Short communication

An unusual *Actinobacillus equuli* strain isolated from a rabbit with Tyzzer’s disease

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Abstract

*Actinobacillus equuli* was isolated in pure culture from the liver and lungs of an adult rabbit with Tyzzer’s disease (*Clostridium piliforme*). Based on the haemolytic features on blood agar plates, a positive reaction in the CAMP-test, hydrolysis of esculin, the inability to ferment L-arabinose, tDNA-PCR and sequencing of the 16S rRNA gene, the isolate was classified as *A. equuli* subsp. *haemolyticus* biovar 1. However, the *aqxA* gene, characteristic for haemolytic *A. equuli* strains, was not detected by PCR.

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*Actinobacillus equuli*, a member of the family Pasteurellaceae, is a small, pleomorphic, non-motile Gram-negative rod. It is the etiological agent of a rare, but frequently fatal septicaemia in neonatal foals also known as “sleepy foal disease” which is spread worldwide (Rycroft and Garside, 2000; Berthoud et al., 2002). Besides this acute form of disease, *A. equuli* can be associated with other types of infections in both foals and adult horses, such as abortion, enteritis, peritonitis, pleuropneumonia, pericarditis, periorchitis and arthritis (Webb et al., 1976; Dill et al., 1982; Al-Mashat and Taylor, 1986; Belknap et al., 1988; Peremans et al., 1991; Collins et al., 1994; Patterson-Kane et al., 2001). *A. equuli* has been subdivided in two different subspecies, designated *A. equuli* subsp. *haemolyticus* (formerly described as Bisgaard Taxon 11), which is haemolytic and enhances the haemolytic effect of staphylococcal β-haemolysin (CAMP-positive) and *A. equuli* subsp. *equuli*, which is non-haemolytic and CAMP-negative (Christensen et al., 2002). The haemolytic strains have mainly been described in horses, but are not specifically associated with “sleepy foal disease”, which is typically linked with the non-haemolytic strains (Kuhnert et al., 2003). *A. equuli* subsp. *equuli* was not only isolated from horses, but has also been associated with abortion and endocarditis in pigs.
(Jones and Simmons, 1971; Werdin et al., 1976). Moreover, the organism was isolated from the nasopharynx, conjunctiva and middle ear of guinea pigs, rats and mice (Lentsch and Wagner, 1980). A. equuli is additionally considered as a potential opportunistic pathogen of humans, especially in immunocompromised people or people who are occupationally exposed to infected horses or pigs (Ashhurst-Smith et al., 1998; Vaz et al., 2001).

In this report, we describe the isolation of an unusual A. equuli strain from the liver and lungs of an adult rabbit with Tyzzer’s disease.

An adult, male pet-rabbit (Oryctolagus cuniculus) weighing 4.1 kg, died after a 4-day history of anorexia and depression. The animal had not been treated with an antimicrobial agent.

At necropsy, a fibrinous pericarditis as well as numerous 1-mm-diameter necrotic foci scattered within the liver parenchyma were observed. Histological examination of haematoxylin-eosin stained sections of formaldehyde (10%) fixed, paraffin embedded liver tissue samples revealed a focal extensive necrotizing hepatitis. In Warthin-Starry-stained sections of the liver, numerous large, elongated bacilli were observed within the cytoplasm of viable hepatocytes at the margins of necrotic foci. The latter finding is diagnostic for Tyzzer’s disease, a rapidly progressing, acute hepatitis caused by Clostridium piliforme (Ganaway et al., 1971; Kelly, 2000).

Liver and lung samples of the animal were bacteriologically examined. Cultures of these samples on Columbia agar with 5% sheep blood (Oxoid, Basingstoke, UK) yielded profuse and pure growth of haemolytic bacteria which produced greyish-white colonies. Gram staining revealed small, pleomorphic Gram-negative rods. The organism was oxidase, catalase, urease and CAMP-positive, but negative for \( L\)-arabinose fermentation. In a bile containing medium, hydrolysis of esculin was observed. Based on these phenotypic characteristics, the isolate was identified as A. equuli subsp. haemolyticus biovar 1 (Christensen et al., 2002).

