



Classification of Porcine Wasting Diseases Using Sound Analysis

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ABSTRACT : This bio-acoustic study was aimed at classifying the different porcine wasting diseases through sound analysis with emphasis given to differences in the acoustic footprints of coughs in porcine circo virus type 2 (PCV2), porcine reproductive and respiratory syndrome (PRRS) virus and *Mycoplasma hyopneumoniae* (MH) - infected pigs from a normal cough. A total of 36 pigs (Yorkshire×Landrace×Duroc) with average weight ranging between 25-30 kg were studied, and blood samples of the suspected infected pigs were collected and subjected to serological analysis to determine PCV2, PRRS and MH. Sounds emitted by coughing pigs were recorded individually for 30 minutes depending on cough attacks by a digital camcorder placed within a meter distance from the animal. Recorded signals were digitalized in a PC using the Cool Edit Program, classified through labeling method, and analyzed by one-way analysis of variance and discriminant analysis. Input features after classification showed that normal cough had the highest pitch level compared to other infectious diseases ($p < 0.002$) but not statistically different from PRRS and MH. PCV2 differed statistically ($p < 0.002$) from the normal cough and PRRS but not from MH. MH had the highest intensity and all coughs differed statistically from each other ($p < 0.0001$). PCV2 was statistically different from others ($p < 0.0001$) in formants 1, 2, 3 and 4. There was no statistical difference in duration between different porcine diseases and the normal cough ($p > 0.6863$). Mechanisms of cough sound creation in the airway could be used to explain these observed acoustic differences and these findings indicated that the existence of acoustically different cough patterns depend on causes or the animals' respiratory system conditions. Conclusively, differences in the status of lungs results in different cough sounds. Finally, this study could be useful in supporting an early detection method based on the on-line cough counter algorithm for the initial diagnosis of sick animals in breeding farms. (**Key Words :** *Mycoplasma hyopneumoniae*, PCV2, PRRS, Pig Cough, Sound Analysis)

INTRODUCTION

Clinically, coughing is the most frequent presenting symptom of many diseases affecting airways and lungs, and is often an early symptom of some diseases (Hischberg and Szende, 1982). Coughing is presented by a sudden air expulsion from the airways which is characterized by a typical sound and this sound is so characteristic that it allows identification of the cough distinguished from other vocal manifestations. The causality of coughing is separated into four common-cough classes respectively labeled as voluntary, chemically induced, acute and chronic (Korpas et al., 1996).

Sound analysis is potentially an observation of considerable importance, because it is a way in which we

can classify and quantify the cough reflex which is one of the most common symptoms of respiratory diseases. Sound production by animals is a candidate bio-signal that can be measured easily at a distance which does not cause additional stress (Aerts et al., 2005). Furthermore, recently, sound analysis has become an increasingly important tool to interpret the behavior, the health condition, and the well-being of animals. Several studies were reported on sound analysis applied to animal sounds in general (Weary and Fraser, 1995; Hayward, 1996; Murray et al., 1998; Van Hirtum and Berckmans, 2002a, 2002b; Madsen et al., 2003; Yeon et al., 2005), to farm animals particularly (Van Compernelle et al., 1992; White et al., 1995; Ikeda et al., 2000; Moreaux et al., 2000; Chedad et al., 2001; Van Hirtum and Berckmans, 2003; Manteuffel et al., 2004) and to pigs specifically (Weary and Fraser, 1997; Chedad et al., 2001; Marchant et al., 2001; Moshou et al., 2001; Van Hirtum and Berckmans, 2003; Aerts et al., 2005; Moura et al., 2008; Ferrari et al., 2008). However the objective registration of cough sound as a diagnostic tool is not yet

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used in current medical practice compared with the registration of the electro-cardiogram (ECG), electro-encephalogram (EEG), electro-myogram (EMG), etc (Moshou et al., 2001).

