Cough Sound Acoustics and Environmental Agents in Modern Swine Farming

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Abstract. Cough sound analysis has been performed in this research for fattening swine respiratory disease assessments. In particular, here are investigated the abiotic environmental factors predisposing animals to diseases. From cough sound recording during 4 fattening cycles it has been possible to evaluate the dynamics both of respiratory diseases and of cough sounds. The bioacoustic analysis investigated features like sound duration, amplitude and frequency and it has been combined with environmental recordings of temperature, relative humidity, gaseous ammonia and particulate matter concentrations. A serological screening has been also performed to evaluate the animal's non specific immunity, useful to evaluate how farming conditions may affect animals welfare and health. The correlations of all parameters allowed estimation of the weight of every factor on the type of respiratory disease and estimation of which are useful acoustics parameters to be monitored for early diagnosis of respiratory hazards.

1 Introduction

In intensive swine farming conditions respiratory disease outbreaks are often linked to housing conditions, environmental predisposing factors and etiologic agents. All these actors make it a multifactor diseases that require punctual observation both on the sanitary and environmental levels. The extremely high number of animals in farms is associated to an increase of diffusible pathologies and to a more difficult controlled environment. Temperature, relative humidity, air ventilation, airborne dust and gaseous pollutants severely affect the respiratory systems of animals. Epidemiologic studies, in the past, had underlined how gaseous ammonia and airborn dust act as a irritative agents and may accumulate in the mucus of respiratory mucosa increasing nasal mucosa lesions and pneumonia incidence which allows insediation of secondary opportunists agents [1], [2].

Other studies about climate influences shows a higher respiratory diseases incidence in wintertime confirmed by slaughterhouse lung scoring. the animals reared in such season suffer ventilation deficiency and show major lung lesions. On the other side a too high ventilation rate generates cold air flows at animal's level affecting

Ferrari S., Costa A., Gusmara C., Sala V. and Guarino M..
Cough Sound Acoustics and Environmental Agents in Modern Swine Farming.
DOI: 10.5220/0003886400300039
In *Proceedings of the International Workshop on Veterinary Biosignals and Biodevices* (VBB-2012), pages 30-39
ISBN: 978-989-8425-94-2

their immune system. Humans can't quantify a continuous observation of animal's health and its relation with environment and abiotic disease causes, and respiratory disease control and prevention are largely realized by drugs administration.

This work aims to show a different integrated approach for respiratory disease diagnosis using bioacoustics as a precision livestock farming continuous monitoring tool together with environmental and sanitary control. Sound analysis has been widely researched and applied in swine and dairy calves farms showing how different cough sounds can be associated to certain type of diseases [3], [4], [5], how cough sounds have different acoustic features compared to other environmental or animal vocalizations and how, for this reasons, they may be used as diagnostic tool [6], [7], [8]. We propose in this paper how cough sound recordings and analysis might allow detection of respiratory disease linked with climate parameters, particulate matter, ammonia concentrations and non-specific immunity parameters [9], [10]. The integration between the studied parameters is useful to understand the environmental causes of respiratory diseases and the acoustics of coughs they generate. This knowledge is important for a better approach to the disease improving management and avoiding unuseful drug approach to diseases and to understand how better environment leads to healthier animals.

2 Materials and Methods

Acoustic and environmental data were collected in Italians swine farming along the seven months of 4 fattening cycles (30-175 kg). All farms showed similar building structures for fattening pigs which were reared on fully slatted concrete floor in multiple boxes 3,5x7m. Two of the cycles (*a* and *b*) were breed in mechanically ventilated compartments while the other two (*c* and *d*) in naturally ventilated piggeries.

2.1 Sound Recordings

Sounds were collected once a week during the whole period (6/7 months) of each fattening cycle. Directional microphones were hanged 1.5 m over the pens to cover the surface of two adjacent boxes. Microphones were connected to a laptop and recordings were supported by Adobe Audition®. The sampling rate was 44.1 KHz, suitable for vocal sounds recordings, and the signals were converted in digital formats (.Wav). Each recording was run for 30 minutes a week and the data were stored on an external hard disk via the pc unit.

2.2 Sound Labeling and Analysis

The audio file playbacks were run in a specifically home designed labeling tool to extract and classify individual sounds on the basis of the amplitude of the sound signal. The system recognizes the sound's energy envelopes, which are indicators of the place where the signals energies are concentrated. When the energy envelope exceeds an automatically selected, environment specific threshold, the corresponding sound is selected. After having completed the sound extraction, the individual sounds were manually labelled to select only cough sounds (figure 1) that will be further analyzed.

