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Coinfection with *Candida tropicalis* and Cytomegalovirus in a Piglet

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Abstract

Chronic mucocutaneous candidiasis caused by *Candida tropicalis* was identified in a 43-day-old female piglet with lesions on body surfaces (face, abdomen, limbs, and hooves) and mucosal surfaces of the oral cavity and stomach (nonglandular region). Cytomegalovirus (CMV) inclusion bodies were observed in the epidermis, apocrine sweat gland, rostral plate eccrine gland, buccal gland, duodenal gland, and renal distal tubule. In addition, adenovirus-like inclusion bodies, *Cryptosporidium*, and *Brachyspira* were detected in the intestine. The presence of these opportunistic pathogens on or in the skin and oral–gastrointestinal mucosa implies that the piglet had immunological defects, especially in mucocutaneous barriers. Thus, CMV may have facilitated the activity of *C. tropicalis* and may have contributed to the persistence of candidiasis.

Discipline: Animal Science
Additional key words: chronic mucocutaneous candidiasis, hereditary immunodeficiency, opportunistic infection, swine

Introduction

In humans, chronic mucocutaneous candidiasis (CMC) is a heterogeneous immunodeficiency disorder, characterized by susceptibility to *Candida* infection of the skin, nails, and mucous membranes (Steensma et al. 2000). Some cases of CMC associated with autoimmune manifestations, particularly endocrine diseases occurring in childhood, are either autosomal dominant or recessive (van de Veerdonk et al. 2011). Coinfection with *Candida* and cytomegalovirus (CMV) is not uncommon in patients who have had a kidney or liver transplant or who have acquired immunodeficiency syndrome (Laine et al. 1992, Olczak-Kowalczyk et al. 2008).

In swine, cutaneous candidiasis is caused by the yeast *Candida albicans* and occurs when the host's resistance is lowered. This disease has been reported in grower pigs that are fed garbage and kept in unsanitary conditions (Cameron 2006). *Candida* fungi also invade internal organs, and chronic gastroenteritis, gastric ulceration, and oropharyngeal infections can occur in piglets aged 7-14 days (Taylor 2006). CMC caused by *C. albicans*, reported in two growing pigs, was believed to be due to an immunosuppressed state associated with postweaning multisystemic wasting syndrome (PMWS) (Zlotowski et al. 2006). Porcine CMV is an immunosuppressive virus that inhibits the functions of macrophages and T lymphocytes; as a result, bacterial secondary infections may increase the mortality rate of animals infected with this virus (Liu et al. 2013, Liu et al. 2014, Yoon et al. 2006). The present study reports a case of CMC in which porcine CMV infection was found in

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various different epithelial cells.

Materials and methods

1. Animals

Four of the 10 crossbred piglets from one litter bred on a regular farm manifested lameness at the age of 1 week and exhibited elevated lesions on the coronary band and bottoms of all their hooves. The animals' condition deteriorated despite antibiotic treatment, and similar lesions appeared on their left forelimbs, abdomen, auricles, mandibles, and rostral plates. Feeding and weight gain remained normal. Due to the expanding dermatosis, the animals were euthanized before weaning, but no postmortem examination was performed. Another female piglet in the same litter developed hoof lesions at the age of 3 weeks and showed a clinical course similar to her littermates with dermatosis. The piglet was euthanized for pathological evaluation at the age of 43 days. Just before death, the white blood cell count was 20,025/μL. Prior to the occurrence of this disease, there were no records of fungal infections, CMV infection, or abnormal births on this farm.

