

Estimating the Effect of Respiratory Disease on Production Performance in Farrow-to-Finish Pig Farms

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Abstract

Background Respiratory disease is one of the most important factors impacting pig production worldwide. However, the literature highlights the multitude of confounding factors complicating the clear attribution of growth impairment to respiratory disease, and the extrapolation of the effects of respiratory disease to a wider population has not been thoroughly researched. The objective of this study was to estimate the impact of respiratory disease on production performance in a subset of 56 Irish farrow-to-finish pig farms. Proxies for respiratory disease status such as serology for four major pathogens (influenza A virus, porcine reproductive and respiratory syndrome virus, *Mycoplasma hyopneumoniae* and *Actinobacillus pleuropneumoniae*), slaughter checks (pleurisy, pneumonia, lung abscesses, pericarditis and liver milk spots) and vaccination information were used as predictors for production performance.

Results The models to estimate production performance from serology, slaughter checks, and vaccination were able to explain the variability of weaner and finisher mortality by 26 and 20%, respectively, and average daily feed intake (ADFI), average daily gain (ADG) and age at slaughter by 47, 40 and 41%, respectively. Feed conversion ratio and sow performance were not explained by the studied predictors.

Conclusions The models fitted, especially those for ADFI, ADG and age at slaughter, emphasize the usefulness of sourcing information at different levels to understand the impact of farm health status on pig performance, and highlight the impact of respiratory disease on production performance.

Background

Respiratory disease is known to be one of the most important factors impacting pig production worldwide. The increase of herd size and stocking densities over the years, coupled with housing pigs indoors translates into higher pressure of infection and higher potential for economic losses [1]. However, the literature describes conflicting information regarding the effects of respiratory disease on performance [2–4]. Many experimental studies have described the influence of specific diseases, such as porcine reproductive and respiratory syndrome (PRRS), or *Actinobacillus pleuropneumoniae* (*A. pleuropneumoniae*) and *Mycoplasma hyopneumoniae* (*M. hyopneumoniae*) infections on farm production performance [3, 5–7], although most highlight the multitude of confounding factors complicating the clear attribution of growth impairment to respiratory disease [3, 4]. The extrapolation of these effects to a wider population at a country level has not been thoroughly researched yet, and in recent cross-sectional studies, the aim was to correlate lung lesion patterns with serology or with risk factors for the development of respiratory infections on farm [8–12], not exploring its impact (of lung lesions or risk factors) on production performance.

At the same time, veterinary practitioners carry out regular diagnostics to monitor the health status of pig farms and the efficacy of disease control measures, for example, vaccination [13]. Slaughterhouse checks, including lung scoring and recording of other lesions e.g. pericarditis and liver milk spots (caused

by *Ascaris suum*) are inexpensive monitoring tools, allowing the collection of data from several farms at one point in space and time [14]. Serology in finisher pigs at slaughter also allows screening for several pathogens and estimating the prevalence of certain infections or the efficacy of vaccination on farm [15, 16]. Combining slaughterhouse checks and serology with information on the vaccination protocols on-farm could be a useful approach to infer the farms' health status regarding respiratory disease.

Thus, the objective of this study is to estimate the effect of respiratory disease on production performance in farrow-to-finish farms using slaughter checks, serology and vaccination information.

Methods

Data on lung lesions, the presence of pericarditis and liver milk spots, and blood samples were obtained in visits to eight slaughterhouses (seven in the Republic of Ireland and one in Northern Ireland, UK) from November 2017 to April 2018, targeting 56 Irish farrow-to-finish pig farms. One batch per farm was assessed. A batch was defined as all the finisher pigs from a given farm killed in a slaughterhouse on the same day. Performance data for 2017 for the participating farms were retrieved from Teagasc e-Profit Monitor (ePM) – a national herd monitoring system. Vaccination data were obtained via telephone calls to farmers and corresponding private veterinary practitioners in April 2018. Additionally, farmers and veterinarians were asked if there were any changes in the vaccination scheme in the previous year.

All the farmers participating in this study provided individual signed consent to the use of the data collected on farm, and to the retrieval of their production data from the Teagasc ePM, according to Teagasc's internal data protection regulation.

