

## Case Report

Aspiration From Dysphagia Associated With the Presence of *Pantoea agglomerans* in a Horse

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**ABSTRACT**

*Pantoea agglomerans* is a gram-negative bacterium that usually lives in symbiosis in soil and plants. However, it may also lead to serious diseases in plants, animals and humans. We describe a case of aspiration from dysphagia associated with the isolation of *P. agglomerans* in a horse admitted to an Italian hospital. The patient was presented to the hospital for the onset of chronic cough after an acute episode of pulmonary hemorrhage during a race recorded two months before and was treated with dexamethasone and penicillin. The cough was accentuated during meals, leading to a suspicion of aspiration from dysphagia. A bronchoalveolar lavage was collected, and cytological examination showed the presence of vegetable fibers, rod bacteria, and a high macrophage population (89%). From the tracheal wash collected for bacteriological culture, *P. agglomerans* was isolated in monoculture, and the antimicrobial susceptibility test revealed that the isolate was susceptible to all tested antimicrobials except sulfonamide. The horse recovered well after 10 days of enrofloxacin treatment. The present report highlights the role of *P. agglomerans* as an opportunistic respiratory pathogen in horses, especially in the presence of plants or their parts.

**Keywords:** *Pantoea agglomerans*, Aspiration from dysphagia, Equine medicine, Equine respiratory disease, Horses

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## Case History

**P***antoea agglomerans* is a ubiquitous, non-encapsulated and non-spore-forming gram-negative rod, usually found in different geographical and ecological sources such as plants, human feces and the environment (Watterson & Stavrinides, 2015). In plants and soil, it has usually been found as a symbiont. Still, it has also been identified as a cause of diseases in several cultivable plants, such as cotton, sweet onion, rice, maize, sorghum, bamboo, walnut, ornamental plants like Chinese taro (*Alocasia cucullata*) and *Gypsophila* (*Gypsophila paniculata*) and in the onion couch (*Arrhenatherum elatius*) (Dutkiewicz et al., 2016). *P. agglomerans* has also been associated with animal infections; however, there are few reports on infectious diseases in vertebrates. It has been isolated from brown and rainbow trout (Carbajal-González et al., 2011). Moreover, *P. agglomerans* strains are commonly found in insects such as bees (Lozo et al., 2015; Piva et al., 2020) and ants (Suen et al., 2010), usually as commensals. In mammals, descriptions of *P. agglomerans* infection are scarce (Dutkiewicz et al., 2016). In cows, this agent has been considered a potential cause of allergic pulmonary disease, whereas, in horses, it has been occasionally described in association with cases of equine abortion and placentitis (Gibson et al., 1982; Henker et al., 2020). In humans, *P. agglomerans* has been associated with infections such as arthritis, septicemia, peritonitis and abscesses, usually after piercing or laceration of the skin with plant material, such as wooden splinters, causing subsequent penetration of the bacteria (Dutkiewicz et al., 2016; Jain et al., 2012; Duerinckx et al., 2008; Rave et al., 2012). It can occasionally cause hospital-acquired infections due to contaminated medical equipment or fluids in immunocompromised individuals (Dutkiewicz et al., 2016). This report describes a case of aspiration from dysphagia associated with the isolation of *P. agglomerans* in a racehorse.

## Clinical Presentation

In August 2020, a 6-year-old male, thoroughbred racehorse had an episode of violent pulmonary hemorrhage after a race. The practitioner, suspecting an exercise-induced pulmonary hemorrhage, administered an ex juvantibus therapy (penicillin 9000 UI/kg for 7 days every 24 h, IM; dexamethasone 0,06 mg/kg IV for one day). One week after the end of the therapy, the horse continued to present cough and nasal discharge. The cough was consistently accentuated during meals, leading to

suspicion of dysphagia, although multiple endoscopic examinations performed at rest did not reveal problems in the guttural pouch or the functionality of the larynx. A bronchoalveolar lavage (BAL) was then collected and sent to a laboratory for cytological assessment. Cytological examination showed the presence of vegetable fibers, considered sample contamination and an inflammatory state. Several airway endoscopies, both at rest and under exercise, were subsequently performed, and only the endoscopy at rest showed the presence of mucus. In October 2020, the patient was admitted to the Veterinary Teaching Hospital of the University of Bologna because of a persistent cough. The horse was clinically healthy on clinical examination except for a slight weight loss and chronic cough. Complete blood count (CBC) analysis showed normal values with hematocrit 46.3% (normal range 31.5%-50.5%), platelets 117.000/mm<sup>3</sup> (normal range 100.000-600.000/mm<sup>3</sup>), hemoglobin 15.6 g/dL (normal range 11-19 g/dL), leukocytes 9.380/mm<sup>3</sup> (normal range 5500-125000/mm<sup>3</sup>). Biochemistry parameters collected following standard procedures described by Kamali Sadeghian et al. (2021) showed no alteration. Endoscopic examination of nerve function revealed no abnormalities, although there was evidence of food material in the trachea. A BAL sample was collected for cytological assessment using direct and sediment smears. The sample showed a high presence of macrophages (89%), with phagocytic activity, mast cells (5%), and lymphocytes (6%). Vegetable fibers and rod bacteria were also reported. Cytological analyses were repeated to ensure that the presence of vegetable fibers was not a sample contamination. Plant fiber and rod bacteria in the cytological examination confirmed the possibility of aspiration from dysphagia, which could lead to a respiratory tract infection. One week later, a tracheal wash sample was collected for bacteriological culture.

