

The first reported case of equine nocardioform placentitis in South Africa

D H Volkmann^a, J H Williams^{b*}, M M Henton^c, J M Donahue^d and N M Williams^d

ABSTRACT

Since the late 1980s a distinct form of focally-extensive mucoid to mucopurulent uterine body chronic placentitis, caused by nocardioform organisms, has been recognised in horses in the USA state of Kentucky and possibly in other areas. This disease has led to increasing numbers of foal losses from late abortions, still-births, prematurity, or early neonatal deaths. The foals are usually not infected, but may be small or emaciated. Modes of infection and transmission are as yet unknown. *Nocardia* spp. and related nocardioform bacteria as causes of equine infertility, endometritis and foal death are briefly reviewed. A case of near full-term abortion involving a Friesian mare in the Pretoria district of Gauteng Province in South Africa during February 2000, with the same placental lesion as described in the Kentucky cases, is presented. Nocardioform organisms were visualised on impression smears and histological sections of affected foetal membranes, and were also cultured. The organism has been identified at the Livestock Disease Diagnostic Center of the University of Kentucky as an *Amycolatopsis* sp. of the less-commonly diagnosed group of nocardioforms causing placentitis in the USA. The organism was cultured from the uterus of the mare 18 days post-foaling, but after a 2-week course of oral trimethoprim and sulphamethoxazole, based on antibiogram sensitivity testing, a uterine flush yielded no growth. A semen sample from the sire of the aborted foal did not yield any Gram-positive filamentous branching bacteria. The mare subsequently conceived to a single insemination.

Key words: abortion, *Amycolatopsis*, antibiogram, chronic placentitis, equine, nocardioform, placental insufficiency, stillbirth, uterine body.

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INTRODUCTION

In South Africa, the bacteria most commonly associated with equine abortion are *Streptococcus zooepidemicus*, *Klebsiella* spp, *Pseudomonas aeruginosa* and *Actinobacillus equuli* (Henton, 1989–1993, unpubl. data). In general, placentitis either results in foetal injury by direct spread of infection to foetal organs, or by disruption of the already tenuous placental-uterine interface with resulting placental separation and insufficiency.

This paper documents the first case of equine nocardioform placentitis reported in South Africa, in which the lesion was a single expanding chronic-active mucopurulent focus at the base of the pregnant horn. It is suggested that this lesion caused terminal placental insufficiency with premature placental separation and

intra-uterine death and abortion of the near-term normal foetus. The foetus was not infected by the organism that caused the placentitis or by any other pathogen. These features distinguish this form of placentitis from the usual pathogenesis of ascending placentitis, which typically affects the cervical area of the placenta, and the diffuse or multifocally distributed placentitis that result from haematogenous spread of organisms from the dam. The method/s and route/s of infection of mares and therefore transmission of the nocardioform organism are as yet unknown. A saline uterine flush from the case report mare post-abortion was positive for the nocardioform organisms, and a single semen sample collected from the sire, to ascertain whether semen could be a possible mode of transmission, was negative. Further follow-up studies of mare and stallion were not performed after the mare had responded to treatment and conceived to a subsequent insemination from a different sire.

CASE HISTORY

In early February 2000, a stillborn Friesian colt foal with its foetal mem-

branes was presented to the Department of Pathology, Faculty of Veterinary Science, University of Pretoria, Onderstepoort, a few hours after it had been expelled by the mare. The mare was then 8–9 years old and had foaled uneventfully 2 years previously. At the time of abortion she showed no signs of illness and had given birth without difficulty. The foetus was between 320 and 330 days old. The sire of the dead foal was a Friesian stallion imported 4 years earlier from The Netherlands.

Macroscopic findings

The foal carcass was presented together with its foetal membranes in a plastic bag. The foal was still encased in its intact amnion except for where the owner had broken it open over the foal's muzzle. The foal was large and appeared to be full term, in good condition and normally formed. *Post mortem* changes were mild. There was meconium staining of the perineum, probably indicating foetal distress or asphyxiation. The external mucosae showed pallor. Before opening the carcass, a blood smear from ear capillaries was negative for blood parasites, the total number of leukocytes appeared slightly lower than expected in the blood smear of a term foal or a normal adult, and of those, neutrophils and lymphocytes were equally represented. The carcass was opened in such a way as to leave organs untouched until sterile specimens of liver, spleen, lung, kidney, small intestine, brain, joint fluid and urachal content had been taken and sent on ice for bacterial culture. Various organs were also sampled in formalin for histopathological examination.

