



Article

Environmental Risk Factors Influence the Frequency of Coughing and Sneezing Episodes in Finisher Pigs on a Farm Free of Respiratory Disease

Joana Pessoa ^{1,2,3,*}, Jordi Camp Montoro ^{1,4}, Telmo Pina Nunes ⁵, Tomas Norton ³, Conor McAloon ², Edgar Garcia Manzanilla ^{1,2} and Laura Boyle ¹

- Pig Development Department, Teagasc Animal and Grassland Research and Innovation Centre, Fermoy, P61 C996 Cork, Ireland; jordi.montoro@teagasc.ie (J.C.M.); egmanzanilla@gmail.com (E.G.M.); laura.boyle@teagasc.ie (L.B.)
- Section of Herd Health and Animal Husbandry, School of Veterinary Medicine, University College Dublin, Belfield, D04 V1W8 Dublin, Ireland; conor.mcaloon@ucd.ie
- M3-BIORES—Measure, Model & Manage Bioresponses, KU Leuven, B-3001 Leuven, Belgium; tomas.norton@kuleuven.be
- ⁴ Animal Nutrition and Welfare Service, Department of Animal and Food Sciences, Universitat Autònoma de Barcelona, Bellaterra, 08193 Barcelona, Spain
- OIISA—Centre for Interdisciplinary Research in Animal Health, Faculty of Veterinary Medicine, University of Lisbon, 1300-477 Lisbon, Portugal; tnunes@fmv.ulisboa.pt
- * Correspondence: joana.pessoa@teagasc.ie

Simple Summary: Inappropriate environmental conditions in pig buildings are detrimental for both pig and farm-staff health and welfare. With ongoing technological developments, a variety of sensor technology is available and can be used to measure environmental conditions such as air temperature, relative humidity, and ammonia and dust concentrations in real time. Moreover, a tool was recently developed to give farmers an objective assessment of pigs' respiratory health by continuously measuring coughing in finisher pigs. This study assessed baseline levels of coughing on a farm free of respiratory disease, and aimed to identify relationships between environmental conditions and coughing frequency in pigs. Six replicates were conducted. Coughing levels in healthy pigs were overall low, and coughing frequency can be predicted by environmental conditions such as high ammonia concentrations and high ventilation rates. Results of this study can be used as guidelines to determine normal coughing levels in healthy pigs, and to calibrate the alarm systems of tools that measure coughing frequency, such as the cough monitor used in this study. The collection and amalgamation of data from a variety of sources related to health, welfare, and performance are important in order to improve the efficiency and sustainability of the pig industry.

Abstract: This study aimed to assess baseline levels of coughing on a farm free of respiratory disease, and to identify relationships between environmental conditions and coughing frequency in finisher pigs. Six replicates were conducted (690 pigs in total). A cross-correlation analysis was performed and lags of the predictor variables were carried forward for multivariable regression analysis when significant and showing r > 0.25. Results show that coughing frequency was overall low. In the first replicate, coughing was best predicted by exposure to higher ammonia concentrations that occurred with a lag of 1, 7, and 15 days (p = 0.003, p = 0.001, and p < 0.001, respectively), while in the sixth replicate coughing frequency was best predicted by the exposure to lower relative humidity and higher ventilation rates with a lag of 7 and 15 days (p < 0.001 and p = 0.003, respectively). Ammonia concentrations varied according to ventilation rates recorded on the same day (r > -0.70). In conclusion, guidelines on coughing levels in healthy pigs and calibration of the alarm systems of tools that measure coughing frequency can be extrapolated from this study. Environmental risk factors are associated with the respiratory health of finisher pigs.

Keywords: ammonia; air quality; precision livestock farming; PLF; porcine respiratory disease complex



Citation: Pessoa, J.; Camp Montoro, J.; Pina Nunes, T.; Norton, T.; McAloon, C.; Garcia Manzanilla, E.; Boyle, L. Environmental Risk Factors Influence the Frequency of Coughing and Sneezing Episodes in Finisher Pigs on a Farm Free of Respiratory Disease. *Animals* 2022, 12, 982. https://doi.org/10.3390/ani12080982

Academic Editor: Annalisa Scollo

Received: 21 February 2022 Accepted: 6 April 2022 Published: 11 April 2022

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Copyright: © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).

Animals 2022, 12, 982 2 of 12

1. Introduction

Respiratory disease remains a major economic and health concern in the pig industry worldwide [1,2]. Although there are several primary and opportunistic pathogens involved in the Porcine Respiratory Disease Complex (PRDC) [3,4], inappropriate thermal and gaseous environments in pig buildings can exacerbate transmission and spread of these pathogens, triggering and/or increasing severity of clinical outbreaks [3,5]. Furthermore, unfavorable environmental conditions act as a stressor and may damage the pigs' respiratory tract [3]. Therefore, they have a detrimental effect on pig health, welfare, and performance [6].

Ammonia (NH₃) is the most common health threatening gas in animal buildings [7]. Like several respiratory pathogens, NH₃ depresses ciliary activity and mucus flow [8], impairing the mucosal clearance system, thus predisposing the respiratory tract of pigs to infections [9]. Another air pollutant that contributes to low air quality in pig buildings is 'dust' or particulate matter (PM) [9]. PM is a mixture of suspended materials, made up primarily of feed particles, dried fecal material, bacteria, and bacterial toxins [10]. These particulates cause inflammation or irritation of the pigs' respiratory epithelium [9].

Temperature and relative humidity can also influence pigs' respiratory health by disrupting the normal respiratory and thermoregulatory behavior of the animals, while contributing to the survival of pathogens [5,6]. Environment-oriented data, specifically data on air temperature and relative humidity, are routinely collected and used to regulate ventilation systems in most intensive pig farms. Unfortunately, the potential of such data to add value to pig health management on farm is rarely exploited [11]. Measurements of NH₃ concentrations on-farm largely rely on portable sensors that give intermittent and short-term readings [11,12]. However, Dräger[®] launched an electrochemical sensor in 2016 that is capable of continuously monitoring NH₃ concentrations without requiring recalibration.