Farm selection and production performance indicators and farm characteristics

The Teagasc ePM is a herd monitoring system available on a voluntary basis to all farmers in the Republic of Ireland. In 2017, it included 107 pig herds, representing over 77,000 sows or 52% of the national commercial sow herd [17]. Participation in the cross-sectional study was offered to all farrow-to-finish pig farmers providing data to the ePM, and 56 farmers participated voluntarily. Farms were recruited through the Teagasc advisory service and represented 29.2% of the national commercial sow herd. Performance data from the year 2017 were retrieved from the Teagasc ePM. Data were collected on farms every trimester with the support of Teagasc advisors and collated into a single database.

The production performance indicators used were percentage of weaner and finisher mortality, number of pigs sold per sow per year, average daily feed intake from weaning to slaughter (ADFI), average daily gain from weaning to slaughter (ADG), feed conversion ratio from weaning to slaughter (FCR) and age at sale.

Blood sampling and pluck examinations at slaughter

In the slaughterhouse, blood was collected from a total of 32 randomly selected pigs per farm at sticking (exsanguination). Samples were transported for analysis to the Blood Testing Laboratory of the Department of Agriculture Food and the Marine (Cork, Ireland). Blood was allowed to clot at room temperature, serum was separated, aliquoted into individually labelled anonymised cryovials and stored at -80°C until required for testing. For analysis, 16 samples per farm for PRRS and *M. hyopneumoniae*, and 32 samples per farm for influenza A virus (IAV) and *A. pleuropneumoniae* were selected. The number of samples analysed for each pathogen was based on preliminary prevalence data obtained on a pilot study.

Pluck (lungs, heart, and liver) examinations were performed by the same veterinarian. For each pig, lung lobes were scored for pneumonia lesions according to the method described by Madec and Derrien [18], with the overall surface affected averaged accounting for lobe weights [19]. The variables prevalence of pneumonia (%) and average surface affected out of pneumonic lungs (%), hereinafter called (lung) surface with pneumonia (%), were used for statistical analysis. Pleurisy was scored in the dorsocaudal lobes using a modified version of the Slaughterhouse Pleurisy Evaluation System (SPES), which was developed by Dottori et al., [20] and described by Merialdi et al., [21]. The scores were 0 (no pleurisy), 2 (focal lesions in one lobe), 3 (bilateral adhesions or monolateral lesions affecting more than 1/3 of the diaphragmatic lobe), and 4 (extensive lesions affecting more than 1/3 of both diaphragmatic lobes). The prevalence of pleurisy (lesions with SPES \geq 2) and the prevalence of scores 3 and 4 (prevalence of moderate or severe dorsocaudal pleurisy) were used for statistical analysis (%). Cranial pleurisy (adhesions between lobes, in the surface of the apical and cardiac lobe, and/or adhesions between the lung and the heart), which would correspond to score 1 of the original SPES, and scars (healing indicative of pneumonic lesions which developed earlier in the pig's life) were recorded as absent or present and used in the analysis. Thus, all pleurisy-related variables were pleurisy, moderate and severe pleurisy and cranial pleurisy; while pneumonia-related variables were pneumonia, lung surface with pneumonia, and scars. Additionally, pericarditis (defined as expansion of the pericardial cavity with inflammatory exudate [22]), liver milk spots (presence of white spots in the liver indicative of transhepatic migration of the larvae of *Ascaris suum* [23]), and lung abscesses (presence of one or more abscesses in the lung) were also recorded as absent or present.

Serology

Seroprevalence of antibodies against IAV, PRRS, *M. hyopneumoniae* and *A. pleuropneumoniae* Apx IV were determined using the following pathogen specific IDEXX ELISA kits (IDEXX Europe B.V., Hoofddorp, The Netherlands), respectively: Influenza A Ab Test (95.3% sensitivity, 99.6% specificity), PRRS X3 Ab Test (for the detection of both the European and North American genotypes with 98.8% sensitivity, and 99.9% specificity), HerdChek® *Mycoplasma hyopneumoniae* Antibody Test (89.4% sensitivity, 99.67% specificity), APP-ApxIV Ab Test (97.8% sensitivity, 100% specificity). Following the manufacturers' recommendations, each pig was considered positive to: IAV if the sample-to-negative (S/N) ratio value was less than 0.60, PRRS if the sample-to-positive (S/P) ratio value was greater or equal to 0.40, *M. hyopneumoniae* if the S/P ratio values were greater than 0.40, and to *A. pleuropneumoniae* if their S/P

ratio values were greater or equal to 0.50. ELISA results were transcribed into three variables per infectious pathogen: farm positivity (farms were considered positive if at least one animal tested positive in the ELISA test), on-farm prevalence (number of pigs positive divided by the total number of pigs tested per farm), and average S/P ratio value or S/N ratio value (the latter in the case of IAV) on farm.