The visceral organs were markedly congested, the splenic white pulp was prominent, there was severe, diffuse congestion of the small intestine and the lungs were even-coloured, meaty in consistency, and sank in water (uninflated). No other abnormalities were found.

The foetal membranes were moderately contaminated by stable bedding (straw). The amnion was normal. The folds of the cervical star were thicker and tougher than usual, suggesting that the allantochorion was inadequately prepared for the delivery of the foetus. Apart from the

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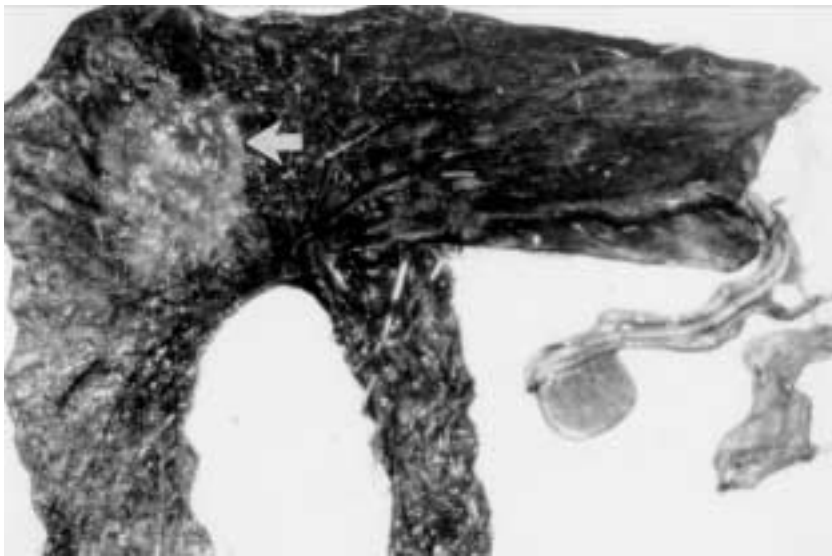


Fig. 1: Foetal membranes with focal mucopurulent placentitis at the base of the pregnant horn (arrow).

well-defined affected area, the allantochorion was evenly covered in dense, well-developed villi. The umbilical cord was moderately twisted, but there were no typical signs of vascular embarrassment. There was a single urachal diverticulum of approximately 10 cm diameter adjacent to the umbilicus.

A well-delineated, circular area of approximately 25 cm diameter covered in necrotic, brown, mucopurulent placental exudate was present at the base of the pregnant horn (Fig. 1). At the centre of the lesion, the villi were flattened or absent.

The blood smear and smears of the placental exudate were stained with Cam's Quick-Stain (C A Milsch), which is a modified Romanovsky stain, and representative pieces from normal, lesion-junction with normal, and affected placenta were sampled in formalin for histopathological examination. A piece of the affected allantochorion, albeit contaminated, was sent on ice for bacteriological culture.

Microscopic findings

Light microscopic examination of foal tissue sections stained with haematoxylin and eosin (H&E) confirmed the macroscopic findings of congestion, and the adrenal cortices and medullae showed microscopic petechiation. The brain, especially in the deeper white matter of the cerebrum and midbrain, showed mild multifocal (only involving some vessels), perivascular or eccentrically-perivascular gliosis, and occasional foci of gliosis not associated with vessels. Some of these foci of gliosis accompanied mild oedema, especially in the mid-brain (probably due to acute hypoxia).

Impression smears from the placental exudate showed many filamentous, branching, beaded organisms resembling *Nocardia* sp., against a background of purulent and mucoid debris and various other bacterial and yeast contaminants. The filamentous organisms occurred singly or in clumps, phagocytosed by neutrophils or lying between cells (Fig. 2).

