SCIENTIFIC FINAL REPORT

on the implementation of the project TE 14/2022 '*Evaluating stress and welfare in cattle and water-buffalo: mapping physiological, behavioural and vocal indicators*' *code* **PN-III-P1-1.1-TE-2021-0027**

Funding:	**State budget**
Programme name in PNCDI III:	**Programme 1 - Development of the national R&D system**
Subprogramme name:	**Subprogramme 1.1 - Human Resources**
Project type:	**Research projects to stimulate young independent teams**
Project title:	**Evaluating stress and welfare in cattle and water-buffalo: mapping physiological, behavioural and vocal indicators**
Total contract value:	**449.704,00 lei**
Contract duration:	**28 months**
Contracting authority:	**Executive Unit for the Financing of Higher Education, Research, Development and Innovation (UEFISCDI)**
Contractor:	**Research and Development Institute for Bovine (RDIB)**
Implementation period	**15.05.2022 - 30.09.2024**
Acronym:	**BovineTalk**
Project code:	**PN-III-P1-1.1-TE-2021-0027**
Contract number:	**TE 14 / 2022**
Principal Investigator:	**Dinu GAVOJDIAN**

AIM of the TE 14/2022 project was: to investigate whether vocal parameters in cattle and water-buffalo, linked with other physiological and behavioural responses, can be indicative of well-being and stress, and whenever these indicators could ultimately be used as tools for assessing objectively animal welfare. To the best of our knowledge, this was the first project to investigate cattle and water-buffalo vocal parameters in order to develop science-based non-invasive welfare indicators.
Our hypothesis was that individual distinctiveness and emotional state of the animals are encoded in their vocalizations, and that bioacoustics profiles of large domestic ruminants can be used as reliable indicators for behaviour, welfare and stress, in various farming contexts.

OBJECTIVES of the BovineTalk project were:
i) use of vocal and infrared-thermography (IRT) parameters in evaluating stress and welfare of cattle;
ii) use of stress biomarkers and accelerometery data in monitoring the health status of cattle;
iii) use of vocal and IRT parameters to assess stress and welfare in water buffalo;
iv) use of stress biomarkers and their correlation with vocal and IRT parameters in water-buffaloes.

ACTIVITIES IMPLEMENTED IN PHASE 1/2022 (reporting date: 31.12.2022):
Activity 1.1 - Collection of sound emissions in cattle and analysis of vocal parameters *(implementation degree 100%)*;
Activity 1.2 - Use of IRT investigation in the assessment of the health status of cattle and water buffalo *(implementation degree 100%)*;
Activity 1.3 - Validation of bioacoustics and IRT indicators in cattle *(implementation degree 100%)*;
Activity 1.4 - Specialisation of the human resources involved in the project through the implementation of a scientific internship *(implementation degree 100%)*;
Activity 1.5 - Dissemination of partial results through the publication of a peer-reviewed scientific article and participation in conferences with presentations *(implementation degree 100%)*.

Quantifiable results phase 1/ Deliverables:
- Research article published in Web of Science indexed journal, from first quartile based on WoS ranking (Q1), Accession Number WOS:000887019100001;
- Research internship in bioacoustics at The National Research Institute for Agriculture, Food and the Environment (INRAE) *Rennes – Saint Gilles, France, 19-30.07.2022;*
- Databases with vocal emissions and infrared thermography images (IRT) in cattle, databases are available as open datasets in public repositories: https://gitlab.com/is-annazam/bovinetalk https://www.frontiersin.org/journals/veterinary-science/articles/10.3389/fvets.2023.1236668/full#supplementary-material;
- Participation to two international conferences to present preliminary data from the BovineTalk project.

ACTIVITIES IMPLEMENTED IN PHASE 2/2023 (reporting date: 31.12.2023):
Activity 2.1 - Analysis of stress biomarkers in cattle *(implementation degree 100%)*;
Activity 2.2 - Use of accelerometer data in health monitoring of cattle *(implementation degree 100%)*;
Activity 2.3 - Correlation of bioacoustics parameters with infrared thermography, physiology and ethology data to validate new welfare indicators in cattle *(implementation degree 100%)*;

Activity 2.4 - Collection of sound emissions and analysis of vocal parameters in water-buffalo *(implementation degree 100%)*;

Activity 2.5 - Use of infrared-thermographic investigations in the assessment of the health status of water-buffalo *(implementation degree 100%)*;

Activity 2.6 - Specialisation of the human resources involved in the project through the implementation of a scientific internship *(implementation degree 100%)*;

Activity 2.7 - Dissemination of partial results through the publication of two peer-reviewed scientific articles and participation in conferences with presentations *(implementation degree 100%)*.

Quantifiable results phase 2/ Deliverables:

- Research articles published in Web of Science indexed journal, from first quartile based on WoS ranking (Q1), Accession Number WOS: 001067218200001, Accession Number WOS: 001126469400001;

- Research internship in bioacoustics at Wageningen University and Research – The Netherlands, 05-09.03.2023;

- Databases with accelerometers behaviour data in cattle;

- Databases with vocal emissions and infrared thermography images (IRT) in water-buffaloes;

- Participation to five international conferences to present preliminary data from the BovineTalk project.

ACTIVITIES IMPLEMENTED IN PHASE 3/2024 (reporting date: 30.09.2024):

Activity 3.1 - Analysis of stress biomarkers in bovine *(implementation degree 100%)*;

Activity 3.2 - Correlation of bioacoustics parameters with thermography and physiology parameters in order to validate new welfare indicators in bovine *(implementation degree 100%)*;

Activity 3.3 - Dissemination of the final results through the elaboration of a scientific article *(implementation degree 100%)*.

Quantifiable results phase 3/ Deliverables:

- Research article published in Web of Science indexed journal, from first quartile based on WoS ranking (Q1), Accession Number WOS: 001161452900001;

- Databases with stress biomarkers in bovine;

- Participation to two international conferences to present data from the BovineTalk project.

RESULTS OBTAINED IN PHASE 1 OF IMPLEMENTATION: RESEARCH ON THE USE OF VOCAL PARAMETERS AND INFRARED-THERMOGRAPHY TO ASSESS STRESS AND WELFARE IN BOVINES

RESULTS PLANNED FOR PHASE 1 (according to the project implementation plan):

- Database of vocal emissions in cattle;

- Database of thermographic images and their correlation with the health status of cattle;

- Scientific internship in bioacoustics and the analysis of vocal parameters in farm species;

- Participation in two international conferences, presenting partial data from the project;

- Scientific article submitted for publication.

The implementation of the BovineTalk project activities planned in phase 1 took place mainly in the Experimental Farm and the Production Systems Laboratory of the Research and Development Institute for Bovine Balotesti, and to a lesser extent in the Experimental Farm of the Research and Development Station for the Buffaloes Sercaia (exclusively for the validation of the infrared thermographic method to assess stress in lactating buffalo cows).

In order to comply with the legislation in force and international good practice on research involving animals, the approval of the RDIB Ethics Committee for monitoring the TE14/2022 project was obtained. Furthermore, all project activities complied with the EU Directive 2010/63 on the protection of animals used for scientific purposes.

The animals involved in the study were as follows:
- multiparous dairy cows, Romanian Black and White HF breed, lactations II-IV, 94 heads;
- un-weaned calves 0-3 months, 25 heads;
- multiparous water buffalo cows of the Romanian Buffalo breed, lactations II-IX, 68 heads.

Vocal emission recording in cattle was performed using the following equipment (Fig. 1):

- Sennheiser MKH 416-P 48 U3 super-cardioid broadcast microphone (40-20,000 Hz);
- Rode NTG2 phantom power microphone (20-20,000 Hz);
- Marantz PMD661MKIII 4-channel audio recorder with file encryption;
- DIGITAL SLR DR-70 audio recorder with 4 channels and linear audio recording.

Contexts studied in the Experimental Farm of RDIB Balotesti:
- isolation of cows and calves (I - visual isolation & II - visual and auditory isolation);
- stress habituation (monitoring of cows and calves in isolation for 4-6 hours);
- positive contexts in cows (return to group, communication with conspecifics, anticipation of feeding);
- positive contexts in calves (return to group, anticipation of feeding, feeding of milk, view of animal-caretaker, housing in group pens);
- positive contexts in heifers 12-18 months (anticipation of feeding, oestrus period, social interactions).

After recording the sounds, the files were tagged according to context and animal, and then analysed using Praat® bioacoustics analysis software. After analysis with the specific software, a sound emission database was built, for each sound emission a number of 24 parameters were calculated.

