105 (2026) 106837

Contents lists available at ScienceDirect

Poultry Science

journal homepage: www.elsevier.com/locate/psj

Full-Length Article

AI-based monitoring of broiler vocal repertoire dynamics reveals robust developmental and diurnal patterns but limited disease sensitivity

Patricia Soster[a,b,*,#], Tomasz Grzywalski[c,#], Camila Lopes Carvalho[a], Imad Khan[a], Pieter Thomas[c], Frank Tuyttens[d,e], Luc Duchateau[f], Isaura Christiaens[b], Maarten De Gussem[g], Paul Devos[c], Dick Botteldooren[c], Gunther Antonissen[a]

[a] Department of Pathobiology, Pharmacology and Zoological Medicine, Faculty of Veterinary Medicine, Ghent University, 9820 Merelbeke-Melle, Belgium
[b] Poulpharm, Izegem, Belgium
[c] Acoustics, Department of Information Technology, Ghent University, Belgium
[d] Department of Veterinary and Biosciences, Faculty of Veterinary Medicine, Ghent University, 9820 Merelbeke-Melle, Belgium
[e] Flanders Research Institute for Agriculture, Fisheries, and Food (ILVO), 9090 Merelbeke-Melle, Belgium
[f] Biometrics Centre, Faculty of Veterinary Medicine, Ghent University, Belgium
[g] Vetworks, Aalter, Belgium

ARTICLE INFO

Keywords:
Poultry
Precision livestock farming
Well-being
Vocalization recognizer
Sound detection

ABSTRACT

Broiler vocalizations may contain relevant information about the welfare and health status; however, there is limited information on how disease affects vocal patterns. This study aimed 1) to investigate whether specific vocalization changes emerge in response to two important poultry diseases: intestinal (*Eimeria* spp.) and respiratory (infectious bronchitis virus, IBV; combined with avian pathogenic *Escherichia coli*, APEC), and 2) to characterize changes in broiler chicken vocalizations over age (14–41 days of age), and across time of the day (morning: 6:00 AM–12:00 PM; afternoon: 12:00 PM–6:00 PM; evening: 6:00 PM–12:00 AM; night: 12:00 AM–6:00 AM). Vocalizations were monitored using an automated AI-based broiler vocalization detector previously developed by our group. A total of 176 Ross 308 broilers were included across four experimental rounds, each consisting of three treatments (control and the two disease challenges) with four replicates each of 11 birds per group. Groups were housed in 3 m^2 pens and continuously recorded from day 14 to day 41 using centrally mounted microphones. Disease challenges were administered on day 14 (*Eimeria* spp.) and day 21 (IBV followed by APEC on day 24). Audio recordings were analyzed using a convolutional neural network trained to detect five vocalization categories: distress calls (DC), short peeps (SP), pleasure notes (PN), warbles (W), and other vocalizations (OV). Vocalizations were aggregated hourly to be evaluated across treatments, age and time of the day. Statistical analyses were performed using mixed-effects models in SAS (PROC MIXED). No significant effects of treatment were detected for all vocalization types ($p > 0.05$). However, significant age-related changes in vocal expression were observed ($p < 0.05$), with DC, SP, and PN decreasing progressively over age, while OV increased. Also, DC, SP, and OV were less frequent during the night from 14 to 28 days of age when compared to the other times of the day ($p < 0.05$), indicating that vocal expression was primarily driven by age- and diurnal period-related changes rather than experimental disease challenge. These results highlight both the potential of vocalization patterns baselines to better understand broilers vocal behavior and current limitations of acoustic monitoring for detecting health disturbances in broiler chickens.

Introduction

Global population growth, rising living standards, and shifting diets are expected to increase demand for animal-derived foods in coming decades (FAO, 2019), driving further intensification of animal production (National Research Council, 2015). Chicken meat is the world's most consumed animal protein, facilitated by minimal religious restrictions and broiler short growth periods, which enables rapid,

* Corresponding author.
E-mail address: Patricia.sosterdecarvalho@gmail.com (P. Soster).
# These authors contributed equally to this work.

https://doi.org/10.1016/j.psj.2026.106837
Received 8 March 2026; Accepted 20 March 2026
Available online 20 March 2026

cost-efficient production (FAO, 2019). Modern systems rear large flocks and process birds at increasingly younger ages. Although automation (such as climate-controlled barns) improves labor efficiency, it can reduce routine human presence and direct welfare observations (Tuyttens et al., 2022). Together with growing public concern for animal welfare, the combination of large flock size, rapid growth, and reduced human oversight complicates early disease detection, limiting opportunities for timely intervention once clinical signs become evident.

Precision Livestock Farming (PLF) enables continuous, real-time monitoring (Wathes et al., 2008; Norton et al., 2019) and requires integrating robust technology with biological and pathological interpretation. Within PLF, bioacoustics is particularly promising because microphones can capture flock vocalizations non-invasively and at scale (Rios et al., 2020). Early poultry sound analysis relied on manual annotation (Manteuffel et al., 2004), whereas deep-learning approaches can now continuously detect and classify complex acoustic patterns over time, even under noisy farm conditions (Cuan et al., 2020; Soster et al., 2025a). Despite advances in PLF technologies, early detection of health challenges in broiler production remains limited.

