

## NON-INVASIVE BLOOD PRESSURE MEASUREMENT IN HEALTHY NEONATAL HOLSTEIN CALVES USING A MOBILE OSCILLOMETRIC DEVICE

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Blood pressure is generally recognized as the fourth vital sign, alongside body temperature, heart rate, and respiratory rate. This study aimed to establish such a reference interval in healthy neonatal calves. Sixty-four healthy Holstein calves, aged 2-18 days were included. Blood pressure and heart rate were measured using an easy-to-operate, non-invasive, mobile oscillometric device that does not require special equipment. Measurements were taken from the right and left forelimbs (arteria radialis) and the tail (arteria coccygea medialis) to determine reference intervals. Additionally, a single blood sample was collected from the jugular vein following blood pressure measurement. Hematological analyses were performed and compared with serum amyloid A concentrations determined by ELISA to verify the clinically healthy status of the calves. Furthermore, N-terminal pro B-type natriuretic peptide, and cardiac troponin I levels were measured by ELISA for neonatal cardiac risk assessment. Systolic (SBP), diastolic (DBP), and mean arterial pressure (MAP) values from both forelimbs were statistically significantly higher than those from the tail ( $P < 0.001$ ). The reference intervals (90% confidence) were as follows: left forelimb – SBP: 123–207 mmHg, DBP: 66–158 mmHg, MAP: 77–176 mmHg; right forelimb – SBP: 116–188 mmHg, DBP: 59–146 mmHg, MAP: 78–172 mmHg; tail – SBP: 89–174 mmHg, DBP: 40–126 mmHg, MAP: 57–133 mmHg. This study defined reference intervals for oscillometric blood pressure in healthy calves using a mobile non-invasive monitor. The tail is recommended as the ideal site for routine measurements due to its consistency and clinical practicality.

**Keywords:** Arterial blood pressure; Cardiac risk assessment parameters; Non-invasive monitoring; Oscillometric methods

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## INTRODUCTION

Blood pressure (BP) is generally regarded as the fourth vital sign, along with body temperature, heart rate, and respiratory rate in humans [1]. The maintenance of these parameters is essential for sustaining life, and therefore, during clinical examinations, it is necessary to first assess these vital signs and ensure they remain within the species-specific reference ranges. Although BP monitoring does not directly indicate organ perfusion, it is considered a rapid and reliable indicator of cardiovascular function [1].

Arterial BP can be measured using either invasive or non-invasive techniques. In veterinary medicine, the gold standard for arterial BP measurement is the direct method, which involves catheterization of an artery. However, this technique is invasive, labor-intensive, and costly, making it unsuitable for routine use in all animals. Moreover, placing an arterial catheter in animals with profuse bleeding or infection risk complicates the use of this method [1]. The oscillometric method, on the other hand, is a non-invasive technique involving the use of mobile BP monitors, which are easy to apply in field conditions. Oscillometric BP devices consist of an appropriately sized cuff, a transducer, a microprocessor, and a display unit. These automated devices function by detecting oscillations within the cuff generated by arterial wall movements [2,3]. The greatest advantage of oscillometric technology over other methods is that it requires minimal training and clinical skill to use. Furthermore, it is portable, automatic, equipped with alarms, and capable of measuring systolic BP (SBP), diastolic BP (DBP), mean arterial pressure (MAP), and heart rate [1].

Blood pressure measurement has become essential in small animal practice due to the high incidence and life-threatening potential of pressure abnormalities [1]. In addition to conventional diagnostic approaches, patient monitoring is commonly used. However, in large ruminant practice, field conditions often hinder patient monitoring, making it difficult to assess vital signs and perform timely interventions. While other vital parameters can typically be assessed in field conditions, BP measurement requires either a monitor, a wall-mounted device, or a mobile BP unit. There are existing BP studies in sheep, goats, cattle, cats, dogs, and newborn foals using various techniques [3-8]. However, the lack of defined BP reference values in calves can result in incomplete clinical interventions in cases with general health deterioration.