Vaccination

The main vaccination protocols on farm were recorded, with special focus on vaccination for IAV, PRRS, *M. hyopneumoniae* and *A. pleuropneumoniae* in sows and in piglets, as present or absent. The variables retained for further analysis were vaccination for IAV and PRRS in sows, and vaccination for *M. hyopneumoniae* and *A. pleuropneumoniae* in piglets.

Statistical analysis

All statistical procedures were performed in R version 3.4.4 [24]. Alpha level for significance and tendency were 0.05 and 0.10, respectively. Production performance indicators were used as dependent variables. Vaccination, serology (farm positivity, on-farm prevalence and average S/P or S/N ratio values), pluck lesions, average herd size and average live weight at slaughter were used as predictors or independent variables. First, a univariate analysis was carried out to study the associations between production performance indicators and each one of the predictors (data not shown). Associations between categorical variables (vaccination and serology positivity) and production performance indicators were tested using the Kruskal-Wallis test. Correlations between serology (on-farm prevalence and average S/P or S/N ratio values), pluck lesions and farm production performance indicators were tested using Spearman's rank correlations. The effect of vaccination, serology and pluck lesions on production performance indicators was estimated through multivariable linear models, including all predictors with a *P*-value below 0.10 in the univariate analysis. A forward regression approach was used to improve the models fitted [*ols_step_forward_p* function from the *olsrr* package in R [25]], using a cut-off value of 0.10 for predictor retention in the model. Two-way interactions were also investigated and retained when relevant. Collinearity among predictors was initially assessed by Spearman's rank correlations and those showing $r_s > 0.70$ were considered collinear. Further checks of collinearity were carried out using Variance Inflation Criterion from the R package *rms* [26]. Collinear variables were removed manually from the multivariable model retaining the one with the highest association to the dependent variable. Normality of the residuals was visually assessed for all the models.

Results

Farm performance and herd characteristics

A summary of the farm herd characteristics and production performance is shown in Table 1. The average herd size of the farms was 789 ± 564 sows, with a range from 109 to 2,498. The average live weight at which pigs were sent to slaughter in these farms was 111 ± 4.9 kg, as per the sale target defined by each farmer. In our sample, pigs were weaned at 29.8 ± 4.27 d of age.

Table 1

Description of herd characteristics and production performance indicators in 56 Irish farrow-to-finish pig farms for the year 2017.

Production data	Mean	± SD	Median	Min	Max	N
Herd characteristics						
Average herd size	789	564.1	659	109	2498	56
Average live weight at slaughter, kg	111	4.9	110	102	121	55
Production performance indicators						
Weaner mortality, %	2.8	1.61	2.7	0.5	8.9	55
Finisher mortality, %	2.0	0.76	1.8	0.9	4.1	55
No. pigs sold /sow-year	26.7	2.23	26.5	21.8	32.0	56
ADFI, g	1740	121.3	1755	1495	2044	54
ADG, g	726	62.6	725	538	903	55
FCR	2.38	0.110	2.38	2.21	2.68	56
Age at sale, days	174	11.8	172	148	208	55
Legend: Data retrieved from the Teagasc e-Profit Monitor; Average herd size – average number of sows; No. pigs sold /sow-year – Number of pigs sold per sow per year; ADFI – Average daily feed intake; ADG – Average daily gain; FCR – Feed conversion ratio.						

Vaccination for IAV, PRRS, M. hyopneumoniae and A. pleuropneumoniae and farm serology results

A total of 39.3 and 42.9% of the farms vaccinated sows for IAV and PRRS, respectively. A total of 73.2% of the farms vaccinated piglets for *M. hyopneumoniae*. *A. pleuropneumoniae* vaccination was only used in five farms (8.9%), all vaccinating weaner pigs.

The results of the serology analysis are shown in Table 2.

Table 2

Prevalence (%) of influenza A virus, porcine reproductive and respiratory virus, *Mycoplasma hyopneumoniae*, and *Actinobacillus pleuropneumoniae* in finisher pigs of 56 Irish farrow-to-finish farms. Samples collected between November 2017 and April 2018.