Coccidiosis is caused by species-specific protozoa of the genus *Eimeria* (*Apicomplexa; Eimeriidae*). In broilers, *E. acervulina, E. maxima*, and *E. tenella* are considered the most relevant, with clinical signs typically emerging from the second week of age onward (Mesa-Pineda et al., 2021). Following enterocyte invasion, Eimeria replicate through multiple intracellular stages that damage the mucosa, increase permeability, and impair digestion and absorption, ultimately reducing productivity (Madlala et al., 2021). Coccidiosis is prevalent in commercial production; even subclinical infections reduce weight gain and feed efficiency, contributing to major global economic losses. Control relies mainly on anticoccidials and vaccination, but drug resistance and variable vaccine efficacy remain key challenges (Mesa-Pineda et al., 2021).

Infectious bronchitis virus (IBV; *Coronaviridae*) primarily causes respiratory disease, with coughing, tracheal rales, nasal discharge, depression, and ocular signs, typically from the third week of age. Signs may be aggravated by secondary avian pathogenic *Escherichia coli* (APEC) infection, leading to colibacillosis and higher mortality (Nordin et al., 2021). IBV is globally distributed and continually evolving, complicating control (Abozeid et al., 2023). Outbreaks reduce performance and increase condemnations and medication costs; despite widespread vaccination, antigenic diversity and incomplete cross-protection allow ongoing circulation of field strains. Together, these two diseases are widespread in commercial production and are linked to immune dysregulation, impaired performance, and reduced welfare.

There is a research gap regarding how broiler vocalization patterns change during illness. Although disease detection in poultry currently relies on indicators such as reduced feed intake, impaired growth, altered behavior, locomotor deficits, or respiratory sounds, these measures often become apparent only after clinical disease is established and typically require manual observation or invasive sampling. Acoustic monitoring offers a continuous, non-invasive, and animal-centered approach that may capture early physiological or behavioral alterations before conventional performance or clinical parameters decline. Previous studies have demonstrated that abnormal respiratory sounds and acoustic features can discriminate healthy from diseased birds. However, existing approaches either focus on general comparisons between healthy and unhealthy chickens using broad vocalization metrics (Huang et al., 2019) or primarily rely on event-based detection, such as coughing (Liu et al., 2020), sneezing (Carpentier et al., 2019), and rales (Rizwan et al., 2016), rather than on systematic characterization of the normal vocal repertoire.

In our previous work, four common broiler vocalization categories were automatically detected using a trained model (Soster et al., 2025b) and evaluated under mid-scale conditions (140 birds/pen; 14 Kg/m^2) during heat stress and platform enrichment (Soster et al., 2025a). Distress calls (DC) are repetitive, high-energy vocalizations linked to stress, whereas short peeps (SP) are low-energy, short-duration calls with descending frequency associated with activity. Warbles (W) are low-energy, bow-shaped elements with ascending/descending frequency, suggested to relate to somnolence, and pleasure notes (PN) are low-energy, short-duration calls with ascending frequency, potentially linked to positive welfare (Marx et al., 2001; Soster et al., 2025b). All remaining sounds were grouped as other vocalizations (OV). While no vocalization changes were observed under heat stress or platform enrichment, clear age- and time-of-day–dependent patterns were detected. However, it remains unclear whether vocalizations exhibit meaningful changes across disease states.

Addressing this gap, the present study used a deliberately small-scale experimental design to reduce simultaneous vocalizations and call overlap, thereby improving detection of the less frequent vocalization types (PN, W and potentially OV). Also, as broiler behavior previously showed change with age and follow diurnal rhythms (Soster et al., 2025), accounting for both age and time of day is important to interpret behavioral metrics and determine baselines. Specifically, the study aimed to (1) evaluate vocalization changes following experimental challenge with two important poultry disease conditions: intestinal (Eimeria spp.) and respiratory (IBV; combined with avian pathogenic Escherichia coli, APEC); and (2) characterize changes in broiler vocalizations with age (14–41 days) and across diurnal periods (morning: 6:00 AM–12:00 PM; afternoon: 12:00 PM–6:00 PM; evening: 6:00 PM–12:00 AM; night: 12:00 AM–6:00 AM) using a broiler vocalization detector developed previously by our group (Soster et al., 2025b).

Material and methods

The experimental protocol for this study was approved by the Ethics Committee of Faculty of Veterinary Medicine and Bioscience Engineering, Ghent University (approval number: 2023-084).

Animals and husbandry

The trial consisted of four rounds, each containing three treatments (one control and two disease models) of groups of 11 chickens (total of 176 Ross 308 broiler chickens). Each group of 11 chickens was housed in one compartment containing a single 3 m^2 pen with clean bedding, one feeder and one tubular water (Fig. 1), ensuring physical separation of groups to prevent cross-contamination. To minimize potential biases due to differences in compartment conditions, a rotation system was employed, whereby treatments were rotated between compartments so that each treatment was represented in every compartment.