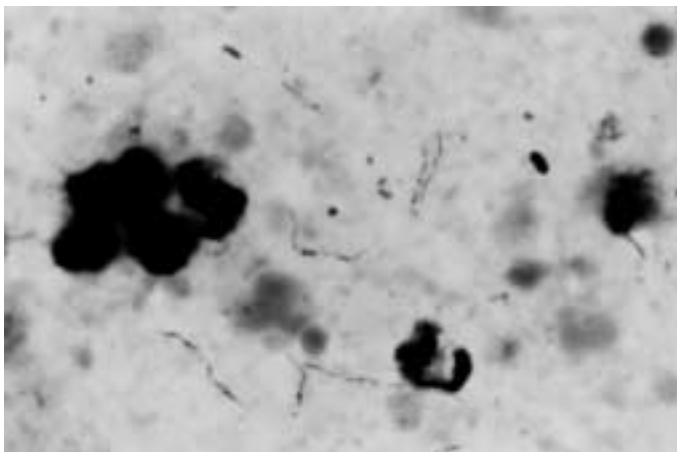


Fig. 2: Placental exudate smear (oil immersion) stained with Cam's Quick Stain, showing filamentous, beaded, branching organisms amongst inflammatory cells and exudate debris.

The H&E-stained tissue sections of the affected allantochorion at the junction with normal tissue revealed an abrupt transition from normal chorion with no inflammatory response and smallish villi to marked acute inflammatory changes. These included subepithelial hyperaemia, occasional petechiae, and infiltration of predominantly lymphocytes and plasma cells, but also scattered macrophages and neutrophils, the latter cell types exhibiting a degree of epithelial exocytosis. The villi at the junction and into the lesion, showed a marked increase in size and number of projections as well as epithelial hyperplasia and degeneration. There was a marked luminal mucopurulent exudate that contained variable numbers and colonies of filamentous, beaded, branching organisms, and the same organisms were evident intracytoplasmically in the villous epithelial cells (Figs 3, 4).

The sections of allantochorion that were stained with Periodic Acid Schiff (PAS) and Gram's stain clearly showed the filamentous branching organisms intracytoplasmically and in the exudate (*i.e.* they were strongly PAS and Gram-positive). Fite's staining method for acid-fast organisms¹³, a modified acid-fast stain developed specifically for *Nocardia* spp. that are usually only partially acid-fast, did not stain the organisms.

Bacteriology

Nocardioform bacteria were isolated only from the allantochorion. Cultures from all foal tissues sampled were negative for these organisms as well as for other pathogens. The *nocardioform* isolate was present in heavy growth, together with mixed contaminants, owing to the external contamination of the foetal membranes. The colonies were yellowish and sunken, and formed a white mycelium on top that became greyish after 3 weeks. Visible colonies were formed at 37 °C within 3 days and grew well on bovine blood tryptose and chocolate agar, casein agar, egg yolk agar, Sabouraud agar and nutrient agar. There was no growth at 42 °C. The organism grew as round white balls in broth, which remained suspended or sank to form a deposit, and these remained intact when shaken. The isolate was Gram-positive, branched, filamentous, catalase positive and remained acid-fast negative, using the cold Kinyoun method, even when cultured on lipid-rich media. The isolate was gelatine hydrolysis, casein hydrolysis, sodium hippurate hydrolysis, urease and alkaline phosphatase positive, it grew on Simmonds citrate, and on 3 % as well as 6 % NaCl agar. It produced only

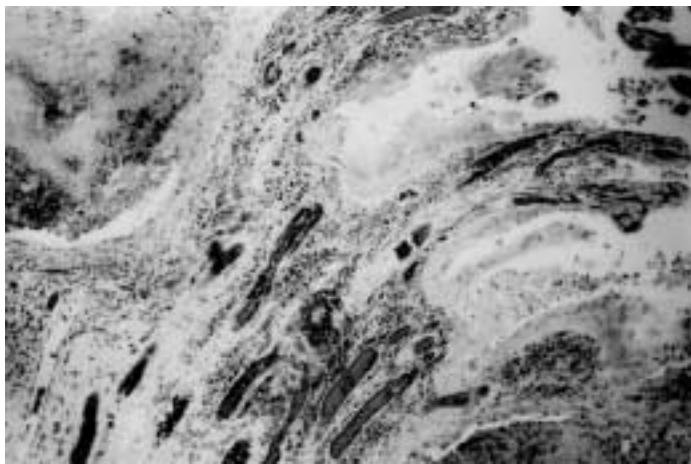


Fig. 3: Section of allantochorion showing active placentitis with a predominantly lymphocyte and plasma cell presence, hyperaemia, villus hypertrophy and luminal mucopurulent exudate (low magnification, H&E).