At this time, most studies primarily related to the sound analysis of pigs are only about the measure conditions for welfare and behavior of animals (Manteuffel et al., 2004) but only a little was done on vocalization for sick animals which could highly affect the rearing of pigs as an example (Chedad et al., 2001; Moshou et al., 2001) and respiratory diseases are widespread causes of mortality and loss of productivity in intensive pig farming. Coughing is one of the symptoms and a central element in screening and diagnosis of common illnesses caused, for example, PCV2, PRRS and MH.

Thus, the objectives of this study were to classify the different kinds of porcine wasting diseases using sound analysis and to determine if there were significant differences in the acoustic features in cough sound among porcine circo virus type 2 (PCV2), porcine reproductive and respiratory syndrome (PRRS) and *Mycoplasma hyopneumoniae* (MH) infected pigs.

MATERIALS AND METHODS

Animal care and management

The experiment was conducted in Pyo Joon Farm, a commercial swine production farm located in Chungnam Province, South Korea. The vaccine program of this farm was PmA, PmD, APP2, App5 and MH and without any history of PCV2 and PRRS vaccination. A total of 36 pigs (Yorkshire×Landrace×Duroc) were used in this study having an average weight ranging between 25-30 kg. Twenty two pigs were housed in a 1.8 m×4.8 m size pen with a room temperature of about 23°C. All pigs were given commercial feed twice a day.

Experimental design

The blood samples of the suspected infected pigs were collected and subjected to serological analysis to determine PCV2, PRRS and MH infection. Those diagnosed PCV2, PRRS and MH infected pigs in this study were considered the infectious group (Group II, III, and IV) and those blood samples with no clinical signs of the infection were considered the normal group (Group I). Take note that in the normal group, we only assumed that this cough is caused by some environmental irritants such as dust, ammonia and other irritants that are usually found in an intensive farm or it may due to other infectious pathogens which are not from PCV2, PRRS and MH.

Disease diagnosis

To diagnose the PCV2, PRRS and MH infection, blood

samples were collected from the *vena cava cranialis* in evacuated test tubes with or without EDTA. Whole blood and serum were centrifuged; the plasma, serum and blood cells were removed, frozen at -20°C and stored for serological diagnosis and DNA extraction. The total DNA from the blood cells was extracted using a QIAmp DNA Mini Kit (Qiagen, USA) in accordance to the manufacturer's instructions. Positive control DNA extracts was acquired from the College of Veterinary Medicine, Gyeongsang National University, Jinju City, Gyeongnam Province, South Korea and Phosphate Buffer Solution (PBS) was used as a negative control. The conventional PCR was performed using primers described previously (Ellis et al., 1999), which amplified the 481-base pair (bp) region from open reading frame (ORF). The forward primer was 5'-CGGATATTGTAGTCTGGTTCG-3' (nucleotide positions 1095-1115), and the reverse primer was 5'-ACTGTCAAGGCTACCACAGTCA-3' (nucleotide positions 1570-1549). PCR amplification of target DNAs was performed in the modified method previously described (Allan et al., 1999), using 2 µl of the total DNA extract with the following modifications: PCR was carried out using Taq PCR master mixer (INTRON Biotechnology, South Korea) scaled down to a final reaction with volumes of 25 µl. The final concentration of reagents for target DNA amplification was: 1.5 mM MgCl₂; 200 µM each dNTP, 0.2 µM in each primer and 0.625 U Taq. The denaturation of template DNA was done one time at 95°C for 15 min, the reaction mixtures were subjected to thermal cycling in a PCR System (Bioneer, South Korea) for 39 cycles in 95°C for 30 s, 55°C for 30 s and 72°C for 30 s. Final elongation was performed at 72°C for 10 min. Amplified products were analysed by electrophoresis with 1.5% (w/v) agarose gels and visualized by staining with ethidium bromide. In the test of PCR amplification, 481-base pair amplified sample were analyzed for PCV2 infection. In order to estimate the respiratory disease like *Bordetella bronchiseptica* (Bb), *Pasteurella multocida* type A (PmA), *Pasteurella multocida* type D (PmD), *Actinobacillus pleuropneumonia* type 2 (App2), *Actinobacillus pleuropneumonia* type 5 (APP5), *Mycoplasma hyopneumonia* (MH) and porcine reproductive and respiratory syndrome virus (PRRS) infection, enzyme-linked immunosorbent assay (ELISA) was used. Moreover, all antigens were acquired from National Veterinary Research and Quarantine Service except for PRRS. All antigens were coated in 96-well micro plate with a coating buffer, incubated at 4°C in 24 h. After incubation, micro plates were washed once with washing buffer (PBS, 0.05% tween20), blocked with blocking buffer (PBS, 2% bovine serum albumin) at 37°C for 1 h. The blocked plates were washed three times with washing buffer (PBS, 0.05% tween20) and the 10 µl of serums were added in each well.

