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A case of *Candida guilliermondii* abortion in an Arab mare

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ABSTRACT

Ascending infections of equine uterus frequently result in placentitis and abortions; most of these infections are bacterial and are less commonly due to fungi. This report describes an abortion case in an Arab mare due to *Candida guilliermondii* that was diagnosed via cytological, histological, cultural and biomolecular assays. The histological lesions found were severe necrotizing placentitis associated with fetal pneumonia. To our knowledge this is the first case of *C. guilliermondii* abortion reported in equine species.

1. Introduction

Abortion, stillbirth and neonatal deaths are an important source of economic loss for an equine industry. As reported in previous studies, the causes of equine abortion vary with geographic area and can change over time [1]. In early studies, infectious agents were reported as major causes of equine reproductive loss [2] whereas later reports indicated that the loss from noninfectious causes, e.g., twinning and stillbirth, surpassed the prevalence of those caused by infectious agents [3]. In mares, most bacterial and fungal infections of the pregnant uterus that result in abortion are attributable to ascending infections via the cervix and vagina following contamination of the external genitalia [4]. Undoubtedly, there are several risk factors such as poor uterine contractility, anatomical malformations and inefficient uterine defense mechanism. This may explain why fungal endometritis is commonly associated with a history of uncorrected anatomical defects that lead to pneumovagina, recurrent persistent post-breeding endometritis and frequent intrauterine antibiotic therapy [5]. The most common source of fungi causing reproductive disease in the mare is probably represented from skin or fecal origin. There are a variety of fungi that have been identified from uterine cultures with *Candida* spp. and *Aspergillus* spp, isolated as the most common yeast and mold, respectively. Although early reports primarily identified *Candida albicans* as the fungal pathogen infecting the reproductive tract of the mare, more recent literature demonstrates that many other *Candida* species and fungal agents such as *Cryptococcus* and *Aspergillus*, can be responsible for equine fungal endometritis or abortion [4,6]. Yeasts of the genus *Candida* are widely distributed in the vegetative parts of plants and soil, as well as external mucosal surfaces (oral cavity, external genitalia) and intestine of domestic animals where they compose the normal microbial flora and are eliminated by excreta [7]. However, despite being saprobes, there have been many reports of disease caused by this microorganism in horse, which can present with different clinical manifestations, related to osteomyelitis [8], ulcerative keratitis [9], arthritis [10] and endometritis [11]. However, while fungal endometritis is well documented in the scientific literature, little is known about the ability of *Candida* to cause placentitis and abortion in mares: only single report was found in literature about an intrauterine inoculation of *Candida parapsilopsis* which resulted in embryonic loss in pony mares [12]. The purpose of this case report is to describe the diagnostic procedures applied in a rare case of placentitis and the subsequent abortion in an Arab mare due to *Candida guilliermondii*. The newly assigned teleomorph species name of *C. guilliermondii* is

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now *Meyerozyma guilliermondii* according to the recent work in 2010 by Kurtzman and Suzuki, but for clarity the species name *Candida guilliermondii* is used throughout this report [13].

