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Livestock Diseases

Avibacterium paragallinarum and *Gallibacterium* spp. coinfection in laying hens with sinusitis and rhinitis[1]

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ABSTRACT.- Rocha P.M.C., Barros M.E.G., Bandeira J.T., Morais R.S.M.M., Braga J.F.V., Souza F.A.L., Mendonça F.S. & Evêncio-Neto J. 2024. ***Avibacterium paragallinarum* and *Gallibacterium* spp. coinfection in laying hens with sinusitis and rhinitis.** *Pesquisa Veterinária Brasileira 44:e07452, 2024*. Laboratório de Diagnóstico Animal, Universidade Federal Rural de Pernambuco, Rua Dom Manuel Medeiros s/n, Dois Irmãos, Recife, PE 52171-900, Brazil. E-mail: joaquim.evenciont@ufrpe.br

Respiratory diseases are responsible for economic losses in the poultry sector. The occurrence of coinfections can aggravate the severity of clinical signs and lesions, such as coinfection with *Avibacterium paragallinarum* and *Gallibacterium* spp. The aim of this study is to describe the epidemiological, clinical and pathological aspects of an outbreak of respiratory disease in laying hens caused by a coinfection by *A. paragallinarum* and *Gallibacterium* spp. in the state of Sergipe, northeastern Brazil. High morbidity and mortality were observed, accompanied by apathy, anorexia, serous nasal discharge and a decrease in egg production (approximately 10%). Five laying hens were selected for general clinical examination and euthanized. Samples of nasal turbinates, infraorbital sinuses, trachea, liver and spleen were collected, fixed in 10% buffered formalin and routinely processed for histopathology. For the microbiological examination, the whole heads of the birds were collected, packed in Nasco® plastic bags, stored at -8°C then sent to a private laboratory for bacterial isolation on Petri dishes containing blood agar and incubated at 37°C in microaerophilic. Grossly, there was a bilateral increase of periorbital volume with complete obstruction of nasal turbinates due to the presence of caseous exudate. Microscopically, there was marked diffuse chronic necrocaseous and granulomatous rhinitis, marked diffuse chronic granulomatous and heterophilic necrotizing sinusitis, focally extense lymphohistiocytic and heterophilic tracheitis with epithelial hyperplasia, loss of cilia and atrophy of mucous glands. In the microbiological examination, *A. paragallinarum* and *Gallibacterium* spp. were identified, and it was concluded that these agents were responsible for the outbreak of rhinitis and sinusitis in the studied birds.

INDEX TERMS: Coinfection, histopathology, infectious coryza, *Avibacterium paragallinarum*, *Gallibacterium* spp., laying hens, poultry.

RESUMO.- [***Avibacterium paragallinarum* e *Gallibacterium* spp. coinfecção em galinhas de postura com sinusite e rinite.**] As doenças respiratórias são responsáveis por perdas econômicas no setor avícola. A ocorrência de coinfecções pode agravar a severidade dos sinais clínicos e das lesões, tais como a coinfecção por *Avibacterium paragallinarum* e *Gallibacterium* spp. Objetivou-se descrever os aspectos epidemiológicos, clínicos e patológicos de um surto de doença respiratória em galinhas poedeiras causado por coinfecção de *A. paragallinarum* e *Gallibacterium* no estado de Sergipe, Nordeste do Brasil. Foram observadas elevada morbidade e mortalidade, acompanhada de apatia, anorexia, secreção nasal serosa e diminuição da produção de ovos (aproximadamente 10%). Cinco galinhas de postura foram selecionadas para exame clínico e eutanásia. Amostras de cornetos nasais, seios infraorbitários, traqueia, fígado e baço foram coletadas, fixados em solução de formalina 10% tamponada e processadas pelo protocolo de histopatologia de rotina. Para o exame

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microbiológico, foram coletadas as cabeças inteiras das aves, acondicionadas em sacos Nasco®, conservadas a -8°C e em seguida enviados para laboratório particular para isolamento bacteriano, em placas de Petri contendo ágar sangue e incubadas a 37°C em microaerofilia. Macroscopicamente, observou-se um aumento de volume periorbital bilateral com completa obstrução dos cornetos nasais devido a presença de exsudato caseoso. Microscopicamente observou-se rinite necrocaseosa e granulomatosa crônica difusa acentuada, sinusite necrotizante granulomatosa e heterofílica crônica difusa acentuada, traqueíte linfohistiocitária e heterofílica focalmente extensa com hiperplasia epitelial, perda de cílios e atrofia de glândulas mucosas. No exame microbiológico identificou-se *A. paragallinarum* e *Gallibacterium* spp. concluindo que esses agentes foram responsáveis pelo surto de rinite e sinusite nas aves estudadas.