With ongoing technological developments, a variety of Precision Livestock Farming (PLF) tools are available [13,14]. In recent years, research efforts developed a tool to help monitor and control respiratory disease on-farm by performing continuous and automated measurements of coughing through the analysis of sound collected within pig buildings using a microphone [15]. A recent study employing such technology identified the need to classify patterns of coughing according to environmental risk factors, and to verify the baseline coughing frequency in healthy pigs [16].

Combining animal- and environment-oriented sensor data for the purpose of strategic decision-making is a promising area of future research [11] and could help address the challenge of the respiratory disease syndromes in pigs [17]. Indeed, the collection and amalgamation of data from a variety of sources (potentially including sensors on-farm) related to biosecurity, health, welfare, and performance should be considered a major priority for the efficiency and sustainability of the pig industry [18].

Therefore, the objectives of this study were (1) to assess baseline levels of coughing on a respiratory disease-free farm, and (2) to assess the relationship between environmental conditions (ammonia, particulate matter, temperature, and relative humidity) and levels of coughing.

2. Materials and Methods

2.1. Animals and Experimental Design

This study took place at the Teagasc Pig Research Facility in Fermoy, Co., Cork, Ireland from March 2019 to January 2020. It received ethical approval from the Teagasc Animal Ethics Committee (Approval number: TAEC 204/2018).

The farm operates as a farrow-to-finish facility with a three-week farrowing batch system. Routine seroprofiles show this farm was negative for Porcine Reproductive and Respiratory Syndrome virus, Influenza A virus, *Mycoplasma hyopneumoniae*, and *Actinobacillus pleuropneumoniae*. Moreover, abnormal cases are sent for analysis after necropsy and are consistently negative for the above-mentioned pathogens.

Animals 2022, 12, 982 3 of 12

Danish Duroc X (Large White \times Landrace) pigs were housed in rooms with 10 pens with fully slatted concrete floors (2.4 \times 4.2 m), containing a wet-dry feeder (MA37, Verba, Sint-Oedenrode, The Netherlands) and one nipple drinker. Water and pelleted feed were provided ad libitum. Temperature was controlled with a temperature-based mechanical ventilation system (Big Dutchman 135pro, Vechta, Germany). Space allowance and mixing procedures are described in [19]. Briefly, space allowance varied between 0.78 m²/pig and 0.96 m²/pig (with 13 and 10 pigs/pen, respectively). In each room, five pens were left as intact litters and the other five pens were mixed balancing by weight and sex.

The rooms were illuminated artificially from 07:00 until 18:00 h and pens were enriched with a 1.20 m larch wood post fixed on one of the walls without impairing on the available floor space.

Three production batches of pigs (690 pigs in total) were each housed in two rooms (rooms A and B; 115 pigs per room) from 11 weeks of age, thus encompassing six replicates. All pigs were identified with ear-tags such that they could be monitored until reaching the target slaughter weight of 110 kg (with 21 to 23 weeks of age).

All seasons within a year were covered. The first batch was reared from March to May (spring), the second from July to September (summer), and the third from October to January (autumn/winter).

2.2. Data Collection

Data on environmental parameters and respiratory health were collected resulting in several datasets originating from different sensors and manual assessments. Moreover, lung lesions were scored at slaughter.

2.2.1. Environmental Data

Environmental sensors were used to record daily measurements (average, minimum, and maximum values) of temperature and relative humidity. Sensors were placed in the center of each room. Temperature sensors were placed at a height of approximately 1.5 m and the relative humidity sensors were placed at approximately 2 m. These sensors were part of the cough monitor (SoundTalks NV, Leuven, Belgium) system.

The ammonia sensors (Dräger Polytron C300 with DrägerSensor NH3-Al, Lübeck, Germany) used in this study were electrochemical sensors that perform continuous long-term ammonia measurements. One NH₃ sensor was placed in each room, following manufacturer's guidelines. It provided data points for ammonia concentrations every 30 s.

Particulate matter (PM_{10} only) concentrations were measured in each room using a hand-held laser particulate counter (PCE-PCO 1, PCE Instruments, Meschede, Germany). This procedure was carried out approximately five days a week between 8 am to 1 pm, every week from the time the pigs entered the house until they were removed for slaughter.

Data on ventilation rates (air exchange in m³ per hour) were recorded for the whole duration of this study (Big Dutchman 135pro and Big Farm Net computer system, Vechta, Germany).

2.2.2. Respiratory Health Data

The cough monitors (SoundTalks NV, Leuven, Belgium) used in this study perform continuous and automated measurements of cough sounds, issuing a Respiratory Distress Index (RDI) that corresponds to the average number of coughs per pig per twenty-four hours. They also generate an automated warning, which is set according to a patented Statistical Process Control algorithm using the history and the variation of the RDI from a specific room.

One cough monitor was installed in each room, following the manufacturer's guidelines. All data collected during this study were stored and accessed using the associated pig respiratory distress monitoring (RDM) software.

For each room, the number of coughs and sneezes were counted over a 5-min period approximately five days a week, until pigs reached the target slaughter weight.

Animals 2022, 12, 982 4 of 12

All assessments were performed following the Welfare Quality Protocol[®] [20] guidelines. The average coughing frequency (CF) was estimated as:

$$CF = \frac{\sum Number \ of \ coughs \ in \ a \ Room}{number \ of \ examined \ pigs \ (n)*total \ time \ of \ observation \ (min.)}$$

Sneezing frequency (SF) was estimated using the same formula. These data were collected at the same time as particulate matter concentrations were recorded.

2.2.3. Slaughterhouse Data

Pigs were sent to the slaughterhouse when they reached 110 kg body weight (3 loads per batch with a one-week interval). A slap number linked to each pig's ear-tag number was used, thus allowing us to match the carcass and offal to the corresponding room. All pluck examinations were carried out by the same trained veterinarian.

