

## Aerossaccolitis and Pneumonia in an Indian Peafowl Caused by *Lactobacillus agilis*

Larissa Caló Zitelli<sup>1,3</sup>, Mônica Slaviero<sup>2,3</sup>, Bruno Albuquerque de Almeida<sup>2,3</sup>, Silvia De Carli<sup>1,3</sup>,  
Emanoelly Machado Sousa da Silva<sup>2</sup>, Fabiana Caroline Zempulski Volpato<sup>4</sup>, Afonso Luis Barth<sup>4</sup>,  
Saulo Petinatti Pavarini<sup>4</sup> & Franciele Maboni Siqueira<sup>1</sup>

### ABSTRACT

**Background:** The peafowl is an ornamental bird that has the habit of eating directly from the earthy soil, which makes this bird more susceptible to endoparasites. One important endoparasite is *Eucoleus contortus*, which leads to inflammatory processes that alter the local microbiota, potentializing disease. By the other way, a member of the bird's microbiota there is the genus *Lactobacillus*, but when occurs some imbalance, these bacteria can overgrowth and even cause some infection. This report describes the pathological and microbiological findings of chronic necrotizing pneumonia and aerossaccolitis caused by *Lactobacillus agilis* in a peafowl, associated with parasitism by *E. contortus*.

**Case:** A peafowl (*Pavo cristatus*), adult, male, who lived on a farm with contact with other species of animal, was submitted to post-mortem examination due to sudden death. This animal lived in an extensive system on the property and was the only one of its species. During the gross evaluation, the air sacs were filled with solid yellowish crumbly material. The same material was observed forming well-defined nodules that occupied > 50% of the lung parenchyma. Histological analysis showed multiple parabronchi dilated and filled with caseous necrosis, characterized by abundant cellular debris and fibrin deposition. These areas were surrounded by the proliferation of fibrous connective tissue and inflammatory infiltrate of macrophages, giant cells, lymphocytes, and plasma cells. The air sacs parenchyma showed fibrin deposition and mixed inflammatory infiltrate. Multiple gram-positive bacilli were observed within the caseous foci in Gram-stained slides. In the crop and esophageal mucosa, cross-sections of filiform nematodes morphologically compatible with *E. contortus* were associated with chronic inflammatory infiltrate and epidermal hyperkeratosis. A lung section was submitted to Gram-Brown-Hopps and Ziehl-Neelsen (ZN) stains for bacterial investigation, and Grocott's methenamine silver (GMS) stain for fungal investigation. Short gram-positive bacilli bacteria are observed within the caseous foci in Gram-stained slides. By the other way, no agents were identified on the ZN and GMS stains. Following the analysis, lung fragments were cultivated at aerobic and microaerophilic conditions on sheep blood agar and McConkey agar. All the microbial cultures were incubated at 37°C to 48 h. Pure culture, in microaerophilic condition, of Gram-positive bacilli, was observed. The isolated bacterium was identified by MALDI-TOF MS as *L. agilis*.

**Discussion:** Although uncommon, *L. agilis* was the single bacterium identified and therefore, associated as a primary cause of necrotic pneumonia and aerossaccolitis in the studied peafowl. The presence of *E. contortus* could induce the aspiration of regurgitated of little amount of material from the gastrointestinal tract with sufficient bacterial load to initiate an infection, but not enough to smother the animal. The gradual aspiration can induce a chronic inflammatory condition. Infections by bacteria from the host microbiota have been observed in animals and humans with immunodeficiency. In summary, both the parasitosis and the inflammation could be resulted by the parasite and the aspiration of gastric product, which probably interfered in the immune response and allowed the overgrowth of *L. agilis*. In the current case report, based on macroscopic, microscopic and bacteriological results, we have provided insights to understand how the parasitosis made possible a pneumonia from a bacterium from the host's microbiota. Finally, to the best of our knowledge, this is the first report of *Lactobacillus agilis* as causal agent of fatal necrotic pneumonia and aerossaccolitis in peafowl.