TERMOS DE INDEXAÇÃO: Coinfecção, histopatologia, coriza infecciosa, *Avibacterium paragallinarum*, *Gallibacterium* spp., galinha de postura, aves.

INTRODUCTION

Gram-negative bacteria *Avibacterium paragallinarum* and *Gallibacterium* spp. are members of the Pasteurellaceae family, both infecting the respiratory tract of chickens (Nascimento et al. 2009, Algammal et al. 2022). *A. paragallinarum* and *Gallibacterium* spp. are pathogens that have a global presence and have been reported in various poultry-producing regions (Deshmukh et al. 2015). It flourishes in environments with overcrowded and stressed birds, and transmission often occurs through direct contact and aerosolized droplets, facilitated by factors such as poor ventilation and transportation stress. Both pathogens can spread through respiratory secretions and contaminated equipment, making it challenging to control in commercial poultry settings (Crispo et al. 2018).

Avibacterium paragallinarum causes infectious coryza in chickens, an acute contagious respiratory disease that leads to decreased egg production, poor growth, and increased mortality rates in affected flocks (Shabbir et al. 2023). *Gallibacterium* spp. is an opportunistic pathogen also causing oophoritis, salpingitis, peritonitis, and enteritis in intensively farmed poultry (Bojesen et al. 2004). When infecting the avian respiratory system, it often causes nasal discharge, sneezing, coughing, and swelling around the eyes and head (Chandravathi et al. 2021).

Among the various species within the *Avibacterium* genus, *A. paragallinarum* stands out as the primary pathogen responsible for causing disease in chickens (Zhu et al. 2022). According to Blackall et al. (1990), *A. paragallinarum* has three different serogroups I, II, and III, as well known as A, C and B, respectively. In the genus *Gallibacterium*, four species are recognized: *G. anatis*, *G. melopsittaci* sp. nov., *G. trehalosifermentans* sp. nov., and *G. salpingitidis* sp. nov., and three genomospecies (Christensen et al. 2003, Bisgaard et al. 2009). *G. anatis* is one of the most important species causing lesions in the respiratory tract, reproductive tract, and related serosal surfaces in poultry (Shabbir et al. 2023).

The potential for simultaneous coinfections involving *A. paragallinarum* and *Gallibacterium* introduces an added dimension of intricacy to the scenario (Paudel et al. 2017a). These coinfections can result in more severe and complicated illness consequences since the interaction between these two pathogens can aggravate clinical signs and have a greater overall influence on poultry health (Mei et al. 2020). Managing and avoiding such coinfections necessitates a thorough understanding of these bacteria's biology, epidemiology, and processes, as well as efficient management techniques to preserve the health and productivity of chicken populations (Paudel et al. 2017b).

To the author's knowledge, reports of *A. paragallinarum* and *Gallibacterium* spp. coinfection in laying hens presenting sinusitis and rhinitis has not been published in Brazil. The aim of this study is to describe the epidemiological, clinical and pathological aspects of an outbreak of respiratory disease in laying hens caused by a coinfection by *A. paragallinarum* and *Gallibacterium* spp. in the state of Sergipe, Northeast region of Brazil.

MATERIALS AND METHODS

Ethical approval. Animal procedures were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Animals and the "Conselho Nacional de Controle de Experimentação Animal" (National Council for Controlling Animal Experimentation – CONCEA), "Ministério da Ciência, Tecnologia e Inovação" (Ministry of Science, Technology and Innovation – MCTI), Brazil.

An outbreak of avian respiratory disease was observed in a Lohmann lineage laying hen farm located in the State of Sergipe, Northeast region of Brazil. Five adult laying hens were randomly chosen, clinically examined and euthanized. Clinical examination was performed according to Beynen et al. (1989) and Andreatti Filho et al. (2020).

Samples of nasal turbinates, infraorbital sinus, trachea, liver and spleen were collected, fixated in 10% formaldehyde in 0.1M phosphate buffer and pH 7.2 and processed routinely, were stained with hematoxylin and eosin (HE) and analyzed histopathologically.

