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H. Moyaert, A. Decostere, M. Baele, K. Hermans, P. Tavernier, et al.. An unusual strain isolated from a rabbit with Tyzzer's disease. *Veterinary Microbiology*, 2007, 124 (1-2), pp.184. 10.1016/j.vetmic.2007.04.013 . hal-00532245

**HAL Id: hal-00532245**

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Submitted on 4 Nov 2010

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## Accepted Manuscript

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PII: S0378-1135(07)00177-0  
DOI: doi:10.1016/j.vetmic.2007.04.013  
Reference: VETMIC 3654

To appear in: *VETMIC*

Received date: 8-1-2007  
Revised date: 30-3-2007  
Accepted date: 4-4-2007

Please cite this article as: Moyaert H., Decostere A., Baele M., Hermans K., Tavernier P., Chiers K., Haesebrouck F., An unusual *Actinobacillus equuli* strain isolated from a rabbit with Tyzzer's disease, *Veterinary Microbiology* (2007), doi:10.1016/j.vetmic.2007.04.013

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

H. Moyaert\*, A. Decostere, M. Baele, K. Hermans, P. Tavernier, K. Chiers, F. Haesebrouck

*Department of Pathology, Bacteriology and Avian Diseases, Faculty of Veterinary Medicine,  
Ghent University, Salisburylaan 133, B-9820 Merelbeke, Belgium*

\* Corresponding author: Mailing address: Department of Pathology, Bacteriology and  
Avian Diseases, Ghent University, Faculty of Veterinary Medicine, Salisburylaan 133, B-  
9820 Merelbeke, Belgium; Tel.: +32-9-2647434; Fax: +32-9-2647494; E-mail:

[Hilde.Moyaert@UGent.be](mailto:Hilde.Moyaert@UGent.be)

26 **ABSTRACT**

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

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48 **Keywords:** *Actinobacillus equuli*, rabbit

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51 *Actinobacillus equuli*, a member of the family Pasteurellaceae, is a small,  
52 pleomorphic, non-motile Gram-negative rod. It is the etiological agent of a rare, but  
53 frequently fatal septicaemia in neonatal foals also known as “sleepy foal disease” which is  
54 spread worldwide (Rycroft and Garside, 2000; Berthoud et al., 2002). Besides this acute form  
55 of disease, *A. equuli* can be associated with other types of infections in both foals and adult  
56 horses, such as abortion, enteritis, peritonitis, pleuropneumonia, pericarditis, periorchitis and  
57 arthritis (Webb et al., 1976; Dill et al., 1982; Al-Mashat and Taylor, 1986; Belknap et al.,  
58 1988; Peremans et al., 1991; Collins et al., 1994; Patterson-Kane et al., 2001). *A. equuli* has  
59 been subdivided in two different subspecies, designated *A. equuli* subsp. *haemolyticus*  
60 (formerly described as Bisgaard Taxon 11), which is haemolytic and enhances the haemolytic  
61 effect of staphylococcal  $\beta$ -haemolysin (CAMP-positive) and *A. equuli* subsp. *equuli*, which is  
62 non-haemolytic and CAMP-negative (Christensen et al., 2002). The haemolytic strains have  
63 mainly been described in horses, but are not specifically associated with “sleepy foal disease”,  
64 which is typically linked with the non-haemolytic strains (Kuhnert et al., 2003). *A. equuli*  
65 subsp. *equuli* was not only isolated from horses, but has also been associated with abortion  
66 and endocarditis in pigs (Jones and Simmons, 1971; Werdin et al., 1976). Moreover, the  
67 organism was isolated from the nasopharynx, conjunctiva and middle ear of guinea pigs, rats  
68 and mice (Lentsch and Wagner, 1980). *A. equuli* is additionally considered as a potential  
69 opportunistic pathogen of humans, especially in immunocompromised people or people who  
70 are occupationally exposed to infected horses or pigs (Ashhurst-Smith et al., 1998; Vaz et al.,  
71 2001).

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

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

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

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

93 Using tDNA-PCR, the organism showed an identical pattern to *A. equuli* and *A. suis*  
94 (Baele et al., 2000). Sequencing of the 16S rRNA gene (Baele et al., 2001) yielded a fragment  
95 of 1214 bp (accession number: EF141826) which showed 99% similarity to *A. suis* and *A.*  
96 *equuli* subsp. *haemolyticus* and 98% similarity to *A. equuli* subsp. *equuli*. Subsequently,  
97 *aqxA*, *apxICA* and *apxIICA* PCR-assays were carried out (Frey et al., 1995; Berthoud et al.,  
98 2002). In these PCR-assays, *A. equuli* subsp. *haemolyticus* CCUG 19799<sup>T</sup>, an *A. suis* field

99 isolate and *A. pleuropneumoniae* serotypes 1-12 reference strains were used as controls. None  
100 of these PCR-assays yielded an amplicon with DNA of the rabbit isolate, while the expected  
101 amplicons were obtained with DNA of the control strains (Frey et al., 1995; Kuhnert et al.,  
102 2003).

103 Within the genus *Actinobacillus*, haemolysis is observed for *A. pleuropneumoniae*, *A.*  
104 *equuli* subsp. *haemolyticus* and *A. suis*. In contrast with *A. pleuropneumoniae* and most of the  
105 *A. equuli* strains, all *A. suis* strains have the ability to ferment L-arabinose. However, these  
106 closely related species cannot unequivocally be separated based on phenotypic characteristics  
107 alone. Additionally, 16S rRNA gene sequence comparison and tDNA-PCR are not able to  
108 separate *A. equuli* subsp. *equuli*, *A. equuli* subsp. *haemolyticus* and *A. suis* at the (sub)species  
109 level. Therefore, PCR typing for RTX (repeat in toxin) genes is recommended (Kuhnert et al.,  
110 2003; Christensen and Bisgaard, 2004). Indeed, *A. equuli* subsp. *equuli* does not contain  
111 *apxICA*, *apxIICA* nor *aqxA* genes. In contrast, *A. equuli* subsp. *haemolyticus* strains contain  
112 the *aqxA* gene and neither *apxICA* nor *apxIICA* genes, while *A. suis* isolates contain *apxICA*  
113 and *apxIICA* but no *aqxA* genes (Kuhnert et al., 2003). *A. pleuropneumoniae* serotypes 1, 5a,  
114 5b, 9, 10, 11 and 14 contain the *apxICA* gene and *apxIICA* is present in all serotypes except  
115 in serotypes 10 and 14 (Frey et al., 1995; Schaller et al., 2001).

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

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

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#### 134 **ACKNOWLEDGMENTS**

135 We are grateful to Serge Verbanck and Arlette Van de Kerckhove for their technical  
136 assistance.

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