After adding serum, the plates were incubated at 37°C for 2 h. After incubation, the plates were washed three times with washing buffer, conjugates (anti-pig IgG peroxidase conjugated, Sigma A 7042) was added, incubated at 37°C for 1 h. After incubation, the plates were washed four times with washing buffer, substrate (OPD solution) was added in each well, incubated at room temperature for 10 minutes, added stopping solution (4 M H₂SO₄) in each well and measured the optical density (OD) using ELISA reader (492 nm). Serological diagnosis for PRRS virus infection was tested through PRRS diagnosis kit (Idexx laboratories, Germany) according to the manufacturer's instructions.

Sample sound collection

The sounds emitted by animals were recorded through a digital camcorder (JVC GR-DVL520A, Japan) which was placed within a meter distance from the sick pig. The observation was done in a field condition. Although pigs were allowed to move around the pen, most of our recordings were done when they were lying on the floor. The recording was done individually from each pig undergoing coughing. Coughs were recorded for around 30 minutes depending on the cough attacks.

The recorded signal was digitalized in a PC with a standard soundcard Realteck AC97 at 16 bits and 44.100 Hz sampling rates using Cool Edit (Adobe, San Jose, CA) program, and grasped types of sounds (cough sounds) (Figures 1 and 2). We also measured the parameters (pitch, intensity, duration, and formants 1, 2, 3 and 4) of the collected sounds using Praat 0.4 (P. Beersman & D. Weenink, University of Amsterdam, The Netherlands) program with a wide band (300 Hz) filter. In these parameters, the duration of call was the duration of a cough, intensity was the degree of strength of sound, the pitch was the relative concept of frequency, an auditory feature of a cough aiming the high and low of sound, and each formant which is known to be characterized by the frequency of the peak, resonance factor and the relative amplitude level of the cough. The sounds collected were classified through a labeling method. Labeling is a manual procedure based on acoustic analysis combined with visual spectral analysis which is used to extract cough sounds from the entire recordings. This means that the entire recorded files are listened to by a human and every sound is marked and described. Labeling was then offline to extrapolate only those sounds that the visual observation of spectrogram and the auditive confirmation of the operator classified as cough attacks.

Statistical analysis

The sounds emitted by pigs were recorded and analyzed to determine whether there are differences between the cough of different kinds of porcine wasting disease. All of

the recordings were extracted to cool edit and sounds which are not considered cough sounds were eliminated including those considered coughing but which were extremely affected by noise. PCV2, PRRS and MH- infected pigs serve as distinctions for specific pigs as experimental units. The acquired data from Normal, PCV2, PRRS and MH parameters which include pitch, intensity, duration, and formants 1, 2, 3 and 4 were compared through one way analysis of variance and discriminant analysis using SPSS 16.0 program. The result of statistic analysis showed that there was no difference in durations among the parameters. Therefore, when discriminating coughs, the duration was excluded.

RESULTS AND DISCUSSION

According to the results in PCR amplification and serological analysis from 36 pig blood samples, 7 samples gave negative results for any viral and bacterial infection classified as Group I. Thirteen (13) samples were positive to PCV2 infection but negative to other infections (Group II), 7 samples were positive to PRRS infection but negative to other infections (Group III), and positive to MH infection but negative to other infections were identified in the 9 samples classified as (Group IV) (Table 1 and Figure 1).

