

## ***Morganella morganii* subsp. *morganii*-associated pneumonia in a Belgian Blue calf**

*Pneumonie geassocieerd met Morganella morganii subsp. morganii bij een Belgisch Witblauw kalf*

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### ABSTRACT

A multiresistant *Morganella morganii* subsp. *morganii* strain was isolated in pure culture from pneumonic lesions of a 3-week-old Belgian Blue calf, originating from a farm coping with chronic diarrhoea and respiratory tract disease as well as increased mortality in 1-3-week-old animals. Additionally, high numbers of *Clostridium perfringens* were isolated from the jejunum and a *Giardia* infection was diagnosed. Moreover, necropsy findings and histological examinations indicated nutritional myodegeneration. *M. morganii* is an opportunistic pathogen that is mainly associated with postoperative and nosocomial infections in immunosuppressed individuals.

### SAMENVATTING

Een multiresistente *Morganella morganii* subsp. *morganii* stam werd in reïncultuur geïsoleerd uit pneumonieletsels bij een drie weken oud Belgisch Witblauw kalf. Het dier was afkomstig van een bedrijf dat kampte met chronische diarree, ademhalingsproblemen en een verhoogde mortaliteit bij 1 tot 3 weken oude dieren. Bij het kalf in kwestie werden ook hoge aantallen *Clostridium perfringens* geïsoleerd uit het jejunum en werd een *Giardia*-infectie gediagnosticeerd. Bovendien wezen de autopsiebevindingen en het histologisch onderzoek op nutritionele spierdystrofie. *M. morganii* is een opportunistische pathogeen die vooral geassocieerd wordt met postoperatieve infecties en ziekenhuisinfecties bij immunodeficiënte individuen.

### INTRODUCTION

Within the Enterobacteriaceae, the genus *Morganella* is a member of the tribe Proteaeae, which also includes the genera *Proteus* and *Providencia*. These organisms are opportunistic pathogens that are ubiquitous in the environment. However, individual case reports and nosocomial-outbreak reports indicate that they are capable of causing major infectious disease problems, mainly in human medicine. The genus *Morganella* consists of one species, *Morganella morganii*, with two subspecies, *morganii* and *sibonii* (Stock and Wiedemann, 1998; O'Hara *et al.*, 2000). It is a Gram-negative, motile bacillus that has been associated with pleuropneumonia in a piglet and a captive jaguar (Ono *et al.*, 2001; Choi *et al.*, 2002). The bacterium has also been isolated from septicaemic reptiles and ocular lesions in seals (Novak and Seigel, 1986; Heard *et al.*, 1988; Thornton *et al.*, 1998). Finally, *M. morganii* has been considered as a possible cause of swollen head

syndrome in a broiler chicken (Tanaka, 1995). Here we describe a case of pneumonia in a calf associated with a multiresistant *M. morganii* subsp. *morganii* strain.

### CASE HISTORY

#### Necropsy

Necropsy was performed on a 3-week-old, female Belgian White Blue calf originating from a farm coping with chronic diarrhoea and respiratory tract disease as well as increased mortality (4 to 5%) in 1 to 3-week-old calves. The animal was healthy at birth, but developed cough and diarrhoea after 10-12 days for which it was treated with colistine sulphate (Promycine<sup>®</sup> bolus, VMD, Arendonk, Belgium) (50.000 IU/kg/day). However, the diarrhoea persisted, the animal lost weight and died. At necropsy, the calf was cachectic and slightly dehydrated. Both lungs had

a mottled appearance due to multifocal areas of severe congestion and alveolar emphysema. The mucosa of the small intestine and colon was congested and the intestinal tract was filled with a mucous content. Within several striated muscles throughout the body, pale and dry areas indicative of muscular degeneration were seen. They were most prominent in the gluteal muscles.

### Histology

Representative tissue samples were fixed in 10% formaldehyde, routinely processed and embedded in paraffin wax. Five-micrometer-thick sections were mounted on glass slides and stained with haematoxylin and eosin. Microscopic examination of the lungs revealed the presence of neutrophils, macrophages and lymphocytes admixed with numerous bacilli within bronchial, bronchiolar and associated alveolar lumina. Multifocally, bacilli seemed attached to the alveolar walls.

In the small intestine, multifocal mucosal necrosis and villous blunting and fusion were seen. Small numbers of rod-shaped bacteria, indicative of *Clostridium* species, were present within the necrotic material. A mixed inflammatory infiltrate was seen throughout the lamina propria.

Several striated muscle fibres and cardiomyocytes were hyalinized, some of which showed fragmentation (degeneration). Numerous striated muscle fibres with central vesicular nuclei indicative of regeneration were seen.