To minimize calf mortality, establishing an appropriate treatment protocol that considers BP values is of critical importance. This study aimed to establish, for the first time, reference values for BP in healthy newborn calves using a non-invasive mobile oscillometric BP device, which has previously demonstrated successful applications in field conditions.

## **MATERIAL AND METHODS**

This study was conducted with the permission of the Erciyes University Animal Experiments Local Ethics Committee (ERUHADYEEK), 23/10, dated January 4, 2023. Informed consent has been obtained for client-owned animals included in this study.

### **Animals**

BP measurements were performed using a mobile oscillometric blood pressure monitor on 64 healthy newborn calves aged between 2 and 18 days, which were brought to the Ruminant Clinic of the Department of Internal Medicine, Faculty of Veterinary Medicine, Erciyes University, for routine check-ups. Among the Holstein calves (n=64) measured with the mobile blood pressure monitor, 42 were female and 22 were male. The calves ranged in age from 2 to 18 days, with a median age of 7 days. Their average body weight was  $51.09 \pm 7.72$  kg.

Measurements were taken from both forelimbs (from the proximal region) and the base of the tail (as close as possible to its root). To determine whether the animals had any congenital circulatory disorders, serum Bovine NT-proBNP and serum Bovine cTn-I levels were measured using ELISA assays. Additionally, to detect any possible infections, a positive acute phase protein (APP), serum Bovine Serum Amyloid A (SAA), was also evaluated using ELISA.

### **Physical examination, inclusion, and exclusion criteria**

Calves exhibiting any clinical signs of illness during the physical examination (such as congenital anomalies, diarrhea, ocular and/or nasal discharge, coughing, lameness, omphalitis, etc.) were excluded. Only clinically healthy calves with vital parameters within normal limits were included for BP measurement. Body temperature, respiratory rate, and heart rate measurements were recorded while the calves were standing and calm, under minimal restraint, and before blood sample collection. Rectal body temperature was measured using a digital rectal thermometer (Kruuse, Denmark) and recorded once the device indicated a stable reading. Respiratory rate and heart rate were determined by auscultation using a stethoscope (Hauptner-Herberholz, Germany) for 60 seconds, and the recorded values were used for analysis.

### **Hematological analyses**

Complete blood count parameters were analyzed from venous blood samples collected in K<sub>3</sub>-EDTA tubes from all healthy calves using the ExigoEos hematology analyzer (Boule Medical, Sweden).

## ELISA analyses

Serum biomarker concentrations of NT-proBNP (Cat. No. CK-bio-26275) (Sensitivity: 1.0 pg/ml, Detection Range: 10–400 pg/ml), cTn-I (Cat. No. CK-bio-26276) (Sensitivity: 10 pg/ml, Detection Range: 20–1000 pg/ml), and SAA (Cat. No. 201-04-0126) (Sensitivity: 0.133 µg/ml, Detection Range: 0.15–40 µg/ml) were measured using bovine-specific ELISA test kits, in accordance with the manufacturers' instructions. Concentrations were determined at 450 nm using a microplate reader (Biotek ELx800 ELISA Reader, USA).

## Blood pressure measurements

Before BP measurements, the calves were allowed to rest for 10 minutes in the examination room without any stress-inducing stimuli. Each calf was kept calm and in the same sternal position during measurement, which was performed by a single experienced and qualified veterinarian. To select the appropriate cuff size, the circumference of the right and left forelimbs and proximal tail region was measured using a flexible tape. Cuffs were then selected according to the manufacturer's guideline chart: <4.05–4.55 cm (pink) and 4.55–5.55 cm (gray). BP values and heart rates were measured using mobile monitors (Pettrust, BioCARE Corporation/Taiwan). For BP measurements from the forelimbs, the bladder of the cuff was placed over the radial artery in the middle of the antebrachium, neither too tightly nor too loosely. For tail measurements, the cuff was placed as close as possible to the base of the tail over the median line of the arteria coccygea medialis. Using the oscillometric method, the device displayed SBP, DBP, MAP, and heart rate. A total of five readings were taken with 15-second intervals between each. The arithmetic mean of these five readings was used for subsequent statistical analyses.

