



NOTE

Pathology

Swine abortion caused by *Candida parapsilosis*

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J. Vet. Med. Sci.

83(11): 1666–1669, 2021

doi: 10.1292/jvms.21-0439

Received: 6 August 2021

Accepted: 5 September 2021

Advanced Epub:

14 September 2021

ABSTRACT. In July 2020, a sow in a breeding herd in the Chiba Prefecture, Japan, suffered abortion. A necropsy revealed pale pulmonary foci scattered in the two fetuses. Histologically, multifocal pulmonary necrosis was detected with numerous yeasts. The yeast was positively stained using the periodic acid-Schiff reaction and Grocott's silver stain. Molecular identification indicated that the yeast was *Candida parapsilosis*. In conclusion, our results suggested that *C. parapsilosis* caused multifocal necrotizing pneumonia in the two fetuses. This study is the first report of a swine abortion with *C. parapsilosis* infection.

KEY WORDS: abortion, *Candida parapsilosis*, pneumonia, swine

Candidiasis is the most common opportunistic yeast infection that can cause superficial or systemic infections in humans [4]. Yeasts of the anamorph genus *Candida* are commensal inhabitants of the alimentary tracts, skin, oral cavity, and external genitalia [2, 4]. The incidence of candidiasis has increased due to the use of immunosuppressive regimens, broad-spectrum antimicrobial agents, and aggressive chemotherapy [20].

Although *C. albicans* accounts for the majority of *Candida* infections, a progressive shift from *C. albicans* to non-*albicans* *Candida* spp. (*C. glabrata*, *C. parapsilosis*, *C. tropicalis* and *C. krusei* (*Pichia kudriavzevii*)) has been observed in several parts of the world [14]. Hence, the non-*albicans* *Candida* spp. have gained importance as pathogens [14].

C. parapsilosis has previously been reported in bovine abortion [8], mastitis [5, 6], and equine endocarditis [3]. Furthermore, seborrheic dermatitis [18], granulomatous rhinitis [10], and urinary tract infections [13] have been reported in dogs and cats. Most of these cases are associated with antibiotic treatment, immunosuppression, and medical treatment, leading to immunosuppression and other infections [12, 13].

Although there have been several reports of candidiasis in humans, their incidence in pigs is rare. There are two main types of candidiasis in pigs; gastrointestinal candidiasis [19] and mucocutaneous candidiasis [21]. However, swine abortions correlated with *Candida* spp. and swine *C. parapsilosis* infections have not been reported. Herein, we describe the first case of a swine abortion caused by *C. parapsilosis* and presents the histopathological characteristics of the fetal lesions.

In July 2020, two sows aborted in a breeding herd, with a scale of 680 sows, 2,800 fattening pigs, and five boars. While the first case of abortion was not examined, the second abortion occurred the next day in three months of the first pregnancy, and the farmer called Chiba Prefectural Chuou Livestock Hygiene Service Office. The sow exhibited clinical symptoms of fever and abortion. The placenta was not available for examination in the present case. The two fetuses (No. 1 and 2) were moved to the Livestock Hygiene Service Office and necropsied. The sows were vaccinated against swine erysipelas, porcine respiratory and reproductive syndrome (PRRS) virus, Japanese encephalitis virus, and classical swine fever virus in the farm. After the two abortion cases, the occurrence of serious infections was not recognized in the farm.

Similar gross lesions were found in the two fetuses. Furthermore, significant gross lesions were limited to the lungs of both fetuses. Necropsy of the fetuses (Fig. 1a) showed multiple, small (<3 mm in diameter), and pale foci in the lung (Fig. 1b). While the contents of the stomach were yellowish and viscid, the colonic contents were yellowish brown. No other gross lesions were observed.