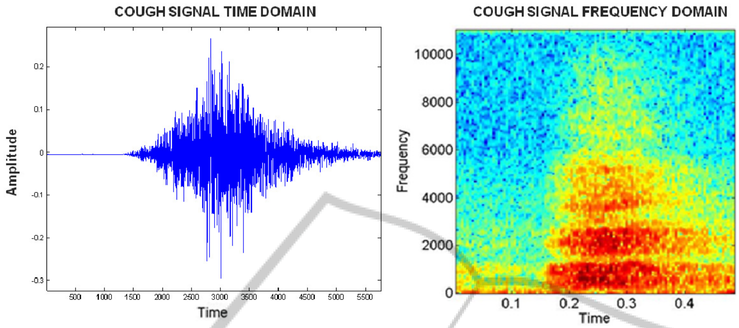

Fig. 1. Pig cough sound wave in time and frequency domain.

The subsequent sound analysis aimed to investigate coughs acoustics features like duration, fundamental frequency, peak frequency and amplitude (Ferrari et al., 2008b). The duration of a sound is defined by an analysis of signal time-series starting from the point in which the sound wave suddenly increases in amplitude until it decreases back to the silence state. The frequency of a sound signal has been studied in terms of its Fundamental Frequency (f0), which is the lowest frequency, or harmonics produced by any particular sound source (Gerhard, 2003) and in terms of peak frequency to keep higher values of energy in within frequency levels.

For the amplitude analysis the Root Mean Square (RMS) is applied. This parameter reflects the fraction of the time in which the signal amplitude is near to its maximum. The ratio between coughs number and minutes of recordings has been also calculated (C/min where C=coughs). For the signal processing we used a homemade program based on Matlab 7.1® to extract sounds characteristics and draw a fast descriptive statistics about the trends of the analyzed sounds.

2.3 Environmental Measures and Air Quality

Temperature and relative humidity were recorded inside the fattening compartments, simultaneously with recordings periods, by air probes portable datalogger. The parameters were collected spot at animal's level to have a more correct recording of the animals feelings and the marked temperature was based on a four spots measurements average. Air quality was assesses by measuring airborn dust in terms of Particulate matter concentrations (PM_{10}) and gaseous ammonia concentrations. PM_{10} were monitored 30 minutes a week by a dust sampler based on combined light scattering nephelometer and filter gravimetric air sampler (Haz Dust-Epam 5000) that allows dust measurements in (mg/m^3). For the ammonia sampling the portable Dräger Chip Measurement System was used for spot gas measurements in the pens at floor and animal's level. The device combines an electronic based analyzer with substance specific chips capillaries filled with a specific reagent for ammonia. All the environmental samplings both microclimate and air quality data, were collected once a week.

2.4 Sanitary Measurements: Non-specific Immune Response

In this research we monitored non-specific factors associated to animal's adaptations to intensive farming conditions as indicators of stress.

The complement system is a group of proteins that when activated lead to target cell lysis and facilitates phagocytosis through opsonisation. The CH50 is a screening assay for the activation of the classical complement pathway. If a complement component is absent, the CH50 level will be zero; if one or more components of the classical pathway are decreased, the CH50 will be decreased and this happens in cases of stress or diseases. Serum Lysozyme (LYS) is part of the innate system that damage bacterial cell walls by catalyzing hydrolysis over peptidoglycans (found in the cell walls of bacteria, especially Gram-positive bacteria). Its concentrations has been assessed by Fluorimetric assays which is capable of detecting enzyme activity in concentrations down to 1 μg/ml. Serum bactericidal activity (SBA) has been also tested by traditional microtiter assay.

2.5 Statistic Analysis

All data collected were submitted to Variance Analysis and to Pearson correlation (SAS 9.2, 2011) to estimate the effects of environmental conditions on respiratory disease in pigs. Analysis of Variance was done to analyze relations of number and acoustics characteristics of coughs with environmental parameters and air quality (T°C, RH, ventilation rates, NH_3 e PM_{10}). The results of this analysis were discriminated according to period of the cycle, season, cycle and typology of farm and the number of coughs per minute were transformed in classes (tab.1).

Table 1. Class grouping of number of coughs recorded per minute.

From (C/min)	To (C/min)	Class
0	0.5	1
0.5	1	2
1	1.5	3
1.5	2	4
…	…	…

A further variance analysis was performed by dividing data coming from different piggery buildings (mechanical ventilation in cycles a and b and natural ventilation for cycles c and d). Further data correlation was assessed by performing a Pearson correlation procedure over the whole dataset to weight the effect of environment on cough acoustics and animal health.