2. Histological, histochemical, and immunohistochemical examination

Tissues were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at 4 μm, and stained with hematoxylin and eosin, Giemsa, or periodic acid–Schiff (PAS) stains. Selected paraffin sections from the skin, oral cavity, stomach, intestine, kidney, and urinary bladder were utilized for immunohistochemistry, which was performed using either the universal immune-enzyme polymer method with a Histofine Simple Stain MAX-PO kit (Nichirei, Tokyo, Japan) or the streptavidin–biotin complex/horseradish peroxidase (SAB) method with a Histofine SAB-PO kit (Nichirei, Tokyo, Japan). Rabbit polyclonal antibodies against porcine CMV (Sekiguchi et al. 2012), *C. albicans* (Biogenesis, Dorset, England), *Brachyspira pilosicoli* (provided by Dr. Tatsuo Ohya), and *Trueperella pyogenes* (provided by Dr. Shotaro Takeuchi) were used as primary antibodies. Antigen retrieval was performed by enzymatic digestion with 0.1% actinase at 37°C for 20 min for CMV or 0.05% pepsin at 37°C for 25 min for others. Antibodies were detected by incubation with aminoethyl carbazole for CMV and 3,3′-diaminobenzidine tetrahydrochloride solution for others.

3. Mycological and virological examination

Materials obtained from limb and auricle lesions were cultured aerobically on potato dextrose agar (Nissui, Tokyo, Japan) with chloramphenicol for 7 days at 25°C. Molecular biological analysis was performed to identity fungal isolates. The fungal DNA was extracted via mechanical disruption with zirconia beads and a QIAamp DNA Mini Kit (Qiagen, Hilden, Germany). The internal transcribed spacer (ITS) regions among the nuclear ribosomal DNA were amplified using the primer pair ITS1 and ITS4 (Hosoya et al. 2010, White et al. 1990). The polymerase chain reaction (PCR) products were sequenced using an Applied Biosystems 3130xl genetic analyzer (Life Technologies Corp., CA, USA), and a basic local alignment search tool (BLAST) analysis of the GenBank database was conducted.

PCR testing for the detection of porcine respiratory and reproductive syndrome virus (PRRSV) (Kono et al. 1996), porcine circovirus type-2 (PCV2) (Kawashima et al. 2003), and classical swine fever virus (CSFV) (Vilček et al. 1994) was performed on tonsil tissues. Jejunal feces were also used for PCR detection of transmissible gastroenteritis virus (TGEV) (Jung & Chae 2005) and porcine epidemic diarrhea virus (PEDV) (Park et al. 2008).

Results

1. Gross pathology

All the hooves and the skin around the left carpal joint was almost completely covered with flattened raised nodules or masses (Fig. 1). Smaller nodules were scattered over all the limbs. Multiple nodules with a thickly keratinized surface layer were present on the abdomen and auricles, and several more were present on the rostral plate and mandible. The buccal mucosa showed thickening with keratosis, roughening, and fissure formation on the surface. Similar findings were observed on the dorsal aspect of the tongue and the proventricular part of the stomach. A 1-cm-diameter abscess was detected in the urinary bladder wall.

2. Histological, histochemical, and immunohistochemical findings

Histologically, fungal organisms characterized as PAS-positive yeast cells and pseudohyphae were observed in the macroscopically visible lesions. In the body surface lesions, organisms were localized on or in hyperkeratotic and acanthotic stratified squamous epithelium, accompanied by neutrophil infiltration (Fig. 2A). Basophilic intranuclear inclusion bodies (CMV inclusion bodies) in greatly swollen epidermal (Fig. 2B) and apocrine sweat gland cells (Fig. 2C) were rarely visible. Contrary to localized fungal lesions on the rostral plate surface, eccrine gland lobules were extensively

infiltrated and replaced by lymphocytes and plasma cells (Fig. 2D), with focal necrosis in some places. Several CMV inclusions were found in acinar and ductal cells (Fig. 2E). Acinar cells with hyperchromatic or pyknotic nuclei were frequently observed, regardless of the presence of inflammatory cells.

As in cutaneous lesions, fungal organisms were visible on or in the keratinized layer of the oral, lingual, and gastric squamous mucosa with hyperkeratosis and hyperplasia. There were few CMV inclusions in the buccal glands and only a moderate stromal infiltration (Fig. 2F). They were extremely rare in the duodenal glands, which had no reactive infiltrates. A few CMV inclusion-bearing giant cells of unknown type were detected in the jejunal lamina propria, hepatic sinusoids, and lung alveolar wall. In the kidneys, CMV inclusion bodies were present in a few distal tubules, some of which were located near or adjacent to lymphocytic and plasmacytic infiltrates or regenerating tubules. An encapsulated abscess in the muscular layer and subserosa of the urinary bladder contained numerous gram-positive diphtheroid bacteria.