## Statistical analyses

Statistical analyses were performed using IBM SPSS Statistics 21.0 software (SPSS Inc., Chicago, IL, USA). Normality of the data was assessed by the Shapiro-Wilk test. Data were expressed as median (min and max) and mean ± standard deviation. Comparisons between groups were conducted using One-Way ANOVA (or the Kruskal-Wallis test as an alternative) and an independent samples t-test (or the Mann-Whitney U test as an alternative). The Bonferroni test was used for post hoc comparisons. Pearson correlation analysis was performed to evaluate linear relationships between variables. A p-value of less than 0.05 was considered statistically significant. Reference intervals were established using a different software (NCSS Statistical Software, NCSS LLC, Kaysville, Utah, USA). Ninety percent confidence intervals (2.5th and 97.5th percentiles) were calculated for the upper and lower reference intervals according to the method recommended by the Clinical and Laboratory Standards Institute (CLSI) [9,10].

## RESULTS

### Physical examination findings

The mean body temperature ( $^{\circ}\text{C}$ ) of healthy calves was  $38.8\pm 0.5$ , the mean respiratory rate (breaths/min) was  $44.9\pm 7.9$ , and the mean heart rate (beats/min) was  $103.5\pm 10.8$ .

### Hematological analyses

The results of the hematological analyses are given in Table S1.

### ELISA analyses

The mean serum SAA concentration ( $\mu\text{g/ml}$ ) in healthy calves was  $0.96\pm 0.27$ , the mean NT-proBNP level was  $159.92\pm 32.05$  pg/ml, and the mean cTn-I level was  $302.69\pm 53.59$  pg/ml.

A low but positive correlation was observed between mean NT-proBNP (pg/ml) and mean cTn-I (pg/ml) levels in healthy calves ( $r=0.350$ ,  $P<0.01$ ) (Table S2).

No correlation was found between mean serum SAA ( $\mu\text{g/ml}$ ) and mean cTn-I (pg/ml) levels ( $r=0.228$ ,  $P=0.07$ ). However, a moderate positive correlation was detected between mean serum SAA ( $\mu\text{g/ml}$ ) and mean NT-proBNP (pg/ml) levels ( $r=0.584$ ,  $P<0.01$ ) (Table S3).

### BP measurement analyses

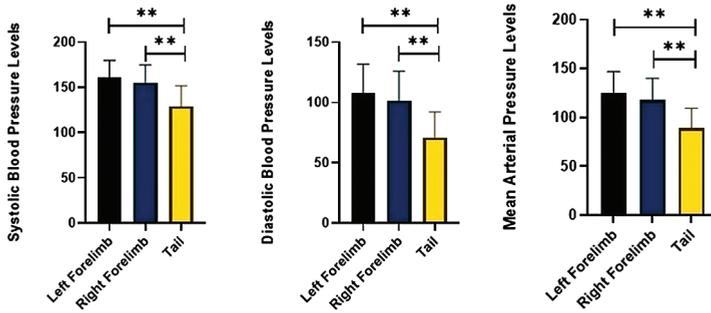
Statistically significant differences were found among blood pressure (BP) measurements taken from different anatomical sites in healthy calves (Table 1).