Figure 1. Vocalization recordings in 0-3 months calves at RDIB Balotesti (left photo) and in lactating cows at INRA*E* France (right photo)

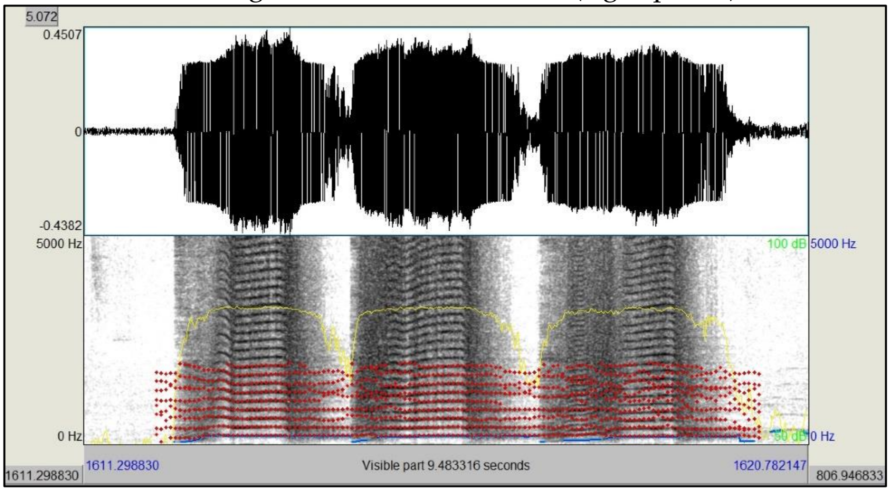

Figure 2. Oscillogram and spectrogram of a sound emission by adult cow, open-mouth vocalization (high frequency call) in negative context (isolation)

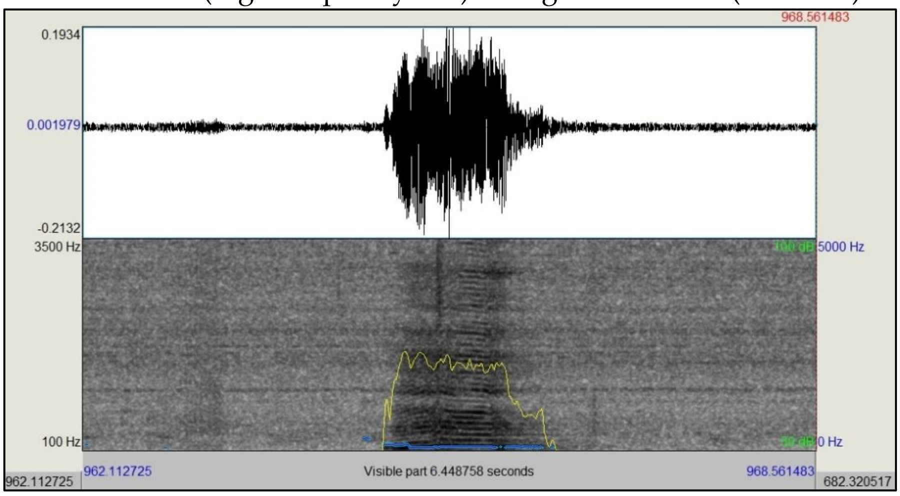

Figure 3. Oscillogram and spectrogram of a sound emission by adult cow, closed-mouth vocalization (low frequency call) in negative context (isolation)

Table 1. Means and dispersion indices for vocal parameters in adult cows (n=10, 10 sounds analysed per animal) subjected to isolation for 4 hours, sounds emitted with open mouth (high frequency calls)

Vocalization parameter	High-frequency calls within the first hour after isolation			High-frequency calls within 3-4 hours after isolation		
	Mean±SEM*	Min.	Max.	Mean±SEM*	Min.	Max.
F0 (Hz)	**183.86±7.24[a]**	165.42	205.31	**196.0±11.0[b]**	170.8	234.3
Max. F0 (Hz)	259.86±7.96[a]	240.88	284.89	250.4±17.1[a]	208.8	293.1
Min. F0 (Hz)	**93.5±11.4[a]**	71.4	136.2	**80.20±7.42[b]**	66.45	108.30
Gama F0	166.39±9.79[a]	144.99	199.78	170.2±21.7[a]	100.5	212.1
Q25% (Hz)	**310.4±49.2[a]**	212.4	431.9	**211.0±10.7[b]**	185.4	247.0
Q50% (Hz)	**439.1±59.9[a]**	275.9	608.1	**360.9±23.5[b]**	297.5	430.1
Q75% (Hz)	**1071.0±230[a]**	561.0	1740.0	**824.0±103.0[b]**	522.0	1116.0
Peak F (Hz)	122.7±11.7[a]	95.2	162.4	119.0±14.0[a]	98.9	173.8
Duration (s)	1.851±0.243[a]	1.370	2.684	1.656±0.177[a]	1.108	2.058
Variability AM	42.15±7.79[a]	21.98	65.82	35.18±8.70[a]	14.16	56.60
Rate AM (s^{-1})	10.856±0.879[a]	8.754	13.410	10.352±0.696[a]	8.577	12.150
Degree AM (dB/s)	3.826±0.506[a]	2.014	4.908	3.461±0.848[a]	1.291	5.101
Harmonicity (dB)	9.59±1.13[a]	5.39	11.69	8.22±1.48[a]	3.57	11.72
Mean F1 (Hz)	**320.4±21.6[a]**	272.5	382.7	**286.8±16.6[b]**	256.0	350.6
Mean F2 (Hz)	603.2±15.4[a]	550.6	638.1	626.8±22.4[a]	551.8	685.6
Mean F3 (Hz)	985.0±26.8[a]	886.5	1042.1	966.8±25.6[a]	882.3	1033.3
Mean F4 (Hz)	1361.7±28.4[a]	1274.3	1438.6	1345.2±20.9[a]	1274.4	1389.0
Mean F5 (Hz)	1724.2±29.2[a]	1625.7	1791.9	1728.0±21.8[a]	1655.4	1770.4
Mean F6 (Hz)	2117.1±28.2[a]	2022.4	2182.4	2119.6±11.5[a]	2081.0	2148.2
Mean F7 (Hz)	2535.6±22.0[a]	2465.4	2583.7	2524.7±10.4[a]	2501.4	2557.9
Mean F8 (Hz)	2853.1±18.6[a]	2807.1	2918.0	2862.1±27.3[a]	2784.9	2935.4
Dispersal (Hz)	361.82±5.05[a]	349.67	376.45	367.90±5.13[a]	355.45	382.77
Wiener entropy	**-1.69±0.22[a]**	-2.22	-1.05	**-1.48±0.07[b]**	-1.72	-1.27

** Note: For means with different superscript the p-value is ≤0.05*

Table 2. Means and dispersion indices for vocal parameters in adult cows (n=10, 10 sounds analysed per animal) subjected to isolation for 4 hours, sounds emitted with closed mouth (low frequency calls)

Vocalization parameter	**Low-frequency calls within the first hour after isolation**			**Low-frequency calls within 3-4 hours after isolation**		
	Mean±SEM	**Min.**	**Max.**	**Mean±SEM**	**Min.**	**Max.**
F0 (Hz)	83.80±2.05^{a}	77.71	89.25	81.10±3.03^{a}	72.35	89.62
Max. F0 (Hz)	95.59±4.41^{a}	85.75	106.61	91.71±5.56^{a}	75.33	104.34
Min. F0 (Hz)	69.91±1.98^{a}	65.63	75.37	66.33±1.39^{a}	61.65	69.01
Gama F0	25.67±5.99^{a}	11.83	40.00	25.38±5.24^{a}	12.82	39.66
Q25% (Hz)	139.1±34.0^{a}	84.0	254.4	136.3±14.1^{a}	110.8	183.5
Q50% (Hz)	307.0±95.0^{a}	102.7	640.9	334.9±69.2^{a}	187.1	567.9
Q75% (Hz)	**1201.0±481.0^{a}**	172.0	2462.0	**883.0±212.0^{b}**	358.0	1544.0
Peak F (Hz)	99.2±13.7^{a}	78.8	153.4	110.5±23.9^{a}	75.2	203.8
Duration (s)	1.082±0.129^{a}	0.784	1.478	1.025±0.060^{a}	0.874	1.202
Variability AM	35.94±8.39^{a}	15.66	66.68	38.50±18.58^{a}	8.86	65.76
Rate AM (s^{-1})	7.58±1.02^{a}	5.10	10.70	7.74±1.18^{a}	5.15	10.70
Degree AM (dB/s)	5.08±1.38^{a}	2.52	9.91	4.86±0.69^{a}	3.10	6.57
Harmonicity (dB)	10.82±2.35^{a}	4.17	16.84	8.01±2.21^{a}	1.20	14.43
Mean F1 (Hz)	304.9±25.1^{a}	234.9	356.7	319.1±32.5^{a}	254.9	440.6
Mean F2 (Hz)	749.5±30.0^{a}	667.4	840.6	730.6±48.6^{a}	578.9	884.4
Mean F3 (Hz)	1211.7±13.6^{a}	1179.2	1241.8	1157.7±26.5^{a}	1073.4	1226.8
Mean F4 (Hz)	1611.1±9.9^{a}	1586.4	1635.4	1575.7±22.3^{a}	1505.3	1636.2
Mean F5 (Hz)	2076.6±39.9^{a}	1986.7	2225.3	2011.5±10.7^{a}	1974.0	2039.7
Mean F6 (Hz)	**2580.5±18.6^{a}**	2512.4	2618.7	**2483.1±9.88^{b}**	2445.2	2503.3
Mean F7 (Hz)	**3058.8±28.9^{a}**	2978.2	3141.1	**2930.0±35.3^{b}**	2856.1	3050.1
Mean F8 (Hz)	3276.4±40.4^{a}	3156.9	3363.9	3252.3±99.7^{a}	2890.2	3476.1
Dispersal (Hz)	424.50±8.3^{a}	400.03	447.00	419.0±18.6^{a}	349.9	454.6
Wiener entropy	-1.59±0.35^{a}	-2.44	-0.74	-1.11±0.13^{a}	-1.44	-0.66

Infrared thermography (IRT) data recording in cattle and water-buffalo was performed using two FLIR® Pro Thermal mobile cameras with a resolution of 19200 pixels, temperature measurement ranges from -20°C to +400°C. Thermal imaging data were stored and processed using specific VividIRTM software.
The collection of thermal imaging data in the dairy cattle and the lactating buffalo cows herds included in the study were aimed at the following contexts: pre- and post-milking; isolation of animals; metabolic diseases; mammary gland diseases and disorders (clinical, sub-clinical mastitis, mechanical lesions); lameness with various aetiologies; calves 0-3 months at neutral temperatures and heat stress (>35°C); oestrus period in cows and heifers; cows in the last 48 hours before calving; before and after weaning of calves; at separation of dam-cow from calf.