The ileum exhibited atrophy and fusion of villi, with increased numbers of lymphocytes, plasma cells, and macrophages in the lamina propria. Epithelial cells occasionally had intranuclear amphophilic inclusion bodies with distinct peripheral halos (Fig. 2G) and rarely weakly basophilic inclusions with or without halos. These were reminiscent of adenovirus inclusions. *Cryptosporidium* organisms were sparsely distributed on the villus surface (Fig. 2G). In the cecum, great numbers of *Brachyspira*-like spiral organisms were present on the mucosal surface and in the upper crypts, but rarely in the lamina propria. *Cryptosporidium* organisms were also observed on the colonic surface epithelium.

Fig. 1. Macroscopic appearance
Raised nodules or masses are observed on the face, auricles, forelimb, and hooves.

Immunohistochemically, fungal organisms, spiral organisms, and diphtheroid organisms were stained with antibodies against *Candida*, *Brachyspira*, and *Trueperella*, respectively (Figs. 2H and 2I). Giant cells with CMV inclusion bodies showed cytoplasmic staining with antibody against porcine CMV (Fig. 2J), and the viral antigen was also observed in necrotic foci in glands of the rostral plate (Fig. 2K).

3. Mycological and virological findings

The targeted ITS region was successfully amplified and sequenced with extracted fungal DNA. The obtained sequences for the hindlimbs, right forelimb, and auricle contained 449, 367, and 368 bp, respectively. These isolates were identified as *C. tropicalis* with BLAST analysis because all sequences exhibited a 100% match of the ITS region to sequences of *C. tropicalis* strain CNRMA10.288 (Accession No: KP131813). The accession numbers registered in DNA Data Bank of Japan were LC649464, LC64965, and LC649466 for the hindlimbs, right forelimb, and auricle, respectively.

PCR testing for PRRSV, PCV2, and CSFV in tissues was negative. PCR for TGEV and PEDV in feces was also negative.

Discussion

In human medicine, *C. tropicalis* has emerged as one of the most important *Candida* species. It is considered the second most virulent *Candida* species, with only *C. albicans* being more virulent (Zuza-Alves et al. 2017). Compared to cutaneous candidiasis in humans, it is much less prevalent in animals. Cutaneous cases caused by *C. albicans* have only rarely been reported in swines (Ramot et al. 2017, Reynolds et al. 1968). Piglets that were experimentally immunosuppressed and infected with *C. albicans* developed extensive and invasive intestinal lesions and disseminated infection to the kidney, lung, liver, spleen, and heart (Andrutis et al. 2000), whereas *C. tropicalis* showed superficial

infections in the case described here. This difference may depend on the degree of immunodeficiency of the host, as well as *Candida* virulence. Different mouse strains show differing susceptibility to *Candida* infection, which could potentially alter virulence results (MacCallum 2012).

In humans, CMC is an immunodeficiency disease, and patients are susceptible to *Candida* fungi (Steensma et al. 2000). In the current case, the presence of *Candida*