Infectious agents	Farms positive, %	On-farm prevalence, % (median; IQR ²)	On-farm serology values ¹ (median; IQR)
Influenza A virus	78.6 (n = 44)	38 (3–66)	0.66 (0.51–0.88)
Porcine reproductive and respiratory syndrome virus	58.9 (n = 33)	50 (0-100)	0.56 (0.01–1.39)
<i>Mycoplasma hyopneumoniae</i>	78.6 (n = 44)	94 (11–100)	1.11 (0.16–1.54)
<i>Actinobacillus pleuropneumoniae</i>	98.2 (n = 55)	88 (59–100)	1.03 (0.65–1.38)
¹ Includes all samples per farm; Sample-to-negative ratio values for influenza A virus; and sample-to-positive ratio for porcine reproductive and respiratory syndrome virus, <i>M. hyopneumoniae</i> and <i>A. pleuropneumoniae</i> .			
² IQR - Interquartile range.			

Pluck lesions

The prevalence of the lung lesions, pericarditis and liver milk spots recorded at slaughter is presented in Table 3. A total of 9,254 plucks were assessed. On average, each farm had 162 ± 52 plucks assessed (range 55–308).

Table 3

Description of the prevalence of lung lesions, pericarditis and liver milk spots in finisher pigs of 56 Irish farrow-to-finish farms (farm prevalences averaged). Pluck lesions were assessed between November 2017 and April 2018.

Slaughter checks (%)	Mean \pm SD	Median	Min	Max
Pleurisy ¹	12.0 \pm 14.15	5.2	0	55.2
Moderate and severe pleurisy ²	9.9 \pm 11.59	4.7	0	44.8
Cranial pleurisy ^{3,4}	14.3 \pm 12.58	9.3	0.9	48.1
Pneumonia ⁵	13.4 \pm 14.21	8.4	0	58.4
Average lung surface with pneumonia ⁵	6.2 \pm 3.88	5.7	0	19.7
Scars ⁴	14.0 \pm 10.80	12.0	0	38.8
Lung abscesses ⁴	0.7 \pm 1.73	0	0	8.8
Pericarditis ⁴	7.4 \pm 4.52	7.1	0	16.6
Liver milk spots ⁴	28.6 \pm 30.94	15.0	0	93.4
¹ Prevalence of dorsocaudal pleurisy as established in the Slaughterhouse Pleurisy Evaluation System (SPES [20–21]).				
² Prevalence of scores 3 and 4 of dorsocaudal pleurisy, using SPES as a reference [20–21]).				
⁴ Scored at slaughter as present or absent.				
⁵ Surface affected averaged accounting for lobe weights [19].				

Estimating the effect of vaccination, serology for IAV, PRRS, M. hyopneumoniae and A. pleuropneumoniae and pluck lesions on production performance indicators

The multivariable linear models fitted for each production performance indicators are presented in Table 4 and were able to explain 8.2 to 47% of variability (as per the adjusted R²). Only those models explaining more than 15% of the variability are shown in the table. Finally, the models for number of piglets per sow per year and FCR only explained 8.2 and 14% of the variability, respectively.

Table 4

Multivariable linear regression modelling of production performance indicators from herd characteristics and vaccination protocols, and serology results and pluck lesions from finisher pigs of 56 farrow-to-finish Irish pig farms. Samples and observations were collated between November 2017 and April 2018.

Models	Predictors	Estimate	SE	P-value
Weaner mortality, %	Intercept	1.16	0.404	0.006
Adjusted R ² = 26%	Scars, %	0.05	0.019	0.019
P-value < 0.001	Pericarditis, %	0.08	0.045	0.085
	Cranial pleurisy, %	0.03	0.017	0.099
Finisher mortality, %	Intercept	1.30	0.204	< 0.001
Adjusted R ² = 20%	Avg. herd size [per 100 sows]	0.04	0.017	0.028
P-value = 0.002	M. <i>hyopneumoniae</i> piglet vaccination: yes	0.43	0.211	0.046
	Lung abscesses, %	0.10	0.053	0.065
ADFI, g	Intercept	614.5	280.31	0.033
Adjusted R ² = 47%	Avg. liveweight at slaughter (kg)	11.5	2.54	< 0.001
P-value < 0.001	M. <i>hyopneumoniae</i> : positive	-86.4	32.18	0.010
	PRRS S/P value	-45.1	17.66	0.014
	Liver milk spots, %	-1.1	0.41	0.010
	Cranial pleurisy, %	-1.8	1.00	0.076
ADG, g	Intercept	231.7	151.23	0.132