Using tDNA-PCR, the organism showed an identical pattern to A. equuli and A. suis (Baele et al., 2000). Sequencing of the 16S rRNA gene (Baele et al., 2001) yielded a fragment of 1214 bp (accession number: EF141826) which showed 99% similarity to A. suis and A. equuli subsp. haemolyticus and 98% similarity to A. equuli subsp. equuli. Subsequently, \( aqxA, apxICA \) and \( apxIICA \) PCR-assays were carried out (Frey et al., 1995; Berthoud et al., 2002). In these PCR-assays, A. equuli subsp. haemolyticus CCUG 19790\(^{T}\), an A. suis field isolate and A. pleuropneumoniae serotypes 1–12 reference strains were used as controls. None of these PCR-assays yielded an amplicon with DNA of the rabbit isolate, while the expected amplicons were obtained with DNA of the control strains (Frey et al., 1995; Kuhnert et al., 2003).

Within the genus Actinobacillus, haemolysis is observed for A. pleuropneumoniae, A. equuli subsp. haemolyticus and A. suis. In contrast with A. pleuropneumoniae and most of the A. equuli strains, all A. suis strains have the ability to ferment \( L\)-arabinose. However, these closely related species cannot unequivocally be separated based on phenotypic characteristics alone. Additionally, 16S rRNA gene sequence comparison and tDNA-PCR are not able to separate A. equuli subsp. equuli, A. equuli subsp. haemolyticus and A. suis at the (sub)species level. Therefore, PCR typing for RTX (repeat in toxin) genes is recommended (Kuhnert et al., 2003; Christensen and Bisgaard, 2004). Indeed, A. equuli subsp. equuli does not contain \( apxICA, apxIICA \) nor \( aqxA \) genes. In contrast, A. equuli subsp. haemolyticus strains contain the \( aqxA \) gene and neither \( apxIICA \) nor \( apxIICA \) genes, while A. suis isolates contain \( apxICA \) and \( apxIICA \) but no \( aqxA \) genes (Kuhnert et al., 2003). A. pleuropneumoniae serotypes 1, 5a, 5b, 9, 10, 11 and 14 contain the \( apxICA \) gene and \( apxIICA \) is present in all serotypes except in serotypes 10 and 14 (Frey et al., 1995; Schaller et al., 2001).

Phenotypically, the rabbit isolate was identified as A. equuli subsp. haemolyticus biovar 1. Contradictorily, since we could not detect amplicons of the \( aqxA \) gene, our isolate would be identified as A. equuli subsp. equuli. The haemolytic activity of our isolate and its ability to enhance the haemolytic effect of staphylococcal \( \beta\)-haemolysin indicate the presence of an RTX-toxin (Devenish et al., 1992). The negative result in the \( aqxA \)-specific PCR might be caused by a DNA-mutation in one of both primer binding sites, resulting in failure of primer annealing and amplification. Alternatively, one might speculate on the presence of a different RTX-toxin in this rabbit-isolate.

Our finding that this A. equuli strain was isolated in pure culture from both the lungs and the liver might
suggest that it contributed to the disease, probably as a secondary invader. Recently, a comparable case has been reported by Meyerholz and Haynes (2005): a 5-year-old pet rabbit died after a 3-day history of anorexia and depression. This rabbit suffered from an *Actinobacillus capsulatus* septicaemia, secondary to the gastric stasis syndrome (“wool block”). Veterinarians and diagnosticians need to be aware of *Actinobacillus* species as a potentially under-diagnosed bacterial pathogen of lagomorphs, especially since *Actinobacillus* spp. may be misidentified as *Pasteurella* spp., which are quite common in rabbits (Lentsch and Wagner, 1980; DeLong and Manning, 1994).

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**References**