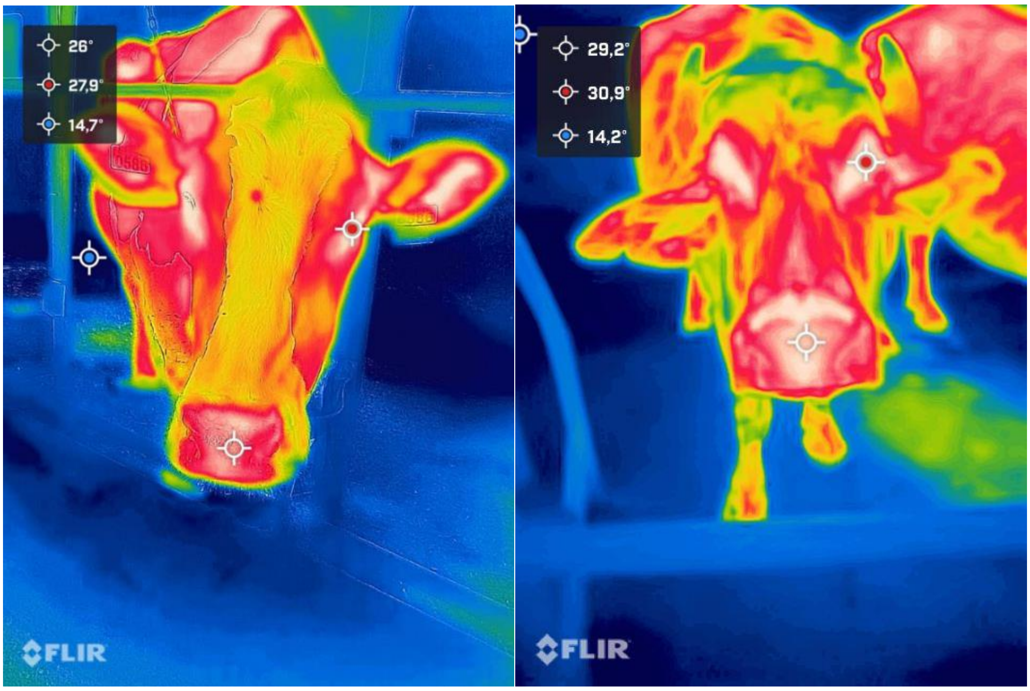

Figure 4. Aspects of the use of infrared thermometry (IRT) in dairy cattle (left photo) and water buffaloes (right photo)

Table 3. Means and dispersion indices for infrared thermal imaging data (IRT) in dairy cows subjected to isolation (n=20), for 4 hours post-milking, with assessment of ocular and nasal regions temperature

Variable	Mean±SEM	DS	CV	Min.	Max.	Q1
IRT nasal region at 0 hours [°C]	27.86±0.546	2.44	8.76	21.60	31.10	26.17
IRT nasal region at 2 hours [°C]	29.87±0.329	1.47	4.93	26.70	32.30	29.07
IRT nasal region at 4 hours [°C]	29.13±0.533	2.38	8.19	22.40	31.90	27.45
Differences 0 vs. 2 hours		*p=0.0055, ***				
Differences 0 vs. 4 hours		*p=0.0698, NS*				
Differences 2 vs. 4 hours		*p=0.5884, NS*				
IRT ocular region at 0 hours [°C]	31.51±0.459	2.05	6.51	26.10	34.90	30.80
IRT ocular region at 2 hours [°C]	32.54±0.295	1.31	4.05	29.20	34.50	32.10
IRT ocular region at 4 hours [°C]	31.74±0.449	2.00	6.33	27.20	34.30	30.40
Differences 0 vs. 2 hours		*p=0.0482, **				
Differences 0 vs. 4 hours		*p=0.4902, NS*				
Differences 2 vs. 4 hours		*p=0.2180, NS*				

*Note: Statistical differences were tested with Mann-Whitney U Test, NS p>0.05; * p≤0.05; ** p≤0.01*

Table 4. Means and dispersion indices for infrared thermal imaging data (IRT) in lactating buffalo cows (n=68), pre- and post-milking, with assessment of ocular and nasal region temperature

Milking temperament	**IRT nasal region [°C]**		**IRT ocular region [°C]**	
	pre-milking	**post-milking**	**pre-milking**	**post-milking**
Cohort	29.33 ± 0.296	29.47 ± 0.392	31.75 ± 0.192	31.74 ± 0.422
Calm	29.46 ± 0.305	29.31 ± 0.532	31.76 ±0.263	31.61 ± 0.591
Nervous	29.02 ± 0.734	29.86 ± 0.420	31.74 ±0.209	32.06 ± 0.289
Differences calm vs. nervous	*p=0.916, NS*	*p=0.712, NS*	*p=0.958, NS*	*p=0.958, NS*

*Note: Statistical differences were tested with Mann-Whitney U Test, NS p>0.05; * p≤0.05; ** p≤0.01*

DVM Madalina Mincu, Young Researcher and PhD student at the Host Institute, member of project no. TE 14/2022, carried out a scientific internship at the National Research Institute for Agriculture, Food and the Environment (INRA*E*) UMR PEGASE Rennes - Saint Gilles, France, in order to specialize in the field of bioacoustics, the internship coordinator on behalf of INRAE was Dr. Céline Tallet.

The programme of the scientific internship was as follows:
- 19 - 26 June: introduction to the study of mammalian vocalizations, study of literature and recordings in the experimental dairy cattle farm La Rheu - INRA*E*;
- 27 - 30 June: analysis of vocal parameters recorded during the first week of the study, using specific software (Praat®);
- 27 June: participation in an online meeting (Zoom platform) with Prof. Elodie Briefer from the University of Copenhagen - Denmark, specialist in vocal communication in cattle, in order to learn the elements of statistics applied to bioacoustics;
- 28 - 29 June: participation (online) in the international conference UFAW2022: Advancing Animal Welfare Science, organised by the International Society of Applied Ethology (ISAE).

Main activities implemented:
- Learning the principles of vocal communication in mammals: what is vocal communication? What is the mechanism of sound production in animals? Source-filter theory of sound production;
- Creation of a database with the vocal repertoire of cattle from the La Rheu experimental farm. Sounds were recorded in 4 different contexts: leaving the pasture, returning from the pasture, in the waiting area of the milking parlour and in anticipation of feeding;
- Analysis of recorded sounds with Praat® software.

<u>RESULTS OBTAINED IN PHASE 2 OF IMPLEMENTATION</u>: RESEARCH ON THE USE OF BIOMARKERS AND SENSOR DATA IN CATTLE HEALTH MONITORING. STUDY ON THE USE OF VOCAL PARAMETERS AND INFRARED THERMOGRAPHY TO ASSESS STRESS AND WELFARE IN WATER-BUFFALOES

<u>RESULTS PLANNED FOR PHASE 2 (according to the project implementation plan):</u>

- Database with stress biomarkers in cattle;

- Database with health behaviour using sensor data in cattle;

- Database with vocal emissions in water-buffalo;

- Scientific internship in bioacoustics and the analysis of vocal parameters in farm species;

- Participation in two international conferences, presenting partial data from the project;

- Publication of two scientific articles in Q1 and/or Q2 WoS ranked journal.

The implementation of the BovineTalk project activities planned in phase 2/2023 took place in the following RD units and commercial farms:

- Experimental Farm and the Cattle Production Systems Laboratory of the Research and Development Institute for Bovine Balotesti (on the following categories: adult cattle, heifers, male and female calves, un-weaned calves 0-3 months);

- Experimental Farm of the Research and Development Station for Water Buffaloe Sercaia – Brasov (on the following categories: adult water-buffaloes, adult water-buffalo bulls, buffalo heifers, male and female buffalo calves, un-weaned buffalo calves 0-3 months);

- Washington State University Knott Dairy Center in Pullman, Washington State, USA (exclusively on lactating dairy cattle);

- SC Agroindustriala Pantelimon SA, Pantelimon, Ilfov, Holstein dairy farm (exclusively on un-weaned dairy calves);

- SC Transylvanian Natural Products SRL, Rupea – Mesendorf, Brasov, water buffalo dairy farm (exclusively on lactating dairy water-buffalo cows).

Vocal emission recordings in both cattle and water-buffaloes were performed using the following equipment:

- Sennheiser MKH 416-P 48 U3 super-cardioid broadcast microphone (40-20,000 Hz);
- Rode NTG2 phantom power microphone (20-20,000 Hz);
- Marantz PMD661MKIII 4-channel audio recorder with file encryption;
- DIGITAL SLR DR-70 audio recorder with 4 channels and linear audio recording.