3 Results

3.1 Sound Analysis

2500 minutes of audio tracks have been collected during the 4 followed fattening cycles. The labeling procedure allowed the extraction of more than 1800 coughs (average of 450 coughs per cycle with min 180 coughs in cycle *a* and max 570 in cycle *b*) (figure 2). The analysis of the acoustic features has been performed over the entire database and the results were grouped per months of the fattening cycles. The results were used in a comparative way from the statistic analysis nevertheless the qualitative analysis of the cough sounds showed some very clear distinction regarding each of the acoustic characteristics (figure 3)

Sound duration showed higher values in cycle *b* (0,49s) and cycle *c* (0,46s) while the lower trend were measured in cycle *d* (0.31s); large length variations were measured in cycle *a* (0,35s± 0,11).

Sound RMS showed no variations among the cycles and among the months in each cycle. On average this features measured 0,21± 0,011. For Peak Frequency higher values were recorded in cycle *c* (1445Hz ±79) and lower values in cycle *a* (776Hz ±200). In each of the four cycles the peak frequency decreased according to the increase of age and weight of the animal. Fundamental Frequency recorded outlier peaks in cycle *d* (3000Hz over an average of 1000 Hz). Large variation was measured for this parameter in all the cycles investigated demonstrated also by the high SD from the average (*a*: 931Hz ±454; *b*: 866Hz± 378; *c*: 1003Hz±276; *d*: 1354Hz± 898).

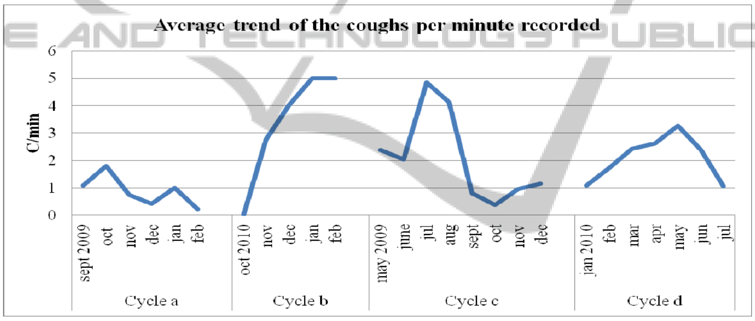

Fig. 2. Average trend of the coughs recorded for the four fattening cycles.

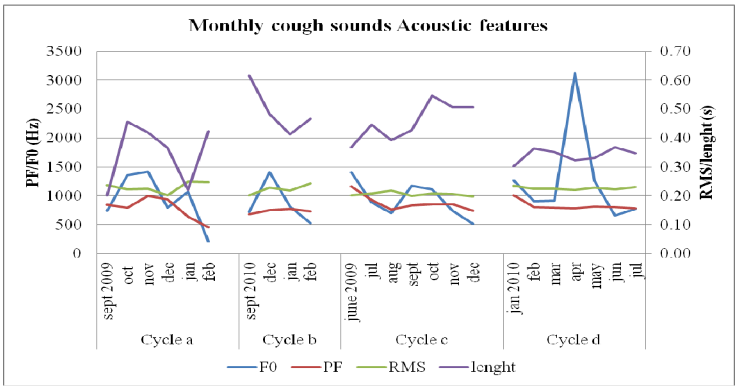

Fig. 3. Trend of the acoustics characteristics studied over the coughs database.

3.2 Environmental Measures and Air Quality (Figure 4 and 5).

Cycle a: this cycle was performed from September 2009 to March 2010. The average monthly temperature recorded ranged from 18 to 25 °C with minimum peaks of 12 ° C recorded in December. Relative humidity ranged between 60 and 80% and the average ventilation rate was 17.907 m^3/h, min. 5.027 and max 30.692. Particulate matter concentrations (PM_{10} mg/m^3) and ammonia concentrations (NH_3, ppm) measured at animal's level showed values included between 0,06 and 1,19 mg/m^3 and between 6 e 15 ppm respectively.
Cycle b: this cycle was performed from October 2010 to February 2011. The records showed typical season trends except for a relative humidity outlier that measured 98%. The average ventilation rate was 21.000 m^3/h, min. 16.800 m^3/h and max. 25.200. In the beginning of the cycle monthly ammonia concentrations averaged 9ppm but reached 27 ppm in February 2011. PM_{10} concentrations were always inferior to 0,6 mg/m^3, showing higher peaks in November.
Cycle c: in this naturally ventilated building microclimate recordings showed similar trends as of external climate. August temperatures reached 30 °C while decreased in winter seasons to a minimum of 15 °C in December. Make up air values were on average 2,6 m/s, min 0,2 m/s and max 5 m/s. Particulate matter concentrations (PM_{10}, mg/m^3) and ammonia concentrations (NH_3, ppm) measured at animal's level showed values included between 0,21 and 0,49 mg/m^3 and between 5.25 and 20 ppm respectively.
Cycle d: from January to July 2010. During the month of May 2010 relative humidity showed a critical trend by decreasing until 40%. Make up air values were on average 1,26 m/s, min 0,2 m/s and max 2,7 m/s. Particulate matter concentrations (PM_{10}, mg/m^3) showed maximum records in March 0,44 mg/m^3. Ammonia concentrations (NH_3, ppm) measured at animal's level showed values included between 4 and 23 ppm with higher values recorded during the first two months of the cycle.