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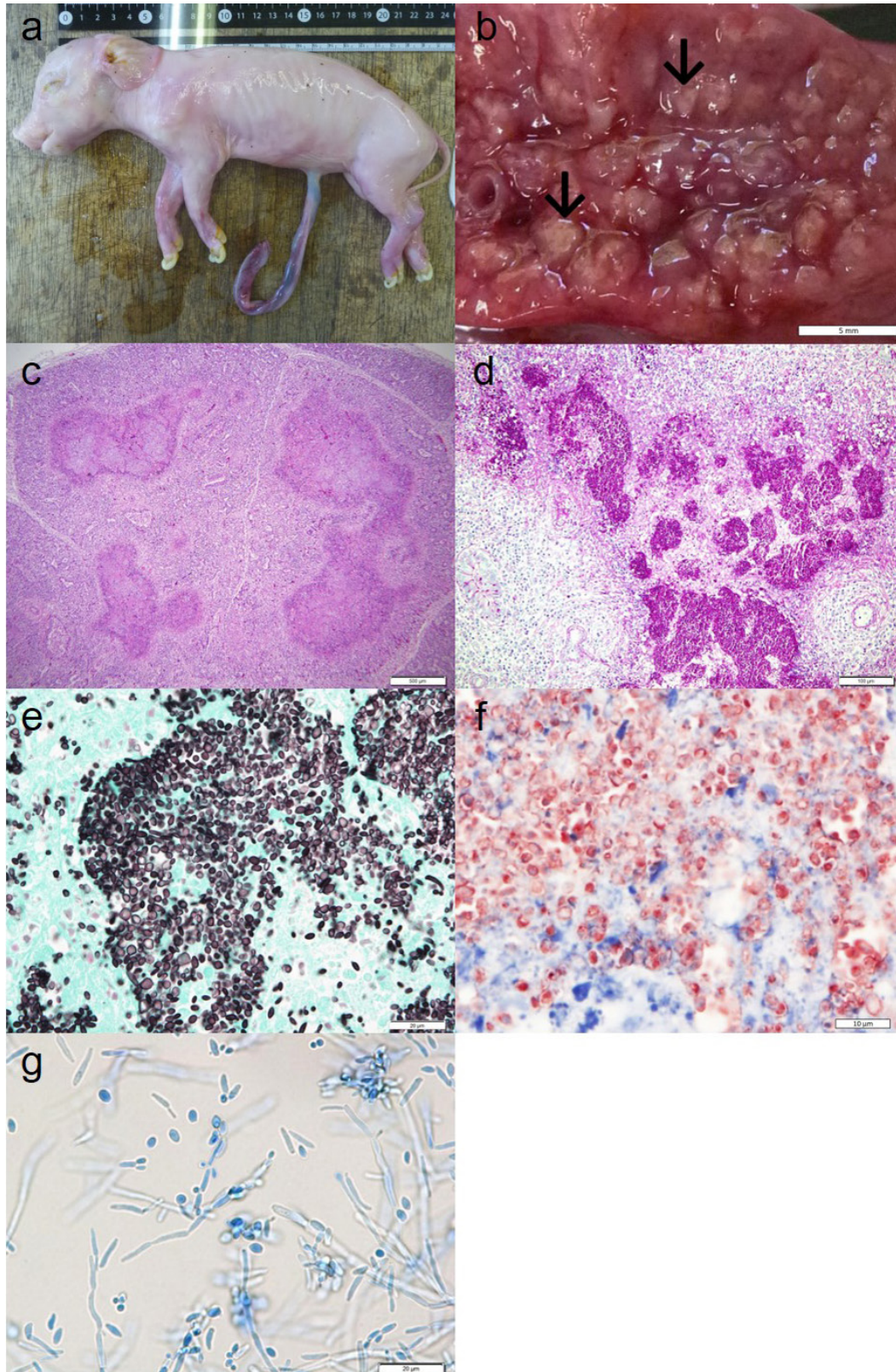