Lung lesion scoring followed the same protocol reported in [16]. In summary, for each pig, individual lung lobes were examined for pneumonia lesions according to the method developed by Madec and Derrien [21]. The scores were 0 (no pneumonia) to 4 (76–100% of the lung lobe affected). Pleurisy was scored on the dorsocaudal lobes using a modified version of the Slaughterhouse Pleurisy Evaluation System [22]. The scores were 0 (no pleurisy), 2 (focal lesions in one lobe), 3 (bilateral adhesions or monolateral adhesions affecting more than 1/3 of the diaphragmatic lobe), and 4 (extensive lesions affecting more than 1/3 of both diaphragmatic lobes). Cranial pleurisy (CP) and pericarditis were recorded as absent (0) or present (1).

Individual cold carcass weights were also recorded.

2.3. Statistical Analysis

R version 4.0.2 (R Core Team, Vienna, Austria) was used for the statistical analyses [23]. Descriptive statistics are presented for all variables assessed.

2.3.1. Sensor-Based Data

For all variables (temperature, relative humidity, ammonia concentrations, ventilation rates and the respiratory distress index), mean, standard deviation, median, minimum, and maximum values were calculated for rooms A and B in each batch.

Daily averages of NH₃ concentrations and of ventilation rates were used.

To assess the relationship between the time series corresponding to temperature, relative humidity, NH₃ concentrations, ventilation rates (the predictors), and the RDI (response variable), variables were first tested for stationarity using the Dickey-Fuller test. If non-stationary, differencing was applied.

Because respiratory health can depend on the exposure to the currents day's environmental conditions (lag 0), but also on exposure on previous days (negative lags), a cross-correlation analyses was carried out in order to gain insight into the relationship between these variables, and to identify lags that may be useful predictors of the RDI. For biological reasoning, only negative lags and lag 0 were considered. Lags of the predictor variables were carried forward for multivariable regression analysis when significant and showing r > 0.25.

Models were constructed using a backward stepwise elimination based on the Akaike information criterion (AIC). Autocorrelation of residuals was assessed using the Breusch-Godfrey test. Only models where no autocorrelation of residuals was detected are presented. Bonferroni correction was applied to correct for multiple testing.

A cross correlation analyses was also carried out to assess the relationship between ventilation rates and NH_3 concentrations.

2.3.2. Manually Collected Data

Because these data were not continuous time series, a different statistical approach was taken. Spearman's rank correlations were performed to examine the associations between

Animals 2022, 12, 982 5 of 12

particulate matter, NH₃ concentrations, temperature, relative humidity, ventilation rates, and coughing and sneezing frequencies.

Day was used as the experimental unit. For sensor-based data, only the days when manual assessments took place were included (to avoid missing data). Alpha level for significance and tendency were 0.05 and 0.1, respectively.

3. Results

The duration of each finisher period was on average 78 (± 1) days. Lung lesions were scored on 506 pairs of lungs (84 \pm 22 pairs of lungs per replicate). In general, no gross pathology was observed in the lungs. However, the prevalence of pericarditis in pigs reared in the first batch in room A was 10%; moreover, 3 \pm 2% of the trial pigs presented liver milk spots.

Table 1 shows descriptive results for all sensor-based variables.

Table 1. Descriptive statistics for three environmental parameters, ventilation flow, and the Respiratory Distress Index (RDI) summarized over the whole time period for each batch and room.

-					
Replicate	[NH ₃] ¹	Temperature °C	Relative Humidity %	Ventilation ²	RDI
		First	batch		
		Roo	om A		
Mean (sd)	11 (4.3)	21 (0.7)	65 (3.9)	43 (18.6)	1.6 (5.22)
Median	10	21	65	39	0.2
(min-max)	(3.2-22.0)	(22.0-22.5)	(56.8–73.5)	(13.8–84.8)	(0-31.87)
		Roo	om B		
Mean (sd)	9 (3.1)	21 (0.7)	68 (3.8)	43 (13.9)	0.4 (0.31)
Median	9	22	68	41	0.25
(min-max)	(2.7-15.7)	(20.0-22.6)	(58.6-74.3)	(20.9–75.5)	(0-1.5)
		Second	d Batch		
		Roo	om A		
Mean (sd)	10 (2.6)	22 (0.6)	69 (4.1)	80 (14.0)	0.2 (0.15)
Median	9	22	69	81	0.11
(min-max)	(2.7-15.9)	(20.3-23.6)	(61.8–78.1)	(28.5–103.0)	(0-0.6)
		Roo	om B		
Mean (sd)	6 (2.2)	22 (0.7)	72 (4.3)	87 (12.7)	0.3 (0.36)
Median	6	22	71	87	0.21
(min-max)	(1.6-13.5)	(20.5-24.2)	(64.1 - 80.7)	(44.7 - 108.0)	(0-2.1)
		Third	Batch		
		Roo	om A		
Mean (sd)	13 (3.6)	20 (1.3)	70 (2.9)	39 (19.0)	0.3 (0.86)
Median	14	19	70	37	0.11
(min-max)	(3.5-21.3)	(18.5-21.5)	(64.2 - 84.5)	(15.1–82.9)	(0-4.71)
		Roo	om B		
Mean (sd)	10 (3.3)	20 (1.6)	72 (3.1)	41 (18.9)	0.2 (0.30)
Median	9	19	72	39	0.11
(min-max)	(3.9-22.5)	(18.5-22.4)	(66.4-88.7)	(15.9-81.2)	(0-1.99)

¹ [NH₃]: ammonia concentrations (ppm); ² m³/h.

Overall, the respiratory distress index remained low throughout the six replicates assessed, with the exception of room A during the 1st batch where the RDI reached 31.9 at the end of the finisher stage (Figure 1).