After recording the sounds, the files were tagged according to context and animal, and then analysed using Praat® bioacoustics analysis software. After analysis with the specific software, a sound emission database was built, for each sound emission a number of 23 parameters were calculated as follows: call type (closed-mouth or low-frequency and open-mouth or high-frequency); Mean F0; Max F0; Min F0; Range F0; Q25%; Q50%; Q75%; Fpeak; sound duration (s); AM var; AM rate; AM extent; harmonicity; F1 mean; F2 mean; F3 mean; F4 mean; F5 mean; F6 mean; F7 mean; F8 mean; formant dispersal and wiener entropy.

Accelerometer (sensor) data in cattle were recorded using: the CowManager® system (CowManager B.V., Harmelen, Netherlands), with ear tag sensors that continuously records animal behaviour, and ear temperature 24 h/day. The measurements of interest were activity (non-active, active and highly-active), eating time, rumination time and ear temperature. The CowManager® sensor is a moulded microchip that has been adapted into a cattle ear identification tag, being fitted with a 3-dimensional accelerometer within the sensor continuously registers the activities of the animal (validated and described in Bikker et al., *J. Dairy Sci.*, 97(5): 2974-2979. https://doi.org/10.3168/jds.2013-7560). Behaviour data was collected using the following equipment: GoPro Hero 10 Black and Set GoPro Hero 10 Black Media Mod sets.

Infrared Thermography (IRT) data were taken using the following equipment: IRT readings were taken using two FLIR ONE Pro LT mobile cameras (19,200-pixel resolution, temperature range -20° to 400°C) and FLIR Systems INC© image processing software. Temperature measuring points were mainly the lacrimal caruncle of the eye in the orbital region (*regio orbitalis*) and at the nasal region (*regio nasalis*), which had been previously validated as thermal windows for water-buffalo, with the IRT pictures being taken (x2/animal/region) from a 0.8-1.2 m distance, and an angle of 90°.

Stress biomarkers were assessed using the following equipment: automated biochemical analyser Spotchem EZ SP-4430 and the enzymatic-immunoassay ELISA system 96 wells (STAT FAX (2200-2600-3200), for hormones detection based on blood biological samples. ELISA interest hormones were the following: cortisol, cattle haptoglobin, cattle interferon gamma, cattle tumour necrosis factor alpha, toll receptor 4, the biochemical indicators were: creatinine, total protein, glucose, glutamyl transaminase, alkaline phosphatase, total cholesterol, total bilirubin, uric acid, ureic acid, fructozamine, gamma glutamyl transpeptidase.

Figure 5. *Video and audio-recordings on pasture (left side) and during feed-anticipation in replacement water-buffalo heifers (right side) at the Water-Buffaloes RD Station in Sercaia*

Results on using sensor (behaviour) data to monitor health in lactating dairy cattle:

The aim of this study was to employ machine learning algorithms based on sensor behaviour data for (1) early-onset detection of bovine digital dermatitis ('lameness', DD); and (2) DD prediction. With the ultimate goal to set-up early warning tools for DD prediction, which would than allow farmers and veterinarians to better monitor and manage DD under commercial settings, resulting in a decrease of DD prevalence and severity, while improving animal welfare.

Data collection occurred over 60 consecutive days at the Washington State University Knott Dairy Center (KDC) in Pullman, Washington, USA. The experimental cattle facility (KDC) houses 180 Holstein pedigreed purebred cows, with lactating animals being housed in a free-stall barn with individual cubicles, using composted manure as bedding. Cows are milked twice per day, using a 6x6 'herring-bone' milking parlour, having *ad libitum* access to two water troughs and are fed a total mixed ration twice per day. The KDC farm practices zero-grazing for lactating cows, with movement alleys and the outside paddock having concrete flooring. While during the dry period the cows are housed on deep-bedded packs with access to grazing areas. Each cow at the KDC experimental farm was fitted with a CowManager® ear tag that continuously records animal behaviour, rumination, and ear temperature 24 hours per day. All behavioural data were calculated as the proportion of time each cow spent exhibiting each behavioural pattern, and computed in hours devoted to that behaviour per 24 hours.

Cattle were enrolled into the study if they met two criteria: 1) no lesions for at least 7 days prior to the first observation of an active lesion and 2) had at least 2 consecutive days of DD lesion observed. During the study, 21 animals developed DD, cows that were between 1st and

5th lactations. Each cow which developed a DD episode was then matched with a healthy counterpart that had the same parity, reproduction status (open/pregnant), and lactation period (early/mid/late). Lactation periods were classified as early (< 100 DIM), mid (101 – 199 DIM), or late (> 199 DIM). Therefore, the final dataset included 21 cows with DD and 21 healthy cows.

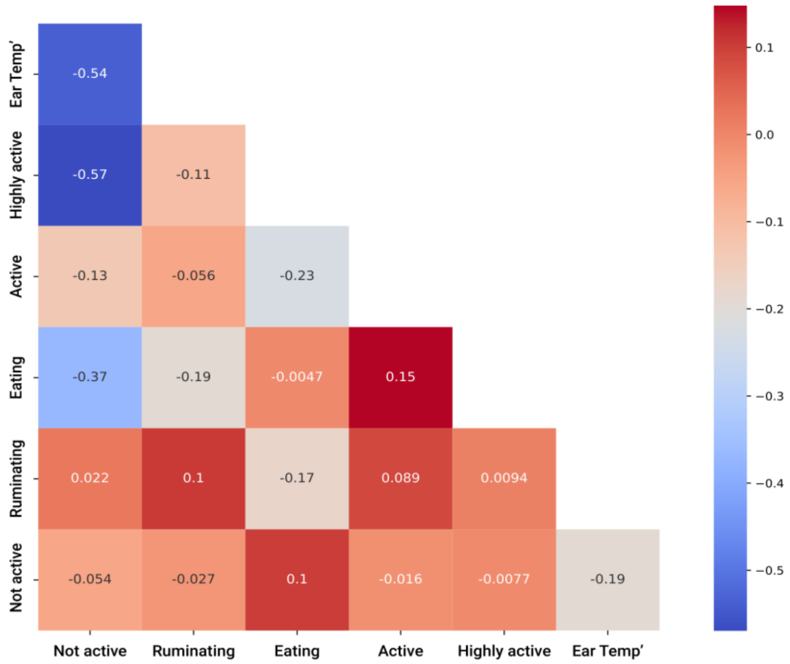

Figure 6. A Pearson coloration matrix between the input features for the disease detection model

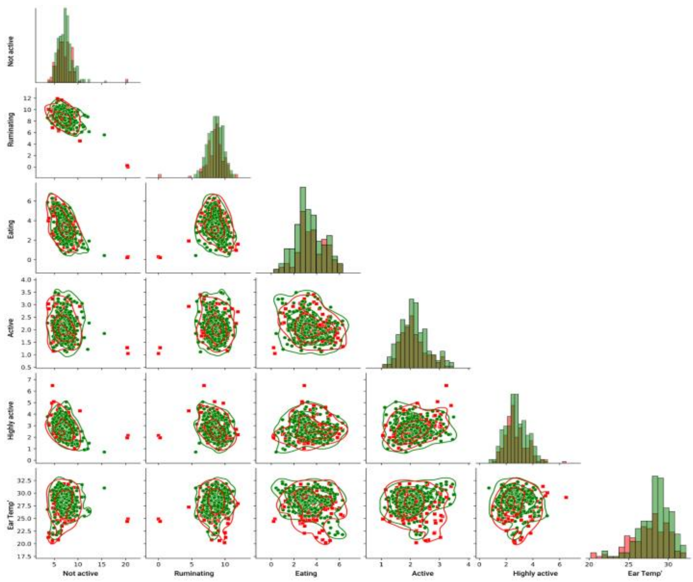

Figure 7. A pair plot between the features of the model, divided by the target features such that the red (square) markers indicate DD sick cows while the green (circle) markers indicate healthy cows. The lines indicate the kernel density estimate of each pair-wise distribution

Detection Machine Learning Model: The first task we addressed was providing a machine learning classifier of whether a specific cow has DD or not on day 0, than described the training process of the machine learning model, after divided the dataset into training and testing cohorts such that the training cohort contained (80%) of the dataset, while the remaining (20%) belonged to the test cohort. Importantly, the distribution of the target feature in both the training and test cohorts, using the Monte-Carlo method, taking the best random split out of n=100 attempts. The training cohort was then used to train the model and the testing cohort was used to evaluate its performance. Importantly, samples from the same individual were either included in the training or testing cohort, in order to avoid potential data leakage between the two. Moreover, to make sure the results were robust, the training cohort was further divided using the k-fold cross-validation method with k=5. Using the training cohort, the Tree-Based Pipeline Optimization Tool (TPOT) automatic machine learning library was used. Formally, given a dataset D∈Rr,c with c∈N features and r∈N samples, TPOT was utilized, that uses a GA-based approach, to generate and test ML pipelines based on the popular scikit-learn library. Formally, the TPOT classifier search method was runed to obtain an ML pipeline that aims to optimize the classifier's mean accuracy over the k folds.

