

Bronchopneumonia in sheep associated with *Providencia stuartii*

Broncopneumonia em ovinos associada a *Providencia stuartii*

Bronconeumonía en ovinos asociada a *Providencia stuartii*

Recebido: 14/11/2021 | Revisado: 22/11/2021 | Aceito: 26/11/2021 | Publicado: 08/12/2021

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Abstract

Bacteria of the genus *Providencia* are opportunistic pathogens in humans, widely distributed in the environment and associated with greater resistance to antibiotics, and this being uncommon the association with clinical diseases. This study reports the isolation of *P. stuartii* in two sheep that presented clinical signs of pneumonia. At necropsy there was severe and acute fibrinopurulent bronchopneumonia. Histologically, there were infiltrated neutrophils and fibrin in the alveolar lumen, and the alveolar septa presented multifocal thickening with moderate proliferation of pneumocytes and mononuclear interstitial infiltrate. *Providencia* sp. was isolated in the microbiological tests of the lung and tracheal secretions. The isolate was subjected to DNA extraction, polymerase chain reaction (PCR) for the 16SrRNA gene and sequencing of genomic DNA, which demonstrated 100% homology with *P. stuartii*. This is the first report of the presence of this microorganism as a cause of interstitial and fibrinopurulent bronchopneumonia in sheep. Therefore, it is suggested that epidemiological surveillance strategies should be carried out in animals to better understand their role in the dissemination of this pathogen.

Keywords: Microbiology; Pathology; Pneumonia; Sequencing; Sheep.

Resumo

Bactérias do gênero *Providencia* são patógenos oportunistas em humanos, amplamente distribuídas no ambiente e estão associadas a maior resistência aos antibióticos, sendo incomum a associação com doenças clínicas. Este estudo relata o isolamento de *P. stuartii* em duas ovelhas que apresentaram sinais clínicos de pneumonia. Na necropsia havia broncopneumonia fibrinopurulenta aguda e grave. Histologicamente, havia neutrófilos infiltrados e fibrina na luz alveolar, e os septos alveolares apresentavam espessamento multifocal com proliferação moderada de pneumócitos e infiltrado intersticial mononuclear. *Providencia* sp. foi isolado nos exames microbiológicos do pulmão e secreções traqueais. O isolado foi submetido à extração de DNA, reação em cadeia da polimerase (PCR) para o gene 16SrRNA e sequenciamento do DNA genômico, que demonstrou 100% de homologia com *P. stuartii*. Este é o primeiro relato da presença desse microrganismo como causa de broncopneumonia intersticial e fibrinopurulenta em ovinos. Portanto, sugere-se que estratégias de vigilância epidemiológica devam ser realizadas em animais para melhor compreensão de seu papel na disseminação desse patógeno.

Palavras-chave: Microbiologia; Patologia; Pneumonia; Sequenciamento; Ovinos.

Resumen

Las bacterias del género *Providencia* son patógenos oportunistas en el ser humano, ampliamente distribuidos en el medio ambiente y asociados a una mayor resistencia a los antibióticos, siendo infrecuente la asociación con enfermedades clínicas. Este estudio reporta el aislamiento de *P. stuartii* en dos ovejas que presentaban signos clínicos de neumonía. En la necropsia había bronconeumonía fibrinopurulenta aguda y grave. Histológicamente había neutrófilos infiltrados y fibrina en la luz alveolar, y los septos alveolares presentaban engrosamiento multifocal con moderada proliferación de neumocitos e infiltrado intersticial mononuclear. *Providencia* sp. se aisló en las pruebas microbiológicas de las secreciones pulmonares y traqueales. El aislado se sometió a extracción de ADN, reacción en cadena de la polimerasa (PCR) para el gen 16SrRNA y secuenciación del ADN genómico, que demostró una homología del 100% con *P. stuartii*. Este es el primer reporte de la presencia de este microorganismo como causa de bronconeumonía intersticial y fibrinopurulenta en ovinos. Por tanto, se sugiere que se lleven a cabo estrategias de vigilancia epidemiológica en animales para comprender mejor su papel en la diseminación de este patógeno.

Palabras clave: Microbiología; Patología; Neumonía; Secuenciación; Ovino.

1. Introduction

The genus *Providencia* comprises gram-negative rods that include ten species: *P. alcalifaciens*, *P. heimbachae*, *P. rettgeri*, *P. rustigianii*, *P. stuartii*, *P. vermicola*, *P. sneebia*, *P. burhododranariae*, *P. thailandensis* (Juneja & Lazzaro, 2009; Muller et al., 1986; Somvanshi et al., 2006) and the new species *P. entomophila* (Ksentini et al., 2019). Unlike other members of the Enterobacteriaceae family, they are characterized by the ability to oxidatively deaminate to the corresponding keto acid and ammonia (Farmer et al., 1985).