Animals 2022, 12, 982 6 of 12

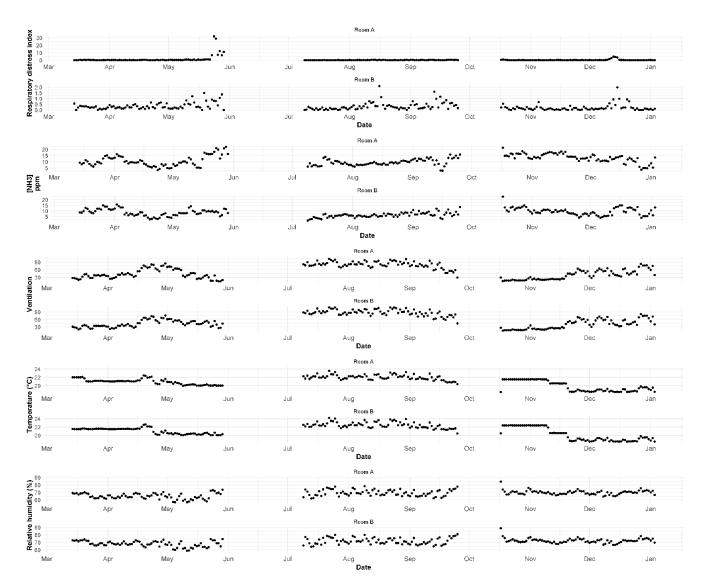


Figure 1. Daily averages of the respiratory distress index, three environmental parameters, and ventilation flow (air exchange in m^3/h).

Daily variation for all sensor-based variables can be seen in Figure 1. In general, higher daily values of NH₃ concentrations were recorded in autumn/winter (3rd batch), followed closely by spring (1st batch). The inverse occurred for ventilation rates, which were higher during summer (2nd batch).

Table 2 shows descriptive results for all manually assessed variables. Overall, coughing and sneezing frequencies were low throughout all batches in the two replicates assessed, although CF was higher in room A during the 1st batch, when it increased by the end of the finisher stage, as did the corresponding RDI values (Figures 1 and 2). Values of particulate matter were consistently low during the summer (Figure 2).

Results on the cross-correlation analysis between environmental conditions and the RDI are shown in Supplementary Material Figure S1.

The multivariable models fitted for the RDI in room A during the 1st batch and in room B during the 3rd batch are presented in Table 3 and were able to explain 47 and 39% of variability, respectively. In room A during the 1st batch the RDI was best predicted by exposure to higher ammonia concentrations that occurred with a lag of 1, 7, and 15 days (p = 0.003, p = 0.001, and p < 0.001, respectively), while in room B during the 3rd batch the RDI was best predicted by the exposure to lower relative humidity and higher ventilation rates with a lag of 7 and 15 days, respectively (p < 0.001 and p = 0.003, respectively).

Animals 2022, 12, 982 7 of 12

Table 2. Descriptive statistics for the concentration of Particulate Matter (PM_{10}) and coughing and sneezing frequencies assessed manually.

Replicate	PM_{10} 1	Coughing Frequency ²	Sneezing Frequency ³
	First l	batch $(n = 33)$	
]	Room A	
Mean (sd)	1346 (621.4)	0.0012 (0.00427)	0.005 (0.0050)
Median (min-max)	1230 (391.3–2924.3)	0 (0-0.021)	0.004 (0-0.0211)
		Room B	
Mean (sd)	1435 (376.6)	0.0011 (0.00397)	0.004 (0.0032)
Median (min-max)	1405 (694.3–2039.7)	0 (0-0.021)	0.004 (0-0.0158)
	Second	l batch (n = 32)	
]	Room A	
Mean (sd)	644 (186.2)	0.0001 (0.00044)	0.001 (0.0022)
Median (min-max)	623 (387.3–1061.0)	0 (0-0.002)	0 (0-0.0088)
		Room B	
Mean (sd)	604 (154.7)	0.0007 (0.00176)	0.002 (0.0035)
Median (min-max)	589 (263.7–923.0)	0 (0-0.007)	0.002 (0-0.0126)
	Third	batch (n = 46)	
]	Room A	
Mean (sd)	1350 (447.0)	0.0005 (0.00097)	0.003 (0-0.0021)
Median (min-max)	1450 (441.0–2231.7)	0 (0-0.004)	0.002 (0-0.0087)
]	Room B	
Mean (sd)	1441 (370.4)	0.0012 (0.00181)	0.002 (0.0026)
Median (min-max)	1365 (763.7–2291.3)	0 (0-0.007)	0.002 (0-0.0139)

¹ μg/m³; ² Number of coughs/pig/min; ³ Number of sneezes/pig/min.

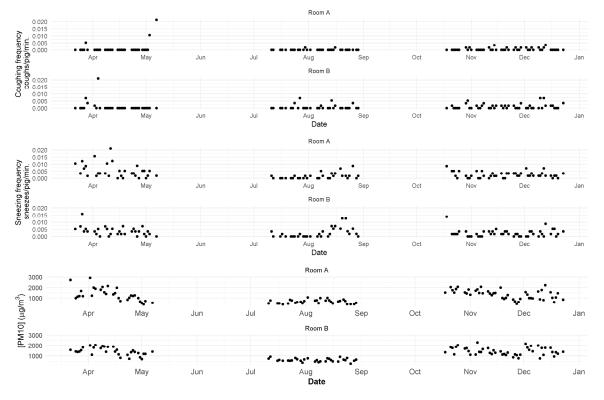


Figure 2. Intermittent measurements of the concentration of Particulate Matter (PM10) and coughing and sneezing frequencies assessed manually.

Animals 2022, 12, 982 8 of 12

Table 3. Multivariable regression models of I	lagged environmental	parameters from the Respiratory
Distress Index.		