**Table 1.** Comparison of BP values of healthy calves at different measurement regions

BP Measurement Regions	SBP (mmHg)	DBP (mmHg)	MAP (mmHg)	Heart rate (bpm)
Left Forelimb	$160.80\pm 18.91^a$	$107.68\pm 24.17^a$	$124.55\pm 22.26^a$	$110.6\pm 21.0^a$
Right Forelimb	$155.49\pm 19.34^a$	$101.70\pm 23.95^a$	$117.77\pm 22.16^a$	$114.1\pm 23.7^a$
Tail	$128.89\pm 22.68^b$	$70.70\pm 21.36^b$	$89.27\pm 19.97^b$	$114.5\pm 21.1^a$
P Values	0.000	0.000	0.000	0.558

Values are expressed as mean $\pm$ standard deviation.

The mean SBP values (mmHg) at the left forelimb ( $160.80\pm 18.91$ ) and right forelimb ( $155.49\pm 19.34$ ) were significantly higher than those measured at the tail ( $128.89\pm 22.68$ ) ( $P=0.000$ ) (Figure 1).



**Figure 1: SBP, DBP, and MAP** values in different measurement regions of healthy calves. Statistically significant at  $P < 0.001$  level\*\*

Similarly, the mean DBP values (mmHg) at the left forelimb ( $107.68 \pm 24.17$ ) and right forelimb ( $101.70 \pm 23.95$ ) were significantly higher than those at the tail ( $70.70 \pm 21.36$ ) ( $P = 0.000$ ) (Figure 1).

The mean MAP values (mmHg) also differed significantly between the left forelimb ( $124.55 \pm 22.26$ ), right forelimb ( $117.77 \pm 22.16$ ), and tail ( $89.27 \pm 19.97$ ) ( $P = 0.000$ ) (Figure 1).

No statistically significant differences were observed in heart rate (bpm) among the left forelimb ( $110.57 \pm 21.03$ ), right forelimb ( $114.10 \pm 23.66$ ), and tail ( $114.48 \pm 21.09$ ) measurements ( $P = 0.558$ ).

In this study, arterial BP and NT-proBNP, cTn-I, and SAA 95% reference interval (RI) values are presented in Tables 2 and 3, respectively.

**Table 2.** Two-Sided 90% Percentile Reference Interval Different Blood Pressure Measurement Regions for SBP, DBP, and MAP Values

Variables		2.5% Lower Reference Limit			97.5% Upper Reference Limit		
		Value	90% Confidence Interval Limits		Value	90% Confidence Interval Limits	
			Lower	Upper		Lower	Upper
Left Forelimb	SBP (mmHg)	126	123	126	207	207	207
	DBP (mmHg)	68	66	68	157	157	158
	MAP (mmHg)	89	88	89	171	171	176
Right Forelimb	SBP (mmHg)	116	116	116	188	188	188
	DBP (mmHg)	59	59	59	146	146	146
	MAP (mmHg)	78	78	78	161	161	172
Tail	SBP (mmHg)	89	89	89	174	174	174
	DBP (mmHg)	40	40	40	126	126	126
	MAP (mmHg)	57	57	57	133	133	133

**Table 3.** Two-Sided 90% Percentile RIs for NT-proBNP (pg/ml), cTn-I (pg/ml) and SAA (µg/ml)

Variables	2.5% Lower Reference Limit			97.5% Upper Reference Limit		
	Value	90% Confidence		Value	90% Confidence	
		Lower	Upper		Lower	Upper
NT-proBNP (pg/ml)	109.45	108.44	109.45	230.50	230.10	230.74
cTn-I (pg/ml)	179.22	177.34	180.78	378.93	377.56	378.93
SAA (µg/ml)	1.03	1.00	1.09	1.39	1.35	1.44

## DISCUSSION

The primary aim of our study was to determine the reference intervals of BP values in healthy neonatal calves. Establishing these values would allow for early diagnosis of hypo/hypertension due to various diseases under field conditions and enable the application of accurate medical interventions, thereby improving survival rates. In our study, non-invasive indirect oscillometric BP measurements (SBP, DBP, and MAP) were performed using a mobile blood pressure monitor in healthy neonatal calves. It was found that the values obtained from the left and right forelimbs were significantly higher than those obtained from the base of the tail ( $P < 0.001$ ). Supporting our findings, Deniz *et al.* [11] reported that both in diarrheic and healthy calves, the mean SBP and DBP values measured from the right and left forelimbs were significantly higher than those measured from the tail base ( $P < 0.001$ ). In the same study, it was also observed that the mean MAP values measured from the forelimbs in healthy calves were significantly higher than those measured from the tail base ( $P < 0.001$ ).