Widely distributed in the environment, species of *Providencia* are important opportunistic pathogens in humans, being associated with higher antibiotic resistance (Wie, 2015). Research related to resistance genes and their environmental dissemination show their importance in public health (Iwata et al., 2020), mainly due to the growth of intrinsic resistance to antibiotics that are considered last-resort treatments and the gene acquisition that code for different enzymes (Liu et al., 2020; Tavares et al., 2015). *Providencia stuartii* is present in the environment, including water and soil, and is also responsible for nosocomial infections (Liakopoulos et al., 2017). In humans, this bacterium has been isolated mainly from patients with chronic urinary tract diseases who are subjected to prolonged use of catheters (Barl et al., 2012; Wie, 2015). However, reports of the presence of renal and hepatic abscesses (Chamberland et al., 2013; Lin et al., 2017), meningitis (Sipahi et al., 2010), endocarditis (Krake & Tandon, 2004), conjunctivitis (Crane et al., 2016) and septic vasculitis (George et al., 2020) have also been described in the literature.

Descriptions in animals are scarce in the literature, being associated with primate sepsis, ulcerative dermatitis and cellulitis in canine and diarrheal swine feces (Almeida et al., 2007; Liu et al., 2016; Papadogiannakis et al., 2007). In addition, *Providencia* spp. were found in retail meats in China and Japan (Di et al., 2018) and Barbour et al (2012) isolated of liver from broiler chicken in Lebanon, which could cause a serious public health problem

Therefore, this report describes the pathological findings, microbiological and molecular analyses of *P. stuartii* present in the lung of sheep with bronchopneumonia, highlighting that animals may be important sources of transmission to humans, in addition to enabling the spread of *Providencia* spp. in food.

2. Methodology

A batch of 150 sheep was purchased and introduced to a property in the municipality of Santo Antônio de Leverger - MT. Within 3 months, some animals showed clinical signs of difficulty breathing and coughing. Of these, two young male sheep, weighing 15 kg and 17.3 kg, presented slightly pale oral and ocular mucosa, evolving to death.

A fragment of the lung and a sample of the tracheal secretion were sent for bacteriological isolation to the Laboratory of Microbiology (HOVET-UFMT), seeded in 8% Sheep Blood Agar, MacConkey Agar and Sabourad Agar, incubated in

aerobic conditions at 37°C for up to 7 days, and after growth, colony identification was performed according to Quinn et al. (2011).

For molecular characterization, the isolate was inoculated in Brain Heart Infusion Broth (BHI) and incubated at 37°C *overnight*. After centrifugation, the precipitate was resuspended in 1 mL lysis buffer (100 mM NaCl, 25 mM EDTA, 100 mM Tris-HCl pH 8.0, 0.5% SDS, 0.1 mg Proteinase K) and DNA was extracted using the phenol-chloroform method, according to Sambrook and Russell (2004). The DNA was resuspended with 50 µl of ultrapure water. To verify the integrity and quality of the extract, the DNA was stained with Gel Red (Biotium), subjected to 1.5% agarose gel electrophoresis at 100 V for 40 min, and visualized on ChemiDoc™ XRS using ImageLab™ software.

PCR was performed to sequence the 16S rRNA, using the oligonucleotide pairs 27F: AGA GTT TGA TCC TGG CTC AG (Lane, 1991) and 1492R: GGT TAC CTT GTT ACG ACT T (Turner et al., 1999), amplifying products of 1424 bp. The reaction was performed with 10 ng genomic DNA, 0.4 pmol oligonucleotides, 0.2 mM dNTPs, 3 mM MgCl₂, 1x PCR buffer 10x (200 mM Tris-HCl pH 8.4 and 500 mM KCl), 1 U of Taq DNA polymerase (*Invitrogen*) and ultrapure water q.s.p. to a final volume of 25 µL. The reactions were amplified in a My Cycler™ thermocycler (Biorad), with an initial denaturation of 5 minutes at 95°C, followed by 35 denaturation cycles for 45 seconds at 95°C, hybridization for 1 minute at 52°C and 1 minute and 30 seconds at 72°C extension and a final extension cycle at 72°C for 7 minutes.