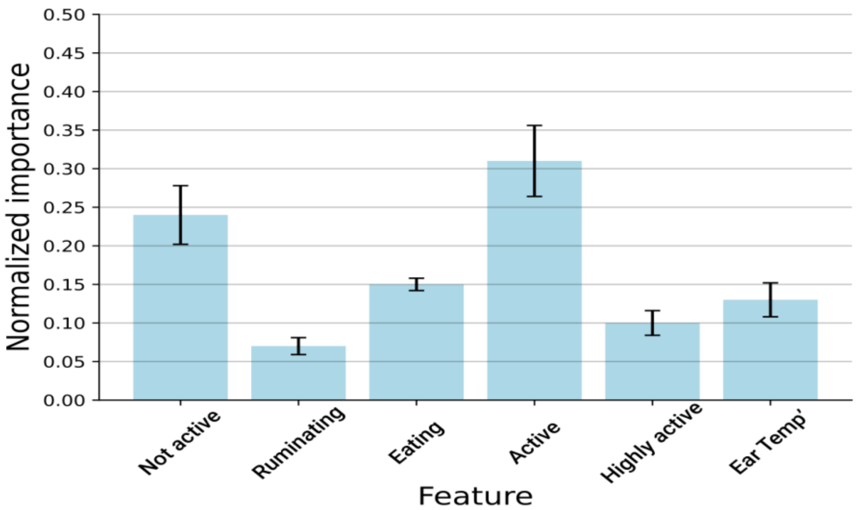

Figure 8. The disease detection model’s feature importance measuring the relative information gain from each feature. The results are shown as the mean ± standard deviation of 5-fold cross-validation performed on the entire dataset

Once the pipeline was obtained, we further aimed to improve the model's performance over the training cohort using the grid-search hyperparameters method such that the hyperparameters value ranges were chosen manually. Finally, the obtained model was evaluated using the testing cohort. This model development process was similar in nature to other recent studies in sensory data of dairy cattle; however, rather than manually testing multiple ML models, we used the automatic machine learning approach, which performed this task more time-efficient.

Prediction Machine Learning Model: Two important concepts in the context of time series forecasting were 'lag' and 'window'. A 'lag' in time series prediction was a way of referencing past data points: e.g., a lag of 1 would mean the previous data point, a lag of 2 would mean the data point two periods back, and so forth. A (rolling) 'window' referred to a fixed-size subset of a time series dataset. The aim was to take a portion of the data of a particular length (window size) and move that data across the time series. Having a window allowed us to create aggregated features such as moving averages, sums, standard deviations, etc. A time-series task with some lag $l \in N$, and window size $w \in N$ has than resulted. In this representation, the disease occurrence prediction takes a binary classification form. However, naturally, the number of negative samples is much larger than the number of possible samples as these occur once for each cow, by definition. Hence, to balance the data, we under-sample the negatively-labeled samples using the K-means method such that the number of clusters equals the number of positive samples. Building on these grounds, the same computational process was repeated as the one used to obtain the disease detection classifier.

In addition, to investigate the influence of the lag and window size parameters, the disease occurrence predictor was obtained for all possible combinations of these parameters. To control the balancing method, the class weights fixing was used, where the number of samples are kept the same but the weight of each label is different to count for the differences in the labels' groups sizes. Both models were implemented using the Python programming language (Version 3.8.1) and set $p \leq 0.05$ to be statistically significant.

The proposed machine learning models might help to achieve a real-time automated tool for monitoring and diagnostic of DD in lactating dairy cows, based on behaviour sensor data in conventional dairy barns environments. Our results suggest that alterations in behavioural patterns at individual levels can be used as inputs in an early warning system for herd management in order to detect variances in health and wellbeing of individual cows.

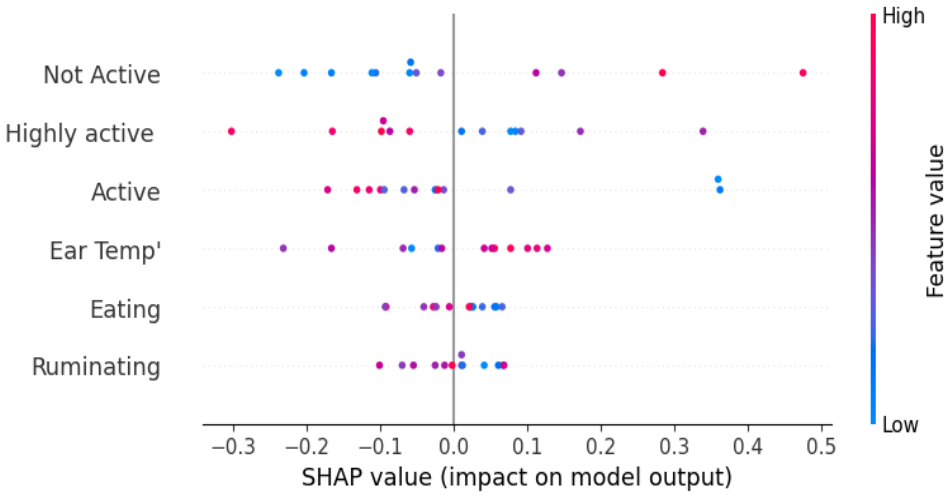

Figure 9. The disease detection model's feature importance measuring the SHapley Additive exPlanations (SHAP) value of each feature

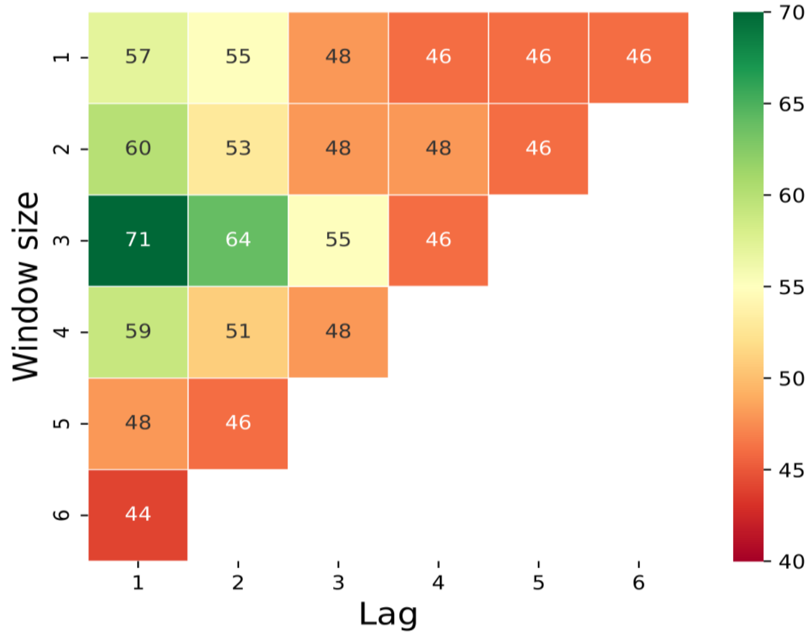

Figure 10. A heatmap of the models' accuracy on the test set (in percentage) as a function of their lag and window size, a 50% accuracy of a binary prediction indicates a random choice, so all results below it shows that the model failed to learn any significant pattern

In conclusion, a machine learning model that is capable of predicting and detecting bovine digital dermatitis in cows housed under free-stall conditions based on behaviour sensor data has been proposed and tested in this exploratory study. The model for DD detection on day 0 of the appearance of the clinical signs has reached an accuracy of 79%, while the model for prediction of DD 2 days prior to the appearance of the first clinical signs has reached an accuracy of 64%.

Results on vocal structure of vocalizations in water-buffaloes:

Table 5. Means and dispersion indices for vocal parameters in adult water-buffalo cows (n=10), sounds emitted with open mouth (HFC) and closed mouth (LFC)

Vocalization parameter	High-frequency calls (HFC)			Low-frequency calls (LFC)		
	Mean±SEM	**Min.**	**Max.**	**Mean±SEM**	**Min.**	**Max.**
F0 (Hz)	**180.6±12.3**[a]	117.0	227.4	**88.44±4.14**[b]	71.64	104.07
Max. F0 (Hz)	**255.13±5.85**[a]	218.94	273.83	**100.21±4.57**[b]	75.78	111.57
Min. F0 (Hz)	**125.4±13.6**[a]	67.3	187.7	**73.16±2.73**[b]	64.42	87.08
Gama F0	**129.7±16.1**[a]	58.2	201.5	**27.05±3.81**[b]	7.98	43.60
Q25% (Hz)	503.0±24.9	330.8	602.6	493.0±29.9	304.9	589.8
Q50% (Hz)	738.1±16.7	636.1	795.8	762.4±40.9	503.6	912.8
Q75% (Hz)	1039.7±39.5	870.9	1305.4	1263.9±63.0	783.0	1519.1
Peak F (Hz)	197.7±13.3	101.1	244.7	162.0±24.2	77.8	284.6
Duration (s)	**1.762±0.248**[a]	0.857	3.249	**0.7317±0.035**[b]	0.581	0.903
Variability AM	48.27±2.90	35.43	59.75	61.95±2.61	47.88	72.34
Rate AM (s^{-1})	9.485±0.795	4.658	13.103	5.997±0.878	1.993	10.515
Degree AM (dB/s)	5.699±0.985	2.969	12.729	5.699±0.985	2.969	12.729
Harmonicity (dB)	**2.259±0.675**[a]	-0.260	4.920	**-0.275±0.435**[b]	-1.410	3.390
Mean F1 (Hz)	409.9±12.8	368.0	487.2	414.24±4.71	388.60	435.97
Mean F2 (Hz)	701.68±7.12	669.94	736.35	743.6±11.1	683.9	787.1
Mean F3 (Hz)	1004.7±10.4	940.2	1037.8	1149.2±15.7	1040.2	1218.8
Mean F4 (Hz)	1291.9±28.6	1146.8	1437.5	1514.7±17.8	1430.1	1641.3
Mean F5 (Hz)	1719.6±20.0	1593.0	1792.1	1910.0±20.8	1797.8	2016.9
Mean F6 (Hz)	2134.0±13.4	2103.5	2206.3	2342.9±20.8	2212.1	2419.3
Mean F7 (Hz)	**2543.0±16.9**[a]	2442.9	2624.6	**2881.4±15.2**[b]	2777.3	2929.0
Mean F8 (Hz)	**2909.8±17.2**[a]	2831.8	2995.3	**3351.3±9.77**[b]	3295.4	3389.1
Dispersal (Hz)	357.13±2.04	346.49	364.79	419.58±1.40	411.33	427.18
Wiener entropy	-0.761±0.070	-1.261	-0.517	-0.647±0.0396	-0.870	-0.448