During the first two weeks, non-vaccinated chicks of the same round were housed together in a separate pen. At the end of the second week, all chickens were weighed and divided into three treatments based on average body weight (similar median body weight between pens). The

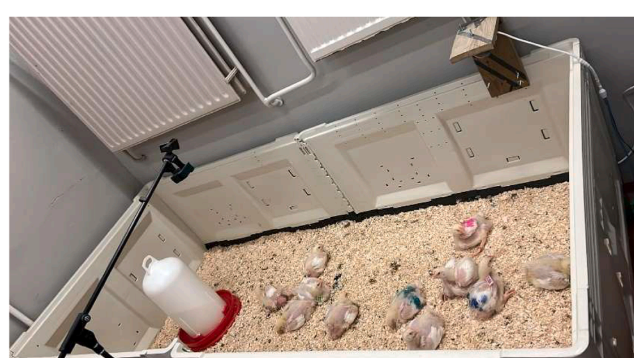

Fig. 1. Setup of one compartment containing a 3 m^2 pen (11 chickens per pen) equipped with one feeder and one drinker. A microphone was positioned centrally for continuous audio recording.

treatments were the following:

- **Treatment 1:** Control group.
- **Treatment 2:** at day 14, chickens were orally infected (1 mL orally via gavage/bird) with *Eimeria spp*. A 10 mL inoculum was prepared containing 15,000 *E. maxima*, 20,000 *E. acervulina*, and 7,000 *E. tenella* oocysts in total.
- **Treatment 3:** at day 21, chickens were challenged with a virulent dose of IBV strain M41 (Titer: 8.3 log10 EID50/mL), 0.1 mL per bird (5 log10 EID50) via eye drops, followed by infection with APEC *E. coli* strain T.01.54, 0.1 mL (5×10^8 CFU) via the oculo-nasal route three days later.

The selected challenge models and doses were based on Poulpharm-established protocols (unpublished), previously shown to reliably induce clinical or subclinical disease in broilers. Oral inoculation with mixed *Eimeria* spp. at day 14 typically results in intestinal lesions and reduced performance within 4–7 days post-infection, with peak clinical impact around days 5–10 (Mesa-Pineda et al., 2021; Madlala et al., 2021). IBV M41 challenge at day 21 induces respiratory signs within 24–72 h, which are frequently exacerbated by secondary APEC infection, leading to colibacillosis and increased morbidity over the subsequent days (Nordin et al., 2021). The diseases were induced at different ages to reflect their typical field occurrence and age-related susceptibility. Age-related periods was divided into four age intervals: period 1 (days 14–20), period 2 (days 21–27), period 3 (days 28–34), and period 4 (days 35–41).

The pens were illuminated with artificial lighting controlled by a 24-hour timer, providing one hour of light and 23 hours of dark in the first week (dark period 00:00–01:00), and 18 hours of light and 6 hours of darkness daily from day 7 (dark period 00:00–06:00), following the management manual (Aviagen, 2024). The temperature regimen followed commercial standards. Feed was obtained from a commercial diet, according to the breed standards and water from the public supply, both provided *ad libitum* during the whole study period. On day 41, all animals were euthanized via intravenous administration of an overdose of sodium pentobarbital 20% (Euthanimal 20%, sodiumpentobarbital, Alfasan, Woerden, The Netherlands), at a dose of 0.5 mL/kg body weight (administered in the wing vein). All animals were checked daily by one of the participating veterinarian researchers throughout the experiment to ensure humane end points would be respected.

Sound recording and analysis

Throughout the trial (days 14–41), sounds from all pens were continuously recorded using a microphone positioned 90 cm above the center of each pen to capture vocalizations from all birds. In Rounds 1–2, recordings were obtained using a low-cost acoustic node equipped with a Knowles FG-23329 microphone (Van Renterghem et al., 2010), operating continuously at a sampling rate of 48 kHz (Soster et al., 2025b). The mounting height balanced (i) relatively uniform recording gain across the $3m^2$ pen and (ii) improved signal-to-noise ratio by limiting airflow noise and wall reflections. The 48 kHz sampling rate, consistent with prior works (Huang et al., 2019; Liu et al., 2020; Lv et al., 2023), adequately resolves the spectral range of chicken vocalizations. Data were automatically stored online.

The device was updated, and in Rounds 3–4 vocalizations were recorded using AudioMoth acoustic loggers (Open Acoustic Devices, UK). Recorders were mounted with the microphone oriented downward and housed in splash-resistant cases to protect against dust and humidity while minimizing acoustic shadowing. AudioMoth units are full-spectrum recorders with on-board pre-amplification and configurable gain; here they captured uncompressed WAV, 16-bit PCM, at 48 kHz, in continuous schedules synchronized to local time. Power was supplied by AA lithium batteries, audio was written to 128 GB microSD cards, and data were offloaded every five days.

Based on Soster et al. (2025c), four well-characterized vocalization types were selected for development of the broiler vocalization detector; additional vocalizations that did not fit these categories were classified as OV. Audio recordings were processed using our custom-built recognizer, described in detail by Soster et al. (2025b). Signal processing and feature extraction followed the workflow outlined by Soster et al. (2025a), ensuring reproducibility and enabling direct comparison across studies. A concise summary of this procedure is provided below.