Literatures on the acoustic features of coughs specifically about coughing which are caused by aging or by microbiological agents are still unknown. Therefore we decided to study the most typical and frequent porcine wasting diseases in pig fattening farming. Sound analysis considered features like pitch, intensity, duration, and formants 1, 2, 3 and 4 of the collected cough samples. Classification results for each of the input features are shown in Tables and Figures. The normal cough had the highest pitch level (325.47±68.01 Hz) compared to those with infectious diseases (PCV2 positive) ($p < 0.002$) but was not statistically different from PRRS and MH - infected pigs (Table 2).

The coughing of pigs with PCV2 was statistically different ($p < 0.002$) from the normal cough and pigs with PRRS but not in MH-infected pigs. The highest level of intensity (85.11±1.51 dB) was found for MH-infected pigs being statistically different from others ($p < 0.0001$) (Figure 4). It also showed that the intensity levels among infectious diseases were statistically different from each other ($p < 0.0001$), moreover in formants 1, 2, 3 and 4, pigs infected with PCV2 were statistically higher compared to others ($p < 0.0001$) (Figure 5). However there was no statistical difference found in duration among the different porcine diseases (PCV2; 96.7±19.26 ms, PRRS; 103.3±25.48 ms, MH; 98.5±19.08 ms) and normal (103.1±21.52 ms) ($p > 0.6863$).

Table 1. The results of PCR amplification and serological test for 36 blood samples from pigs

Diseases*	Samples	PCV2 ¹	PRRS ²	MH ²	PmA ²	PmD ²	App2 ²	App5 ²	Bb ²
Group I	1	-	-	-	-	-	-	-	-
	2	-	-	-	-	-	-	-	-
	3	-	-	-	-	-	-	-	-
	4	-	-	-	-	-	-	-	-
	5	-	-	-	-	-	-	-	-
	6	-	-	-	-	-	-	-	-
	7	-	-	-	-	-	-	-	-
Group II	8	+	-	-	-	-	-	-	-
	9	+	-	-	-	-	-	-	-
	10	+	-	-	-	-	-	-	-
	11	+	-	-	-	-	-	-	-
	12	+	-	-	-	-	-	-	-
	13	+	-	-	-	-	-	-	-
	14	+	-	-	-	-	-	-	-
	15	+	-	-	-	-	-	-	-
	16	+	-	-	-	-	-	-	-
	17	+	-	-	-	-	-	-	-
	18	+	-	-	-	-	-	-	-
Group III	19	+	-	-	-	-	-	-	-
	20	+	-	-	-	-	-	-	-
	21	-	+	-	-	-	-	-	-
	22	-	+	-	-	-	-	-	-
	23	-	+	-	-	-	-	-	-
	24	-	+	-	-	-	-	-	-
	25	-	+	-	-	-	-	-	-
Group IV	26	-	+	-	-	-	-	-	-
	27	-	+	-	-	-	-	-	-
	28	-	-	+	-	-	-	-	-
	29	-	-	+	-	-	-	-	-
	30	-	-	+	-	-	-	-	-
	31	-	-	+	-	-	-	-	-
	32	-	-	+	-	-	-	-	-
	33	-	-	+	-	-	-	-	-
	34	-	-	+	-	-	-	-	-
	35	-	-	+	-	-	-	-	-
	36	-	-	+	-	-	-	-	-

* Group I (normal), Group II (PCV2 positive), Group III (PRRS positive), Group IV (MH positive).

¹ PCR amplification test was estimated by target gene amplification under the agarose gel electrophoresis.

² All serological tests were estimated according to manufacturer's instructions.