## Diagnostic Testing

A sample of BAL was collected for routine aerobic and anaerobic bacterial culture. After 24 h of incubation in aerobic and anaerobic bacterial cultures, small colonies in monoculture were isolated on blood agar base plates with 5% horse blood under aerobic conditions. The isolate was identified as *P. agglomerans* using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) (MALDI biotyper, Bruker Inc., USA) using the Bruker Biotyper version 3.0 software. The identification of *P. agglomerans* was also confirmed by amplification of a portion of the 16S rRNA gene by PCR using generic primers (1492-r; p27-f) and shared 99% identity with that of *P. agglomerans* (Ac-

cession Number CP031649.1). The bacterial strain was subjected to antimicrobial susceptibility testing using the disk diffusion method (CLSL, 2020). The antimicrobial susceptibility test revealed that the isolate was sensitive to amikacin (30 µg), ampicillin (10 µg), amoxicillin-clavulanate (30 µg), cefazolin (30 µg), enrofloxacin (5 µg), marbofloxacin (5 µg), tetracycline (30 µg), trimethoprim-sulfamethoxazole (1.25/23.7 µg), gentamicin (10 µg), streptomycin (10 µg) and ceftiofur (30 µg). However, the isolate was resistant to sulfonamide (300 µg). Therefore, the horse was treated with 5 mg/kg of enrofloxacin once daily, for 10 days, without performing other tests (x-rays, ultrasound examination) for cost reasons. At the end of the treatment, the horse was discharged with complete remission of clinical symptoms. After the treatment, CBC analysis was repeated, with no evidence of alterations (hematocrit 39.9%, platelets 187.000/mm<sup>3</sup>, hemoglobin 14.2 g/dL, leukocytes 5.970/mm<sup>3</sup>).

## Assessments

Regarding respiratory tract infections, *P. agglomerans* has been described in humans and horses. In humans, among opportunistic bacteria, *P. agglomerans* is one of the most isolated species in immunodeficient patients showing respiratory symptoms, as confirmed by Flores Popoca et al. (2012), who isolated *P. agglomerans* in human respiratory secretions. In horses, Gibson et al. (1982) identified *P. agglomerans* as a possible cause of equine abortion based on positive cultures from aborted fetuses associated with the presence of inflammatory lesions, mostly appearing as interstitial pneumonia (Gibson et al., 1982). Singh et al. (2004) also described an outbreak of equine abortion due to *P. agglomerans*, highlighting the signs related to interstitial pneumonia. Bond et al. (2017) described horses' upper and lower respiratory tract microbiota. They demonstrated that 50 genera in the respiratory microbiota of horses were the most representative, with *Sphingomonas* and *Pantoea* being the most abundant (Bond et al., 2017). In our case, the initial differential diagnosis could have included equine herpesvirus, as suggested by Raofi et al. (2020). The final diagnosis was made by cytological assessment, with vegetable fibers and rod bacteria, as well as by bacterial culture, with the evidence of a *P. agglomerans* monoculture, and the clinical symptoms led to suspicion of aspiration secondary to dysphagia, although no further investigations to find the cause were performed to determine the cause. Multiple endoscopies performed under exercise and at rest are part of the regular racehorse examination (Mokhber Dezfouli et al., 2019).

The first treatment with dexamethasone effectively controlled airway inflammation and improved pulmonary function (Léguillette et al., 2017). Still, it could have caused immunosuppression within the respiratory tract (Cohn, 1991), promoting the overgrowth of specific bacteria. In our case, *P. agglomerans* probably entered the respiratory tract through vegetable fibers (Büyükcamlı et al., 2018; Cheng et al., 2013). The isolated strain of *P. agglomerans* showed a high antimicrobial sensitivity profile following the findings of Cheng et al. (2013) and Cruz et al. (2007) and the proper antibiotic treatment led to complete recovery.

In conclusion, *P. agglomerans* is a well-known agent of opportunistic infections in humans and animals, including horses, especially in immunocompromised individuals. The present study reports the possibility of *P. agglomerans* causing aspiration from dysphagia in horses in particular conditions, which has already been reported in humans (Cruz et al., 2007), highlighting the importance of focusing on its pathogenic potential. This issue should be especially considered when the clinical picture contains some association with the presence of plants or their parts.

## Ethical Considerations

### Compliance with ethical guidelines

All ethical principles are considered in this article. The participant and his family were informed of the purpose of the research and its implementation stages. They were also assured about the confidentiality of their information. They were free to leave the study whenever they wished, and the research results would be available to them if desired. A written consent has been obtained from the subject. Principles of the Helsinki Convention was also observed.

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### Authors' contributions

Conceptualization: Silvia Piva, Riccardo Rinnovati, and Elisabetta Mondo; Methodology: Elisabetta Mondo, Alessandro Illuzzi, Riccardo Rinnovati, and Silvia Piva; Validation: Silvia Piva, Federica Giacometti, Federica Savini, Federico Tomasello, and Riccardo Rinnovati; Formal analysis: Elisabetta Mondo, Silvia Piva, and Raffaele Scarpellini; Investigation: Elisabetta Mondo, Raffaele Scarpellini, Riccardo Rinnovati, Alessandro Illuzzi, and

Silvia Piva; Resources: Silvia Piva, and Elisabetta Mondo; Data curation: Raffaele Scarpellini, Riccardo RInnovati, Silvia Piva, and Elisabetta Mondo; Original draft preparation: Elisabetta Mondo; Review and editing: All authors. Visualization: Raffaele Scarpellini; Supervision, project administration and funding acquisition: Silvia Piva.

### Conflict of interest

The authors declared no conflict of interest.

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