Fig. 1. **a.** No gross lesions were visible at necropsy in fetus No. 1. **b.** Pale foci were scattered in the fetal lung (arrows) tissue section in the fetus No. 1. Bar=5 mm. **c.** Multifocal necrosis was randomly diffused in the fetal lung of the fetus No. 1. Hematoxylin and eosin staining. Bar=500 μ m. **d.** Periodic acid-Schiff positive yeasts were observed in the pulmonary necrosis using periodic acid-Schiff reaction in the fetus No. 1. Bar=100 μ m. **e.** Yeasts were stained in the pulmonary necrosis using Grocott's silver staining in the fetus No. 1. Bar=20 μ m. **f.** Yeasts reacted with anti-*Candida albicans* antibody in the pulmonary necrosis observed in the fetus No. 1 by immunohistochemical staining. Bar=10 μ m. **g.** *Candida parapsilosis* isolated from the lung of fetus No. 1 grows in a pseudohyphal form or exists in a yeast phase under lactophenol cotton blue stain. Bar=20 μ m.

At necropsy, the lung, stomach, colon, liver, spleen, kidney, heart, and brain tissue samples from the two fetuses were fixed in 10% neutral-buffered formalin. The fixed tissues were embedded in paraffin wax, sectioned (~3 µm), and stained with hematoxylin and eosin for histological examination. Gram, periodic acid-Schiff (PAS) reaction, and Grocott's silver staining were also performed on the lung, stomach, and colon samples from the two fetuses. For immunohistochemical (IHC) examination, the formalin-fixed paraffin-embedded sections of the lung, stomach, and colon were deparaffinized and incubated with 3% hydrogen peroxide in methanol solution to suppress endogenous peroxidase activity. Antigen retrieval was conducted using 0.1% actinase E solution in phosphate-buffered saline at 37°C for 10 min. After treating the sections with 10% normal goat serum to block non-specific reactions, the sections were incubated with rabbit polyclonal antibodies against *C. albicans* (Biogenesis, Dorset, UK), anti-*Aspergillus* (Clone Mab-WF-AF-1; Dako, Carpinteria, CA, USA) and anti-*Rhizomucor* (Clone WSSA-RA-1; Dako) mouse monoclonal antibodies for 1 hr. Sections were incubated with a secondary antibody (Histofine Simple Stain MAX-PO Multi; Nichirei Bioscience Inc., Tokyo, Japan) for 30 min at room temperature, and then treated with aminoethyl carbazole substrate solution (Histofine Simple Stain AEC solution; Nichirei Bioscience Inc.) at room temperature. Finally, the sections were counterstained with hematoxylin.

The histopathological findings were also similar between the two fetuses. Multifocal necrosis was randomly diffused in the lungs (Fig. 1c). These foci were circumscribed by an acellular eosinophilic necrotic material and epithelioid cells. Additionally, the foci contained colonies composed of myriads of round to oval yeast that were 3–5 µm wide. The yeast colonies were strongly stained by PAS (Fig. 1d) and Grocott's silver stain (Fig. 1e). Bacteria were not detected in the lungs by Gram staining. IHC staining with *C. albicans* antiserum revealed positive reactions in the yeasts in the necrotic lesions (Fig. 1f), whereas that with both *Aspergillus* and *Rhizomucor* was found to be negative. The polyclonal antibodies against *C. albicans* were not absorbed, and they cross reacted with other *Candida* spp. Bronchiolitis with several neutrophils and lymphocytes as well as thickening of the interstitium with fibroblasts, macrophages, and lymphocyte infiltration were also observed in the lung.

In the PAS reaction, Grocott's silver stain, and IHC, the gastric and colonic contents contained yeast-like fungi, but histologically, the mucous membrane was intact. Barring the fetal pulmonary tissues, slight neutrophilic and lymphocytic infiltration was observed in the liver and epicardium. However, no fungal colonies were detected in the histological assessment.