Giguère *et al.* [4] evaluated the accuracy of two different automatic indirect oscillometric monitors for measuring MAP in anesthetized neonatal foals. Measurements obtained from the coccygeal, metatarsal, and median arteries revealed that SBP values ranged from 33 to 145 mmHg, with a mean of  $85.7 \pm 27.2$  mmHg, and DBP values ranged from 17 to 75 mmHg, with a mean of  $39.5 \pm 11.8$  mmHg. They concluded that both monitors, when equipped with cuffs placed over the coccygeal or dorsal metatarsal arteries, were acceptable for measuring MAP in foals. Several studies have reported BP values in normal dogs and cats [8,12,13]. These studies have demonstrated that different measurement methods can yield variable results even within the same species.

In the present study, the BP values measured on the tail were recorded as follows: SBP  $128.89 \pm 22.68$  mmHg, DBP  $70.70 \pm 21.36$  mmHg, and MAP  $89.27 \pm 19.97$  mmHg. Naseri *et al.* [14], in a limited number of healthy calves, reported tail BP measurements of 126 (110–165) mmHg for SBP, 77 (64–116) mmHg for DBP, and 93 (79–132) mmHg for MAP, which are consistent with our findings. In contrast to our results, Deniz *et al.* [11] reported significantly lower tail BP measurements in healthy calves,

with SBP, DBP, and MAP values of  $111.40 \pm 18.31$  mmHg,  $51.13 \pm 9.86$  mmHg, and  $70.67 \pm 9.28$  mmHg, respectively. This discrepancy may be attributed to age-related differences in BP values, as the median age of calves in their study was approximately twice that of calves in our study. Additionally, differences in sample size and handling techniques during BP measurement across various animal species can lead to variability in results. This highlights the importance of standardizing measurement techniques in veterinary practice [6]. Moreover, it has been documented that BP measurements vary with age in humans and show small age-related increases in dogs, with consistent findings also reported in cats [6].

In our study, the mean body temperature of healthy calves was  $38.8 \pm 0.5^\circ\text{C}$ , the mean respiratory rate was  $44.9 \pm 7.9$  breaths per minute, and the mean heart rate was  $103.5 \pm 10.8$  beats per minute. These values are consistent with the reference ranges for vital signs in calves reported by Abdisa [15].

The hematological findings obtained in healthy calves are in agreement with the data reported by Knowles *et al.* [16] and Wood [17].

Serum amyloid A (SAA) is an important acute phase protein (APP) used to determine the severity of inflammation in cattle [18]. In our study, serum SAA levels were measured in order to detect any inflammatory condition in the calves included in the study and to investigate possible correlations with other cardiac biomarkers. Previous studies have shown that calves suffering from various diseases exhibit higher SAA levels than healthy control calves [18-22]. In our study, the mean SAA concentration in healthy calves was  $0.96 \pm 0.27$   $\mu\text{g}/\text{mL}$ . Despite the low concentration of SAA in healthy calves, it has been reported that the concentration may increase up to tenfold during inflammation, making SAA a highly sensitive APP for detecting inflammatory responses [23]. The serum concentration of SAA, an  $\alpha$ -globulin, has been reported to be  $<24$   $\mu\text{g}/\text{ml}$  in healthy cattle [24,25], which aligns with the SAA levels detected in our healthy calves. There are also academic studies in the literature that report various concentrations of SAA in calves [22,26-29]. Overall, the SAA levels reported in these studies are consistent with our findings. It is believed that variations in SAA measurements among calves may be influenced by factors such as age and type of commercial ELISA kit used. Considering the mean SAA levels observed in the healthy calves in our study, and in line with the hematological findings, it can be concluded that no signs of disease or inflammation were present in the animals included. This indicates that the target population of healthy calves was accurately selected and that the BP values and cardiac biomarker levels (NT-proBNP and cTn-I) obtained in the study are reliable.