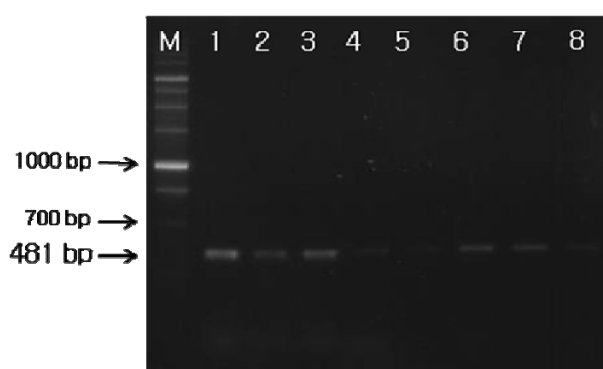


Figure 1. PCR amplification for PCV2. Viral DNA was extracted from whole blood and PCR amplification was performed. The 481 base pair amplified product was shown in agarose gel electrophoresis. M; marker, lane1; positive control, lane 2-8; PCV2 infected samples.

Moreover, the discriminant result among the measured parameters indicated that it is possible to discriminate the different porcine wasting diseases parameters like pitch, intensity and formants 1, 2, 3 and 4. However, among the

Table 2. Pitches (Hz) of the major porcine wasting diseases

Diseases*	No. of pigs	Pitch (Hz)
Group I	7	325.47±68.01 ^{1a}
Group II	13	197.01±115.35 ^b
Group III	7	301.88±80.84 ^a
Group IV	9	269.86±98.88 ^{ab}
p-value		p<0.002

* Group I (normal), Group II (PCV2 positive), Group III (PRRS positive), Group IV (MH positive).

¹ Mean±SD.

^{a, b} Values with different superscript letters are significantly different in p<0.002.

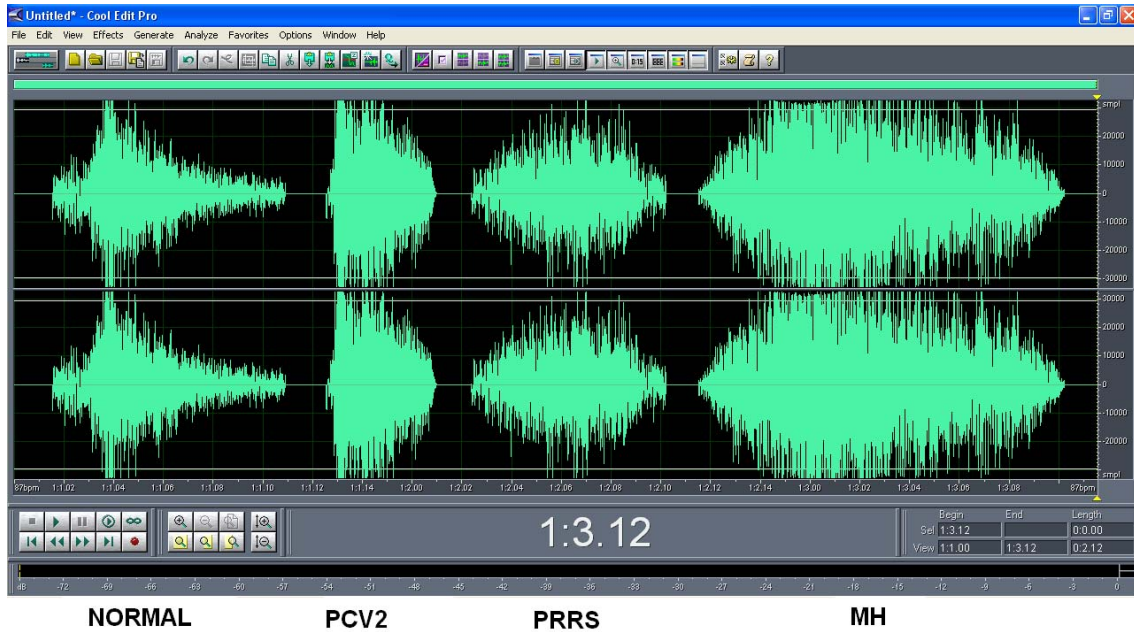


Figure 2. Spectrums of the different cough sounds acquired from normal, PCV2, PRRS and MH samples, respectively.

measured parameters, only cough duration was excluded because findings did not show statistical difference among treatments ($p>0.6863$).