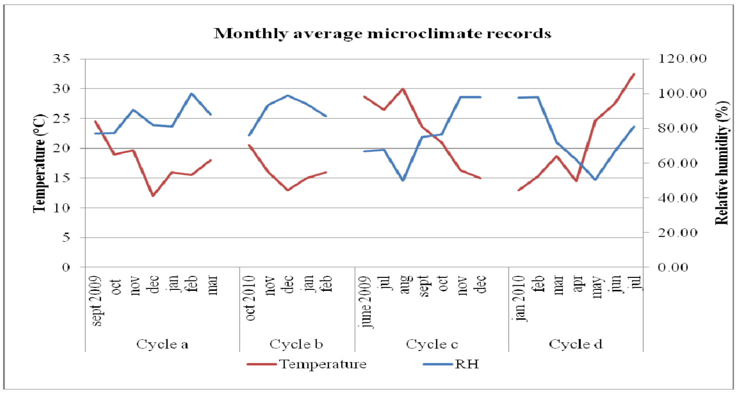

Fig. 4. Trends of the microclimate parameters studied during the four cycles.

3.3 Non-specific Immunity (Tab. 2)

Cycle a: the CH50 level was found to be low from the beginning of the trial and to further decrease along the months of fattening cycle. This shows animal's stress towards livestock conditions. On the other side the higher values of SBA showed positive effective reaction of pigs' immune systems to infections even thought continually exposed to respiratory pathogens, as confirmed by the increase of serum lysozyme concentration.

Cycle b: lower CH50 and SBA levels have also been found in this cycle showing how the intensive swine farming environment is stressful and adversely affects their immune system. The severe animal's exposition to pathogens is also confirmed, especially for the 1st phase of the fattening cycle, by higher values of serum lysozyme.

Cycle c: the CH50 values were low. SBA were constant along the 2/3 of the cycle except for an increasing trend in the last phase. Also the lysozyme concentration was very high along the whole period showing how stress may interfere with local immune reactivity.

Cycle d: this cycle showed the best sanitary and environmental status of animals. CH50 values increased along the screening period showing animal adaptation and comfort in relation to the better environment. In this farm animals had also lower serum lysozyme values and higher SBA showing a minor stress from pathogens exposition.

Table 2. Serum non specific immunity assessment among the four cycles.

Cycle	sampling	CH 50/100 mcl	SBA	LYS (u/ml)
	T1	38,17	67,40 %	84,27
a	T 2	35,38	75,46%	123,87
	T 3	28,96	69,32 %	N.D.
b	T 2	35,40	64,42%	124,69
	T 3	17,69	59,25%	85,84
	T1	25,17	63,63%	196,81
c	T2	32,80	67,42%	117,55
	T3	26,14	80,20%	167,41
	T1	30,62	62,47%	147,83
d	T2	73,06	27,58%	102,35
	T3	72,28	31,60%	138,71

3.4 Statistical Analysis

From the statistic analysis, over 10000 datasets were collected during the 4 cycles. The number of coughs every 30 minute, expressed in C/min classes, was positively influenced by the grouping of relatively acceptable environmental parameters.

Variance analysis performed over the dataset collected in all the swine cycle showed:

- Fundamental frequency (F0) seasonal variation ($P < 0.01$) being averagely higher in summer periods.
- Fundamental frequency (F0) positive variation among increased C/min ($P < 0.05$).
- Peak frequency (PF) does not show significant differences nor variations among seasons and cycles ($P = 0.68$).

- Single cough sounds length is influenced by seasonal changes ($P < 0.05$) being shorter in wintertime.
- The number of C/min is strictly linked to ammonia concentrations ($P < 0.05$).
- Higher number of C/min when PM_{10}< 59 mg/m^3
- Higher number of C/min when NH_3 ranged from 7 to 20 ppm.