For the microbiological examination, five whole heads from birds with apparent clinical signs were collected and stored in a sterile bag for sampling, conserved at -20°C and sent to the microbiology laboratory for bacterial isolation. The samples were seeded in Petri dishes containing blood agar with stria of NAD-factor-producing *Staphylococcus aureus*, incubated at 37°C in microaerophilia for 24 hours. Both *Avibacterium paragallinarum* and *Gallibacterium* spp. were initially cultivated on the same plate. After the first incubation, colonies with different morphologies were replicated and then phenotypically classified.

RESULTS

High morbidity (50%) and mortality (3%) was observed. The main clinical signs were similar, differing only in severity. Most cases consisted of apathy, anorexia, serous nasal discharge and a decrease in egg production (approximately 10%). More severe cases included mucocatarrhal nasal discharge, increased infraorbital sinus volume, and decreased egg production (approximately 10%). Grossly, there was a bilateral increase of periorbital volume with complete obstruction of nasal turbinates due to the presence of caseous exudate, which caused light dyspnea (Fig.1-2).

Microscopically, these lesions consisted of a large amount of amorphous eosinophilic material composed of cellular debris (caseous necrosis), surrounded by epitheliod macrophages. The nasal mucosa was intensely thickened, due to an inflammatory infiltrate composed of lymphocytes, histiocytes and a few

heterophils, with diffuse epithelial loss. There was a focal fibrin deposition on the mucosa, as well as mucous gland atrophy and discrete to moderate multifocal fibroplasia. There was diffuse hyperemia in the dermis, diffuse histiolymphocytic vasculitis and fibrin deposition, showcasing a marked diffuse chronic necrocaseous and granulomatous rhinitis (Fig.3).

In the infraorbital sinus, distension was observed, caused by a large amount of caseous necrosis surrounded by multinucleated giant cells and some macrophages. In the adjacent mucosa, there was epithelial loss and intense proliferation of fibrous connective tissue, in addition to a moderate number of lymphocytes, macrophages and some heterophils and discrete to moderate mucosa-associated lymphoid tissue hyperplasia. Discrete to moderate multifocal hyperemia and focal moderate hemorrhage, characterizing a marked diffuse chronic granulomatous and heterophilic necrotizing sinusitis were also observed (Fig.4 and 5).

In the trachea, there was a focally extensive mucous thickening due to inflammatory infiltrate, composed of lymphocytes, macrophages and heterophils, in addition to moderate lymphoid tissue hyperplasia and discrete hyperemia, as well as an area with discrete focal hemorrhage, mucous gland atrophy, cilia loss and epithelial hyperplasia.

Avibacterium paragallinarum and *Gallibacterium* spp. were isolated and identified in the microbiological examination of the nasal turbinates.

DISCUSSION

Herein, a coinfection by *Avibacterium paragallinarum* and *Gallibacterium* spp. in laying hens that presented clinical signs of infectious coryza was demonstrated for what seems to be the first time in the Northeast region of Brazil. Low biosecurity measures provide an ideal environment for *Gallibacterium* spp. and *A. paragallinarum* proliferation. The primary route of both microorganisms is through the respiratory tract (Bisgaard 1977, Bojesen et al. 2003).