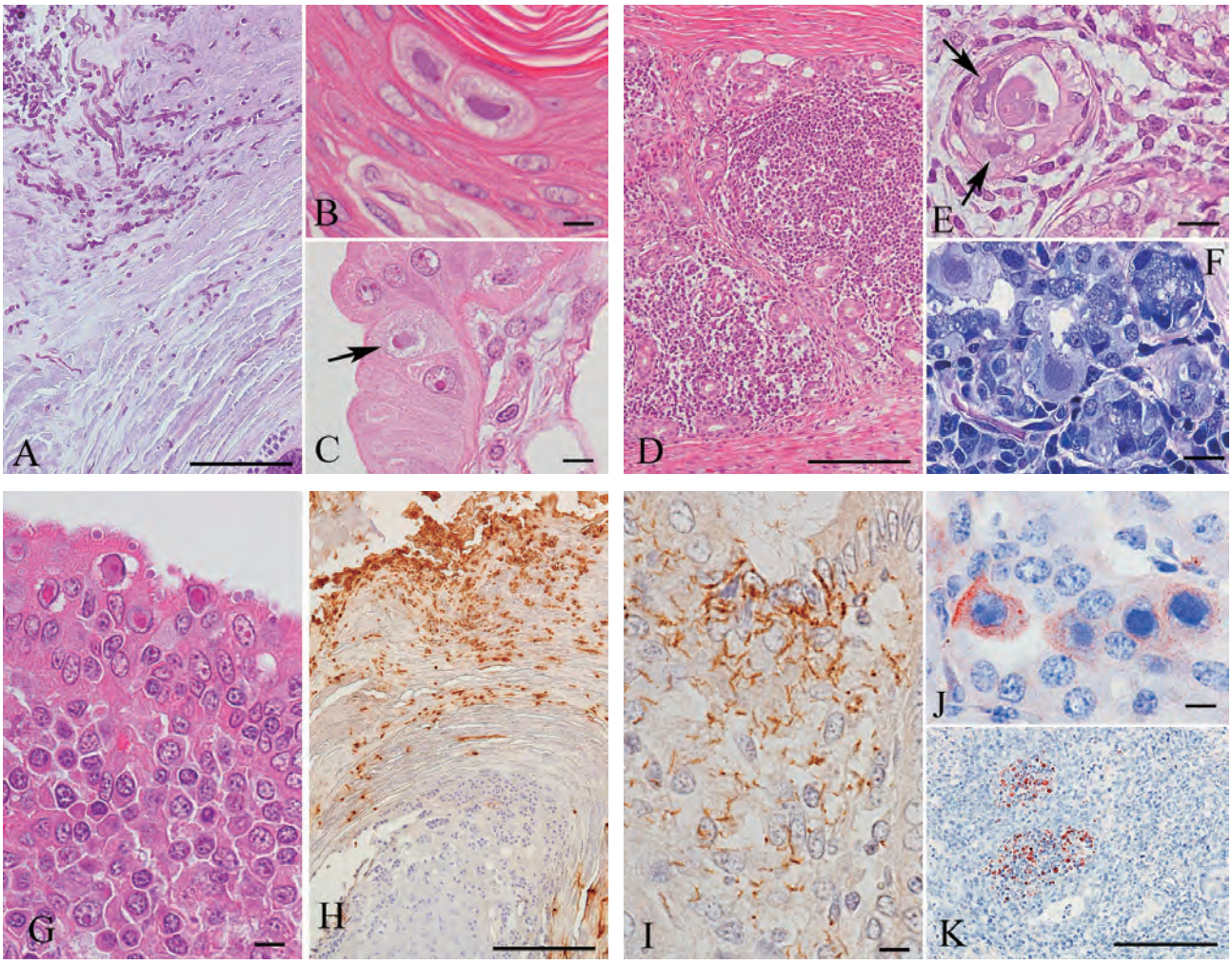

Fig. 2. Histology and immunohistochemistry

(A) Skin of the abdominal wall. Yeast cells and pseudohyphae staining red are visible in the hyperkeratotic layer with cell debris (higher left), and neutrophil infiltrates or accumulations are observed in the acanthotic layer (lower right). Periodic acid–Schiff staining. Bar = 50 μm. (B) Skin of the lower abdomen. Basophilic inclusion bodies are observed in the enlarged nuclei of two keratinocytes. Hematoxylin and eosin (HE) staining. Bar = 5 μm. (C) Submandibular skin. The arrow indicates an apocrine gland cell with an intranuclear inclusion body. HE staining. Bar = 5 μm. (D) Gland of the rostral plate. Lymphocytic and plasmacytic infiltration predominates in this field, and many acini have been lost. HE staining. Bar = 100 μm. (E) Gland of the rostral plate. In an acinus, megalocytic cells have irregularly shaped inclusion bodies (arrows), and the stroma is edematous with inflammatory cells. HE staining. Bar = 10 μm. (F) Buccal gland. Intranuclear inclusions are seen in large acinar cells with abundant cytoplasm (upper left and center). Stromal infiltration is mild. Giemsa staining. Bar = 10 μm. (G) Ileum. Adenovirus-like intranuclear inclusion bodies are observed in villus epithelial cells with *Cryptosporidium* organisms on the apical surface. The lamina propria is full of inflammatory cells (lower half). HE staining. Bar = 5 μm. (H) Skin of the abdominal wall. Fungal organisms staining with anti-*Candida* antibody are within the hyperkeratotic layer. Streptavidin–biotin complex/horseradish peroxidase (SAB) and detection with 3,3′-diaminobenzidine tetrahydrochloride (DAB). Bar = 100 μm. (I) Cecum. Spiral organisms staining with polyclonal antisera against *Brachyspira* show invasion into the lamina propria. SAB and detection with DAB. Bar = 5 μm. (J) Kidney. Distal tubule epithelial cells with intranuclear inclusion bodies show positive cytoplasmic staining for cytomegalovirus (CMV). Universal immune-enzyme polymer (UIP) and detection with aminoethyl carbazole (AEC). Bar = 5 μm. (K) Gland of the rostral plate. CMV-positive deposits are detected in necrotic foci. UIP and detection with AEC. Bar = 100 μm.

species was demonstrated on various body surfaces as well as in the oral cavity and gastric mucosa. Moreover, CMV, *Cryptosporidium* and *Brachyspira* infections and adenovirus-like inclusion bodies were observed in the current case. The presence of these opportunistic pathogens suggests that the piglet was immunologically defective, especially in mucocutaneous barriers, and the diagnosis of CMC was made. In reported CMC cases of two piglets, the cause of immunodeficiency was PMWS