small amounts of hydrogen sulphide, detectable by lead acetate, and litmus milk became alkaline and was hydrolysed. It was nitrate, starch hydrolysis, lecithinase and lipase negative. The isolate oxidised glucose only weakly, and no change was detectable in phenol red broth containing glucose or other sugars. There was only weak hydrolysis of aesculin.

Sequencing, using 16S rDNA, indicated that the isolate was *Amycolatopsis* sp. It had 97–98 % homology with *A. azurea*, *A. alba* and *A. coloradensis*.

The isolate was sensitive to ciprofloxacin, trimethoprim and sulphonamides, and resistant to penicillin, methicillin, streptomycin, chloramphenicol, neomycin, nitrofurans, erythromycin, spectinomycin, clindamycin, bacitracin, amikacin, colistin and kanamycin.

A single semen sample collected from the imported sire of the aborted foal shortly after the discovery of the described nocardioform as the cause of the placentitis, was submitted for culture to ascertain whether the sire's semen could

have been a possible source of infection, but yielded no similar organisms. This was done since the mode of transmission and placental infection by this group of organisms in horses is as yet undiscovered. No further investigations relating to the stallion were possible.

Four days after her foal heat the mare's uterus was flushed with sterile saline and a return-flow sample was submitted for bacterial culture. The same nocardioform organisms were isolated in small numbers, but there was no cytological evidence of endometritis. Based on the previously performed antibiogram the mare received a 14-day course of an oral combination of trimethoprim and sulphamethoxazole (Purbac DS, Lennon). Another flush, performed 7 days after the end of the treatment, yielded no bacterial growth. The mare was inseminated with semen from a different stallion during her next oestrus and conceived.

DISCUSSION

Nocardia spp. have been recorded rarely worldwide as sporadic causes of equine

infertility³, endometritis^{12,15} and abortion², and amongst the genital flora of clinically healthy mares⁴. Typing of a related organism isolated from the lungs and amnion of an aborted foetus revealed it to be closely related to *Rhodococcus rubropertinctus*⁵. Placentitis caused by a branching, beaded, filamentous nocardioform actinomycete that is Gram-positive and non-acid-fast, and related to *Nocardia* spp, emerged as a new disease in the late 1980s that causes abortion and stillbirths in Kentucky, USA, and possibly in other areas^{6,8,9,17,18}. In the 1998 foaling season in Kentucky, over 100 cases of nocardioform placentitis were diagnosed¹⁷.

The infection has typically been associated with late abortions, stillbirths and early neonatal deaths. Most foals, however, remain uninfected despite the placental infection^{6,17}. The aborted foetus may be emaciated and growth retarded, or premature delivery of a weak live foal may occur^{6,18}. Foals may also be normal¹⁸. Most mares show no outward sign of infection, but some mares may show premature mammary development with lactation and occasionally a vaginal discharge¹⁸. The placentitis is typically focal to focally extensive, chronic or chronic-active, the area involved being covered with thick, tenacious brown mucoid exudate, and the location is predominantly at the base of the horns or at the junction of the horns and the body^{6,8,9,17}. This distinguishes it from the typical and most common pathogenesis of ascending placentitis affecting the cervical area of the placenta and from diffuse or multifocal placentitis resulting from haematogenous spread of organisms^{17,18}.

The chorionic villi at the centre of the lesion are typically necrotic, reduced in size or absent, with a chronic inflammatory cell response of mainly lymphocytes and plasma cells. The periphery of the lesion is acutely inflamed, with villi being intact and epithelial cells showing degenerative changes and intracellular invasion by the bacteria. Deeper structures are not invaded by the organisms¹⁷.

