

EFFECT OF BACTERIAL AGENTS OF PORCINE RESPIRATORY DISEASE COMPLEX ON PRODUCTIVE INDICES AND SLAUGHTER WEIGHT

IMPACTO DE AGENTES BACTERIANOS DO COMPLEXO DE DOENÇAS RESPIRATÓRIAS DE SUÍNOS (CDRS) NOS ÍNDICES ZOOTÉCNICOS E NO PESO AO ABATE EM SUÍNOS EM FASE DE TERMINAÇÃO

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Abstract

Porcine respiratory disease complex comprises the interaction of two or more infectious agents. The major bacterial agents involved were investigated in 115 finishing pigs at a farm in São Paulo State, Brazil: *Actinobacillus pleuropneumoniae* (serology, bacterial culture, and multiplex PCR), *Mycoplasma hyopneumoniae* (Mhyo) (nested PCR), *Pasteurella multocida* (multiplex PCR), *Haemophilus parasuis* (PCR multiplex), and *Streptococcus* sp. (bacterial culture). Macroscopic and microscopic lung lesions were evaluated, and zootechnical indices were recorded. Mhyo occurred in 113 animals (98.3%), seventeen of which were co-infected with *Streptococcus* sp. The finding of emphysematous lung was associated with significantly lower final and carcass weight at slaughter. Although vaccinated against Mhyo with an inactivated immunogen, almost 100% of the animals were infected. Mhyo infection with and without *Streptococcus* sp. co-infection was related to lung lesions of varying degrees and lower slaughter and carcass weight.

Keywords: differential diagnosis, *Mycoplasma hyopneumoniae*, pleuropneumonia, *Streptococcus* sp., swine

Resumo

O Complexo de Doenças Respiratórias em suínos (CDRS) compreende a interação de dois ou mais agentes infecciosos, manejo e ambiente. Os principais agentes bacterianos causadores de CDRS foram investigados em 115 suínos em fase de terminação em uma granja no Estado de São Paulo, Brasil: *Actinobacillus pleuropneumoniae* (sorologia por ELISA, cultivo bacteriano e PCR multiplex), *Mycoplasma hyopneumoniae* (Mhyo) (nested PCR), *Pasteurella multocida* (PCR multiplex), *Haemophilus parasuis* (PCR multiplex), e *Streptococcus* sp (cultivo bacteriano). Foram avaliadas lesões pulmonares macroscópicas e microscópicas, e impacto nos índices zootécnicos e no aproveitamento de carcaça. Houve positividade ao Mhyo em 113 animais (98,26%), destes houve infecção associada ao *Streptococcus* sp ao Mhyo em 14,78% (17) animais. O pulmão enfisematoso

diminuiu significativamente o peso no final da terminação e o peso da carcaça. Apesar de vacinados com imunógeno inativado contra Mhyo, quase 100% dos animais estavam infectados e as lesões mais observadas foram broncopneumonia purulenta e pleurite. A infecção de Mhyo associado ou não ao *Streptococcus* sp causou lesões pulmonares em diferentes graus, menor peso ao abate e da carcaça.

Palavras-chave: suínos, pleuropneumonia, diagnóstico diferencial, *Streptococcus* sp, *Mycoplasma hyopneumoniae*.

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Introduction

Respiratory diseases are a critical issue in intensive pig rearing⁽¹⁾. Confined populations are subjected to an environment that may be stressful and reduce immunity, contributing to the occurrence of respiratory disease⁽²⁾. Pneumonias result in low zootechnical indices, high pharmaceutical costs, and carcass condemnations at the slaughterhouse, where, in Brazil, approximately 50% of animals present some type of lung injury, with these lesions accounting for 50% of all carcass condemnations⁽³⁾. The more intensive the management system, the higher the costs of production⁽⁴⁾.

The etiology of respiratory problems in pigs is complex, usually involving interaction of two or more infectious agents⁽⁵⁾, bacterial and/or viral, in addition to factors such as handling and environment, affecting pigs in the growth stages and their value at slaughter^(6, 7).

Porcine respiratory disease complex (PRDC) describes a syndrome that encompasses several bacterial agents, including *Mycoplasma hyopneumoniae* (Mhyo), the agent of enzootic pneumonia in swine; *Pasteurella multocida*, responsible for pleurites and which, together with *Bordetella bronchiseptica*, causes atrophic rhinitis; *Actinobacillus pleuropneumoniae*, causing swine pleuropneumonia; *Haemophilus parasuis*, the agent of Glasser's disease; and *Streptococcus suis*, causing pneumonia and pleuritis⁽⁷⁾.