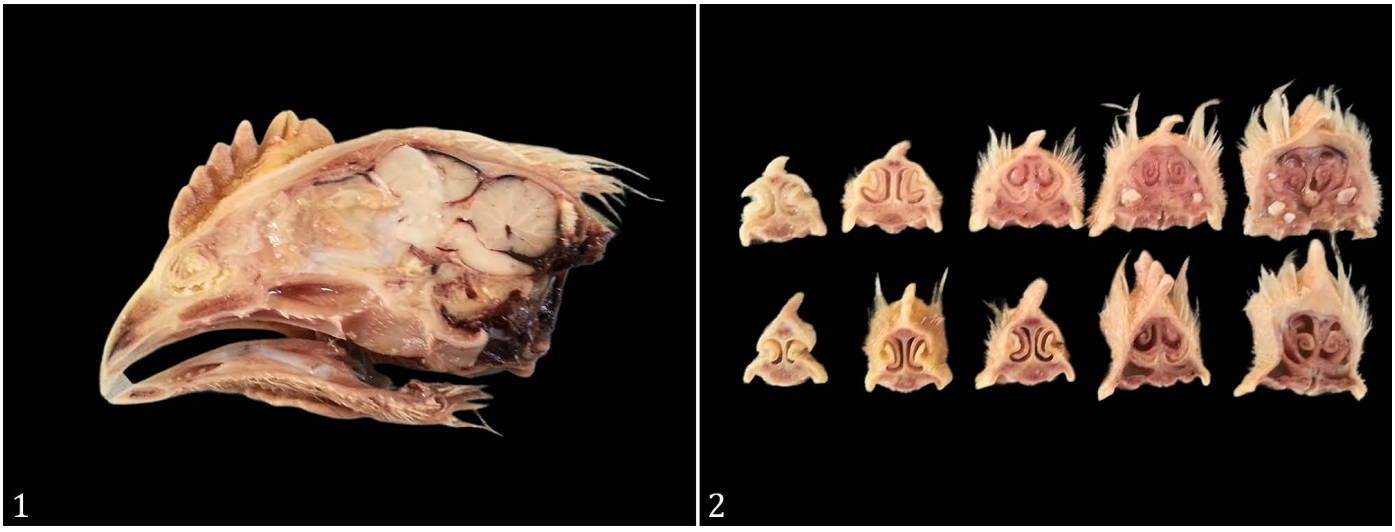

Fig.1-2. Gross lesions of coinfection *Avibacterium paragallinarum* and *Gallibacterium* spp. in laying hens. (**1**) Sagittal section of laying hen head: the nasal sinus is completely obstructed by caseous exudate. (**2**) Cross-section of the laying hen head: nasal turbinates deformation with the presence of caseous exudate, also in the infraorbital sinus.

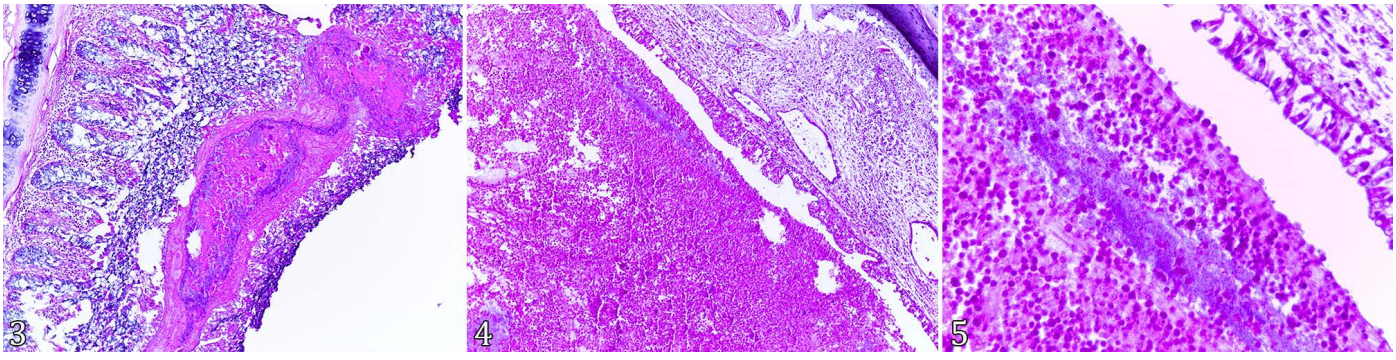

Fig.3-5. Microscopic lesions of coinfection *Avibacterium paragallinarum* and *Gallibacterium* spp. in laying hens. (**3**) Infraorbital sinus, nasal cavity. A large amount of caseous necrosis with intralesional bacteria myriad, epithelial loss and proliferation of fibrous connective tissue. HE, obj.10x. (**4**) Same section as Figure 3. Caseous necrosis formed by heterophils and cellular debris. Characterizing a marked diffuse heterophilic necrotizing sinusitis. HE, obj.40x. (**5**) Nasal turbinates. A large amount of amorphous eosinophilic material composed of cellular debris (caseous necrosis), surrounded by epitheliod macrophages. The nasal mucosa was thickened due to an inflammatory infiltrate with diffuse epithelial loss. There was also a focal fibrin deposition on the mucosa. HE, obj.10x.

The pathogenesis of *A. paragallinarum* and *G. anatis* are similar; both pathogens enter the blood after colonizing the respiratory mucous membranes (Ali et al. 2013) or reproductive organs (Paudel et al. 2014). Balouria et al. (2018) demonstrated that just 12 hours after inoculation, there is superficial colonization of the respiratory tract by *A. paragallinarum*. Within 48 to 72 hours, there is infiltration into subcutaneous tissue, in addition to this form of infection. It is believed that *G. anatis* can also infect a bird's reproductive tract ascending from the cloaca (Bojesen et al. 2003, Neubauer et al. 2009).