* *Note: For means with different superscript and **in bold** the p-value is ≤0.05*

Table 6. Means and dispersion indices for vocal parameters in young water-buffalo heifers (6-8 months of age, n=10), sounds emitted with open mouth (high frequency calls) and closed mouth (low frequency calls)

Vocalization parameter	**High-frequency calls (HFC)**			**Low-frequency calls (LFC)**		
	Mean±SEM	**Min.**	**Max.**	**Mean±SEM**	**Min.**	**Max.**
F0 (Hz)	**129.5±25.1[a]**	85.2	172.0	**84.31±5.70[b]**	67.44	91.70
Max. F0 (Hz)	**235.2±41.9[a]**	152.6	289.0	**95.62±8.44[b]**	70.73	106.23
Min. F0 (Hz)	69.82±2.98	65.64	75.58	62.609±0.868	60.985	64.544
Gama F0	**165.3±41.7[a]**	84.4	223.4	**33.01±8.95[b]**	7.13	45.05
Q25% (Hz)	**339.8±77.0[a]**	244.7	492.2	**264.7±90.2[b]**	138.9	527.7
Q50% (Hz)	729±134	556	993	724±155	404	1146
Q75% (Hz)	**1298±288[a]**	986	1873	**1748±103[b]**	1497	2001
Peak F (Hz)	73.29±3.01	68.27	78.68	64.39±4.16	57.95	76.54
Duration (s)	**1.982±0.360[a]**	1.509	2.689	**0.998±0.099[b]**	0.787	1.228
Variability AM	36.03±3.25	32.29	42.51	42.11±6.56	32.31	60.97
Rate AM (s^{-1})	9.526±0.927	8.290	11.341	9.73±1.38	6.99	12.35
Degree AM (dB/s)	**3.899±0.664[a]**	2.847	5.128	**4.88±1.42[b]**	2.73	8.73
Harmonicity (dB)	**6.20±2.27[a]**	1.69	8.89	**3.170±0.670[b]**	2.340	5.170
Mean F1 (Hz)	322.77±6.07	311.60	332.47	341.1±11.1	323.4	370.4
Mean F2 (Hz)	**762.76±8.65[a]**	748.09	778.05	**860.3±17.3[b]**	823.5	897.5
Mean F3 (Hz)	1058.8±20.1	1030.7	1097.8	1194.6±3.82	1189.2	1205.6
Mean F4 (Hz)	**1396.3±41.9[a]**	1336.1	1476.9	**1628.2±22.9[b]**	1572.7	1667.7
Mean F5 (Hz)	**1758.3±24.4[a]**	1717.1	1801.4	**2039.8±22.0[b]**	1986.9	2085.0
Mean F6 (Hz)	**2104.8±16.7[a]**	2077.3	2135.1	**2522.1±28.3[b]**	2438.1	2557.3
Mean F7 (Hz)	**2553.5±16.7[a]**	2530.7	2585.9	**2977.1±41.1[b]**	2857.3	3037.8
Mean F8 (Hz)	**2900.0±4.19[a]**	2891.8	2905.8	**3390.7±33.6[b]**	3304.8	3458.8
Dispersal (Hz)	**368.17±0.530[a]**	367.12	368.79	**435.65±6.03[b]**	419.20	447.79
Wiener entropy	**-0.55±0.178[a]**	-0.843	-0.229	**-0.87±0.0378[b]**	-0.972	-0.789

Table 7. Means and dispersion indices for vocal parameters in water-buffalo calves (0-3 months of age, n=10), sounds emitted with open mouth (high frequency calls) and closed mouth (low frequency calls)

Vocalization parameter	**High-frequency calls (HFC)**			**Low-frequency calls (LFC)**		
	Mean±SEM	**Min.**	**Max.**	**Mean±SEM**	**Min.**	**Max.**
F0 (Hz)	**124.72±9.66[a]**	90.39	212.31	**95.14±1.84[b]**	93.30	96.97
Max. F0 (Hz)	**176.9±14.2[a]**	101.3	265.1	**103.54±0.172[b]**	103.37	103.71
Min. F0 (Hz)	98.38±6.48	66.36	167.97	83.08±9.61	73.48	92.69
Gama F0	**78.6±12.3[a]**	12.1	165.6	**20.46±9.78[b]**	10.68	30.24
Q25% (Hz)	591.2±64.0	360.9	1274.2	611.2±56.2	555.0	667.4
Q50% (Hz)	987.9±87.8	534.6	1919.4	1092.5±24.5	1068.0	1117.0
Q75% (Hz)	1591±142	1121	3085	1655.0±7.09	1647.9	1662.1
Peak F (Hz)	**124.8±19.7[a]**	10.4	221.4	**99.73±3.72[b]**	96.01	103.46
Duration (s)	**1.1586±0.0910[a]**	0.8030	1.9520	**0.8505±0.0275[b]**	0.8230	0.8780
Variability AM	55.62±3.10	41.93	74.75	53.26±4.64	48.62	57.90
Rate AM (s^{-1})	7.610±0.330	4.546	9.341	6.51±1.39	5.12	7.90
Degree AM (dB/s)	7.649±0.763	5.433	15.477	8.73±2.57	6.15	11.30
Harmonicity (dB)	**7.92±1.15[a]**	0.76	15.12	**4.52±1.39[b]**	3.13	5.91
Mean F1 (Hz)	400.2±16.0	345.7	541.4	377.3±26.1	351.2	403.4
Mean F2 (Hz)	752.2±22.9	578.1	879.9	864.9±22.9	842.0	887.8
Mean F3 (Hz)	1082.9±14.8	1001.5	1208.1	1206.7±27.5	1179.2	1234.1
Mean F4 (Hz)	1406.3±16.6	1296.2	1574.8	1573.8±21.6	1552.2	1595.4
Mean F5 (Hz)	1778.1±12.9	1727.4	1875.8	1928.5±16.2	1912.3	1944.7
Mean F6 (Hz)	**2155.1±17.7[a]**	2082.7	2314.6	**2426.4±26.9[b]**	2399.5	2453.3
Mean F7 (Hz)	**2546.7±11.8[a]**	2478.3	2622.8	**2860.5±34.1[b]**	2826.4	2894.6
Mean F8 (Hz)	**2922.3±12.6[a]**	2810.9	2997.5	**3240.7±25.2[b]**	3215.5	3265.9
Dispersal (Hz)	360.29±2.99	341.54	376.68	409.06±0.131	408.93	409.19
Wiener entropy	-1.273±0.071	-1.7580	-0.8400	-1.035±0.171	-1.205	-0.864

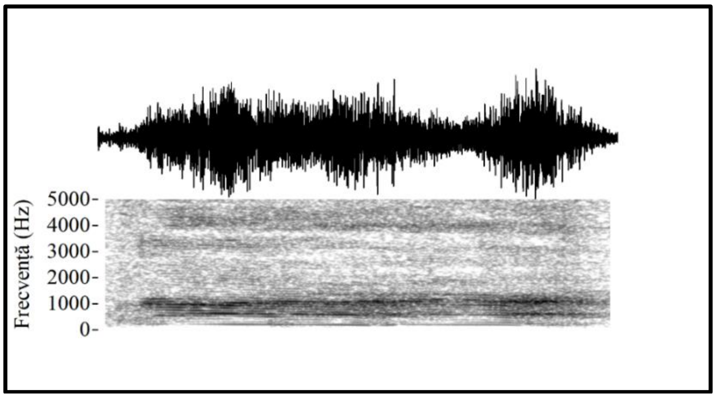

Figure 11. Oscillogram and spectrogram of an adult water-buffalo cow sound emission, high-frequency vocalization (HFC), sound emitted with mouth opened

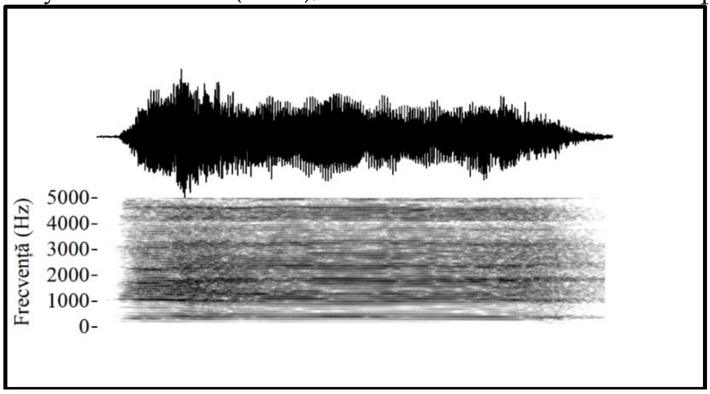

Figure 12. Oscillogram and spectrogram of a water-buffalo calf sound emission, high-frequency vocalization (HFC), sound emitted with mouth opened