Troponin, a protein located in the thin filaments of all striated muscles, plays a key role in the contraction and relaxation of these muscles [30]. Cardiac troponins are used as gold-standard blood biomarkers in humans due to their high sensitivity and specificity for detecting myocardial degeneration [31]. Among these, cardiac troponin I (cTn-I) has been identified as the most sensitive and commonly used troponin in

veterinary clinical practice [32]. In a study conducted across six different species (dog, cat, horse, cattle, rat, and rabbit), elevated serum cTn-I concentrations were observed following cardiac injury, which frequently occurred as a result of various heart diseases or as a consequence of systemic diseases not primarily involving the cardiovascular system [33]. The clinical use of cTn-I in bovine practice is versatile, having been evaluated in ruminant species suffering from cardiac disorders such as pericarditis and endocarditis, as well as in neonatal calves with congenital heart defects, myocarditis, or other non-cardiac conditions [34]. While troponin concentration in animals is a good indicator of the severity of myocardial injury, it does not provide information about the underlying cause, as it cannot differentiate between primary cardiac diseases and secondary cardiac involvement in non-cardiac conditions [35]. Therefore, in our study, in addition to cTn-I, another key cardiac biomarker NT-proBNP was also measured. In a previous study, Güneş *et al.* [36] reported that the mean cTn-I concentration in healthy cattle was 0.052 ng/ml. Ayvazoğlu *et al.* [37], in a study on neonatal calves with diarrhea, found a statistically significant difference in cTn-I levels between the diarrheic and control groups, with the control calves having a mean cTn-I level of  $0.04 \pm 0.00$  ng/ml. These reported concentrations are considerably lower than those observed in our study. Başbuğan *et al.* [38] reported mean cTn-I levels of 0.19 ng/ml in healthy calves aged between 0 and 6 months, which are closer to the values obtained in our research. However, these studies do not provide specific information regarding whether the age range of the calves was comparable to that of our study population. In the present study, the mean cTn-I concentration observed in 64 healthy calves was  $302.69 \pm 53.59$  pg/ml. The differences between our findings and those of previous studies may be attributed to variations in the age ranges of the calves and differences in the commercial ELISA kits used for measurement. Furthermore, due to the lack of studies that report cTn-I concentrations in healthy calves with a similar age range and sample size, the average cTn-I levels identified in our study may be considered original and valuable.

In recent years, natriuretic peptides have become important tools for the diagnosis and monitoring of cardiac conditions [39,40]. In response to pressure or volume overload, ventricular myocardium releases the NT-proBNP hormone, which remains elevated in the bloodstream for up to 60–120 minutes [41]. This neurohormone is primarily secreted from the left cardiac ventricle in response to volume and pressure overload [33,42]. Currently, NT-proBNP is the most widely used natriuretic peptide in veterinary medicine [33,39,40]. In our study, NT-proBNP levels were measured with two primary goals: first, to identify any potential cardiac circulatory disorders in calves and exclude affected animals from the study, thereby ensuring the reliability of blood pressure values measured in healthy calves; and second, to address the gap in the literature regarding reference ranges for NT-proBNP levels in healthy calves. Previous studies have shown that calves with respiratory diseases [43] and neonatal diarrhea [37] exhibit higher NT-proBNP concentrations compared to healthy calves. Mueller *et al.* [42] reported that in humans, NT-proBNP levels up to 300 pg/mL are

not indicative of heart failure, and that age-specific thresholds of 450 pg/mL, 900 pg/mL, or 1800 pg/mL can be used. Moreover, Ayvazoğlu *et al.* [44] noted that cardiac biomarker values, including cTn-I and NT-proBNP, do not show significant variation by breed or age, and that these differences were statistically insignificant. Additionally, the mean NT-proBNP levels detected in all healthy calves were found to be consistent with those reported in the literature.