The lung, gastric and colonic contents, liver, spleen, kidney, heart, and brain tissue samples was cultured in an aerobic condition on blood agar (Eiken Chemical Co., Ltd., Tokyo, Japan) and deoxycholate-hydrogen sulfide-lactose agar (Eiken Chemical Co., Ltd.) at 37°C for two days. The isolates obtained were inoculated onto potato dextrose agar (PDA; Nissui, Tokyo, Japan) containing 50 µg/ml chloramphenicol and incubated at 37°C. The microscopic examination of fungal growth was carried out with lactophenol cotton blue stain. Yeast was isolated only from the lung (Fig. 1g) and gastric and colonic contents. The isolate was characterized by pseudohyphae and round or cylindrical yeast cells, including budding. No bacteria were isolated from these organs. These yeasts were identified as *C. parapsilosis* using the API 20 C AUX system (BioMérieux, Tokyo, Japan).

The isolates were identified as *C. parapsilosis* through sequencing and basic local alignment search tool analysis. The sequences of the internal transcribed spacer (ITS) region (using the primers ITS1 and ITS4) [17] and large subunit ribosomal DNA (28S rDNA; using the primers LROR and LR5) [11, 16] showed a 100% match of the sequences of *C. parapsilosis* type strain CBS 604 (accession number: MH545914.1).

To detect PRRS virus, reverse transcriptase polymerase chain reaction was performed with the lung sample. The result was negative.

These results confirmed a *C. parapsilosis* infection in the aborted fetuses and provided an explanation for the occurrence of necrotizing pneumonia and subsequent abortion. Four cases of bovine abortion caused by *C. parapsilosis* have previously been reported [8]. While the characteristic pathological finding in the present case indicated severe necrotizing pneumonia, mild bronchiolitis or pneumonia has been reported in the bovine fetal lung [8]. Additionally, in cattle, severe placentitis was detected with a large number of yeasts in the trophoblasts, and the pathogenesis of uterine infection is assumed to be hematogenous [8]. Unfortunately, the placenta was not examined in this case.

In humans, an invasive disease with *C. albicans* is transmitted vertically from mother to child around the time of birth [15]. In contrast, *C. parapsilosis* is frequently transmitted horizontally via contaminated external sources, such as medical devices or fluids, the hands of health care workers, prosthetic devices, and catheters [15]. *C. parapsilosis* can be recovered from the soil, skin, and mucosa of humans and animals and may cause opportunistic infections [2, 4]. In the present case, multifocal necrosis was diffusely detected with *Candida* antigens in the lungs of fetal pigs. Histologically, yeast isolates were found in the lungs and gastric and colonic contents that were in contact with the amniotic fluid. These results corresponded with the results of the microbiological examination. Thus, the ubiquitous yeasts detected in the two fetuses led to the abortion, and the source of infection might be amniotic fluid. However, it was not possible to determine whether the amniotic fluid contamination resulted due to a bloodstream infection or an ascending infection.

In humans, the most common risk factors for candidiasis include ageing, malnutrition, obesity, diabetes, and immune deficiency [9]. Fetal candidiasis is rarely reported and is usually associated with low birth weight and premature birth [15]. Furthermore, in humans, pregnancy increases the incidence of female vaginal candidiasis [7]. During pregnancy, estrogen levels increase, reducing the ability of vaginal epithelial cells to inhibit the growth of *C. albicans* [7]. In humans, pregnancy is a risk factor for candidiasis. Therefore, there is a possibility that it is also a risk factor in pigs.

To identify the species of *C. parapsilosis*, we recommend sequencing the ITS and 28S rDNA regions. Identifying clinically important yeasts by sequencing the ITS and D1/D2 regions is a rapid and reliable alternative to conventional identification methods using isolation, culture, fungal morphology, and phenotypic test [1].

In conclusion, a swine abortion due to *C. parapsilosis* was revealed in this report. While *Candida* spp. are known to cause opportunistic infections, there have been no reports of their association with abortions in animals with the exception of cows. Thus, our study provides important insights into swine abortion that will benefit both veterinarians and farmers.

POTENTIAL CONFLICTS OF INTEREST. The authors have nothing to disclose.

ACKNOWLEDGMENTS. We thank Mr. M. Kobayashi and Ms. M. Shimada for their assistance in the histopathological assessment. In addition, we thank Mr. S. Saitou for his useful advice on this paper.

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