Porcine respiratory disease complex is responsible worldwide for economic losses related to mortality and condemnation of slaughtered carcasses⁽¹⁾. Because condemnation has significant impact on profits, research with respect to differential diagnoses of lung lesions could facilitate correlation of lesions with the causal agent to aid in development of disease preventive treatments and cures^(1, 8).

In view of the economic losses that respiratory disease represents to pork production, it is essential that the bacterial agents triggering this process be identified. The goal of this work was to determine if bacterial agents of PRDC are related to zootechnical indices of pigs at slaughter.

Materials and methods

This work was approved by the Ethical Committee on Animal Experimentation of the Biological Institute (CETEA-IB) and registered under protocol 130/13 in compliance with the ethical principles in animal experimentation, adopted by the Brazilian Society of Science in Laboratory Animals/Brazilian College of Animal Experimentation (SBCAL/COBEA).

A pork production unit with a full-cycle breeding system was selected from São Paulo State. The breeding was carried out extensively in the gestation phase, semi-intensively in the maternity phase, and was intensive in the other phases.

The animals were crosses of Landrace and Large White breeds and identified by Australian marking and weighed at birth. A balanced feed formulated with Nucleo and Premix (Agrochemical) was provided. Facilities were cleaned and disinfected daily. The piglets were vaccinated with chemically inactivated *Mycoplasma hyopneumoniae* bacterin (Respisure® One) and/or Circumvent® PCV M (inactivated *Mycoplasma hyopneumoniae* + porcine circovirus ORF2) and against pasteurellosis, paratyphoid, erysipelas, atrophic rhinitis, leptospirosis, and colibacillosis (Suivem®).

Data of birth and finished weight were collected at the farm, while warm cleaned carcasses were weighed at the slaughterhouse.

At slaughter, samples of whole blood, lung fragments, and mediastinal lymph nodes were collected from 115 pigs. Fragments of lung and mediastinal lymph nodes with macroscopic lesions were divided, with a portion kept refrigerated for bacterial isolation and another fixed in 10% buffered formalin for histology.

For the sero-epidemiological investigation of *A. pleuropneumoniae*, the ELISA test for antibody detection (IDEXX APP-ApxIV Ab Test, IDEXX Laboratories Inc, USA) was used.

The histological procedures consisted of dehydration, diaphanization, paraffin embedding, microtomy, and hematoxylin and eosin staining⁽⁹⁾.

Bacteria isolated from lung and lymph nodes samples were suspended in sterile 0.85% saline solution, and 1 mL of the suspension was added to 3 mL brain heart infusion broth for enrichment. Ten µL of the enriched suspension was seeded in 5% sheep blood agar and incubated for 48 hours at 37°C. In samples demonstrating bacterial growth, morphological characteristics of colonies including size, shape, color, and presence and type of hemolysis were recorded. Colonies were Gram stained and examined microscopically for morphology and Gram staining characteristics⁽¹⁰⁾. Bacterial species were identified by specific biochemical tests^(10, 11). Identification of *Streptococcus* was to genus.

Multiplex PCR was used to detect *A. pleuropneumoniae*, *P. multocida*, and *H. parasuis*, and nested PCR for Mhyo. The DNA extraction was performed on lung and lymph node samples using a commercial kit (Quick-gDNA™ MiniPrep, Uncapped Columns, Zymo Research, USA).

For the confirmation of molecular analyses, DNA extraction was conducted by PCR for the β-actin gene⁽¹²⁾. This protocol uses swine-specific primers (actin F-TGAGACCTTCAACACGCC/actin R-ATCTGCTGGAAGGTGGAC), with a size of 745 base pairs (bp). For the PCR reaction, 2.5 µL of extracted DNA was added to 22.5 µL PCR Mix containing 1.25 U Taq DNA polymerase, 200 µM of each deoxynucleotide, 1X PCR Buffer (20 mM Tris-HCl, pH 8.4, 50 mM KCl), 0.75 mM MgCl₂, and 10 pmol of each primer. The amplification process was carried out in a thermocycler under the following conditions: 95°C for 5 minutes; 39 cycles of 95°C for 30 seconds, 56°C for 45 seconds, and 72°C for 45 seconds; and a final extension of 72°C for 5 minutes.