The employed model comprises eleven two-dimensional convolutional layers followed by a one-dimensional convolutional layer, totaling around 1.2 million parameters. It processes log-mel spectrograms of audio to capture both spectral and temporal features of broiler vocalizations. The output layer predicts six classes (five vocalization types and background noise) and includes an auxiliary age-prediction branch, improving robustness by leveraging age-dependent pitch changes.

The network was first pre-trained on 100,000 bird and fowl samples from AudioSet (Gemmeke et al., 2017) and then fine-tuned on a dedicated broiler dataset. This dataset was derived from recordings of ten Ross 308 broilers (days 1–36), automatically preselected with the PANN model (Kong et al., 2020) and manually labeled into five categories (Soster et al., 2025b). The final dataset comprised 2,559 labeled samples balanced across the lifecycle, supplemented with non-animal AudioSet noise to improve generalization. The model achieved balanced accuracies of 91.1% across all sound classes and 89.4% when discriminating specific vocalization types, with particularly high performance for DC (97.1%) and PN (98.5%).

For the present recordings, processing involved: (1) resampling to 8 kHz, (2) 500 Hz high-pass Butterworth filtering, (3) spectral-gating noise suppression (Sainburg et al., 2020), (4) log-mel spectrogram conversion (64 filters, 50 Hz–8 kHz, 100 fps), and (5) classification with the trained model. Predictions were generated every 240 ms, thresholded at 0.7 probability, and converted into per-minute vocalization durations by multiplying detection counts by frame length. Hourly aggregation was applied to reduce variability, with results expressed in seconds of vocalizations per minute.

Data analysis

The effect of disease, age period, and day time period on vocalization rates was analyzed using a mixed model with pen as random effect (SAS version 6.4), with disease group, age period, and day time period and the two way interactions, as fixed effects factors, and further including round as blocking factor. Relevant pairwise comparisons were performed using contrasts. Statistical significance was set at $p < 0.05$ with adjustment for pairwise comparisons by Tukey's method.

Results

No effects of disease were observed on any vocalization category in any period of age ($p > 0.05$, see Table 1). However, significant effects of

Table 1
Mean vocalization rates (s/min) for the different disease groups considering the respective post-challenge evaluation periods: *Eimeria* spp. (14–41 d) and infectious bronchitis virus + *E. coli* (21–41 d).

Intervention	Distress Call	Short peep	Pleasure Notes	Warbles	Other Vocalizations
Control	1.381	2.589	0.189	0.033	1.060
Eimeria spp.	1.490	2.448	0.324	0.060	1.023
IBV+ *E. coli*[1]	1.381	1.838	0.266	0.010	1.092
SE	0.305	0.461	0.045	0.015	0.100
p-value	0.577	0.673	0.258	0.199	0.965

SE: Standard error.
[1] IBV+ *E.* coli: infectious bronchitis virus combined with avian pathogenic *Escherichia coli.*

age period were observed for all vocalization types ($P \leq 0.019$). DC and SP decreased progressively with age ($P < 0.001$; Table 2; Fig. 2), with the highest frequencies in period 1 (days 14–20) and the lowest in period 4 (days 35–41). PN and W also declined over time ($P < 0.001$ and $P = 0.019$, respectively), showing reduced expression in the later periods. In contrast, OV increased from period 1 to period 2 and remained elevated through periods 3 and 4 ($P < 0.001$), with no significant differences among the last three periods. Time of day significantly affected DC ($P < 0.001$), SP ($P = 0.001$), and OV ($P < 0.001$), see Table 3; Fig. 3. All three were significantly lower during the night compared to morning, afternoon, and evening, which did not differ from each other. PN ($P = 0.136$) and W ($P = 0.240$) were not influenced by time period.

Discussion

In the present study, despite using intestinal and respiratory infection models, no significant disease effects were observed for any vocalization category. This contrasts with prior "vocalization-based" approaches that detect labeled abnormal respiratory events, such as sneezes (Carpentier et al., 2019), rales during infectious bronchitis challenges (Carroll et al., 2014; Rizwan et al., 2016), or cough/snore segments extracted from commercial-house recordings (Liu et al., 2020), rather than shifts in the normal vocal repertoire. By design, the present research quantified four common broiler vocalization categories plus an "other" class to capture subtle, naturally evolving changes in vocal output, an approach that may be less sensitive when disease signatures manifest primarily as rare, event-like abnormal sounds representing only a small fraction of total acoustic activity.

Vocalization patterns were driven primarily by age and time of day, with these factors exerting a stronger influence on overall vocal output than the disease challenges. This mirrors our previous findings (Soster et al., 2025a), where vocal activity was similarly structured by age and diurnal patterns, with limited modulation by changes such as heat stress or platform enrichment. Across both studies, SP and DC predominated, likely due in part to higher acoustic energy and thus greater detectability. Importantly, even with markedly reduced call overlap in the present design (11 birds/microphone vs. around 70 birds/microphone previously), W and PN remained rare, supporting the interpretation that these calls are less prevalent across the broiler production cycle rather than merely masked in larger groups. Vocalizations were also more frequent during the daytime than at night, consistent also with prior observations by Du et al. (2018) and Soster et al. (2025a), likely reflecting the lighting schedule applied in the experimental setup.