In our study, a low-level but statistically significant positive correlation was observed between the mean NT-proBNP (pg/mL) and mean cTn-I (pg/mL) levels in healthy calves ( $r=0.350$ ,  $P<0.01$ ). Similarly, Değirmençay [43] reported a statistically significant, low-level positive correlation between NT-proBNP and cTn-I levels in calves ( $r=0.370$ ,  $P<0.05$ ). Furthermore, Ayvazoğlu *et al.* [37] found a statistically significant, high-level positive correlation between NT-proBNP and cTn-I levels ( $r=0.685$ ,  $P<0.01$ ). In light of the findings from various studies, it can be concluded that the reference levels for NT-proBNP and cTn-I determined in our study are reliable.

The main limitation of this study is that repeated BP measurements could not be performed on the same day or on subsequent days in the calves whose blood pressure values were measured using a mobile oscillometric device, which is a convenient method for field conditions.

## CONCLUSION

In addition to hematological parameters, SAA levels were found to be consistent with the complete health profile of the calves, and the BP measurements obtained from healthy calves included in the study were shown to be suitable for establishing reference intervals. For the first time, BP values in healthy neonatal calves were measured using a non-invasive mobile oscillometric blood pressure monitor, and reference ranges were established. Considering the measurements taken from different anatomical sites, it was concluded that tail-based measurements provided more reliable results due to their ease of use and consistency in successive readings. Furthermore, this study established reference intervals for the cardiac biomarkers cTn-I and NT-proBNP in healthy calves for the first time. The fact that the cTn-I and NT-proBNP values identified in healthy calves were consistent with the existing literature supports the reliability of BP measurements obtained via mobile devices. These findings suggest that such measurements can be successfully used to detect hypotension and hypertension in diseased calves and can serve as valid indicators to guide appropriate medical interventions.

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### **Authors' contribution**

ET designed and directed the project. VG and GE planned the study. ET collected samples and performed blood pressure measurements. RT drafted the manuscript. All authors helped with the research and analysis. ET and VG critically revised the manuscript and provided supervision. All authors contributed to the writing of the manuscript and approved the final version.

### **Declaration of conflicting interests**

The authors declare no potential conflict of interest for this study.

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### **Data availability**

All data generated or analyzed during this study are available from the corresponding author on reasonable request.

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## **NEINVAZIVNO MERENJE KRVNOG PRITISKA KORIŠĆENJEM MOBILNOG OSCILOMETRIJSKOG UREĐAJA KOD ZDRAVE NEONATALNE TELADI RASE HOLŠTAJN**

