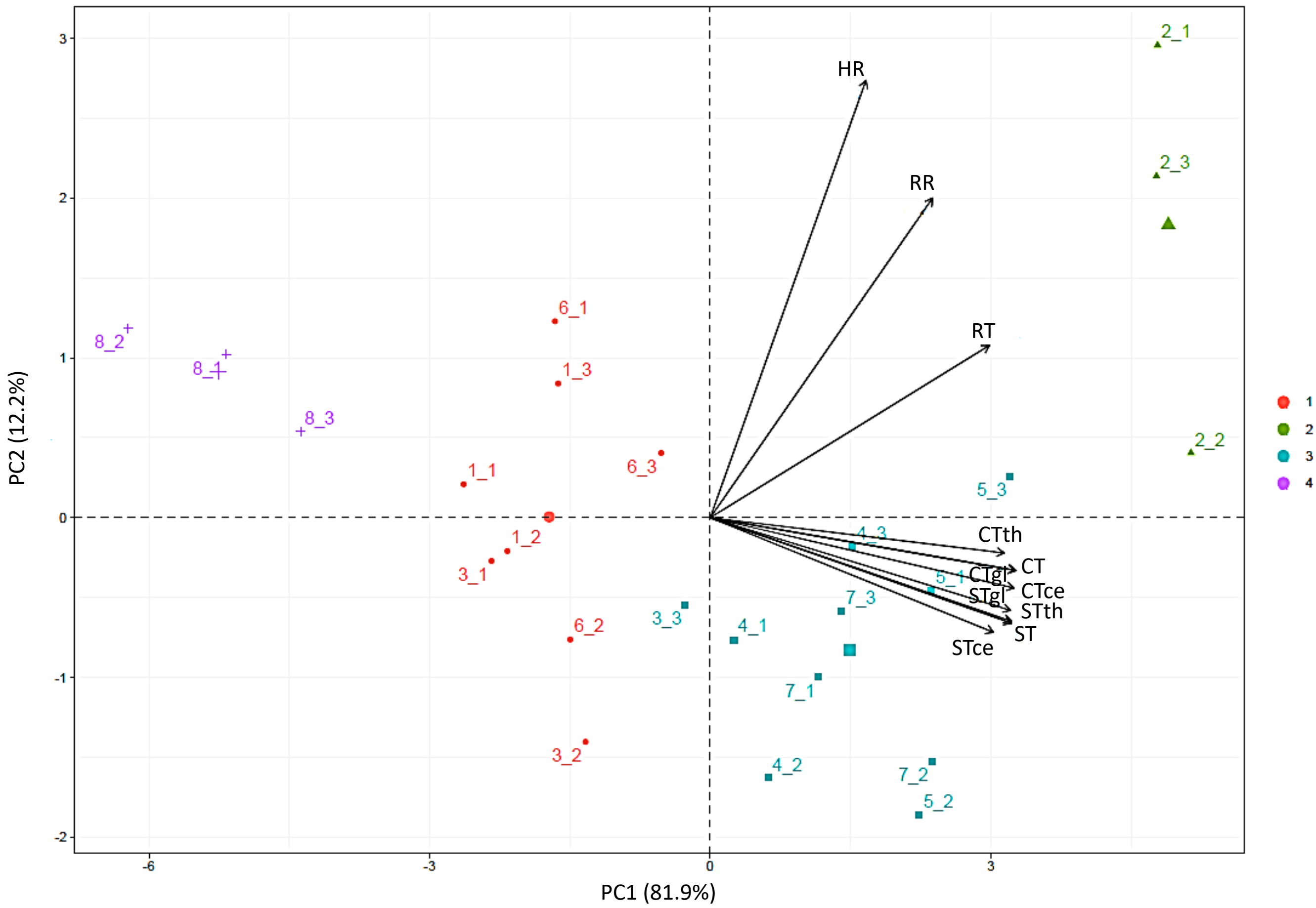

Figure 1. Bidirectional projection of the original variables to form principal components 1 (81.9 %) and 2 (12.2 %) and orthogonal interactions.

reduces the thermal gradient between the animal and the environment, hindering heat exchange through sensitive mechanisms, causing the latent heat exchange mechanisms to be activated (RR). Also as a consequence, there is an increase in HR in an attempt to minimize the difference between the animal × ambient gradient, with increased blood flow through peripheral vasodilation, to reduce the use of RR, as this is a costly mechanism for the organism vital functions (Mascarenhas et al., 2023).