A further shared temporal feature was the progressive increase in OV with age, suggesting either an expansion of the vocal repertoire as broilers mature or a shift toward call types not captured by the four well-characterized categories. In this context, less frequent and currently unclassified sounds may still be informative. Grzywalski et al. (2025) identified 10 additional vocalization clusters from over 7,000 previously categorized "other" sounds, indicating that broilers produce a broader repertoire than commonly recognized. Thus, while disease effects were not detected in the dominant call categories, subtle health- or welfare-related signals may reside within the OV fraction, highlighting the importance of future work explicitly characterizing these clusters and linking them to clinical, behavioral, and environmental measures.

Beyond call frequency or category occurrence, future work could also examine intra-vocalization acoustic features (differences within calls), such as spectral complexity or entropy. Although such analyses were not performed in the present study, they may provide additional sensitivity to detect disease-related changes that are not apparent at the level of vocalization category counts. For example, Mahdavian et al. (2021) showed that acoustic features extracted from individual broiler vocalizations (particularly wavelet entropy) were responsive to respiratory disease challenges, enabling discrimination between healthy and infected birds even when observable vocal changes were limited.

Although the main broiler vocalization categories were carefully defined in this study, uncertainty remains regarding their precise biological significance. Mapping temporal patterns may support testable correlations with health, behavior, and environment, but confirming biological meaning will ultimately require targeted experiments to determine the contexts in which vocalization rates reliably increase or decrease. Finally, although a growing body of research suggests that sound analysis could support animal welfare monitoring, relatively few

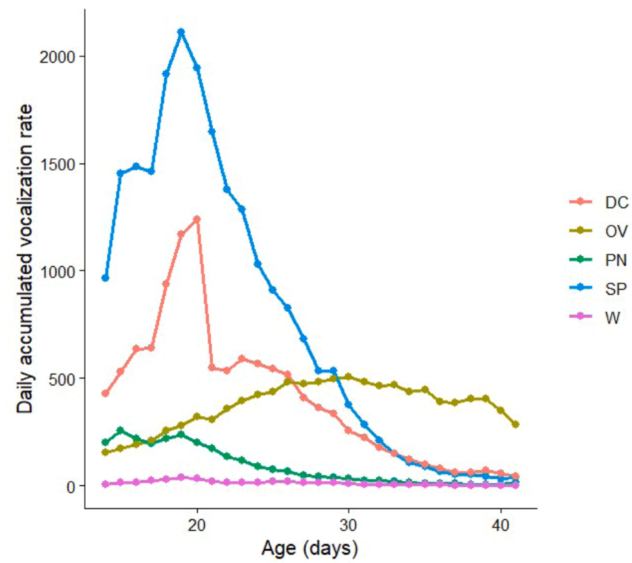

Fig. 2. Daily aggregated vocalization frequency (s/min) across broiler age (14–41 days) pooled across all treatment groups, shown by vocalization category: short peep (SP), distress call (DC), other vocalizations (OV), pleasure notes (PN), and warbles (W).

Table 2
Comparison of mean vocalization rates (s/m) between age periods (period 1: days 14–20, period 2: days 21–27), period 3: days 28–34), and period 4: days 35–41). Means with the same letter within the same column do not differ significantly from each other.

Age Period	Distress Call	Short peep	Pleasure Notes	Warbles	Other Vocalizations
1	2.400[a]	5.111[a]	0.706[a]	0.060[a]	0.721[a]
2	1.643[b]	3.018[b]	0.266[b]	0.041[ab]	1.130[b]
3	0.608[cd]	0.817[cd]	0.070[bc]	0.020[bc]	1.254[b]
4	0.223[d]	0.152[d]	0.023[c]	0.005[c]	1.112[b]
SE	0.211	0.442	0.073	0.013	0.084
p-value	<0.001	<0.001	<0.001	0.019	<0.001

SE: Standard error.

Table 3
Comparison of mean vocalization rates (s/m) between day time periods (morning: 6:00 AM–12:00 PM), afternoon: 12:00 PM–6:00 PM, evening: 6:00 PM–12:00 AM, and night: 12:00 AM–6:00 AM). Means with the same letter within the same column do not differ significantly from each other.

Time period	Distress call	Short peep	Pleasure notes	Warbles	Other vocalizations
Night	0.608[a]	1.161[a]	0.187	0.02140	0.732 [a]
Morning	1.375[b]	2.631 [b]	0.301	0.03521	1.143 [b]
Afternoon	1.439 [b]	2.652 [b]	0.264	0.03202	1.169 [b]
Evening	1.423 [b]	2.572 [b]	0.296	0.03714	1.185 [b]
SE	0.171	0.319	0.039	0.009184	0.065
p-Value					
Period of day	<0.001	0.001	0.136	0.240	<0.001
Disease	0.546	0.645	0.253	0.194	0.972

SE: Standard error.