In the multiplex PCR, specific primers used for each agent were *A. pleuropneumoniae* AP-IVF: ATA CGG TTA ATG GCG GTA ATG G/AP-IVR, ACC TGA GTG CTC ACC AAC G⁽¹³⁾; *P. multocida* KMT1 T7: ATC CGC TAT TTA CCC, AGT GG/KMT1 SP6: GCT GTA AAC GAA CTC GCC AC⁽¹⁴⁾; and *H. parasuis* HPS-F: GTG ATG AGG AAG GGT GGT, GT/HPS-R: GGC TTC GTC ACC

CTC TGT (15) amplifying a segment of 346, 460, and 821 bp, respectively. These primers were used in multiplex PCR as in Hričinová et al.⁽¹⁶⁾.

As a positive control of *A. pleuropneumoniae*, standard samples of serotypes I, III, and lyophilized Va were used (Empresa Irfa Química e Biotecnologia Industrial Ltda)⁽¹⁷⁾. For positive control of *H. parasuis* a sample from the Department of Veterinary Preventive Medicine and Animal Health, FMVZ USP, São Paulo, SP, Brazil was used, and *P. multocida* strain INCQS 00096 (ATCC 6530) was obtained from the Reference Microorganism Collection in Sanitary Surveillance, CRMVS, FIOCRUZ-INCQS, Rio de Janeiro, RJ. The negative sample was ultra-pure water. Samples were amplified using 10 µL extracted DNA plus 40 µL of the reagent mixture containing 1.25 U Taq DNA polymerase, 1X PCR Buffer (10 mM Tris-HCl, pH 8.0, 50 mM KCl), 200 µM of each deoxynucleotide, 2 mM MgCl₂, and 30 pmol of each primer. The amplification process was performed in a thermocycler under the following conditions: denaturing at 95°C for 5 minutes followed by 29 cycles of 94°C for 30 seconds, 58°C for 30 seconds, and 72°C for 45 seconds; followed by final extension at 72°C for 7 minutes.

The nested PCR for Mhyo used external primers A-F: GAGCCTTCAAGCTTCACCAAGA/B-R: GTGTTAGTGA CTTTGGCCACC⁽¹⁸⁾ and internal C-F: ACTAGATAGGAAATGCTCTAGT/D-R: GTGGACTACCAGGGTATCT⁽¹⁹⁾ with fragments of 649 bp and 352 bp, respectively. For the PCR, the reagent solution of the mix consisted of 1.0 U Taq DNA polymerase; 10 pmol of each primer (Mhyo A-F and Mhyo B-R); 200 µM of each dNTP; 0.75 mM MgCl₂; 1X PCR Buffer (20 mM Tris-HCl pH 8.4, 50 mM KCl), and 5 µL extracted DNA. Mhyo DNA (Department of Preventive Veterinary Medicine and Animal Health, FMVZ USP, São Paulo, SP, Brazil) was positive control, and the negative control was ultra-pure water. Conditions were 95°C for 3 minutes followed by 35 cycles of 95°C for 1 minute, 64°C for 1 minute, 72°C for 90 seconds, and a final extension of 72°C for 5 minutes. In the nested PCR, the reagent solution was identical to PCR, replacing only the primers (Mhyo C-F and Mhyo D-R) and reducing the hybridization temperature from 64°C to 60°C.

For biomolecular reactions, the amplified products were examined under ultraviolet light (UV) transillumination after GelRed™ stained 1% agarose gel electrophoresis. The amplified fragments were compared to the molecular size standard (100 bp, DNA Ladder, Invitrogen) arranged in the gel with the analyzed samples. The gel image under UV light was recorded in PhotoDocumentor coupled to a computer.

The analysis of variance (ANOVA) considered mixed infection of *Streptococcus* sp. and Mhyo and Mhyo alone. We evaluated the zootechnical variables birth weight (PN), weight gain to slaughter (GMD), final weight (PF), and carcass weight (PCAR) relative to sex, presence/absence of disease, age in days, and macro- and micro-scopic lesions. Data were analyzed in a completely randomized design. The mixed model was used and the random effects of batch and residue were analyzed with the MIXED procedure in SAS v. 9.3⁽²⁰⁾. When significant, the means of zootechnical variables were compared using Fisher's least significant difference test (DIFF option of the LSMEANS command). In all analyses, the significance was declared at $p \leq 0.05$.