PC2, in turn, is associated with the variables ST, STce, STth, STgl, CT, CTce, CTth, and CTgl, arranged on the vertical axis of the factorial plane, with the HR variable arranged in the opposite quadrant. The grouping of these variables and the arrangement in the factorial plane suggest the existence of opposing forces among these variables (PCs 1 and 2). In fact, the heart rate of the animals rises there is an increase in the values of pelt and coat surface temperature, because there is an increase in blood flow caused by a higher HR, increases peripheral vasodilation of the animal, contributing to the dissipation of heat by non-evaporative mechanisms (Pantoja et al., 2017).

However, the greater the peripheral vasodilation, it may cause the animal to lose fluid through this process, which may promote hemoconcentration, not by increasing the number of red blood cells, but by decreasing the plasma volume, leading to an increase in some hematimetric values, so the higher the level of stress suffered by the animals, the higher the values described by the blood count, which can be explained by the loss of fluid evaporatively, high respiratory rate, and high sweating (Miranda et al., 2018; Mascarenhas et al., 2023).

The increased heat perceived by the animals body causes behavioral, physiological, and metabolic reactions, which in turn affect reproductive success. As a result of exposure to high ambient temperatures, sheep trigger a number of physiological mechanisms that allow the body to adapt to adverse environmental conditions, such as increases in core temperature, respiratory rate, and heart rate (McManus et al., 2020).

As the protection afforded by the skin and coat is not sufficient to prevent heat gain, evaporative heat loss pathways, such as thermal polypnea, are activated (Titto et al., 2016). Physiological changes from thermal stress also include increased body temperature, decreased voluntary food intake, impaired immunity, altered electrolyte and blood pH levels, impaired endocrinological and reproductive functions, decreased cellular energy, altered nutrient metabolism, disruption of gastrointestinal epithelium structure and function, and altered normal microbiota (Binsiya et al., 2017).

The primary automatic responses of an animal under heat stress are sweating and panting. However, the transpiration of the animal depends on the condition of the sweat glands, the quantity and whether they are active, and also takes into account the hair coverage of the animals (Mascarenhas et al., 2023).

Respiration rate has been described as the physiological variable most sensitive to heat stress and one of the most useful. Changes in respiratory rate precede changes in other physiological variables (rectal temperature, sweating, or heart rate) during thermal stress (Pantoja et al., 2017). As it was observed in the study that with increasing RT the RR increased.

The magnitude of such an increase is greater and begins at relatively lower ambient temperature in phenotypes that are poorly adapted to heat (Slimen et al., 2019). Several studies show that within and between breed variations in respiratory rate responses to heat stress are useful for identifying phenotypes that are more susceptible to thermal stress.

Conclusions

The physiological variables of the animals showed an interaction between them, where each of these responses is dependent on each other, and the susceptibility of these physiological responses to climate change. Understanding the functioning of the physiological mechanisms for heat dissipation, it is possible to adapt the animal management in order to increase production without increasing the caloric increase causing stress to the animal.

Compliance with Ethical Standards

Author contributions: Conceptualization: NMHM, BBS; Data curation: NMHM, BBS; Investigation: NMHM, DAF, JVF; Metodology: NMHM, DAF; Project administration: NMHM, BBS; Resources: NMHM, DAF; Supervision: DAF, ANLC, BBS; Validation: DAF, ANLC, BBS; Visualization: NMHM, DAF; Writing - original draft: NMHM, JVF; Writing - review and editing: DAF, ANLC.

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