

**Aspiration from Dysphagia Associated with the Presence of *Pantoea*
agglomerans in a Horse**

**Elisabetta Mondo¹, Riccardo Rinnovati¹, Raffaele Scarpellini ^{*1}, Alessandro Illuzzi²,
Federica Giacometti¹, Federica Savini¹, Federico Tomasello¹, Silvia Piva¹**

1-Department of Veterinary Medical Sciences, University of Bologna, Bologna, Italy

2-Equivet Roma Hospital, Via di Torre di Sant'Anastasia, Rome, Italy

Abstract

Pantoea agglomerans is a gram-negative bacterium that usually lives in symbiosis in soil and plants. However, it may lead to important disease in both plants and animals, including humans. We describe one 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, treated with dexamethasone and penicillin. The cough was accentuated during meals, leading to the suspect of aspiration from dysphagia. A bronchoalveolar lavage (BAL) was collected, and the cytological examination showed the presence of vegetable fibers, rod bacteria and high macrophages 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 every tested antimicrobial, except of sulfonamide. The horse recovered well after a 10 days treatment with enrofloxacin. The present reports highlights the role of *P.agglomerans* as an opportunistic respiratory pathogen in horses, especially in the presence of plants or part of these.

Keywords: *Pantoea agglomerans*; aspiration from dysphagia; equine medicine; equine respiratory

disease; horses

Case History

Pantoea 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 environment (Watterson and Stavrinides, 2015). In plants and soil, it has usually been found as a symbiont, but 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 paniculate*), and in the onion couch (*Arrhenatherum elatius*) (Dutkiewicz et al., 2016). *P.agglomerans* has also been associated to infections in animals, but there are only a few reports on infectious diseases in vertebrates. It has been isolated from brown and rainbow trout (Carbajal-González et al., 2011), from the digestive tract of dunnocks (*Prunella modularis*) (Kiskova et al., 2012) and it has been reported as a cause of hemorrhagic disease in dolphinfish (Hansen et al., 1990). Moreover, *P.agglomerans* strains have been found commonly 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* infections are scarce (Dutkiewicz et al., 2016). In cows, this agent has been

considered as a potential cause of allergic pulmonary disease, whereas in horses it has been occasionally described in association with cases of equine abortion and placentitis (Pomorski et al., 1993, Gibson et al., 1982; Henker et al., 2020; Smith et al., 2003). In humans, *P.agglomerans* has been associated to infections such as arthritis, septicemia, peritonitis, and abscesses, usually after a piercing or laceration of the skin with a 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 also be an occasional agent of hospital-acquired infections, mostly due to the contamination of 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-years 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 7days every 24 h IM; Dexamethasone 0,06 mg/kg IV for one day). One week after the end of therapy, the horse continued to present cough and nasal discharge. The cough was consistently accentuated during meals, leading to the suspect of dysphagia, although multiple endoscopic examinations executed at rest did not reveal problems in guttural pouch or in the functionality of the larynx. A

bronchoalveolar lavage (BAL) was then collected and the sample was sent to a laboratory for cytological assessment. Cytological examination showed, in addition to an inflammatory state, the presence of vegetable fibers that were considered a sample contamination. 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 the persistent cough. On the clinical examination, the horse was clinically healthy 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/mm³ (normal range 100.000-600.000/mm³), hemoglobin 15.6 g/dl (normal range 11-19 g/dl), leucocytes 9.380/mm³ (normal range 5500-125000/mm³). Endoscopic examination of nerve function did not reveal any abnormalities, although there was evidence of food material in the trachea. A BAL sample was collected for cytologic assessment by direct and sediment smear. The sample showed a high macrophages presence (89%), with phagocytic activity, mast cells (5%), lymphocytes (6%); vegetable fibers and rod bacteria presence were also reported. Cytological analyses were repeated to be sure that the presence of vegetable fibers was not a sample contamination. The presence of plant fiber and rod bacteria in the cytological examination confirmed the possibility of an aspiration from dysphagia, that potentially could lead to a respiratory tract infection. A week later, a sample of tracheal wash was collected for bacteriological culture.

Diagnostic testing

A sample of BAL was collected for routine aerobic and anaerobic bacterial culture. After 24h of incubation in aerobic and anaerobic bacterial cultures, small colonies in monoculture were isolated on Blood agar base plates with 5% horse blood (Oxoid) in 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* (Accession Number CP031649.1). The bacterial strain was subjected to an antimicrobial susceptibility test, performed by the disc diffusion method (Wayne, 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), and trimethoprim- sulfamethoxazole (1.25/23.7 µg), gentamicin (10 µg), streptomycin (10 µg), ceftiofur (30 µg), while 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 total remission of clinical symptoms. After the treatment, CBC analysis was

repeated, with no evidence of alterations (hematocrit 39.9%, platelets 187.000/mm³, hemoglobin 14.2 g/dl, leucocytes 5.970/mm³).

Assessments

For what concerns respiratory tract infections, this *P.agglomerans* has been described both 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 also confirmed by Flores Popoca et al. (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 on the basis of positive cultures from aborted foetus associated with the presence of inflammatory lesions, mostly appearing as interstitial pneumonia (Gibson et al., 1982). Singh et al. too, describing an outbreak of equine abortion due to *P.agglomerans*, highlighted signs related to interstitial pneumonia (Singh et al., 2004). Bond et al. (2017), in a study aimed to described upper and lower respiratory tract microbiota in horses, demonstrated that in the respiratory microbiota of horses fifty genera were the most representative, with *Sphingomonas* and *Pantoea* being the most abundant (Bond et al., 2017). In our case, the diagnosis was performed 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 suspect an aspiration secondary to dysphagia, although no further investigations to find the cause were performed.

The first treatment with dexamethasone was effective at controlling airway inflammation and improving pulmonary function (Léguillette et al., 2017), but it could have caused immunosuppression within the respiratory tract (Cohn, 1991), promoting the overgrowth of specific bacteria, in our case *P.agglomerans* that probably entered in the respiratory tract throughout vegetable fibers (Büyükcım et al., 2018; Cheng et al., 2013). The isolated strain of *P.agglomerans* showed a high antimicrobial sensitivity profile in accordance with the findings of Cheng et al. and Cruz et al. (Cheng et al., 2013; Cruz et al., 2007) and the proper antibiotic treatment led to fully recovery.

In conclusion, *P.agglomerans* appears to be a relative well-known agent of opportunistic infections, both in humans and animals, including horses, especially in immunocompromised individuals. The present study reports the possibility of *P.agglomerans* to cause aspiration from dysphagia in horses in particular conditions, as well as has been already reported in humans (Cruz et al., 2007), highlighting the importance to focus on its pathogenic potential. This should be especially considered when the clinical picture contains some kind of association with the presence of plants or part of these.

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