**Keywords:** respiratory infection, ornamental birds, secondary infection, parasitosis.

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<sup>1</sup>Laboratório de Bacteriologia Veterinária (LaBacVet), <sup>2</sup>Setor de Patologia Veterinária (SEPAVET) & <sup>3</sup>Programa de Pós-Graduação em Ciências Veterinárias (PPGCV), Faculdade de Veterinária (FaVet), Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre, RS, Brazil. <sup>4</sup>Laboratório de Pesquisa em Resistência Bacteriana (LABRESIS), Hospital de Clínicas de Porto Alegre (HCPA), Porto Alegre. CORRESPONDENCE: F.M. Siqueira [franmaboni@gmail.com]. LaBacVet - FaVet - UFRGS. Av. Bento Gonçalves n. 9090. CEP 91540-000 Porto Alegre, RS, Brazil.

## INTRODUCTION

The indian peafowl (*Pavo cristatus*) is an ornamental bird popular in many countries around the world [9,13]. Peafowl diet is based on vegetables, fruit, flowers, seeds, and insects and earthworms [16]. The diet and how this bird has contact with the earth and fecal material of other animals make verminosis a common cause of health problems [18].

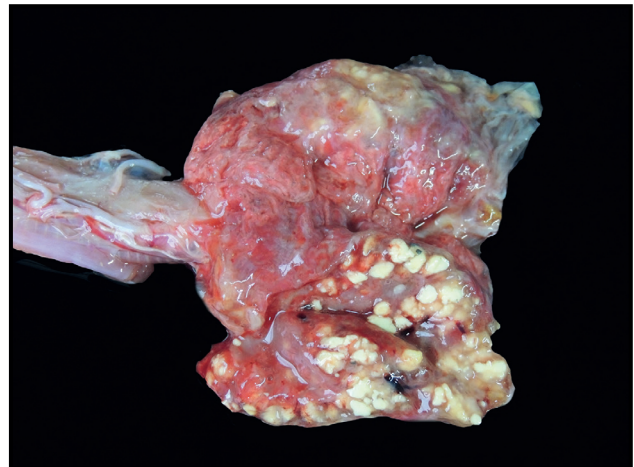
*Eucoleus contortus*, before named *Capillaria contorta*, is an important parasite of birds [11,15]. This filiform nematode is cosmopolite and can infect many bird families, where the Galliformes is the most frequently affected [7,11,14]. The infection can occur due to fecal-oral route or via earthworms [2,8]. The parasite *E. contortus* usually affects the gastrointestinal mucosa and respiratory tract mucosa. It has been observed parasitizing birds and mammals [7]. When parasitized, the bird may show weakness, apathy, anorexia, weight loss, cachexia, and sudden death [8,11,15]. Additionally, *E. contortus* can induce regurgitation and inflammatory processes in the host [11,14].

The genus *Lactobacillus* had great importance in the bird's gastrointestinal microbiota. They are facultative anaerobic or microaerophilic gram-positive bacilli, commensal, that normally are associated to animal health. However, *Lactobacillus* promoting animal disease can occur when microbiota imbalance is triggered [3,6,12]. Thus, this report describes the pathological and microbiological findings of chronic necrotizing pneumonia and aerossaccolitis caused by *Lactobacillus agilis* in an indian peafowl, associated to *Eucoleus contortus* parasitism.

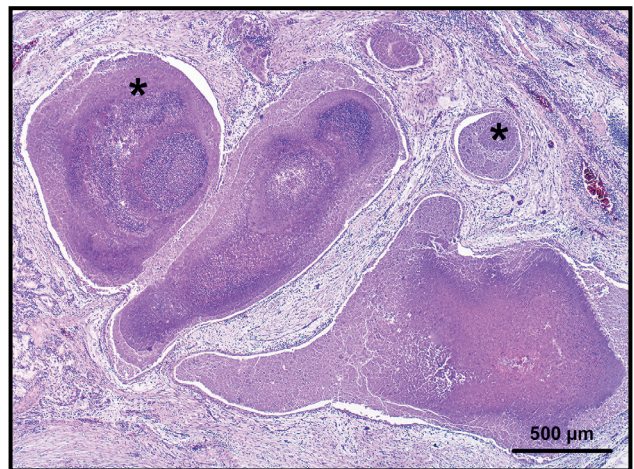
## CASE

An indian peafowl (*P. cristatus*), adult, male was submitted to *post mortem* examination due to sudden death. The history included living in an extensive breeding with other species (birds and mammals), emaciation and ataxia, but on the last day it remained in recumbency until death. Despite living with other birds, it was the only one of its specie on the farm.