Legend: ADFI – Average daily feed intake; ADG – Average daily gain; FCR – Feed conversion ratio; Avg. herd size – Average herd size (No. of sows); M. *hyopneumoniae* piglet vaccination – On-farm piglet vaccination for M. *hyopneumoniae*; M. *hyopneumoniae*: positive – Farms seropositive to M. *hyopneumoniae* based on at least one animal testing positive by ELISA; PRRS S/P value – average on-farm sample-to-positive (S/P) ratio values for PRRS determined by ELISA; PRRS: positive – Farms seropositive to PRRS based at least one animal testing positive by ELISA; SE – Standard error.

Models	Predictors	Estimate	SE	P-value
Adjusted R ² = 40%	Avg. liveweight at slaughter, kg	4.9	1.36	< 0.001
P-value < 0.001	PRRS: positive	-31.4	13.64	0.025
	Cranial pleurisy, %	-2.0	53.74	< 0.001
Age at sale, d	Intercept	161.0	2.49	< 0.001
Adjusted R ² = 41%	Avg. herd size [per 100 sows]	0.5	0.23	0.041
P-value < 0.001	PRRS S/P value	4.5	1.78	0.014
	Cranial pleurisy, %	0.4	0.10	< 0.001
<p>Legend: ADFI – Average daily feed intake; ADG – Average daily gain; FCR – Feed conversion ratio; Avg. herd size – Average herd size (No. of sows); M. <i>hyopneumoniae</i> piglet vaccination – On-farm piglet vaccination for M. <i>hyopneumoniae</i>; M. <i>hyopneumoniae</i>: positive – Farms seropositive to M. <i>hyopneumoniae</i> based on at least one animal testing positive by ELISA; PRRS S/P value – average on-farm sample-to-positive (S/P) ratio values for PRRS determined by ELISA; PRRS: positive – Farms seropositive to PRRS based at least one animal testing positive by ELISA; SE – Standard error.</p>				

Discussion

The objective of this study was to estimate the impact of respiratory disease on production performance indicators. It is accepted that both slaughter checks and serology are mostly related to the health status of the pig by the end of the finisher stage. However, some of the lesions found at slaughter were related to group mortality in the weaner stage. The prevalence of scars was related to higher weaner mortality, which is compatible with the nature of these lesions. Scars are healed pneumonia lesions, most probably occurring in weaner or early finisher stages. The tendencies found for cranial pleurisy and pericarditis were also biologically tenable as higher cranial pleurisy and pericarditis reveal on-farm health issues such as bacterial polyserositis, driving general mortality up especially in weaners. The time distance between the appearance of pericarditis and pleurisy and its finding at the abattoir is not well defined in the literature and needs to be investigated in future research to confirm their relationship to weaner mortality.

Vaccination for M. *hyopneumoniae* was related to higher finisher mortalities. This association is likely to be explained by the higher health status of farms free from M. *hyopneumoniae* which, therefore, were not vaccinating for that pathogen. In general, vaccination for M. *hyopneumoniae* and PRRS were related to worse production performance indicators in the univariate analysis, showing that vaccines are in place when there are issues that affect performance. The number of farms affected by these pathogens but not vaccinating was low, which makes it difficult to estimate the effect of vaccination in positive farms. M. *hyopneumoniae* infections are also relevant due to the aggravation of the lung lesions with secondary infections, which are commonly linked to lung abscesses [27, 28]. Finisher mortality was also related to

the size of the herd. Agostini et al. [29] reported similar results and suggested that in bigger farms, less attention may be paid to individual finisher pigs.

Farms slaughtering pigs at higher live weights had increased ADFI. This finding makes sense as it is well known that the ADFI of pigs increases as they grow. Positivity to *M. hyopneumoniae* and the level of antibodies for PRRS were both related to a decrease in ADFI. Both diseases are known to be among the main ones affecting performance in pig herds [6, 7, 27]. Of the studied lesions, the prevalence of cranial pleurisy and liver milk spots decreased ADFI. Pleurisy is known to cause respiratory distress to the pig and as an inflammatory process should be expected to reduce intake. The liver milk spots are highly suggestive of infection by *Ascaris suum* [30, 31], which is also related to decreased ADFI and ADG [32–34].