Hence, those bacteria reached different organs, producing lesions, such as rhinitis with other respiratory manifestations and a notable decline in laying performance, 5-10% egg drop (Ali et al. 2013, Paudel et al. 2014). Most clinical signs, gross and microscopic lesions seen here, were severe. They consisted mainly of apathy, anorexia, decreased egg production, mucocatarrhal nasal discharge, increased infraorbital sinus volume and marked diffuse chronic necrocaseous and granulomatous rhinitis. In these cases, it has been reported that coinfection with *A. paragallinarum* and other pathogenic agents, such as *G. anatis*, which is usually found on the respiratory and reproductive tract of commercial birds, leads to the increase in the severity of infectious coryza in birds (Paudel et al. 2017a, Crispo et al. 2018, Mei et al. 2020).

The clinical signs described in this study were also found by Silva et al. (2014) in broiler chicken and laying hens in Pernambuco infected by *A. paragallinarum*. There are also reports (Nascimento et al. 2009, Freitas et al. 2020) where carriers of the chronic form of infectious coryza presented nasal discharge, edema and facial obstruction, leading to loss of zootechnical performance and mortality. In seven days, the *A. paragallinarum* can surpass epithelial and immunological defenses of the respiratory tract of birds, triggering the clinical signs (Balouria et al. 2018).

The performance loss was also observed by Blackall & Terzolo (1995); the decrease in egg production was 40%. This is justified by a delay in the ovarian follicular hierarchy found. However, there were no signs of reproductive tract infection, which differs from the observations of Bojesen et al. (2008), who found lesions resulting from infection by *Gallibacterium* spp., usually purulent salpingitis and oophoritis and, in chronic cases, generalized infections or coelomites, were observed. These cases are often associated with *Escherichia coli* growth (Persson & Bojesen 2015).

The histopathological lesions observed in nasal turbinates and trachea corroborate the description of Droual et al. (1990), who emphasize the presence of mucopurulent exudate, deciliation and inflammatory infiltrate. In a study of experimental infection with *A. paragallinarum*, Diptesh et al. (2020) describe similar lesions in chickens and Japanese quail. However, the inflammatory response and severity of clinical signs were smaller in this species when compared with chickens.

Nascimento et al. (2009) observed that in chronic infectious coryza cases, there was degeneration, mucous and glandular epithelium hyperplasia and heterophil infiltration on the lamina propria, and nodular or diffuse lymphoid cells infiltrate on infraorbital sinus, similar to the findings on nasal turbinates, infraorbital sinus and trachea. These lesions also match with findings of nasal inoculation of *G. anatis* only within 24 hours of agent inoculation (Zepeda et al. 2010).

It is believed that the products of these infiltrating cells, mainly the free radicals, are responsible for the vascular lesions, cell damage and extensive necrosis observed in the cases of infectious coryza (Nascimento et al. 2009, Diptesh et al. 2020). After rhinitis development, the bacteria present in the respiratory tract can enter the bloodstream, being able to reach other organs such as the liver and heart (Ali et al. 2013).

Ali et al. (2013) and Zepeda et al. (2010) found lymphocytic hepatitis after *A. paragallinarum* and *G. anatis* inoculation, respectively. Although no focus on hepatitis was found, areas of diffuse discrete microvacuolar degeneration with individual cell necrosis were observed, alterations also described by Zepeda et al. (2010).

No reports of spleen lesions due to infection with *A. paragallinarum* were found. However, in birds infected with *G. anatis,* it was described the presence of necrotic cells and eosinophilic and basophilic aggregates (Bojesen et al. 2004), like the alterations described in this study. Bojesen et al. (2004) verified the presence of *G. anatis* in the spleen utilizing *in situ* hybridization. Neubauer et al. (2009) proved that the *G. anatis* present in the respiratory tract is the same as found in the spleen, therefore justifying that diverse organs can suffer colonization by *G. anatis* and generate lesions during bacteremia.

CONCLUSION

The association of clinical signs, macroscopic lesions, and histopathological and bacterial isolation allowed the first diagnosis of sinusitis and rhinitis caused by *Avibacterium paragallinarum* associated with *Gallibacterium* spp. in Brazil's Northeast.

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Conflict of interest statement.- The authors declare that there is no conflict of interest.

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