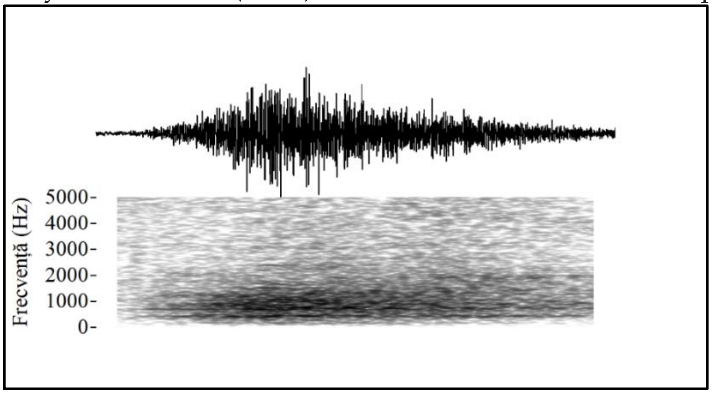

Figure 13. Oscillogram and spectrogram of an adult water-buffalo cow sound emission, low-frequency vocalization (LFC), sound emitted with mouth closed

RESULTS OBTAINED IN PHASE 3 OF IMPLEMENTATION: STUDY ON THE USE OF STRESS BIOMARKERS AND THEIR CORRELATION WITH VOCAL AND THERMOVISION PARAMETERS IN BOVINE

RESULTS PLANNED FOR PHASE 3 (according to the project implementation plan):

- Database with stress biomarkers in bovines;

- Database of thermographic images and their correlation with the health status of bovines;

- Scientific article published.

The implementation of the BovineTalk project activities planned in phase 3/2024 took place in the following RD units and commercial farms:

- Experimental Farm and the Cattle Production Systems Laboratory of the Research and Development Institute for Bovine Balotesti (on the following categories: adult cattle 92 heads, un-weaned calves 0-3 months, 24 heads);

- Experimental Farm of the Research and Development Station for Water Buffaloe Sercaia – Brasov (on the following categories: adult water-buffaloes 42 heads, un-weaned buffalo calves 0-3 months 14 heads);

- Washington State University Knott Dairy Center in Pullman, Washington State, USA (exclusively on lactating dairy cattle, 42 heads);

- Tech4Animals Laboratory of the University of Haifa, Israel (online-only collaboration, consisting of access to the laboratory's computer infrastructure and training in the application of machine learning processes in the study of animal behaviour).

Stress biomarkers were assessed using the following equipment: automated biochemical analyser Spotchem EZ SP-4430 and the enzymatic-immunoassay ELISA system 96 wells (STAT FAX (2200-2600-3200), for hormones detection based on blood biological samples. ELISA interest hormones were the following: cortisol, cattle haptoglobin, cattle interferon gamma, cattle tumour necrosis factor alpha, toll receptor 4, the biochemical indicators were: creatinine, total protein, glucose, glutamyl transaminase, alkaline phosphatase, total cholesterol, total bilirubin, uric acid, ureic acid, fructozamine, gamma glutamyl transpeptidase.

Infrared Thermography (IRT) data were taken using the following equipment: IRT readings were taken using two FLIR ONE Pro LT mobile cameras (19,200-pixel resolution, temperature range -20° to 400°C) and FLIR Systems INC© image processing software and a IRT gun HTI HT-19, XT (pixel-resolution de 49152). Temperature measuring points were mainly the lacrimal caruncle of the eye in the orbital region (*regio orbitalis*) and at the nasal region (*regio nasalis*), which had been previously validated as thermal windows for water-buffalo, with the IRT pictures being taken (x2/animal/region) from a 0.8-1.2 m distance, and an angle of 90° (figure 14).

This study applied deep learning-based computer vision techniques for early onset detection and prediction of lameness in cattle using infrared thermography (IRT) data (Figure 14). We investigated the role of various inputs for these tasks, including thermal images of cow feet, statistical colour features extracted from IRT images, and manually registered temperature values. Our models achieved performances of above 81% accuracy on lameness detection on 'day 0' (first appearance of clinical signs), and above 70% accuracy prediction of lameness two days prior to the first appearance of clinical signs (Table 8). Moreover, current findings indicate that the use of IRT images in conjunction with AI based predictors show real potential for developing future real-time automated tools to monitoring DD in dairy cows.

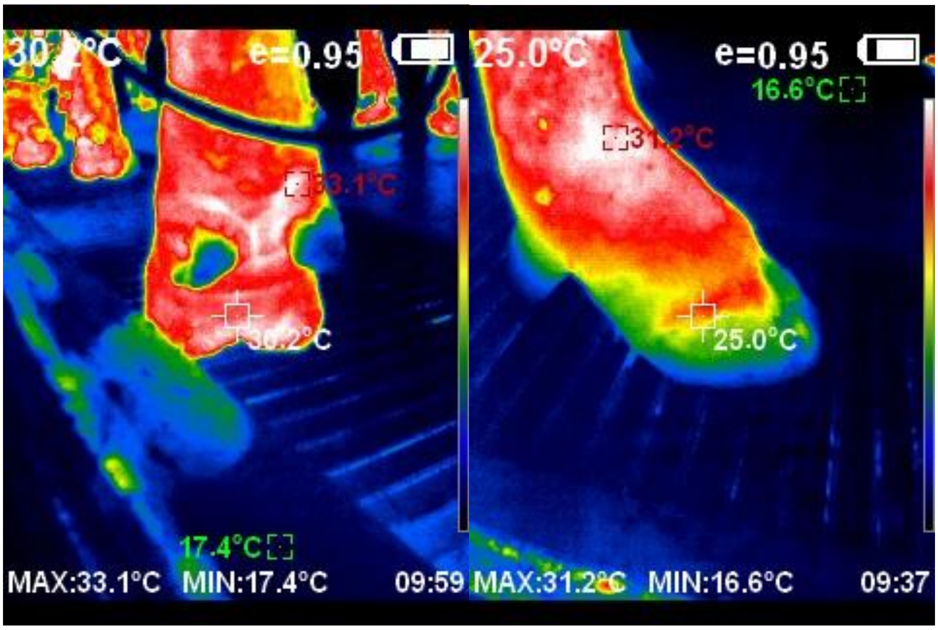

Figure 14. *Using infrared thermography (IRT) to monitor lameness in dairy cows, heel angle (left side) and coronary band (right side)*

Table 8. Onset detection and prediction classification results for lameness in dairy cattle, based on machine learning approaches using IRT data

Pipeline	Angle	Features	Accuracy	Precision	F1	Sensitivity	Specificity
Onset detection	Coronary band	All	0.8115	0.7969	0.8160	0.8361	0.7869
		Only Embedding	**0.8196**	**0.8095**	**0.8226**	**0.8361**	**0.8033**
		Only Additional	0.4754	0.4727	0.4483	0.4262	0.5246
	Heel	All	0.6667	0.6129	0.7308	0.9048	0.4286
		Only Embedding	**0.6786**	**0.6230**	**0.7379**	**0.9048**	**0.4524**
		Only Additional	0.6190	0.5926	0.6666	0.7619	0.4762
Prediction	Coronary band	All	0.6000	0.5882	0.6250	0.6667	0.5333
		Only Embedding	0.6333	0.6429	0.6207	0.6000	0.6667
		Only Additional	**0.7000**	**0.6875**	**0.7097**	**0.7333**	**0.6667**
	Heel	**All**	**0.6500**	**0.6364**	**0.6667**	**0.7000**	**0.6000**
		Only Embedding	0.6500	0.7143	0.5882	0.5000	0.8000
		Only Additional	0.6500	0.6667	0.6316	0.6000	0.7000

PUBLICATION, DISSEMINATION AND COMMUNICATION OF PROJECT RESULTS:

• Research articles published [4 articles published]:

Gavojdian D., Mincu M., Lazebnik T., Oren A., Nicolae I., Zamansky A., 2024, BovineTalk: machine learning for vocalization analysis of dairy cattle under the negative affective state of isolation, *Frontiers in Veterinary Science*, **11:1357109**, DOI: 10.3389/fvets.2024.1357109 (eISSN 2297-1769, impact factor 3,20, Q1 in 'Veterinary Sciences' WoS category);

Mincu M., Nicolae I., **Gavojdian D.,** 2023, Infrared thermography as a non-invasive method for evaluating stress in lactating dairy cows during isolation challenges, *Frontiers in Veterinary Science*, **6;10:1236668**, DOI: 10.3389/fvets.2023.1236668 (eISSN 2297-1769, impact factor 3,20, Q1 in '*Veterinary Sciences'* WoS category);

Magana J., **Gavojdian D.,** Menachem Y., Lazebnik T., Zamansky A., Adams Progar A., 2023, Machine Learning Approaches to Predict and Detect Early-Onset of Digital Dermatitis in Dairy Cows using Sensor Data, *Frontiers in Veterinary Science,* **10:1295430**, DOI: 10.3389/fvets.2023.1295430, (eISSN 2297-1769, impact factor 3,20, Q1 in *'Veterinary Sciences'* WoS category);

Mincu M., Gavojdian D., Nicolae I., Olteanu A.C., Bota A., Vlagioiu C., 2022, Water Buffalo Responsiveness during Milking: Implications for Production Outputs, Reproduction Fitness and Animal Welfare, *Animals*, **12(22), 3115**; DOI: 10.3390/ani12223115 (ISSN 2076-2615, impact factor: 3,231, Q1 in WoS 'Agriculture, Dairy & Animal Science' and 'Veterinary Sciences' categories).