The differences of the acoustic footprints of coughs in PCV2, PRRS and MH infected pigs and from normal cough were also primarily investigated in this study. We assumed that the sound from the lungs of different kinds of porcine wasting diseases and healthy lungs are different because of the variation of sound source, which takes places only if there is disease. Accordingly, changes in the character of the

cough sound can reflect a pathological situation in the lungs (Leith, 1977; Corrao et al., 1979; Hirschberg and Szende, 1982; Korpas et al., 1987; 1993; Braga and Allegra, 1989; Piirila and Sovijarvi, 1995) and the differences between the two classes of cough (infectious or healthy) is that in one case the status of the respiratory system is damaged, and in the other case it is not.

The observed differences of the acoustical patterns (Figures 2 and 3) acquired in this study might be explained by the mechanisms of cough sound creation in the airways.

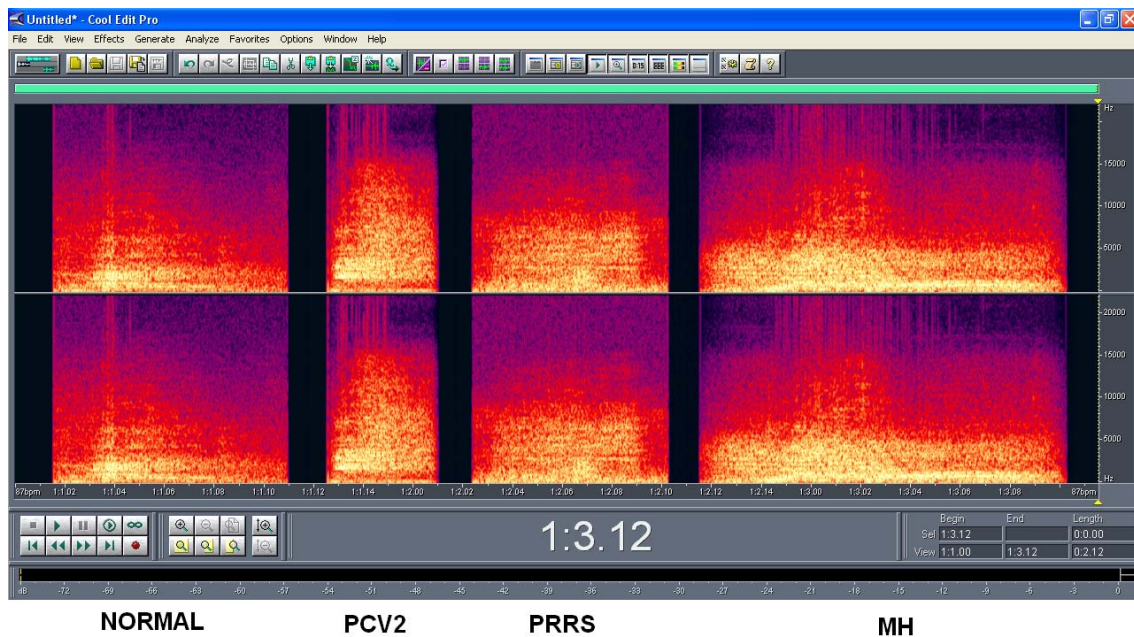


Figure 3. Spectrograms of the different cough sounds acquired from normal, PCV2, PRRS and MH samples, respectively.

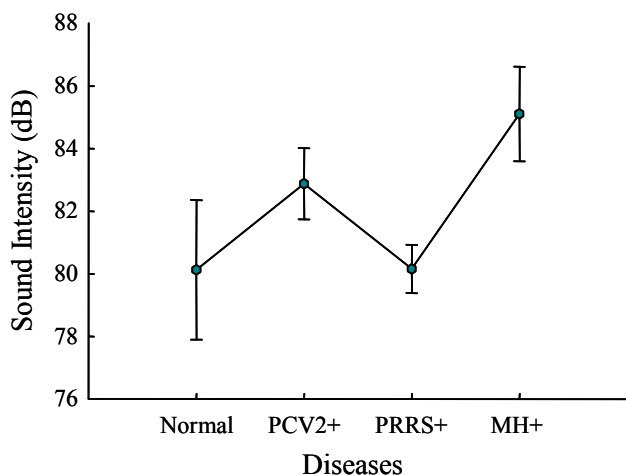


Figure 4. Sound intensities of major porcine wasting diseases (dB).