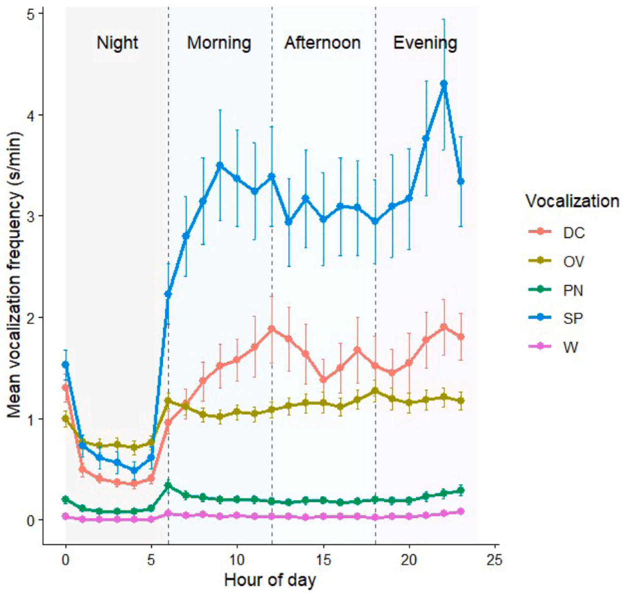

Fig. 3. Mean hourly vocalization frequency (s/min) across the 24h-cycle, pooled across all treatments and ages (14–41 d), for each vocalization category (short peeps, SP; distress calls, DC; other vocalizations, OV; pleasure notes, PN; and warbles, W). Points represent hourly means calculated across rounds and pens, with error bars indicating standard error of the mean (SE). Shaded background regions indicate time-of-day periods: night (0:00–6:00), morning (6:00–12:00), afternoon (12:00–18:00), and evening (18:00–24:00).

studies have progressed through the full pathway of method development, biological validation, and evaluation under real-world commercial conditions. While scientific output in this field has increased markedly in recent years (Soster et al., 2025c), substantial additional work remains necessary before acoustic monitoring can be regarded as a consistently accurate and reliable tool for on-farm welfare assessment.

Some other limitations need to be considered. Relatively large standard errors were observed across several vocalization categories. Although mixed-model analysis did not reveal significant disease effects, the variability within pens may have reduced statistical power to detect subtle differences. Another important consideration concerns the experimental disease models used. While the infection protocols were designed to reliably induce clinical or subclinical disease under controlled conditions, experimental challenges may not fully replicate the complexity of natural infections in commercial settings. Field infections typically involve variable pathogen loads, co-infections, environmental stressors, and fluctuating management conditions, which may amplify or modify acoustic responses. Moreover, in the present study, disease confirmation was not quantified using additional biological parameters such as lesion scoring, serology, pathogen load quantification, or performance metrics. Without concurrent confirmation of disease severity at the individual or pen level, it remains difficult to determine whether the absence of vocalization differences reflects true acoustic resilience or limited clinical expression in this experimental context.

Temporal alignment between peak disease expression and the predefined age periods may also have influenced the results. Age was categorized into weekly intervals, and vocalizations were aggregated hourly to reduce variability. However, if peak pathological expression occurred at narrower time windows that did not align precisely with these period boundaries, disease-related acoustic effects may have been diluted when averaged across broader intervals.

Finally, disease-specific expectations regarding vocal signatures merit further reflection. For respiratory diseases such as IBV, one would theoretically expect the occurrence of distinct abnormal sounds (e.g., rales, sneezes, cough-like events), as demonstrated in previous studies focusing on event-based detection. In contrast, it remains unclear whether intestinal infections or locomotor disorders would be expected to produce distinct, category-level vocal changes. There is currently limited literature linking gut health or lameness in broilers to measurable alterations in spontaneous vocal repertoire. It is plausible that such conditions primarily affect behavior (reduced movement, altered feeding patterns) rather than directly modifying call production. Consequently, acoustic changes in these contexts may be subtle, indirect, or confined to rare vocal subtypes not captured within the dominant categories analyzed here.

Conclusions

This study provides a detailed characterization of broiler vocalization dynamics across the production cycle and daily periods, while testing their sensitivity to three major poultry disease models. No significant effects of Eimeria spp. or infectious bronchitis virus combined with avian pathogenic *Escherichia coli* were detected at the level of vocalization category frequencies. In contrast, vocal output was primarily shaped by age and time of day, with progressive changes observed. SP and DC decreased progressively over time, with the lowest levels in periods 3–4. PN was also lower in periods 3–4 than in period 1. W showed no consistent temporal or disease-related differences. In contrast, OV increased steadily from period 1 through periods 2–4. Vocalization rates were lowest at night compared with other periods of the day.

These findings indicate that, under the present experimental conditions, disease-related acoustic signatures were not expressed as shifts in the major vocalization classes and were instead dominated by strong developmental and diurnal structure. This contrasts with earlier work focusing on isolated abnormal respiratory events and suggests that repertoire-level approaches may have limited sensitivity when pathological sounds are rare relative to overall acoustic activity. At the same time, several limitations should be considered when interpreting the absence of disease effects. The relatively large variability across pens may have reduced statistical power to detect subtle acoustic changes, and controlled challenge models may not fully reproduce the complexity of natural infections in commercial settings, where heterogeneous pathogen exposure, co-infections, and environmental stressors may amplify acoustic responses. In addition, disease confirmation was not quantified using complementary biological or performance measures, limiting the ability to link acoustic output to disease severity. Finally, weekly age-period binning and hourly aggregation may have diluted transient disease effects if peak expression occurred over narrower windows that did not align with period boundaries.