Results and discussion

ELISA serology is simple and highly sensitive⁽²¹⁾. None of the pigs showed *A. pleuropneumoniae* antibodies. Some animals may present negative serology and carry *A. pleuropneumoniae* in the nasal cavities and tonsils without showing clinical signs, evidence that the agent can colonize the upper respiratory tract without inducing seroconversion^(22,23). For this reason, bacteriological analyses were conducted, also resulting in no PCR isolation or detection of *A. pleuropneumoniae*.

Multiplex PCR was negative for *A. pleuropneumoniae*, *P. multocida*, and *H. parasuis*⁽¹⁵⁾, and no animals presented macroscopic lung lesions characteristic of these diseases. This may have been due to the sanitation practices of the farm.

Bacterial genera were isolated from lung and lymph node samples (Table 1), including the only genus with significance to respiratory diseases in swine, *Streptococcus*, with 17 of the 115 (14.8%) animals positive. Diagnosis by bacteriological culture is a challenge due to the extensive use of antibiotics to control disease in pigs and may be a major reason for lack of isolation of bacterial strains involved in respiratory problems. High antibiotic use may increase the occurrence of bacterial resistance to antimicrobials.

Table 1. Bacteria isolated from pulmonary lymph nodes and/or fragments of lungs with microscopic lesions

Species isolated	Isolates n (%)
<i>Bacillus</i> sp.	46 (29.4)
<i>Staphylococcus intermedius</i>	35 (22.3)
<i>Escherichia coli</i>	30 (19.1)
<i>Streptococcus</i> sp.	17 (10.8)
Enterobactéria	12 (7.6)
<i>Pantoa agglomerans</i>	5 (3.2)
Gram negative non-fermenter	5 (3.2)
<i>Serratia liquefaciens</i>	2 (1.3)
<i>Staphylococcus aureus</i>	2 (1.3)
<i>Trueperella pyogenes</i>	1 (0.6)
<i>Morganella morganii</i>	1 (0.6)
<i>Enterobacter aerogenes</i>	1 (0.6)
Total	157 (100)

Table 2 summarizes macroscopic lesions in lung of the 17 pigs positive for both *Streptococcus* sp. and Mhyo pathogens.

Macroscopic lesions lung and heart of the 113 Mhyo-positive animals were consistent with those previously reported associated with Mhyo^(2, 8, 24) (Table 2).

Table 2. Pulmonary macroscopic lesions observed in mixed *Streptococcus* sp. + Mhyo infected pigs, Mhyo-positive/*Streptococcus* sp. negative, and total Mhyo-positive.

Macroscopic pulmonary and cardiac* lesions	Positive <i>Streptococcus</i> sp. and Mhyo n (%)	Positive Mhyo, negative <i>Streptococcus</i> sp. n (%)	Total positive Mhyo n (%)
Marbling	10 (58.8)	62 (54.9)	72 (63.7)
Edema	2 (11.8)	31 (27.4)	33 (29.2)
Hemorrhage	2 (11.8)	12 (10.6)	14 (12.4)
Friable	4 (23.5)	9 (7.9)	13 (11.5)
Paleness	4 (23.5)	8 (7.1)	12 (10.6)
Emphysema	0	7 (6.2)	7 (6.2)
Atrophy	0	4 (3.5)	4 (3.5)
Petechiae	1 (5.9)	2 (1.8)	3 (2.6)
Pleural adherence	0	1 (0.9)	1 (0.9)
Pericarditis*	0	1 (0.9)	1 (0.9)
Total	17/115 (14.8)	113/115 (98.3)	113/115 (98.3)

Mhyo = *Mycoplasma*

Mean PF (65.84 ± 4.91 kg) ($p=0.0041$) and PCAR (49.46 ± 4.42 kg) ($p=0.0012$) was significantly lower in the animals that presented pulmonary emphysema compared to animals without macroscopic lesions in lungs (PF 80.70 kg and PCAR 64.65 kg) (Table 3). Other macroscopic lesions showed no correlation with performance.

Adequate respiratory capacity is essential for the growth and fattening of pigs⁽²⁵⁾, with respiratory disorders being one of the most important problems in modern pork production due to the economic loss caused by mortality and condemnation of carcasses⁽¹⁾.

Table 3. Final weight and carcass weight relative to emphysematous lung

Zootechnical Index Kg	No emphysematous lung	Emphysematous lung	Pr > F
PF (Mean \pm SD)	80.70 \pm 1.26	65.84 \pm 4.91	0.0041
PCAR (Mean \pm SD)	64.65 \pm 1.13	49.46 \pm 4.42	0.0012

PF = weight of the pig at slaughter

PCAR = warm carcass weight

ANOVA in SAS v. 9.3⁽²⁰⁾, alpha error 5%

The main microscopic lesions in the 17 pigs with mixed *Streptococcus* sp. x Mhyo infection are presented in Table 4.