Models	Predictors	Estimate (SE)	<i>p</i> -Value
	Intercept	-0.01 (0.426)	0.976
Respiratory Distress	$[NH_3]^1 \log -1$	0.58 (0.184)	0.003 *
Index	$[NH_3] lag -7$	0.70 (0.209)	0.001 *
(1st batch—room A) Adj. $R^2 = 47\%$	$[NH_3] lag -8$	0.51 (0.210)	0.020
	$[NH_3]$ lag -15	0.98 (0.240)	<0.001 *
	$RH^{2} lag -3$	-0.32(0.127)	0.014
Respiratory Distress	Intercept	-0.00 (0.033)	0.982
Index	RH lag -7	-0.07(0.016)	<0.001 *
(3rd batch—room B)	RH lag -8	0.03 (0.016)	0.028
Adj. $R^2 = 39\%$	Ventilation flow lag—15	0.01 (0.004)	0.003 *

¹ Ammonia concentrations; ² Relative humidity; * Indicates significant variables after Bonferroni corrections.

Regarding the cross-correlation analysis assessing the relationship between ventilation rates and NH_3 concentrations, Figure 3 shows strong (r > -0.70) negative associations on lag 0, meaning that NH_3 concentrations varied according to the ventilation flow recorded on the same day. However, during the 2nd batch, this association was lower (in room A) or not present (in room B).

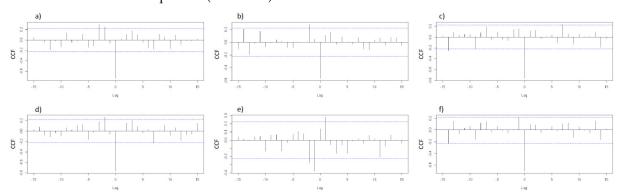


Figure 3. Cross-correlation function for ventilation rates and NH₃ concentrations. Graphs (**a–c**) correspond to the first, second, and third batches of room A, respectively. Graphs (**d–f**) correspond to the first, second, and third batches of room B, respectively. Blue lines indicate the significance threshold. CCF: Cross-correlation function. Blue lines indicate statistically significant associations.

The findings from the univariable analysis of environmental conditions and coughing and sneezing frequencies assessed manually are presented in Figure 4.

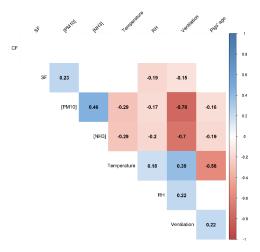


Figure 4. Spearman rank correlations between coughing and sneezing (CF and SF, respectively), concentrations of ammonia and particulate matter ((NH₃) and (PM₁₀), respectively), temperature, relative humidity (RH) and ventilation flow. Blank cells correspond to non-significant results (p > 0.050).

Animals 2022, 12, 982 9 of 12

There were no correlations between CF and environmental conditions. SF had a weak positive association with PM (p < 0.001), whereas PM was negatively correlated with ventilation flow (p < 0.001).

4. Discussion

In the current study, we aimed to explore how coughing frequency varied throughout the finisher stage on a farm free of respiratory disease, and to assess the relationship between environmental conditions within pig houses and coughing levels.

The findings of our study show that the frequency of coughing measured both by the cough monitor and manually were mostly low. Several studies measured the RDI in farms with a high prevalence of lung lesions [15,16] and/or in farms positive for different pathogens associated with the Porcine Respiratory Disease Complex [16,24]. When compared to our study, others generally reported higher values of the RDI, where it reached ≈ 10 [15,16] and ≈ 23 [24]. However, these studies also report low coughing levels throughout the finisher stage. Indeed, Polson et al. [24] associated an increase in coughing with positivity to Influenza A virus. A recent randomized control trial where pigs were inoculated with *Mycoplasma hyopneumoniae* (9 pigs per group with different ratios of inoculated pigs; control group: n=3 pigs) reported RDI varying from 0.01 to 0.17 [25]. Higher coughing frequencies were reported in the groups where more inoculated pigs were present. However, the authors suggest that these low RDI values may be the result of the small number of pigs in each group, since the cough monitors were designed for larger pig populations [25].

Although more research is needed to understand how different coughing patterns associate with different pathogens, our study indicates that RDI values between 0–4 can be considered normal for intensive, indoor commercial farms.

However, we also recorded RDI values of \approx 32 at the end of the finisher stage in one batch (first batch in room A). When exploring the relationship between environmental risk factors and coughing frequency, our model was able to explain 47% of the variability. Interestingly, in this case, the RDI was best predicted by the exposure to higher concentrations of ammonia occurring with a lag of 1, 7, and 15 days. In Figure 1, we can see that, by the end of finisher stage, a steep increase in NH₃ precedes an increase in the RDI. To our knowledge, this is the first study evaluating the relationship between continuous measurements of coughing frequency and NH₃ concentrations. However, in 1969, Stombaugh et al. [8] reported that at 100 and 150 ppm, pigs coughed three times more than those exposed to lower ammonia concentrations (10 and 50 ppm). Still, several other studies showed that (1) ammonia concentrations over 35 ppm induce inflammatory reactions in the respiratory mucosa of animals ([26] cited in [17]); (2) pleurisy is positively correlated with NH₃ concentrations above 25 ppm [27]; (3) pigs exposed to NH₃ concentrations varying from 0.6–37 ppm show small pathological changes in their respiratory tract [28]; and (4) that pigs show a preference for clean air when compared to air with varying NH₃ concentrations [29–31]. Moreover, high concentrations of NH₃ are relevant not only to animal health and welfare, but also to occupational health and safety of farm staff [32,33]. Although there are no legal requirements regarding concentrations of ammonia in pig buildings, studies recommend that levels should be kept below 25 ppm, and ideally below 10 ppm [10,27]. In our study, maximum daily averages reached 22.5 ppm, with mean daily averages varying between 6–13 ppm. Overall, we recorded lower NH₃ concentrations during summer, which is concordant with a trend reported by Chantziaras et al. [17]. Moreover, in agreement with several studies, NH₃ concentrations were higher during the autumn/winter periods [17,34-36]. Interestingly, our cross-correlation analysis shows that higher NH₃ concentrations were significantly associated with decreased ventilation rates, a link that was suggested but not statistically proven [17,36]. However, this correlation was less apparent during summer. It is possible that emptying of slurry tanks in the farm during this time of the year can explain this finding.