Importantly, the observed expansion of unclassified vocalizations with age, together with emerging evidence that broilers produce a broader acoustic repertoire than currently recognized, highlights the potential value of deeper characterization of these “other” sounds. Subtle health- or welfare-related information may reside within these less frequent vocalizations or within intra-call acoustic features not captured by category-based analyses. Future work should therefore integrate detailed acoustic feature extraction with clinical, behavioral, and environmental measures to improve biological interpretability and disease sensitivity.

CRediT authorship contribution statement

Patricia Soster: Writing – original draft, Visualization, Methodology, Investigation, Conceptualization. **Tomasz Grzywalski:** Writing – original draft, Visualization, Formal analysis, Conceptualization. **Camila Lopes Carvalho:** Methodology, Investigation. **Imad Khan:** Methodology, Investigation. **Pieter Thomas:** Methodology, Investigation. **Frank Tuyttens:** Writing – review & editing, Supervision, Project administration, Methodology, Investigation. **Luc Duchateau:** Formal analysis. **Isaura Christiaens:** Methodology, Investigation. **Maarten De Gussem:** Supervision, Project administration, Funding acquisition. **Paul Devos:** Supervision, Funding acquisition. **Dick Botteldooren:** Supervision, Methodology, Funding acquisition. **Gunther Antonissen:** Writing – review & editing, Supervision, Project administration, Methodology, Investigation, Funding acquisition.

Disclosures

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

This study was conducted as part of the imec ICON project WISH, a collaborative initiative involving both academic researchers and industry partners. The WISH project receives co-funding from imec and financial support from Flanders Innovation & Entrepreneurship under project number HBC.2021.0664.

References

Abozeid, H.H., 2023. Global emergence of infectious bronchitis virus variants: evolution, immunity, and vaccination challenges. Transbound. Emerg. Dis. 2023, 1144924. https://doi.org/10.1155/2023/1144924.

Aviagen, 2024. Ross 308 broiler: performance objectives. Retrieved March 27, 2025, from. https://en.aviagen.com/brands/ross.

Carpentier, L., Vranken, E., Berckmans, D., Paeshuyse, J., Norton, T., 2019. Development of sound-based poultry health monitoring tool for automated sneeze detection. Comput. Electron. Agric. 162, 573–581. https://doi.org/10.1016/j.compag.2019.05.013.

Carroll, B.T., Anderson, D..V., Daley, W., Harbert, S., Britton, D.F., Jackwood, M.W., 2014. Detecting symptoms of diseases in poultry through audio signal processing. In: Wang, Z., Li, G., Juang, F. (Eds.), IEEE International Conference on Acoustics, Speech, and Signal Processing (ICASSP) 2022. Piscataway, NJ, USA. IEEE, pp. 1132–1135. https://doi.org/10.1109/ICASSP43902.2022.9746290.

Cuan, K., Zhang, T., Huang, J., Fang, C., 2020. Detection of avian influenza-infected chickens based on a chicken sound convolutional neural network. Comput. Electron. Agric. 178, 105688. https://doi.org/10.1016/j.compag.2020.105688.

Du, X., Lao, F., Teng, G., 2018. A sound source localization analytical method for monitoring the abnormal night vocalisations of poultry. Sensors 18 (9), 2906. https://doi.org/10.3390/s18092906.

FAO, 2019. OECD-FAO Agricultural Outlook 2019–2028. OECD Publishing, Paris, France. Food and Agriculture Organization of the United Nations: Rome, Italy.

Gemmeke, J. F., Ellis, D. P., Freedman, D., Jansen, A., Lawrence, W., Moore, R. C., Ritter, M., 2017. AudioSet: an ontology and human-labeled dataset for audio events. In: 2017.

Grzywalski, T., Soster, P., Hou, Y., Thomas, P., Tuyttens, F.A.M., Dedeurwaerder, A., De Gussem, M., Devos, P., Antonissen, G., Botteldooren, D., 2025. Identification and characterization of clusters of potentially new vocalizations in broiler chickens using advanced acoustic analysis. Poult. Sci. 104, 105769.

Huang, J., Wang, W., Zhang, T., 2019. Method for detecting avian influenza disease of chickens based on sound analysis. Biosyst. Eng. 180, 16–24. https://doi.org/10.1016/j.biosystemseng.2019.01.004.

Kong, Q., Cao, Y., Iqbal, T., Wang, Y., Wang, W., Plumbley, M.D., 2020. PANNs: large-scale pretrained audio neural networks for audio pattern recognition. IEEE/ACM Trans. Audio Speech Lang. Process. 28, 2880–2894.

Liu, L., Li, B., Zhao, R., Yao, W., Shen, M., Yang, J., 2020. A novel method for broiler abnormal sound detection using WMFCC and HMM. J. Sens. 2020, 2985478. Article ID.