Amplification products, stained with Gel Red (Biotium), were subjected to 1.0% agarose gel electrophoresis at 100 V for 90 min, visualized on ChemiDoc™ XRS using ImageLab™ software, and purified using Illustra™ ExoProstar™ enzyme, following the manufacturer's instructions. Subsequently, sequencing was performed using an ABI-PRISM 3500 Genetic Analyzer (Life Technologies Corporation, USA).

3. Results

After referral for necropsy, two samples were collected from the sheep for histopathological and microbiological analysis. In the post-mortem examination, the presence of yellowish mucous material was observed in most of the bronchial tree, obliterating the bronchial lumen. The pleural surface contained random reddish multifocal areas (“marbled pattern”), affecting 60% of the organ, with consolidation and the presence of fibrin.

Histological changes were similar in both animals, with thickened alveolar septa, associated with moderate proliferation of pneumocytes and mononuclear interstitial infiltrate. In the bronchiolar and alveolar lumen, there was a variable neutrophil infiltration associated with fibrin aggregates and cellular debris, in addition to the occasional necrosis of the alveolar septa and the rare presence of syncytial cells, lymphocytes and plasma cells. Occasionally there were fibrin thrombi in the vascular lumen.

In the two lung samples, bacteria with phenotypic characteristics similar to *P. stuartii* were identified. The sequences obtained were compared and deposited into the GenBank database using the Basic Local Alignment Search Tool (BLAST) program (<http://www.ncbi.nlm.nih.gov/blast/Blast.cgi>) on the NCBI server (<http://www.ncbi.nlm.nih.gov/BLAST>) and the result demonstrated 100% identity with *P. stuartii*. The sequences were deposited at GenBank under the number MN733989.

4. Discussion

Respiratory diseases are common in domestic animals and they generate economic impacts related to productive loss, such as reduced weight gain and growth, drug costs and death (Azizi et al., 2013). Pneumonia in sheep can occur in lambs and adult animals depending on the existence of predisposing factors, it being considered a common disease (Azizi et al., 2013; Glendinning et al., 2017). Factors such as age, geographic location, environmental stressors, climate, nutritional

status, immunosuppression, unhygienic conditions, managing system can contribute to the development of this disease (Azizi et al., 2013; Bell, 2008; Kalogianni et al., 2020). In sheep, the recent transport history can be considered a stress factor that predisposes to pneumonia (Brogden et al., 1998; Chakraborty et al., 2014; Scott, 2011). Reports of pneumonia in sheep caused by different etiologies have already been described in the literature (Azizi et al., 2013; Bell, 2008; Carmo et al., 2020; Cid et al., 2019; Jaý & Tardy, 2019; Kalogianni et al., 2020), however there are no reports of *P. stuartii*.

The macroscopic and histological changes found in the lungs of the sheep were compatible with interstitial and fibrinopurulent bronchopneumonia (Ackermann & Brogden, 2000). Sepsis in primates by *P. stuartii*, with the presence of fibrin and edema in the bronchi and bronchioles, has also been described by Liu et al. (2016). Histologically, there was intense neutrophil infiltration and exudation of fibrin in the alveoli, bronchi and bronchioles. Exudate is accompanied by necrosis and degenerate neutrophils (Hussain et al., 2017). These microscopic findings were present in the sheep, indicating fibrinopurulent bronchopneumonia. Tracheitis and acute suppurative bronchopneumonia have been reported in lambs associated with bacterial infections (Azizi et al., 2013; Hussain et al., 2017).

In the two lung samples, bacteria with phenotypic characteristics similar to *P. stuartii* (O'Hara et al., 2000) were isolated and their identity was confirmed molecularly (Ovchinnikova et al., 2013). The source of infection was not determined in these cases; however, it may have originated from the environment since it is found in soil, water and manure.

As an opportunistic pathogen in humans and animals, *P. stuartii* can be associated with primary and secondary infections (O'Hara et al., 2000). This microorganism has rarely been described in the veterinary literature, with no reports on the presence of this microorganism as a pathogen in respiratory tract infections of sheep to date.

5. Conclusion

Descriptions of respiratory diseases are common in farm animals, however, in sheep there is no pneumonia associated with *P. stuartii*. This etiological agent is an important public health pathogen that is associated with antibiotic resistance and it is widely distributed in the environment. There have been few studies on *P. stuartii* in animals, and this is the first report of the presence of this microorganism as a cause of interstitial and fibrinopurulent bronchopneumonia in sheep, confirmed by pathological findings, microbiological and molecular analyses. Therefore, it is suggested that epidemiological surveillance strategies should be carried out in animals to better understand their role in the dissemination of this pathogen.

Acknowledgments

We are grateful to the Coordination of Higher Education Personnel (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – CAPES) for the concession of a doctoral scholarship.

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