Animals 2022, 12, 982 10 of 12

Furthermore, for the trial pigs reared during autumn/winter (3rd batch in room B) we also found that the RDI was best predicted by the exposure to lower relative humidity and higher ventilation rates with a lag of 7 and 15 days, respectively (adjusted $R^2 = 39\%$). As reviewed by Boyle et al. [37] there is strong evidence that high ventilation rates, i.e., draughts are risk factors for respiratory disease. Indeed, draughts are associated with increased frequencies of coughing and sneezing [38], and with the prevalence of pleurisy [39]. Conflicting with our results, high relative humidity is associated with respiratory disease [40], although most studies were carried out in farms where disease was present, meaning that this association may be an indirect one, as relative humidity facilitates dissemination of bacterial pathogens [6].

Regarding particulate matter, we recorded concentrations varying between 264 to 2924 $\mu g/m^3$. These results are in line with the values reported in other studies carried out in finisher farms [10,41]. Furthermore, the majority of microorganisms are present in particles of size 10 μm [10], thus we restricted our measurements to PM₁₀.

Considering the manual assessments carried out in this study, we only found a weak positive correlation between sneezing frequency and particulate matter. It is well established that dust irritates pigs' respiratory epithelium [9], thus this association is coherent. However, we also found strong negative associations between particulate matter and ventilation flow, which is not in agreement with the literature [42]. Indeed, we recorded the lowest concentrations of PM during autumn/winter (when ventilation rates were lowest). Nevertheless, Wang et al. [43] suggest that as ventilation rates increase, PM is better mixed with air within an airspace, meaning that higher ventilation leads to a decrease of the overall mean particulate matter concentration. Still, the fact that we did not measure PM continuously is a limitation of this study.

Ultimately, our results suggest that coupling continuous environmental-oriented data and animal-oriented data may be useful to better understand pigs' respiratory health, and as suggested by Chantziaras et al. [17] could help to elucidate the complexity of the Porcine Respiratory Disease Syndrome.

5. Conclusions

To our knowledge, this is the first study to investigate coughing in a farm where there were no pathogens associated with the PRDC. Results of this study can be used as guidelines on coughing levels in healthy pigs, and to calibrate the alarm systems of tools that measure coughing frequency, such as the cough monitor used in this study.

Furthermore, we show that environmental risk factors are to some extent associated with the respiratory health of pigs, thus we suggest that information collected on these risk factors should be used to help with decision-making processes on farm. We highlight the importance of continuously measuring ammonia concentrations, and urge for the integration of sensor technology and ventilation systems.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/ani12080982/s1, Figure S1: Cross-correlation function for ammonia concentrations (A), temperature (B), relative humidity (C), and ventilation rate and the Respiratory Distress Index. Graphs (i–iii) correspond to the first, second, and third batches of room A, respectively. Graphs (iv–vi) correspond to the first, second, and third batches of room B, respectively. Blue lines indicate the significance threshold.

Author Contributions: J.P.: Study design, data collection, curation, statistical analysis and interpretation; manuscript writing and reviewing. J.C.M.: Data collection and manuscript reviewing. T.P.N.: Statistical analysis and interpretation, manuscript reviewing. C.M.: Manuscript reviewing. E.G.M.: Data collection, study design, and manuscript reviewing. T.N.: Manuscript reviewing. L.B.: Study design, funding acquisition, data collection, manuscript reviewing and editing. All authors have read and agreed to the published version of the manuscript.

Funding: J.P. and J.C.M. were supported by the Teagasc Walsh Scholarship fund grant-in-aid projects reference 0165 and 0415, respectively.

Animals 2022, 12, 982 11 of 12

Institutional Review Board Statement: The animal study protocol received ethical approval from the Teagasc Animal Ethics Committee (TAEC 204/2018).

Informed Consent Statement: Not applicable, as this research did not involve any humans.

Data Availability Statement: The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Acknowledgments: We would like to thank Anais Gonzalez, Agathe Levacher, and Juan Manuel Ortiz for their invaluable help with data collection.

Conflicts of Interest: The authors declare no conflict of interest.