• Research articles under revision [one article]:

Feighelstein M., Mishael A., Malka T., Magana J., Zamansky A., Adams- Progar A., **Gavojdian D**., AI-Based Prediction and Detection of Early- onset of Digital Dermatitis in Dairy Cows using Infrared Thermography, Submission ID 646a4af5-ddf2-418c-8679-4ab4bedc94ee, Scientific Reports (ISSN 2045-2322, impact factor: 3,800, Q1 in WoS 'Multidisciplinary Sciences' category).

• Participation to international conferences** [9 abstract papers published and presented]:**

Gavojdian D., Mincu M., Nicolae I., Lazebnik T, Zamansky A., A Deep-Learning Model to Identify Call-type and Individuality of Dairy Cattle, *Book of Abstracts of the 74th Annual Meeting of the European Federation of Animal Science (EAAP 2024)*, 1 – 5 September 2024, Florence - Italy, vol. 29, ISBN 978-90-8686-936-7, pp. 675 (poster presentation);

Gavojdian D., Mincu M., Nicolae I., Artificial intelligence approaches to evaluate individuality in cattle based on vocal emissions, *Book of Abstracts International Conference 'Agriculture for Life, Life for Agriculture'* - Section 4 Veterinary Medicine, 6-8 June 2024, Bucharest – Romania, ISSN 2457-323X, pp. 47 (oral presentation, invited as plenary);

Gavojdian D., Mincu M., Ber V., Nicolae I., Could low frequency calls be indicative of stress and negative arousal states in cattle? *Book of Abstracts Animal Resources Bioengineering - Multidisciplinary Conference on Sustainable Development*, 25 – 26 May 2023, Timisoara - Romania, ISSN 2821-4293, pp. 36 (poster presentation);

Gavojdian D., Mincu M., Nicolae I., **Constantin T.,** Effects of isolation on high frequency calls parameters in dairy cows – partial results, *Book of Abstracts International Conference 'Agriculture for Life, Life for Agriculture'* - Section 4 Veterinary Medicine, 8-10 June 2023, Bucharest – Romania, ISSN 2457-323X, pp. 80 (poster presentation);

Mincu M., Gavojdian D., Nicolae I., Grigore D.M., Enculescu M., Vlagioiu C., Chute score influence on production and reproduction outputs in dairy cattle, *Book of Abstracts International Conference 'Agriculture for Life, Life for Agriculture'* - Section 4 Veterinary Medicine, 8-10 June 2023, Bucharest – Romania, ISSN 2457-323X, pp. 98 (poster presentation);

Mincu M., Nicolae I., **Gavojdian D.,** Is milking reactivity of water buffalo cows influenced by the production system? *Book of Abstracts 56th Congress of the International Society for Applied Ethology - ISAE 2023*, 1-5 August 2023, Tallinn - Estonia, pp. 175 (oral presentation);

Gavojdian D., Mincu M., Nicolae I., Evaluation of infrared thermography as a non-invasive method for measuring stress in dairy cows during isolation, *Book of Abstracts 56th Congress of the International Society for Applied Ethology - ISAE 2023*, 1-5 August 2023, Tallinn - Estonia, pp. 190 (oral presentation);

Gavojdian D., Mincu M., Evaluating Cattle Welfare Throughout the use of Behavioural and Vocal Indicators: A Review, *Book of Abstracts Animal Resources Bioengineering - Multidisciplinary Conference on Sustainable Development* (pp. 46), 26-27 May 2022, Timisoara Romania (oral presentation);

Mincu M., Gavojdian D., Cattle Vocal Parameters as Non-Invasive Animal Welfare Indicators: Potential Uses and Current Developments, *Book of Abstracts Anthrozoology Symposium 5th Edition – Non-human Animals in Open Societies* (pp. 22), 4-5 November 2022, Iasi – Romania (oral presentation).

• ***Research internships for young researchers*** *[two research study-visits]*:

INRAE scientific internship 19-30.06.2022: DVM Mincu M., member of the project no. TE 14/2022 as PhD student, carried out a scientific internship at the National Research Institute for Agriculture, Food and the Environment (INRAE) UMR PEGASE Rennes - Saint Gilles, France, in order to specialize in the field of bioacoustics, internship coordinator Dr. Céline Tallet;

Scientific internship **Wageningen University and Research** - The Netherlands, 05-09.03.2023: DVM Mincu M., member of the project no. TE 14/2022 as PhD student, carried out a scientific internship 'The fundamentals of animal emotions', in order to specialize in the field of bioacoustics and animal ethology.

• Awards:

Nicolae Teodoreanu award of the Academy of Agricultural and Forestry Sciences (ASAS) for the paper "Water Buffalo Responsiveness during Milking: Implications for Production Outputs, Reproduction Fitness, and Animal Welfare" published in the journal Animals, authors: Mincu M., Gavojdian D., Nicolae I., Olteanu A.C., Bota A., Vlagioiu C.;

UEFISCDI prize code PN-IV-P2-2.3-PRECISI-2023-70608, awarded for the paper "Water Buffalo Responsiveness during Milking: Implications for Production Outputs, Reproduction Fitness, and Animal Welfare" published in the journal Animals, authors: Mincu M., Gavojdian D., Nicolae I., Olteanu A.C., Bota A., Vlagioiu C.

• Scientific communications (outreach activities):

Presentation of BovineTalk project results by Gavojdian D. at the workshop "*The Study of Farmed Animals Bioacoustics*", organized by INRAE, Paris- France, 28-30.08.2024 (oral presentation);

Presentation of BovineTalk project results by Gavojdian D. at the conference "*1st Meeting of the European Network on Livestock Phenomics*", organized by the University of Bologna in collaboration with the COST EU-LI-PHE project, Bologna - Italy, 28-30.08.2024 (poster presentation);

Presentation of BovineTalk project results by Gavojdian D. at the Annual Session of Scientific Communications of the Research and Development Institute for Bovine Balotesti, 08.12.2023 (oral presentation);

Presentation of BovineTalk project results by Gavojdian D. at the Department of Animal Science of Washington State University, Pullman - USA, 23.02.2023 (oral presentation, invited);

Presentation of BovineTalk project results by Gavojdian D. at the workshop organized by the Doctoral School of Veterinary Medicine of USAMV Bucharest, 03.06.2022 (oral presentation, invited).

Tabel 9. Project indicators/deliverables

Indicator	Planned	Achieved
Number of research articles published in Web of Science (Q1 and Q2) ranked journals	4 (Q1 or Q2)	4 (Q1) *+ 1 article under revision*
Number of participations to international conferences	4	9
Number of research internships in bioacoustics for young researchers	2	2
Number of communications of the projects results (outreach activities)	2	5
Databases (vocalisations, accelerometery and infrared thermography)	5	5

ESTIMATED IMPACT OF THE PROJECT:

BovineTalk represents the first research project at the national level, and among the few existing efforts at the international level, to study bioacoustics parameters in cattle and water-buffalo species. Following the implementation of the project, the following sets of results were obtained (selection):

- bioacoustics (vocal communication) parameters in cattle and water-buffalo species were characterized by using specific hardware (infra- and ultra-sound microphones) and software (Praat DSP and scripts). The machine learning models developed and tested achieved an accuracy rate between 87.2 and 89.4% for classifying the types of vocalizations emitted in different contexts, and an accuracy rate of 72.5% for individual vocal fingerprint identification in bovine;
- the use of infrared thermography (IRT) was validated as a non-invasive method to assess signs of social stress in cattle, representing the first study of its kind, the results demonstrating that the method is a suitable tool for the assessment and grading of stress in livestock, with two 'thermal windows' validated, namely *regio orbitalis* and *regio nasalis*. These results can allow objective assessment of affective states in the future;
- IRT monitoring techniques in cattle, in combination with the use of machine learning processes, allow the detection of certain disease states with high accuracy (>80%) and the prediction of disease onset (>70%) with 2-4 days before the appearance of first clinical signs. These results can allow better monitoring of animal health and early diagnosis of some diseases (e.g. lameness, mastitis, metabolic diseases);
- disease diagnosis based on sensor data (accelerometers) in cattle is possible by using machine learning processes, based on the analysis of changes in behavioural patterns, and can be used in particular in the detection (79% accuracy) and prediction (64% accuracy) of lameness. These results are important because lameness health issues have a prevalence of more than 20%, with major negative effects on animal health, productivity and welfare.

In conclusion, the results of the *BovineTalk* project have demonstrated the feasibility of using bioacoustics (vocal communication) parameters in cattle and water-buffaloes to assess welfare and affective states of the two species. Complementary to bioacoustics, infrared thermography (IRT), accelerometer data (sensors) and artificial intelligence (machine learning & machine vision) techniques were tested and validated in order to develop new non-invasive objective criteria for the assessment of welfare and health of bovines.

BovineTalk project PI,
Dr. **Dinu GAVOJDIAN**