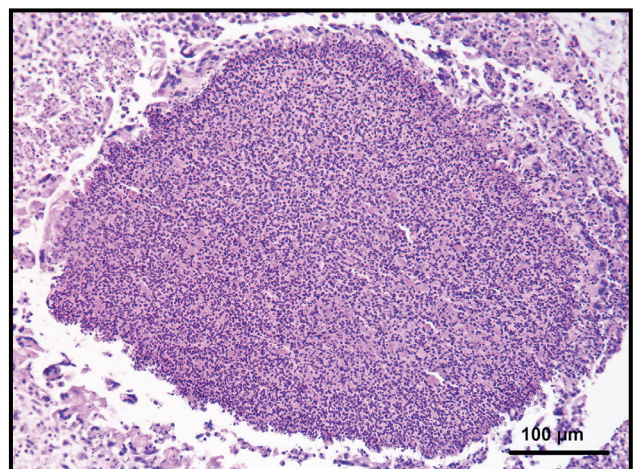
During gross evaluation, pectoral muscle showed severe atrophy, highlighting keel shape. The air sacs were filled with solid yellowish crumbly material (Figure 1). The same material was observed forming well-defined nodules that occupied > 50% of the lung parenchyma (Figure 2). Multiple tissue samples were collected, fixed in 10% neutral buffered formalin,



**Figure 1.** Indian peafowl, lung, necrotizing pneumonia. Symmetrical yellowish areas, predominantly in the lateral and caudal regions of the lungs. On the cut surface, the lung exhibited multiple multifocal to coalescent yellowish nodules.



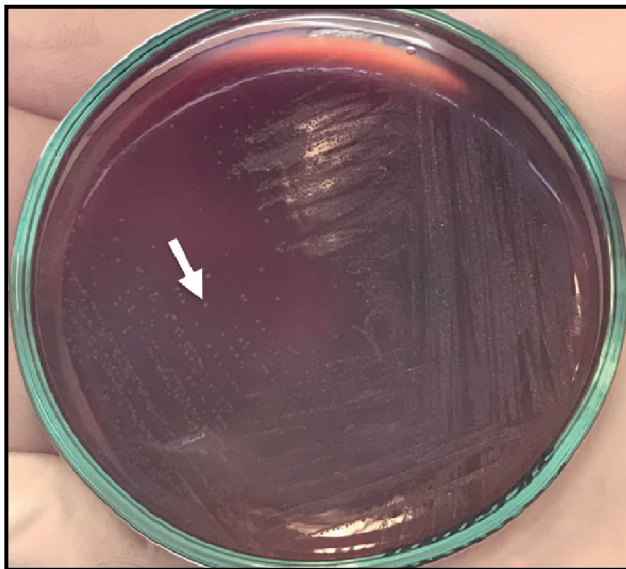
**Figure 2.** Indian peafowl, lung, necrotizing pneumonia. The parabranchi are filled and often expanded by strongly eosinophilic material associated with debris and inflammatory cells (asterisk). [HE; 40x].



**Figure 3.** Indian peafowl, lung, necrotizing pneumonia. Necrosis of the parabranchial lining epithelium, which is replete with strongly eosinophilic material associated with cellular debris and inflammatory cells, also seen in the interstitium, expanded by fibrous connective tissue. [HE; 200x].



**Figure 4.** Indian peafowl, esophagus, parasitary esophagitis. Several cross-sections of *Eucoleus contortus* in esophageal mucosa associated with parakeratotic hyperkeratosis of stratified squamous epithelium (asterisk). Foci of inflammatory cells in the submucosa are also observed (headarrow). [HE; 200x].