characterized by depletion of T and B lymphocytes (Zlotowski et al. 2006). The underlying immunodeficiency was not clear in the present case, but four siblings derived from the same litter had exhibited closely similar clinical findings. Although histopathological examination was not performed for these animals, the possibility of autosomal dominant familial candidiasis remains (van de Veerdonk et al. 2011) and must be considered in future case studies.

Human CMV infection results in leucopenia and impaired macrophage function and increases the pathogenic potential of bacteria. This virus also causes epithelial cell damage (erosions, ulcerations, and focal necrosis of the oral mucosa), leading to deep pathogenic invasion (Olczak-Kowalczyk et al. 2008). Similarly, murine CMV infection plays an important role in enhancing the susceptibility of mice to infection by *Pseudomonas aeruginosa*, *Staphylococcus aureus*, or *C. albicans*, and a striking synergistic effect on mortality is observed in mice coinfected with the virus and either of these bacteria or fungi (Hamilton et al. 1976). Coinfection of porcine CMV and other viruses often occurs in immunosuppressed swine, and various bacterial infections can occur following porcine CMV infection (Liu et al. 2013, Liu et al. 2014, Yoon et al. 2006). In the current case, CMV infection may have facilitated the activity of *C. tropicalis*. CMV inclusions or antigens were detected in epithelial cells of the skin and oral mucosa, with reactive lymphocyte and plasma cell infiltration and accumulation; thus, the virus and reactive infiltrates capable of causing epithelial cell damage may have played a significant role in eliciting and sustaining candidiasis.

Saliva is believed to be the major source of murine CMV for horizontal spread, and productive infection is confined to glandular epithelial cells of the salivary glands for a prolonged period of time in an immunocompetent host (Krmpotic et al. 2003). Lifelong latent infection with porcine CMV occurs in congenitally infected pigs, and latently infected pigs can shed viruses when stressed (Shibahara et al. 2012). In the piglet in the present study, the eccrine gland of the rostral plate was most severely inflamed and focally necrotic, with CMV inclusion bodies in the acini and ducts. The gland may have been a persistent source of CMV for spreading to other body sites (both skin and upper alimentary tract) or neighboring piglets. The microorganisms described here are commensal or opportunistic, but a piglet with nonfatal immunodeficiency, like the present case, poses a risk of continuously disseminating microorganisms to other animals.

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