References

- 1. Nathues, H.; Alarcon, P.; Rushton, J.; Jolie, R.; Fiebig, K.; Jimenez, M.; Geurts, V.; Nathues, C. Cost of porcine reproductive and respiratory syndrome virus at individual farm level—An economic disease model. *Prev. Vet. Med.* 2017, 142, 16–29. [CrossRef] [PubMed]
- 2. Calderón Díaz, J.A.; Fitzgerald, R.M.; Shalloo, L.; Rodrigues da Costa, M.; Niemi, J.K.; Leonard, F.C.; Kyriazakis, I.; García Manzanilla, E. Financial Analysis of Herd Status and Vaccination Practices for Porcine Reproductive and Respiratory Syndrome Virus, Swine Influenza Virus, and Mycoplasma hyopneumoniae in Farrow-to-Finish Pig Farms Using a Bio-Economic Simulation Model. Front. Vet. Sci. 2020, 7. [CrossRef] [PubMed]
- 3. Brockmeier, S.L.; Halbur, P.G.; Thacker, E.L. Porcine Respiratory Disease Complex. In *Polymicrobial Diseases*; Brogden, K., Guthmiller, J., Eds.; ASM Press: Washington, DC, USA, 2002.
- 4. Sibila, M.; Pieters, M.; Molitor, T.; Maes, D.; Haesebrouck, F.; Segalés, J. Current perspectives on the diagnosis and epidemiology of Mycoplasma hyopneumoniae infection. *Vet. J.* **2009**, *181*, 221–231. [CrossRef] [PubMed]
- 5. Huynh, T.T.T.; Aarnink, A.J.A.; Verstegen, M.W.A.; Gerrits, W.J.J.; Heetkamp, M.J.W.; Kemp, B.; Canh, T.T. Effects of increasing temperatures on physiological changes in pigs at different relative humidities. *J. Anim. Sci.* 2005, 83, 1385–1396. [CrossRef]
- 6. Brumm, M.C. Effect of Environment on Health. In *Diseases of Swine*, 11th ed.; Zimmerman, J.J., Karriker, L.A., Ramirez, A., Schwartz, K.J., Stevenson, G.W., Zhang, J., Eds.; Wiley-Blackwell: Hoboken, NJ, USA, 2019; pp. 50–58.
- Cargill, C.F.; Murphy, T.; Banhazi, T. Hygiene and Air Quality in Intensive Housing Facilities in Australia. In *Animal Production in Australia*; Revell, D.K., Taplin, D., Eds.; Australian Society of Animal Production: Adelaide, Austrilia, 2002; Volume 24, pp. 387–393.
- 8. Stombaugh, D.P.; Teague, H.S.; Roller, W.L. Effects of Atmospheric Ammonia on the Pig. J. Anim. Sci. 1969, 28, 844–847. [CrossRef]
- 9. Yaeger, M.J.; Van Alstine, W.G. Respiratory System. In *Diseases of Swine*, 11th ed.; Zimmerman, J.J., Karriker, L.A., Ramirez, A., Eds.; Wiley-Blackwell: Hoboken, NJ, USA, 2019; pp. 393–407.
- Michiels, A.; Piepers, S.; Ulens, T.; Van Ransbeeck, N.; Del Pozo Sacristan, R.; Sierens, A.; Haesebrouck, F.; Demeyer, P.; Maes, D. Impact of particulate matter and ammonia on average daily weight gain, mortality and lung lesions in pigs. Prev. Vet. Med. 2015, 121, 99–107. [CrossRef]
- 11. Pineiro, C.; Morales, J.; Rodriguez, M.; Aparicio, M.; Manzanilla, E.G.; Koketsu, Y. Big (pig) data and the internet of the swine things: A new paradigm in the industry. *Anim. Front.* **2019**, *9*, 6–15. [CrossRef]
- 12. Dennier, S. Permanent long-term surveillance of ammonia in livestock—possibilities of a novel sensor technology. In Proceedings of the International Conference on Production Diseases in Farm Animals, Bern, Switzerland, 27–29 June 2019; p. 70.
- 13. Norton, T.; Chen, C.; Larsen, M.L.V.; Berckmans, D. Review: Precision livestock farming: Building 'digital representations' to bring the animals closer to the farmer. *Animal* **2019**, *13*, 3009–3017. [CrossRef]
- 14. Larsen, M.L.V.; Wang, M.; Norton, T. Information Technologies for Welfare Monitoring in Pigs and Their Relation to Welfare Quality®. *Sustainability* **2021**, *13*, 692. [CrossRef]
- 15. Hemeryck, M.; Berckmans, D.; Vranken, E.; Tullo, E.; Fontana, I.; Guarino, M.; van Waterschoot, T. The Pig Cough Monitor in the EU-PLF project results and multimodal data analysis in two case studies. In Proceedings of the 7th European Conference on Precision Livestock Farming (EC-PLF), Milan, Italy, 15–17 September 2015; pp. 147–155.
- 16. Pessoa, J.; Rodrigues da Costa, M.; Garcia Manzanilla, E.; Norton, T.; McAloon, C.; Boyle, L. Managing respiratory disease in finisher pigs: Combining quantitative assessments of clinical signs and the prevalence of lung lesions at slaughter. *Prev. Vet. Med.* **2021**, *186*, 105208. [CrossRef]
- 17. Chantziaras, I.; De Meyer, D.; Vrielinck, L.; Van Limbergen, T.; Pineiro, C.; Dewulf, J.; Kyriazakis, I.; Maes, D. Environment-, health-, performance- and welfare-related parameters in pig barns with natural and mechanical ventilation. *Prev. Vet. Med.* 2020, 183, 105150. [CrossRef] [PubMed]
- 18. Pandolfi, F.; Edwards, S.A.; Maes, D.; Kyriazakis, I. Connecting Different Data Sources to Assess the Interconnections between Biosecurity, Health, Welfare, and Performance in Commercial Pig Farms in Great Britain. *Front. Vet. Sci.* **2018**, *5*, 41. [CrossRef] [PubMed]
- 19. Camp Montoro, J.; Pessoa, J.; Solà-Oriol, D.; Muns, R.; Gasa, J.; Manzanilla, E.G. Effect of Phase Feeding, Space Allowance and Mixing on Productive Performance of Grower-Finisher Pigs. *Animals* **2022**, *12*, 390. [CrossRef] [PubMed]

Animals **2022**, 12, 982 12 of 12

20. Dalmau, A.; Velarde, A.; Scott, K.; Edwards, S.; Butterworth, A.; Veissier, I.; Keeling, L.; Overbeke, G.; Bedaux, V. Welfare Quality®Assessment for Pigs (Sows and Piglets, Growing and Finishing Pigs); Welfare Quality®Consortium: Lelystad, The Netherlands, 2009.