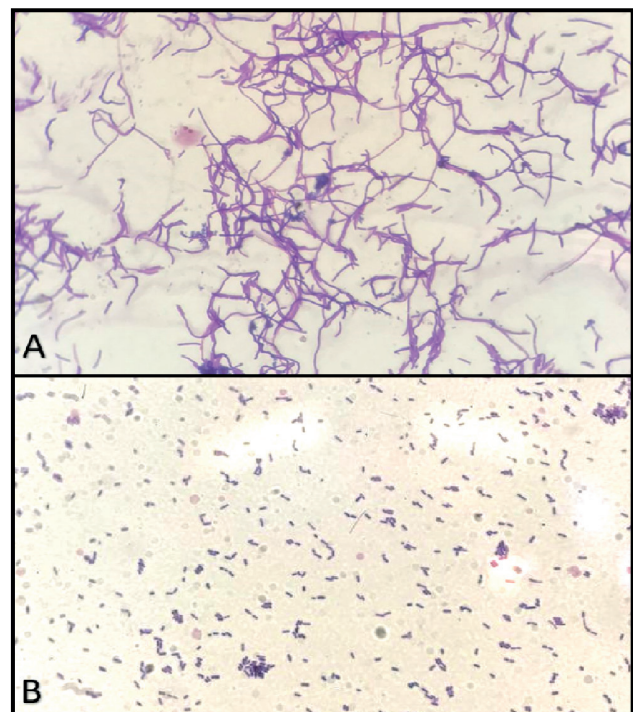


**Figure 5.** *Lactobacillus agilis* culture. Colonies in sheep blood agar, after 48 h of cultivation at 37°C. The arrow points to the small, round, and transparent colony.

routinely processed for histopathology, and stained with hematoxylin<sup>1</sup> and eosin (HE)<sup>2</sup>. Lung sections were also stained with Gram-Brown-Hopps (gentian violet<sup>3</sup>, potassium iodide<sup>2</sup>, acetone<sup>2</sup>, basic fuchsin<sup>4</sup>, picric acid<sup>1</sup> and acetone-xylene solution<sup>2</sup>) and Ziehl-Neelsen (fenicade fuchsin<sup>4</sup>, acid alcohol<sup>4</sup> and methylene blue stain<sup>4</sup>) for bacterial investigation, and with Grocott's methenamine silver (GMS)<sup>5</sup> for fungal investigation. Histological analysis showed multiple parabronchi dilated and filled with caseous necrosis, characterized by abundant cellular debris, degenerate heterophils, and fibrin deposition. These areas were surrounded by proliferation

of fibrous connective tissue and inflammatory infiltrate of macrophages, giant cells, lymphocytes, and plasma cells (Figure 3). These inflammatory cells were also observed distended the pleura surface, associated with fibrin deposition. Multiple short gram-positive bacilli bacteria are observed within the caseous foci in Gram-stained slides. No infectious agents were identified on the Ziehl-Neelsen and GMS histochemical stains. The air sacs parenchyma showed fibrin deposition and mixed inflammatory infiltrate. Additionally, the crop and esophageal mucosa showed multiple cross-sections of filiforms nematodes covered by a thin eosinophilic cuticle and presented a pseudocoelomatic cavity, coelomyarian musculature, with digestive, and reproductive structures, sometimes with bioperculated eggs (morphologically compatible with *E. contortus*). These nematodes were often associated with epidermal parakeratotic hyperkeratosis and chronic inflammatory infiltrate (lymphocytes and plasma cells) in the submucosa (Figure 4).