The models for ADG and age at sale were very similar. Positivity for PRRS and the prevalence of cranial pleurisy were both related to lower ADG and higher age at sale. PRRS is the main disease affecting growth of pigs with (post-outbreak) estimated costs of \$17.7 USD per pig in farrow-to-finish farms [35]. Our findings confirm the relevance of PRRS as an important factor affecting performance also in Irish conditions. Pleurisy is also known to result in important production losses. In the UK, 10% pleurisy prevalence at batch level was estimated to cost approximately 226p (GBP) per slaughter pig [36]. In all the models described in this study, cranial pleurisy showed better predictive values than average dorsocaudal pleurisy or moderate to severe dorsocaudal pleurisy lesions. However, these variables were highly correlated and could be used interchangeably in the models. Although cranial pleurisy may not necessarily be linked to a particular disease, dorsocaudal pleurisy is in general related to *A. pleuropneumoniae* [21] which is very prevalent in Irish pig farms as shown in this study. Taking into account the low use of vaccination for *A. pleuropneumoniae* in Ireland, the situation could be improved with wider use of vaccination [2, 37, 38], which in turn, would result in a reduction in the use of antibiotics. The only difference for the models for ADG and age at sale was that ADG increased as weight at slaughter was higher but age at sale was more affected by herd size. It is well known that ADG increases as the pig increases in size, thus it makes sense that selling bigger pigs improves ADG. On the other hand, a worsening in performance as the size of the herd increases has been reported previously. In a study analysing production parameters and production cost over time (2010–2014) in Spain, Racadembosch et al. [39] concluded that herd size affected negatively most performance indicators, including ADG in nursery and finishing stages, as also found in this other study [40].

The models fitted explained a significant percentage of the variability for weaner mortality, finisher mortality, ADFI, ADG, and age at sale. It is interesting to notice that the models could be used to explain almost the double of the variability in ADFI, ADG, and age at sale when compared to the variability of weaner and finisher mortality. The understanding of the morbidity and mortality of disease, especially in the absence of secondary infections, could explain the impact on performance without necessarily causing increased mortality. However, the number of pigs sold per sow per year and FCR did not produce good models. The pigs sold per sow per year were included as an indicator of sow productivity to study the effects of respiratory disease in sow performance. However, in this study, no significant effects were

found. On the other hand, FCR was only affected negatively by herd size and cranial pleurisy, but these effects only accounted for 14% of the variability. This result suggests that disease clearly affects the growth rate and feed intake of pigs but does not necessarily make production less efficient in terms of feed use.

One limitation of this study is that serology and slaughterhouse assessments correspond to one batch of each farm, instead of including multiple batches to account for a representative sample of the farm. In fact, a minimum of two batches per farm were assessed at slaughter and a maximum of five batches per farm were assessed throughout the study period, but the reasoning to use data from one batch solely was to assure that serology results were a perfect match to the pluck lesions assessed on the same day. Pluck lesions' averages for the multiple batches assessed per farm were compared to the values of the batch used for these analyses and only minimal differences were found, mainly in the prevalence of liver milk spots (data not shown). Finally, the production performance figures accounted for the whole year of 2017, as opposed to referring to the batches assessed at slaughter.

Conclusions

The models to estimate production performance from vaccination, serology and slaughter checks were able to explain the variability of ADFI, ADG and age at slaughter by 47, 40 and 41%, respectively. However, FCR and sow performance were not greatly affected by the studied predictors. Serology and lesion scores at slaughter are useful tools to understand and monitor the impact of the farm health status on its production performance.

Declarations

Ethics approval and consent to participate

All the farmers participating in this study gave individual signed consent to the use of the data collected on farm and at slaughter, and to the retrieval of their production data from the Teagasc e-ProfitMonitor according to Teagasc's internal data protection regulation.

Consent for publication

Not applicable.

Availability of data and material

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

MRC: data collection, curation, statistical analysis and interpretation; manuscript writing and reviewing. AR, MT and JG: manuscript writing and reviewing. RMF: laboratorial analysis, manuscript writing and reviewing. HOS: funding acquisition, manuscript reviewing. EGM: study design, funding acquisition, data collection, statistical analysis and interpretation, manuscript writing, reviewing and editing. All authors read and approved the final manuscript.

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