Some studies have also examined the characteristics of sounds according to their waveforms, finding that the signal envelope appears to differ between different diseases (Kelemen et al., 1987; Korpas et al., 1987). A related technique is to characterize a cough sound by the total energy, which is the integral of the sound intensity (Salat et al., 1986; Korpas et al., 1987). One study showed a significant difference in this quantity between asthmatic and non-asthmatic patients. One approach in describing the spectral character of a cough sound is to obtain the average spectrum of the cough. This appears to differ somewhat between asthmatic and non-asthmatic (Piiirila and Sovijarvi, 1989; Debreczeni et al., 1990). Another approach is to compute a series of spectra to illustrate the evolution of spectral components with time (Piiirila and Sovijarvi, 1989;

Toop et al., 1989). Olia et al. (1993) studied the spontaneous and voluntary cough sounds of patients with chronic lung diseases with the help of Fast Fourier Transform (FFT) analysis, concluding that the cough sound presents a marked heterogeneity; however in most cases, it is possible to identify on the spectrogram the first explosive phase characterized by a rapid transient increase in acoustic energy and rapid changes in energy content at various frequencies.

Widdicombe (1987) has summarized information about sound analysis and concluded that the tussiphonogram may be of considerable value in identifying the mechanisms of airway pathology present in respiratory diseases. This kind of approach may also give information about the pathophysiological mechanisms of coughing by indicating the appearance of tissue in the airway which could lead to a certain pattern of cough. Similarly, it may give information about the behavior of the glottis and whether glottis function behaves differently in different airway pathologies.

However, despite the positive findings of the frequency distribution of the cough sound, it is very variable between subjects and within the same subject in different conditions (Olia et al., 1993). This heterogeneity of the characteristics of cough sounds may constitute a severe obstacle in its evaluation. According to Thorpe et al. (1992), the other difficulty in classifying cough sounds into different types corresponding to different diseases is that the sound can vary in many different ways not at all of which are relevant to changes induced by disease (e.g. sensitivity of equipment, technique of registration, etc).

In veterinary medicine, the serological test is a general and broad method of diagnosing infection. In many

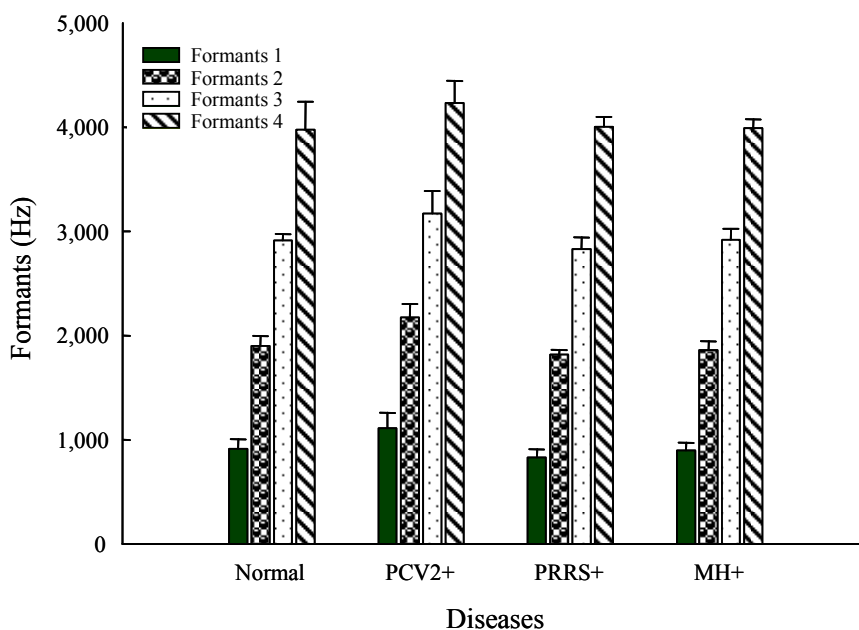


Figure 5. Formants (Hz) 1, 2, 3 and 4 of the major porcine wasting diseases.