In the Mhyo-positive animals, the predominant microscopic lesions were consistent with Mhyo as described by other authors^(8, 24) (Table 4).

Table 4. Microscopic pulmonary lesions in *Streptococcus* sp. x Mhyo-positive pigs, Mhyo-positive, and *Streptococcus* sp. negative, and total Mhyo-positive

Microscopic lung injuries	Positive <i>Streptococcus</i> sp and Mhyo n (%)	Positive Mhyo and negative <i>Streptococcus</i> sp n (%)	Total animals positive Mhyo n (%)
Thickening of interlobular pleura	17 (100.0)	91 (80.5)	108 (95.6)
Thickening of visceral pleura	12 (70.6)	67 (59.3)	79 (69.9)
Interstitial fibrin	17 (100.00)	90 (79.6)	107 (94.7)
Thickening of alveolar septa	17 (100.00)	89 (78.8)	106 (93.8)
Interstitial mononuclear inflammatory infiltrate	15 (88.2)	91 (80.5)	106 (93.8)
Peribronchiolar mononuclear inflammatory infiltrate	14 (82.3)	90 (79.6)	104 (92.0)
Emphysema	13 (76.5)	81 (71.7)	94 (83.2)
Cellular debris in lumen of bronchioles	7 (41.2)	68 (60.2)	75 (66.4)
Edema	12 (70.6)	57 (55.4)	69 (61.1)
Pulmonary mass	6 (35.3)	52 (46.0)	58 (51.3)
macrophages	6 (35.3)	42 (37.2)	48 (42.5)
Neutrophils in parenchyma and lumen of bronchioles	6 (35.3)	40 (35.4)	46 (40.7)
Interstitial hemorrhage	4 (23.5)	32 (28.3)	36 (31.9)
Total positive/total examined	17/115 (14.8)	113/115 (98.3)	113/115 (98.3)

Mhyo = *Mycoplasma*

Macroscopic and microscopic examination demonstrated Mhyo association with pulmonary lesions with and without co-infection of *Streptococcus* sp. Mhyo was identified by nested PCR in 98.26% of the pigs with mild lung lesions and those with moderate to severe lung lesions and pericarditis. Since the pigs were vaccinated with the inactivated Mhyo pathogen as piglets, the presence of Mhyo DNA may indicate sub-clinical infection (colonization) in cases of minimal lung lesions, with the agent present in insufficient quantities to cause disease⁽²⁶⁾. This result demonstrated the presence of active infection on the farm, since many pigs vaccinated for Mhyo presented compatible lesions, including one animal showing adherence of the pleura and pericardium in the costal gradil, serious chronic lesions.

Mhyo causes enzootic pneumonia of swine, a catarrhal bronchopneumonia considered one of the major infectious diseases in pigs worldwide and causing significant economic losses⁽²⁹⁾. Mhyo control consists of biosecurity measures and vaccination. Vaccination is the primary control measure recommended for enzootic pneumonia, but immunization may fail due to differences between the vaccine and the field strains. The commercial vaccines available in Brazil consist of inactivated whole Mhyo cells of a strain isolated in England, and it is questionable whether this strain has similar characteristics to those circulating in Brazilian swine herds and in other parts of the world^(30,31). These

vaccines have been shown effective in reducing clinical signs but confer only partial protection against the development of lesions and transmission of the agent. They do not prevent colonization of Mhyo in the respiratory tract, as observed in the present study, and the vaccine efficacy is in question⁽²⁷⁾. The infection pressure may reach the point at which the vaccine does not generate sufficient immunity to neutralize the infection. A study in Minas Gerais, Brazil demonstrated the genetic diversity of Mhyo in field samples from pig lungs⁽²⁸⁾. The samples were categorized into 30 genetic groups showing broad distribution in the studied regions. Histology identified lesions suggestive of enzootic pneumonia in swine. The results indicate that several variants of Mhyo circulate in swine herds, suggesting its broad genetic diversity even within a specific region. The authors concluded that it is necessary to investigate protective antigens and strategies in the elaboration of vaccine prototypes against Mhyo infection.