Lv, M., Sun, Z., Zhang, M., Geng, R., Gao, M., Wang, G., 2023. Sound recognition method for white feather broilers based on spectrogram features and the fusion classification model. Measurement 222, 113696.

Madlala, T., et al., 2021. Understanding the interactions between *Eimeria* infection and gut health in chickens. Front. Vet. Sci. 8, 751481. https://doi.org/10.3389/fvets.2021.751481.

Mahdavian, A., Minaei, S., Marchetto, P.M., Almasganj, F., Rahimi, S., Yang, C., 2021. Acoustic features of vocalization signal in poultry health monitoring. Appl. Acoust. 175, 175. https://doi.org/10.1016/j.apacoust.2020.107756.

Manteuffel, G., Puppe, B., Schön, P.C., 2004. Vocalization of farm animals as a measure of welfare. Appl. Anim. Behav. Sci. 88, 163–182. https://doi.org/10.1016/j.applanim.2004.02.012.

Marx, G., Leppelt, J., Ellendorff, F., 2001. Vocalisation in chicks (Gallus gallus domesticus) during stepwise social isolation. Appl. Anim. Behav. Sci. 75, 61–74. https://doi.org/10.1016/S0168-1591(01)00180-0.

Mesa-Pineda, C., et al., 2021. Chicken coccidiosis: from the parasite lifecycle to control strategies. Front. Vet. Sci. 8, 787653. https://doi.org/10.3389/fvets.2021.787653.

National Research Council, 2015. Critical Role of Animal Science Research in Food Security and Sustainability. The National Academies Press, Washington, DC. https://doi.org/10.17226/19000.

Nordin, N., Mohd Sani, N.I., Abdul Kadir, A., Shaari, R., Mohamed, M., Hanif Reduan, M. F., Nordin, M.L., 2021. Infectious bronchitis associated with Escherichia coli infection in commercial broiler chickens: a case report. J. Adv. Vet. Anim. Res. 8 (1), 101–104. https://doi.org/10.5455/javar.2021.h491.

Norton, T., Chen, C., Larsen, M.L.V., Berckmans, D., 2019. Review: precision livestock farming: building 'digital representations' to bring the animals closer to the farmer. Animal 13 (12), 3009–3017. https://doi.org/10.1017/S175173111900199X.

Rios, H.V., Waquil, P.D., Soster de Carvalho, P., Norton, T., 2020. How are information technologies addressing broiler welfare? A systematic review based on the Welfare Quality® assessment. Animals 10 (2), 331. https://doi.org/10.3390/ani10020331.

Rizwan, M., Carroll, B., Anderson, D., Daley, W., Harbert, S., Britton, D., Jackwood, M., 2016. Identifying rale sounds in chickens using audio signals for early disease detection in poultry. Comput. Electron. Agric. 128, 0–4. https://doi.org/10.1016/j.compag.2016.08.006.

Sainburg, T., Thielk, M., & Gentner, T. Q. (2020). Finding, visualizing, and quantifying latent structure across diverse animal vocal repertoires. In F.E. Theunissen (Ed.), PLoS Computational Biology, 16(10). https://doi.org/10.1371/journal.pcbi.1008235.

Soster, P.(a), Grzywalski, T., Buyse, K., Thomas, P., Lopes Carvalho, C., Khan, I., Khalfi, B., Tuyttens, F., De Gussem, M., Devos, P., Botteldooren, D., Antonissen, G., 2025. Influence of age, time of day, and environmental changes on vocalization patterns in broiler chickens. Poult. Sci. 104, 105298.

Soster, P.(b), Gzywalski, T., Hou, Y., Thomas, P., Dedeurwaerder, A., De Gussem, M., Tuyttens, F., Devos, P., Botteldooren, D., Antonissen, G., 2025. Automated detection of broiler vocalizations: a machine learning approach for broiler chicken vocalization monitoring. Poult. Sci. 104, 104962. https://doi.org/10.1016/j.psj.2025.104962.

Soster, P.(c), Devos, P., Smets, R., Tuyttens, F.A.M., Andretta, I., Buyse, K., De Gussem, M., Antonissen, G., 2025. A systematic review linking welfare and automated analysis of acoustic signals in broiler chickens and layers. World's Poult. Sci. J. https://doi.org/10.1080/00439339.2025.2543042.

Tuyttens, F.A.M., Molento, C.F.M., Benaissa, S., 2022. Twelve threats of precision livestock farming (PLF) for animal welfare. Front. Vet. Sci. 9, 889623. https://doi.org/10.3389/fvets.2022.889623.

Van Renterghem, T., Thomas, P., Dominguez, F., Dauwe, S., Touhafi, A., Dhoedt, B., Botteldooren, D., 2010. On the ability of consumer electronics microphones for environmental noise monitoring. J. Env. Monit. 13, 544–552. https://doi.org/10.1039/c0em00532k.

Wathes, C.M., Kristensen, H.H., Aerts, J.-M., Berckmans, D., 2008. Is precision livestock farming an engineer's daydream or nightmare, an animal's friend or foe, and a farmer's panacea or pitfall? Comput. Electron. Agric. 64 (1), 2–10. https://doi.org/10.1016/j.compag.2008.05.005.