Table 3. Discrimination result of the different parameters of cough sounds that include pitch, intensity, formants 1, 2, 3 and 4 acquired from the different porcine wasting diseases

Analysis case processing summary			
Unweighted cases		N	Percent
Valid		76	98.7
Excluded	Missing or out-of-range group codes	1	1.3
	At least one missing discriminating variable	0	0.0
	Both missing or out-of-range group codes and at least one missing discriminating variable	0	0.0
Total		1	1.3
Total		77	100.0

serological diagnostic methods, ELISA is a useful and highly sensitive method for diagnosis. Nevertheless, the most precise method for diagnosing infection is isolation and the identification of pathogens. Even though the serological diagnostic method is useful, the nonspecific reaction or cross reaction with other pathogen can possibly occur. For these reasons, using pathogenic artificial infection and specific instruments may need to confirm our results. In the condition of field farms, there are many factors that can cause pneumonic disease. In this study, we tested the major pathogenic disease in field farm such as PCV2, PRRS, *Pasteurella multocida* type A (PmA), *Pasteurella multocida* type D (PmD), *Actinobacillus pleuropneumonia* type 2 (App2), *Actinobacillus pleuropneumonia* type 5 (APP5), and MH. There are many factors that causes pneumonia in intensive farming, therefore a wide range test is needed to confirm our results.

According to the report of Korpas et al. (1996), the first cough sound creation of healthy subjects is created in place of constitutional airway narrowings and bifurcation, where linear air stream becomes turbulent, which produces vibrations in airway and surrounding lung tissue. In pathological conditions the airway narrowings are multiplied due to the bronchoconstriction, fibrosis, local thickenings of airway walls (inflammation, oedma, mucus collection) resulting to a more extended and folded sound. We assumed that the differences of the cause of the different porcine wasting diseases (PCV2 virus; PRRS-virus; MH-bacteria) will damage differently in lungs that might result in different acoustical patterns of cough. Yanagihara et al. (1966) who studied the simultaneous changes of the flow rate, glottal function and cough sound creation reported that the cough sound reveals its pattern and this typical pattern has also been described repeatedly by others (Korpas et al.,

1982; 1992; 1996; Young et al., 1987; Thorpe et al., 1992) despite the different techniques of registration. The respiratory system is the source of cough sounds and its alterations give different sound source (Ferrari et al., 2008). According to Korpas et al. (1993), secretions in the airways influence the character of cough sounds. Moreover, Piirila and Sovijarvi (1995) observed that subjects with diseases such as mucus or chronic bronchial obstruction show multiple flow spikes and long cough sequences.

SUMMARY

After manual labeling, a comparison between the cough of different porcine wasting diseases was done using sound analysis by investigating physical features of sound wave forms of cough. The findings of this work showed the possibility of discriminating cough sounds of different porcine wasting diseases from sound analysis. Pigs with PCV2 were easily distinguished among other porcine wasting diseases in analysis of formants 1, 2, 3, and 4. Significant differences were also observed in the pitch and intensity of the cough. No statistical difference was observed regarding the cough duration. These findings indicate the existence of acoustically different cough patterns according to the different cause or physical condition of the animal's respiratory system and we found out that the different status of lungs provide different cough sounds.

This bio-acoustic study can allow a design for a detection method based on the on-line cough counter algorithm to recognize sick animals in breeding farms since sound analysis has the ability to provide additional, useful, non-invasive, objective and quantitative information about animal health and care. It could be a candidate in developing an automatic on-line monitoring tool as a biomarker to indicate the presence of porcine wasting diseases in a farm. Specifically, our work would like to introduce the characterization features of pig cough caused by specific agent in terms of acoustical parameters for it to be a useful tool in typical respiratory diseases studies in piggeries and in order to improve the database of labeled pig coughs.

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