### Microbiology

A pure culture of Gram-negative rods was isolated from the lungs on Columbia agar with 5% sheep blood (Oxoid). The isolate was subsequently identified as *Morganella morganii* by means of tDNA-PCR, as described before (Marien *et al.*, 2006). Sequencing of the 16S rRNA gene (Baele *et al.*, 2001) revealed a fragment of 1487 bp (accession number: EF455493), which showed 99% similarity to *M. morganii*. Based on its inability to ferment trehalose, the isolated strain was classified within the *M. morganii* subsp. *morganii* subspecies (O'Hara *et al.*, 2000). Antimicrobial susceptibility testing using a disk diffusion assay on Iso-Sensitest-agar plates (Oxoid) with Neosensitab tablets (Rosco) revealed that the isolate was susceptible to ceftiofur, neomycin, apramycin and spectinomycin, intermediately susceptible to enrofloxacin, and resistant to ampicillin, amoxicillin-clavulanic acid, colistin, tetracyclines, flumequine, gentamicin, sulfonamides and trimethoprim.

From the jejunum, high numbers of *Clostridium perfringens* ( $> 10^7$  colony forming units/gram intestinal content) were isolated on Columbia agar with 5% sheep blood (Oxoid) after anaerobic incubation. Many *Giardia* cysts were found in the faeces using a previously described diagnostic protocol (Geurden *et al.*, 2004) based on the commercially available Merifluor *Cryptosporidium*/*Giardia* assay (Meridian Diagnostics

Inc.). Bovine viral diarrhoea virus was not detected in the blood by PCR (Adiavet BVD, Adiagene) and rotavirus and coronavirus, *Cryptosporidium parvum* and F5-positive enterotoxigenic *Escherichia coli* (Digestive Elisa Kit, Bio-X) were not detected in the faeces by Enzyme-Linked Immunosorbent Assays carried out according to the manufacturer's recommendations.

### DISCUSSION

*M. morganii* is a common member of the intestinal microbiota of humans, mammals and reptiles and is also found in water, soil and sewage. It is an opportunistic pathogen usually involved in postoperative and nosocomial settings, especially causing urinary tract and wound infections in humans (Tsanaktsidis *et al.*, 2003; Golubić-Ćepublic *et al.*, 2004). To the authors' knowledge, this is the first description of *M. morganii* subsp. *morganii*-associated pneumonia in a calf. *M. morganii* is intrinsically resistant to ampicillin, amoxicillin, amoxicillin-clavulanic acid, first and second generation cephalosporins, sulfamethoxazole, and polymyxins. Resistance to the  $\beta$ -lactam antibiotics mentioned is due to inducible chromosomal ampC  $\beta$ -lactamases. Intrinsic resistance to polymyxins is a characteristic of all Proteaceae (Stock and Wiedemann, 1998). However, the isolate in the present study also showed acquired resistance to trimethoprim, flumequine, gentamicin and tetracyclines and it displayed intermediate susceptibility to enrofloxacin. This high resistance rate may hamper treatment of affected animals.

The pale foci observed at necropsy in the muscles and the results of histological examinations of the skeletal and cardiac muscles are pathognomonic for nutritional myodegeneration (white muscle disease) due to vitamin E and/or selenium deficiency. Selenium and vitamin E are vital compounds of the mammalian cell's defence against oxidative damage, and deficiency or suboptimal supplementation of these nutrients may adversely affect productivity and disease resistance (Van Metre and Callan, 2001; Menzies *et al.*, 2004).

In the present study, many *Giardia* cysts were detected in the faeces of the calf. Since *Giardia* is known to cause disease in young calves, with acute to chronic diarrhoea and ill thriving, the parasite might account for the chronic diarrhoea problem on this farm, possibly resulting in a lowered health status and higher susceptibility to secondary infections (Olson *et al.*, 2004).

### CONCLUSION

On the basis of the combined results of the necropsy and the histological, bacteriological and parasitological investigations, the calf was diagnosed with a multifocal pneumonia due to *M. morganii* subsp. *morganii* and a *C. perfringens*-enteritis. The cachexy, the vitamin E and/or selenium deficiency, and, to a lesser extent, the *Giardia* infection may have resulted in immunosuppression, probably allowing *M. morganii* to sneak in and cause the disease. Likewise, *C.*

*perfringens*, a common intestinal inhabitant, may substantially multiply in a gut with altered microbiota (Songer, 1996).

#### ACKNOWLEDGMENTS

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

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#### Uit het verleden

### VACCA - VACCINIA - VACCIN

We staan er niet bij stil. Vaag weten we het, maar we verdringen het in onze pseudo-wetenschappelijke dromen: het woord *vaccin* is afgeleid van de nederige koe, in het Latijn: *vacca*. Met de enting in 1796 van het jongetje James Phipps met vocht uit de koepokken (*vaccinia*) van de koe met de verrukkelijke naam Blossom overgedragen op het melkmeisje Sarah Neames, gaf de simpele plattelandsarts Jenner (geen veearts ...) de geneeskunde het eerste effectieve middel tegen een verschrikkelijke ziekte. Om dat echt te begrijpen moet men weet hebben van de complete machteloosheid, verhuld in de meest ingewikkelde theorieën (het liefst in het Latijn), die de geneeskunde tot dan toe kenmerkte. De niet-universitair opgeleide chirurgijn barbiers konden wel wat bereiken met zalven, snijden en verbinden, maar de heren doktoren hielden het bij piskijken, geleerde praat verkondigen en honoraria incasseren.

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