Lung fragments were subjected to bacterial culture in sheep blood agar<sup>6</sup> plates (aerobic and microaerophilic conditions) and McConkey agar<sup>1</sup> plates (aerobic conditions) at 37°C. After 48 h, pure small, round and transparent colonies in blood agar plates,



**Figure 6.** Morphotintorial presentation of the isolate. A- Gram staining performed on bacteria from colonies cultivated on blood agar under microaerophilic condition showing Gram-positive filamentous bacilli. B- Gram stain performed on bacteria from colonies grown on blood agar under aerobic atmosphere.

from both microaerophilic and aerobic conditions, with a zone of hemolysis (Figure 5). There was no significant growth in the McConkey agar plate. Gram staining showed gram-positive bacilli whereas Catalase test was negative. In the microaerophilic condition, the bacterium was filamentous (Figure 6A), whereas in aerobic condition it did not present filaments (Figure 6B). The isolate was identified by biochemical tests and confirmed as *Lactobacillus agilis* by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS)<sup>7</sup>.

## DISCUSSION

The diagnosis of *L. agilis* as primary microbiological cause of the chronic necrotizing pneumonia and aerossaculitis in the studied peafowl was based on the pathological and microbiological findings. Bacterial pneumonia in birds is often associated with pathogenic bacteria such as *Staphylococcus* spp. or *Enterococcus* spp. [14]. However, in the present report case the microscopic and microbiological results indicated an uncommon bacteria triggered pneumonia, *L. agilis*, that belong to the bird's microbiota [3].

The genus *Lactobacillus* is present in the gastrointestinal mucosa, colonizing and competing with other microorganisms. However, these bacteria can cause diseases in cases of microbiota imbalance [4,5]. The animal of the reported case was parasitized by *E. contortus*. When this parasite settles in the glottis, crop and esophagus, it causes regurgitation and difficulty in swallowing. Due to this phenomenon, *Lactobacillus* and other bacteria, food contents, saliva and nasal secretions could be aspirated. As consequence, lung lesions resulting of pathogenic bacteria favored by parasitism were previously reported [7,14,15]. Thus, in the present case, the inflammation caused by the parasitism and the aspiration of secretions may have favored the colonization of the pulmonary parenchyma by *L. agilis* from the gastrointestinal tract.

The reported animal showed a chronic bacterial pneumonia. *Lactobacillus* can also modulate inflammatory when naturally present in the microbiota. However, it is not known whether this modulation is

maintained in the case of secondary erratic infection [6,12,10]. Parasitic, in turn, have ability to modulate the host inflammatory response also predisposed to unnoticed and chronic disease [10,17], and induce an eosinophilic response rather than a massive acute response [1]. Consequently, aspiration could be slow and gradual, allowing the pneumonia to evolve chronically.

Despite not having manifested respiratory or gastrointestinal signs, the peafowl showed cachexia with accentuated loss of muscle mass. This suggests a chronic disease, and probably feathers and extensive breeding prevented clinical signs from being noticed. The cachexia and pathological lesions found were compatible with the transit that *E. contortus* have through the gastric system. Additionally, parasite movement and egg deposition in the glottis could induce an inflammatory response and mucous production. The parasite movement and inflammatory process could impact bacteria clearance in gastrointestinal and respiratory systems [11,14,15].

In conclusion, although uncommon, *Lactobacillus* spp. should be considered as a possible cause of necrotic pneumonia and aerossaculitis in peafowl, especially in animals with parasitism. From this point of view, it is essential to work on the parasitic prevention of birds raised in extensive breeding. Finally, to the best of our knowledge, this is the first report of *Lactobacillus agilis* as causal agent of fatal necrotic pneumonia and aerossaculitis in peafowl.

## MANUFACTURERS

<sup>1</sup>Éxodo Científica. Sumaré, SP, Brazil.

<sup>2</sup>Neon Comercial. Suzano, SP, Brazil.

<sup>3</sup>Dinâmica Química. Indaiatuba, SP, Brazil.

<sup>4</sup>Produtos Citológicos Soldan (Pró-cito). Porto Alegre, RS, Brazil.

<sup>5</sup>Grupo Erviegas. Indaiatuba, SP, Brazil.

<sup>6</sup>Kasvi - Produtos e Equipamentos para Laboratórios. São José dos Pinhás, PR, Brazil.

<sup>7</sup>Microflex LT. Bruker Industry, Leipzig, Germany.

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