The group of organisms involved have been identified as nocardioform Actinomycetes and include bacteria belonging to at least 3 different genera. In Kentucky, most of the isolates (over 60 %) were in the genus *Saccharothrix* and probably constitute a single species. The remaining isolates probably belong to 1 or more species in the genera *Amycolatopsis* or *Streptomyces*. All the strains isolated had some common characteristics. They were aerobic, catalase-positive, non-acid-fast, Gram-positive filamentous bacteria that branched extensively in

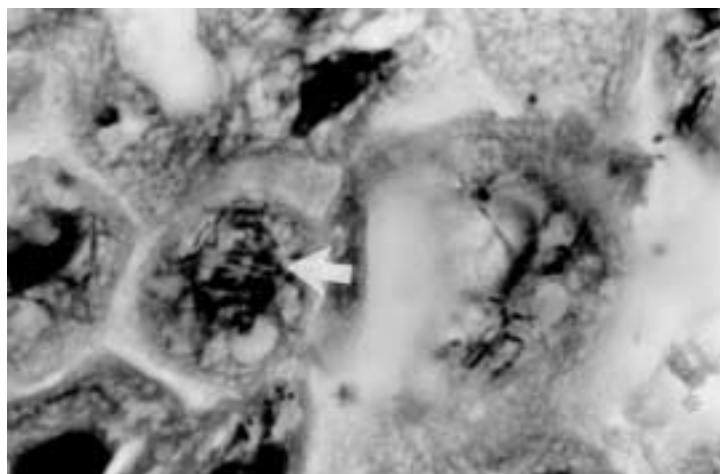


Fig. 4: Section of allantochorionic villus epithelium with intra-cellular nocardioform organisms (arrow), and epithelial necrosis with luminal sloughing (oil immersion, H&E).

tissues and on artificial media. They grew on a wide variety of media, but not Sabouraud dextrose or MacConkey agar, and took 48–72 hours of incubation at 36 °C to form visible colonies^{7,10,11,14}. All the strains had a very characteristic pungent odour that is usually associated with the various species of aerobic actinomycetes.

The foal documented in this report died close to term due to asphyxiation and foetal distress caused by placental insufficiency and separation attributable to the focally-expansile area of nocardioform placentitis. The visceral congestion, uninflated lungs, microscopic adrenal haemorrhages and cerebral gliosis with mild malacia support this conclusion¹. Prematurity was suspected owing to the apparently slightly low white cell count and lack of predominance of neutrophils in the foal's blood smear, as compared to expected blood smear findings from normal full-term foals and adult horses¹⁶. The foal itself was not infected by the nocardioform organism. The impression smears from the placental exudate, the histopathological findings, intra-cellular presence of the same filamentous beaded branching organisms in the epithelial cells of the chorionic villi and in neutrophils, with specific staining characteristics (PAS- and Gram-positive, and non-acid-fast), together with the specific culture requirements and characteristics, confirm the diagnosis^{7,10,11,14}.

The sire of the aborted foal had sired more than 100 foals, covering resident and visiting mares, mostly Friesian but including other breeds, in the 4 years since his arrival in South Africa. The rates of conception, abortion, stillbirth and neonatal death for the stud were not recorded and previous foetal losses were never investigated.

Amycolatopsis was created as a genus in 1986¹¹, for certain organisms previously classified as *Nocardia orientalis*, *Nocardia mediterranei*, *Nocardia rugosa* and *Nocardia sulphurea*. The genus contains species that have been isolated from soil, vegetable matter and clinical specimens, and are of importance in antibiotic production¹¹. This isolate conformed biochemically most closely to *A. alba*¹⁴, but differed from

it by not hydrolysing starch. The single isolate of *A. alba* described, was isolated from soil¹⁴, and was not associated with disease. The isolate differed on a number of tests from *A. azurea* and *A. coloradensis*¹⁰. As the sequencing data showed good homology with *A. azurea*, *A. alba* and *A. coloradensis*, it may represent *A. alba* or a new species. The type strain of *A. alba* was isolated from a mixture of unknown soils by an American company screening bacteria for antibiotic production¹⁴. Whether *Amycolatopsis* is common in South African soils is unknown. The fact that the organism responsible for this abortion was of the less-commonly diagnosed group of *Amycolatopsis* spp. in Kentucky, and not the *Saccharothrix*-related species, suggests that this condition may not escalate into a huge problem in South Africa. It is more likely that this isolate is comparable to the other, previously reported isolated cases of nocardioform reproductive disease.

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