- 21. Madec, F.; Derrien, M. Frequence, intensite et localisation des lesions pulmonaires chez le porc charcutier: Resultats d'une premiere serie d'observations en abattoir. In Proceedings of the Journees de La Recherche Porcine, Paris, France; 1981; pp. 231–236.
- 22. Dottori, M.; Nigrelli, A.D.; Merialdi, G.; Gozio, S.; Bonilauri, P.; Cominotti, F. Proposta di un nuovo sistema di punteggiatura delle pleuriti suine in sede di macellazione. La griglia S.P.E.S. (Slaughterhouse Pleuritis Evaluation System). *Large Anim. Rev.* **2007**, *13*, 161–165.
- 23. R Core Team. R: A Language and Environment for Statistical Computing; R Foundation for Statistical Computing: Vienna, Austria, 2020.
- 24. Polson, D.D.; Playter, S.; Berckmans, D.; Stoffel, A.; Quinn, B. Classification of cough patterns in growing pigs using continuous sound monitoring and an algorithm-based respiratory distress index. In Proceedings of the European Symposium of Porcine Health Management, Barcelona, Spain, 9–11 May 2018.
- Silva, A.P.S.P.; Storino, G.Y.; Ferreyra, F.S.M.; Zhang, M.; Fano, E.; Polson, D.; Wang, C.; Derscheid, R.J.; Zimmerman, J.J.; Clavijo, M.J.; et al. Cough associated with the detection of Mycoplasma hyopneumoniae DNA in clinical and environmental specimens under controlled conditions. *Porc. Health Manag.* 2022, 8, 6. [CrossRef]
- 26. Johannsen, U.; Erwerth, W.; Menger, S.; Neumann, R.; Mehlhorn, G.; Schimmel, D. Experimentelle Untersuchungen zur Wirkung einer chronischen aerogenen Schadgasbelastung des Saugferkels mit Ammoniak unterschiedlicher Konzentrationen 3. Mitteilung: Licht-und elektronenmikroskopische Untersuchungen zur Pathologie und Pathogenese der chronischen aerogenen Ammoniaksch" adigung des Respirationsapparates beim Ferkel. J. Vet. Med. 1987, 34, 260–273.
- Donham, K.J. Association of environmental air contaminants with disease and productivity in swine. Am. J. Vet. Res. 1991, 52, 1723–1730.
- 28. Done, S.H.; Chennells, D.J.; Gresham, A.C.; Williamson, S.; Hunt, B.; Taylor, L.L.; Bland, V.; Jones, P.; Armstrong, D.; White, R.P.; et al. Clinical and pathological responses of weaned pigs to atmospheric ammonia and dust. *Vet. Rec.* 2005, 156, 71–80. [CrossRef]
- 29. Jones, J.B.; Burgess, L.R.; Webster, A.J.F.; Wathes, C.M. Behavioural responses of pigs to atmospheric ammonia in a chronic choice test. *Anim. Sci.* **1996**, *63*, 437–445. [CrossRef]
- Jones, J.B.; Webster, A.J.F.; Wathes, C.M. Trade-off between ammonia exposure and thermal comfort in pigs and the influence of social contact. *Anim. Sci.* 1999, 68, 387–398. [CrossRef]
- 31. Smith, J.H.; Wathes, C.M.; Baldwin, B.A. The preference of pigs for fresh air over ammoniated air. *Appl. Anim. Behav. Sci.* **1996**, 49, 417–424. [CrossRef]
- 32. Lühken, E.; Nicolaisen, T.; Risch, B.; Volkmann, N.; Schnier, S.; Schulz, J.; Kemper, N. Comparison of Two Free-Farrowing Systems and a Conventional Farrowing Crate System with Special Regard to Air Hygiene. *Agriculture* **2019**, *9*, 12. [CrossRef]
- 33. Cole, D.; Todd, L.; Wing, S. Concentrated swine feeding operations and public health: A review of occupational and community health effects. *Env. Health Perspect* **2000**, *108*, 685–699. [CrossRef] [PubMed]
- 34. Van Ransbeeck, N.; Van Langenhove, H.; Demeyer, P. Indoor concentrations and emissions factors of particulate matter, ammonia and greenhouse gases for pig fattening facilities. *Biosyst. Eng.* **2013**, *116*, 518–528. [CrossRef]
- 35. Blunden, J.; Aneja, V.P.; Westerman, P.W. Measurement and analysis of ammonia and hydrogen sulfide emissions from a mechanically ventilated swine confinement building in North Carolina. *Atmos. Environ.* **2008**, 42, 3315–3331. [CrossRef]
- 36. O'Shaughnessy, P.T.; Achutan, C.; Karsten, A.W. Temporal variation of indoor air quality in an enclosed swine confinement building. *J. Agric. Saf. Health* **2002**, *8*, 349–364. [CrossRef]
- 37. Boyle, L.A.; Edwards, S.A.; Bolhuis, J.E.; Pol, F.; Šemrov, M.Z.; Schütze, S.; Nordgreen, J.; Bozakova, N.; Sossidou, E.N.; Valros, A. The Evidence for a Causal Link Between Disease and Damaging Behavior in Pigs. *Front. Vet. Sci.* **2022**, *8*, 771682. [CrossRef]
- 38. Scheepens, C.J.M.; Tielen, M.J.M.; Hessing, M.J.C. Influence of daily intermittent draught on the health status of weaned pigs. *Livest. Prod. Sci.* **1991**, 29, 241–254. [CrossRef]
- 39. Fablet, C.; Marois, C.; Dorenlor, V.; Eono, F.; Eveno, E.; Jolly, J.P.; Le Devendec, L.; Kobisch, M.; Madec, F.; Rose, N. Bacterial pathogens associated with lung lesions in slaughter pigs from 125 herds. *Res. Vet. Sci.* **2012**, *93*, 627–630. [CrossRef]
- 40. Done, S.H. Environmental factors affecting the severity of pneumonia in pigs. Vet. Rec. 1991, 128, 582–586. [CrossRef]
- 41. Van Ransbeeck, N.; Van Langenhove, H.; Van Weyenberg, S.; Maes, D.; Demeyer, P. Typical indoor concentrations and emission rates of particulate matter at building level: A case study to setup a measuring strategy for pig fattening facilities. *Biosyst. Eng.* **2012**, *111*, 280–289. [CrossRef]
- 42. Stärk, K.D. The role of infectious aerosols in disease transmission in pigs. Vet. J. 1999, 158, 164-181. [CrossRef] [PubMed]
- 43. Wang, X.; Zhang, Y.; Zhao, L.; Riskowski, G. Effect of Ventilation Rate on Dust Spatial Distribution in a Mechanically Ventilated Airspace. *Trans. ASAE Am. Soc. Agric. Eng.* **2000**, 43, 1877–1884. [CrossRef]