Molecular techniques have demonstrated high genetic diversity⁽³⁰⁻³⁶⁾, proteomic variation⁽³⁷⁾, and differences in virulence of Mhyo strains⁽³⁸⁾.

Weight gain at slaughter, PF, and PCAR were significantly higher ($p \leq 0.05$) in the pigs with mixed *Streptococcus* sp. and Mhyo (PCAR) infection compared to Mhyo only, while there was no association with PN (Table 5). The mean PN of the animals with mixed infection was 1.91 kg and of animals positive only for Mhyo was 1.75 kg. The low number of pigs without Mhyo infection in the studied facility did not allow analysis of variance to compare the productive indices of a negative group with the animals infected by one or both agents.

Table 5. Zootechnical indices of animals positive for Mhyo and those infected by *Streptococcus* sp. and Mhyo

Zootechnical indices (kg)	Positive only to Mhyo Mean \pm SD	Positive to Mhyo and <i>Streptococcus</i> sp Mean \pm SD	Pr > F
PN	1.75 \pm 0.03	1.91 \pm 0.08	0.0633
GMD	0.56 \pm 0.01	0.61 \pm 0.02	0.0030
PF	78.42 \pm 1.34	87.65 \pm 3.19	0.0088
PCAR	62.57 \pm 1.23	70.12 \pm 2.92	0.0190

Mhyo = *Mycoplasma*

Analysis of variance by SAS v. 9.3; PN = birth weight, GMD = weight gain to slaughter, PF = final weight (137 days), PCAR = warm carcass weight. Fisher's least significant difference test. Significance level = $p \leq 0.05$.

Embrapa⁽³⁹⁾ established the zootechnical indices of mean weight of piglet at birth 1.4 kg, ideal >1.5 kg; mean weight of pigs at slaughter (133 days), 78 kg, ideal >83 kg; at 140 days >85 kg, ideal >90 kg; at 147 days 92 kg, ideal >97 kg; at 154 days 98 kg, ideal >103 kg.

Both the Mhyo- and Mhyo + *Streptococcus* sp.-positive animals presented PF (mean 137 days) consistent with Embrapa's criteria⁽³⁹⁾. The studied farm did not operate as an intensive system of commercial production on an industrial scale. Embrapa⁽³⁹⁾ did not discuss pathogens causing PRDC in the farms evaluated.

We were able to verify that the vaccine against swine mycoplasmosis was not effective in the prevention of Mhyo infection or of chronic lesions. The development of effective immunogens and diagnostic tests using strains circulating in Brazil is essential.

The prevalence and economic impact of respiratory disease in pigs was evaluated in 62 farms in the

states of Rio Grande do Sul, Santa Catarina, and Paraná⁽²⁵⁾ with examination of 3,788 pigs for the frequency and extent of pneumonia, and 3837 heads for frequency and severity of destruction of the nasal turbinates. Pneumonia was diagnosed in 2079 (54.9%) animals, and atrophic rhinitis was recorded in 1894 (49.4%), with a mild degree of injury in 42.6% of cases of pneumonia and 32.4% in atrophic rhinitis. Although most of the pigs did not show obvious clinical signs, there was a reduction in average daily weight gain of 6% with atrophic rhinitis and of 3 to 8% with pneumonia, demonstrating, as in our study, the impact of respiratory diseases on productivity.

In a study of clinical cases of respiratory disease in finished pigs⁽⁴⁰⁾, animals with respiratory clinical signs were necropsied for macroscopic evaluation and for histological and microbiological analysis. Bacterial isolation was performed for bacteria *Mycoplasma hyorhinis* and immunohistochemistry for Influenza A, swine circovirus type 2, and *Mycoplasma hyopneumoniae*. Suppurative bronchopneumonia and pleuritis were the main respiratory lesions found, and *Mycoplasma hyopneumoniae* and *Pasteurella multocida* type A were the most prevalent pathogens. *Pasteurella multocida* type A was associated with a greater extent of lung lesions. In 58% of samples, more than one infectious agent was identified, showing the importance of multiple agents in the endemism of respiratory disease in pigs.

Multiple pathogens increase the challenge of controlling infections, and it is essential to identify agents and jointly evaluate pathology, etiology, and clinical status of finished pigs. The quantification of production losses is necessary to support the development of prophylactic measures in the farms^(40, 41).

Conclusion

The presence of Mhyo with or without *Streptococcus* sp. was sufficient to cause macroscopic and microscopic lesions and lead to productive losses related to slaughter weight and carcass weight.

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