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Krvni pritisak se generalno prepoznaje kao četvrti vitalni znak, pored telesne temperature, srčane frekvencije i frekvencije disanja. Cilj ove studije bio je da se uspostavi referentni interval kod zdravih novorođenih teladi. U studiju je uključeno 64 zdrava teleta holštajnske rase, uzrasta od 2 do 18 dana. Krvni pritisak i srčana frekvencija mereni su pomoću jednostavnog za rukovanje, neinvazivnog, mobilnog oscilometrijskog uređaja koji ne zahteva posebnu opremu. Merenja su obavljena na desnoj i levoj prednjoj ekstremitetu (arteria radialis) i repu (arteria coccygea medialis) kako bi se odredili referentni intervali. Pored toga, uzorak krvi je uzet iz jugularne vene nakon merenja krvnog pritiska. Izvršene su hematološke analize, i upoređene sa koncentracijama amiloida A u serumu određenim ELISA metodom kako bi se potvrdio klinički zdrav status teladi. Nivoi N-terminalnog pro-B-tipa natriuretskog peptida i srčanog troponina I su mereni ELISA metodom za procenu neonatalnog srčanog rizika. Vrednosti sistolnog (SBP), dijastolnog (DBP) i srednjeg arterijskog pritiska (MAP) iz oba prednja ekstremiteta bile su statistički značajno više od onih iz repa. Referentni intervali (90% pouzdanosti) bili su sledeći: leva prednja noga – sistolni krvni pritisak: 123–207 mmHg, dijastolni krvni pritisak: 66–158 mmHg, srednji krvni pritisak: 77–176 mmHg; desna prednja noga – sistolni krvni pritisak: 116–188 mmHg, dijastolni krvni pritisak: 59–146 mmHg, srednji krvni pritisak: 78–172 mmHg; rep – sistolni krvni pritisak: 89–174 mmHg, dijastolni krvni pritisak: 40–126 mmHg, srednji krvni pritisak: 57–133 mmHg. Ova studija je definisala referentne intervale za oscilometrijsko merenje krv-

nog pritiska kod zdravih teladi korišćenjem mobilnog neinvazivnog monitora. Rep se preporučuje kao idealno mesto za rutinska merenja zbog svoje doslednosti i kliničke praktičnosti.

Supplementary files

**Table S1.** Hematological values of healthy neonatal holstein calves

Variables	Healthy Calves (n= 64)
WBC ( $10^9/L$ )	7.31±1.62
Lymph ( $10^9/L$ )	3.95±1.03
Mon ( $10^9/L$ )	0.6 (0.0-0.9)
Gran ( $10^9/L$ )	3.0 (0.9-4.9)
Lymph %	45.4 (5.3-88.5)
Mon %	9.77±3.25
Gran %	44.7 (6.8-85.1)
RBC ( $10^{12}/L$ )	6.35±0.84
HGB (g/dl)	9.54±1.27
HCT %	29.32±4.66
MCV (fL)	43.34±3.62
MCH (pg)	14.66±1.38
MCHC (g/dl)	37.62±3.44
RDWa (fL)	21.73±5.01
PLT ( $10^9/L$ )	586.87±172.42
MPV (fL)	11.8 (4.6-49.0)

Values are expressed as mean±standard deviation (**Gran**: Granulocyte; **HCT**: hematocrit; **HGB**: Hemoglobin; **Lymph**: lymphocyte; **MCH**: mean corpuscular hemoglobin; **MCHC**: mean corpuscular hemoglobin concentration; **MCV**: mean corpuscular volume; **MPV**: mean platelet volume; **Mon**: Monocyte; **PLT**: platelet; **RBC**: Erythrocyte; **RDW**: Erythrocyte distribution width; **WBC**: Leukocyte) and median (min-max) (Mono, Gran, Lym%, Gran%, MPV).

**Table S2.** Pearson correlation table between NT-proBNP (pg/ml) and cTn-I (pg/ml)

		cTn-I (pg/ml)
NT-proBNP (pg/ml)	Pearson Correlation	0.350**
	Sig. (2-tailed)	.005

**\*\***. Significance of correlation  $P < 0.01$ . **cTn-I**: cardiac troponin I; **Nt-proBNP**: N-terminal pro-B-type natriuretic peptide

**Table S3.** Pearson correlation table between SAA ( $\mu\text{g/ml}$ ) and NT-proBNP (pg/ml), and cTn-I (pg/ml)

		NT-proBNP (pg/ml)	cTn-I (pg/ml)
SAA ( $\mu\text{g/ml}$ )	Pearson Correlation	0.584**	0.228
	Sig. (2-tailed)	.000	.070

**\*\***. Significance of correlation  $P < 0.01$ . **cTn-I**: cardiac troponin I; **Nt-proBNP**: N-terminal pro-B-type natriuretic peptide; **SAA**: serum amiloid A