2. Case

A 10-year-old Arab mare had failed to conceive for two consecutive years (2010–2011). In 2011, the mare was twice treated locally with ampicillin for an endometritis due to *Streptococcus equi* subsp. *zooepidemicus*. In July 2012, she was presented for routine transrectal ultrasonographic examination of the reproductive tract before planned breeding with frozen semen. The mare had poor perineal conformation with 50% of the vulva dorsal to the ischiatic arch. A reproductive examination was carried out, including bacteriological and mycological tests of a uterine swab and low volume uterine lavage. No inflammatory cells or fungal organisms were seen on cytology and no bacterial growth was detected after a 48 h aerobic culture. In August 2012, the mare underwent artificial insemination with frozen semen and pregnancy was confirmed 17 days after breeding via transrectal ultrasonographic examination. Because the mare had a poor vulvar conformation, a Caslick's procedure was performed. During the first months of gestation, on physical examination the mare appeared bright, was alert and was in good body condition but at approximately the sixth month of gestation premature udder development was observed and interpreted as a warning sign of placentitis and abortion. Transrectal ultrasound revealed increased thickness of the combined uterus and placenta with evidence of chorioallantoic edema but no placental separation. Abortion occurred the day after presentation, on 18th February 2013 (day 0). The aborted fetus was in good condition. The placenta appeared thickened with a gritty appearance and covered by a dense brown exudate and necrotic material, mostly around the cervical star (Fig. 1). A direct swab from the placental surface was carried out and cytological slides immediately air dried. No gross lesions in the fetus were visible at necropsy. Smears from uterine exudates were stained with May Gruenwald–Giemsa (MGG) and observed under a light microscope. The samples were highly cellular with blue proteinaceous ground and a mixed inflammatory population composed of degenerate and hypersegmented neutrophils and large macrophages. The cytoplasm of the macrophages was filled by dozens of 3–6 μm wide round to oval bodies with thin body walls and bluish cores; some yeasts were in active budding with occasional pseudohyphae formation (Fig. 2). The organisms were consistent with yeast-like fungus. Immediately, samples from the placenta and other fetal organs were submitted to the diagnostic laboratory for evaluation. Tissues were fixed in buffered 10% formalin for approximately 48 h before preparing histological sections which were subsequently stained (day +2). Histologically, the placenta was markedly thickened with edema of the lamina propria and diffuses infiltration by lymphocytes, plasma cells, and macrophages. The villous surface of the allantochorion was covered by acellular eosinophilic necrotic material containing bluish nuclear debris and colonies composed of myriads of round to oval yeast, 4–5 μ wide, pale staining, with thin body walls (blastospheres) occasionally seen in the cytoplasm of macrophages and neutrophils. Occasional yeast showed peripheral budding with rare short chains of blastospheres (pseudohyphae) (Fig. 3A and B). The yeast was strongly stained by Periodic-Acid–Schiff (PAS) and Grocott's silver stain. In fetal tissues, a lymphocytic and macrophagic infiltrate was seen in the interalveolar septa in the lung and the periportal areas in the liver but no fungal bodies were seen histologically. The other organs were unremarkable. The same day of abortion, the placenta, lung, liver, spleen, kidney, and stomach contents were cultured for aerobic bacteria

Fig. 1. Macroscopic placenta appearance. Chorionic surface of the placenta is covered by a thick brown exudates and yellowish necrotic areas.

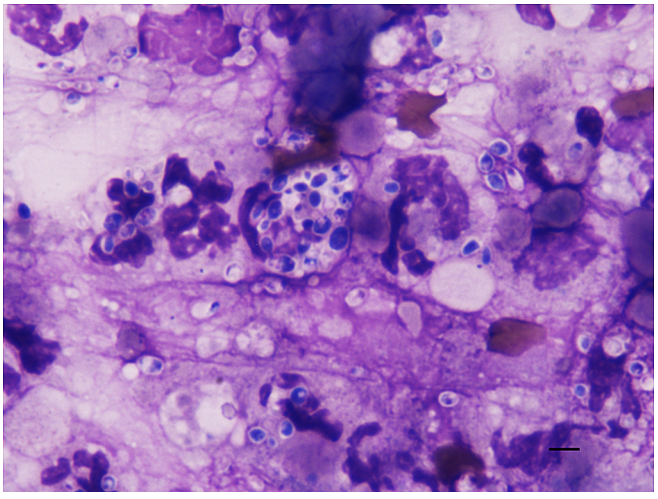

Fig. 2. Cytological appearance of the direct placental swab. Non-degenerated neutrophils and macrophages containing many round to oval, 4–6 μ wide, bluish yeasts with thin body walls and occasional narrow base budding (May-Grünwald–Giemsa, bar: 10 μ).

and fungi on blood agar, MacConkey agar, mannitol salt agar and Sabouraud dextrose agar + chloramphenicol at 37 °C in 5–10% CO_2 for 3 days. A heavy growth of yeast with the morphology of *Candida* was isolated from all our samples and non-pathogenic bacterial growth was obtained from these organs (day +3). Identification of the yeast was based on its morphology on Sabouraud dextrose agar: the colonies were flat, glossy, smooth-edged and cream-colored (Fig. 4). A lactophenol cotton blue preparation from the cultured material was performed and the microscopic examination of this isolate revealed oval and elongated yeast cells of various sizes. *Candida* cells formed clusters of yeast cells with relatively few short pseudohyphae having small groups of blastoconidia at the septa. No true hyphae were produced. Moreover, a biochemical profile was obtained using a commercial yeast identification kit API-ID 32C system and Vitek-2 system was performed, in accordance with the manufacturer's instructions (bioMérieux, S.P.A.). These two biochemical identification methods were not able to discriminate between *Candida famata* and *C. guilliermondii*, giving for each species a probability score of 50%. Another technique used for a rapid and reliable