Considering the two main typologies of buildings and the different environment:

a) In mechanically ventilated buildings:
- Higher serum lysozyme concentrations at lower temperatures ($P < 0.05$).
- Higher serum lysozyme concentrations at higher ammonia concentrations ($P < 0.01$).
- Lower CH50 concentrations at higher ammonia concentrations ($P <0.01$).
- Higher PM_{10} and NH_3 concentration at lower air make ups ($P <0.01$).
- Higher serum lysozyme concentrations at higher PM_{10} ($P < 0.01$).
- Lower SBA at higher PM_{10} and NH_3 concentrations ($P < 0.05$).

b) In naturally ventilated buildings:
- Higher serum Lysozyme concentrations at lower temperatures ($P < 0.001$).
- - Lower SBA at lower temperatures ($P < 0.01$).
- - Higher serum Lysozyme concentrations at higher relative humidity ($P < 0.05$).
- - Higher length of coughs at lower relative ($P < 0.01$).
- - Higher serum Lysozyme concentrations at higher PM10 ($P < 0.05$).
- - PM10 >550 µg/m3 increases C/min(> 4) ($P < 0.05$).
- - NH3 <7 ppm limited number of coughs (C/min < 2) ($P < 0.05$).
- - Higher NH3 > 20 ppm increase the number of C/min($P < 0.05$).
- - Higher NH3 > 20 ppm inhibit cough mechanism (C/min < 2) ($P < 0.01$).

The results from Pearson correlation explain:
- Increased C/min in cycle *b* (+ 63 %, $P < 0.01$) for higher PM_{10} and lower ventilation rates.
- Increase C/min is positively associated to increase of NH_3 (< 20ppm) (+ 76 %, $P < 0.001$).
- Increase C/min is positively associated to increase of serum Lysozyme (+ 64 %, $P < 0.01$).
- Length of cough < 0,41s positively associated with increase of Lysozyme and decrease of SBA.
- Length of cough <0,31s positively associated to environmental stress (e.g. NH_3) ($P < 0.05$).
- Increase of animals' age corresponds to increase of sound duration (+ 19 %, $P < 0.001$).
- Increase of NH_3 concentrations shorter cough sound length.(-14 %, $P <0.01$)
- Increase of NH_3 concentrations increases the fundamental frequency (+ 16.%, $P < 0.05$).
- Increase of animals' age corresponds to decrease of the peak frequency.
- Increase of animals' age corresponds to decrease of fundamental frequency.
- Increase of animals' age corresponds to decrease of number of coughs recorded in 30 min.

4 Discussion and Conclusions

Cough sounds quantity and quality can be influenced by environmental factors in different ways: dust, ammonia levels, temperature and HR act on the respiratory system modifying the structures involved in cough generation. Interesting finding of this study is the potential of sound analysis to distinguish, from the length of cough sounds, abiotic or biotic causes as demonstrated from the statistic analysis which recognizes in longer cough signals an etiologic involvement. Here also, the consequences of ammonia and dust on the respiratory system are investigated and the effect of rare lung clearance capacity, due ammonia exposure, is confirmed by the decrease of the number of coughs per minute over 20ppm and from sounds shorter duration indicating reaction failure of the respiratory system.

The study confirms with scientific objective signs (like C/min) that environmental problems and ventilation rates are also very important for animal's health status since we observed the increase of coughs in presence of low temperature or bad air quality and the increase of serum Lysozyme and decrease of CH50 and SBA that show distress in animals and deficiency in their immune systems. From this evaluation glances the importance of how good maintenance of healthy environment may prevent and reduce the amount of respiratory diseases and how sound analysis may give us a precise instrument to monitor continually health status from the counting and analysis of cough sounds signals. The results of this study showed that the proved integrated diagnostic method, could be efficiently used by farmers, as an early warning of the presence of environmental predisposing factors of respiratory disease. By using a set of microphones the whole farm area will be easily scanned for respiratory diseases in a non invasive way, the system will be included of the cough recognition algorithm and of sound filtering systems e.g. bandstop to avoid ventilation sounds disturbances. The information collected about the biotic or abiotic nature of coughs will be important for correct health interpretations, treatment avoiding, reducing expensive medical costs and improving piggeries environment management. In the same way, the combination of bioacoustics with sanitary screenings like bacteriology or specific immunity would complete the diagnosis, identifying the major etiological agents of disease. Bioacoustics integrated method helped to get more knowledge of the major factors involved in intensive swine farming respiratory diseases.

Aknowledgements

This work has been founded by the Italian Ministry of University and Research (MIUR) in the project "Integrazione di Sistemi tecnologici innovatvi per il monitoraggio a distanza di animali" belonging to National Interest research programs (PRIN 2008).

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