A piglet model for studying Candida albicans colonization of the human oro-gastrointestinal tract

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Abstract

Pigs from a variety of sources were surveyed for oro-gastrointestinal (oro-GIT) carriage of Candida albicans. Candida albicans-positive animals were readily located, but we also identified C. albicans-free pigs. We hypothesized that pigs could be stably colonized with a C. albicans strain of choice, simply by feeding yeast cells. Piglets were farrowed routinely and remained with the sow for 4 days to acquire a normal microbiota. Piglets were then placed in an artificial rearing environment and fed sow milk replacer. Piglets were inoculated orally with one of three different C. albicans strains. Piglets were weighed daily, and culture swabs were collected to detect C. albicans orally, rectally and in the piglet’s environment. Stable C. albicans colonization over the course of the study did not affect piglet growth. Necropsy revealed mucosally associated C. albicans throughout the oro-GIT with the highest abundance in the esophagus. Uninoculated control piglets remained C. albicans-negative. These data establish the piglet as a model to study C. albicans colonization of the human oro-GIT. Similarities between oro-GIT colonization in humans and pigs, as well as the ease of working with the piglet model, suggest its adaptability for use among investigators interested in understanding C. albicans-host commensal interactions.

Introduction

Normally, Candida albicans is a commensal fungus, harmlessly colonizing the human oro-gastrointestinal tract (oro-GIT; reviewed by Koh, 2013). Disruption of the host’s immune defenses, compromise of GIT integrity or alterations in the GIT microbiota can cause commensal fungal cells to turn into a deadly disease-causing inoculum. Extraintestinal translocation of C. albicans is a key part of this process.

While considerable effort has been devoted to understanding C. albicans pathogenesis, study of C. albicans colonization has come into vogue more recently. Mouse models are used most frequently for these analyses. Koh (2013) presented a thorough and insightful review of murine models for studying GIT colonization. The most commonly used model involves antibacterial treatment of adult mice because alteration of the GIT microbiota composition is required for C. albicans colonization. Candida albicans is introduced by drinking water or oral gavage, and fungal persistence is monitored in fecal pellets. While this model is easily adapted to many laboratories, the natural resistance of the murine GIT to C. albicans colonization leads to concerns about the artificiality of this approach.

Pigs are frequently used as human biomedical models. Andrutis et al. (2000) discussed selection of pig models for the study of bacterial, viral and parasitic enteric pathogens, because of similar pathologies produced in pigs and humans. They used gnotobiotic piglets as a model for C. albicans GIT colonization and dissemination. Perhaps gnotobiotic piglets were sought because of the conventional idea that C. albicans is associated with all domestic animals (reviewed by Wrobel et al., 2008), thereby requiring extra effort to derive a C. albicans-negative animal. Pigs were delivered by Cesarean section and housed in microbiological isolators to prevent introduction of normal microbiota. Immunosuppressive treatments caused
disseminated disease that was similar to that in humans. While impressive, this gnotobiotic piglet model was labor-intensive, making it difficult to adapt for widespread use.

The work described here investigates use of routinely farrowed piglets as a model for C. albicans colonization. Study parameters were selected to promote acquisition of normal microbiota. Non-intensive housing methods were designed, with the intention of facilitating broad use of the model among investigators who study C. albicans–host interactions associated with oro-GIT colonization.

Materials and methods

Detection of yeasts in the pig microbiota

Nine pigs, from seven different farms, were submitted for necropsy to the Veterinary Diagnostic Laboratory (VDL) at the University of Illinois and sampled to detect yeasts. Swabs from the oral cavity, large colon and rectum were plated on Sabouraud agar (L−1: 40 g glucose, 10 g peptone and 20 g Bacto agar) with chloramphenicol (20 μg mL−1; Sab + Cml), and incubated for 24 h at 37°C. Pure cultures were derived for colonies with yeast-like cellular morphology. Each isolate was streaked onto CHROMagar Candida (www.chromagar.com) and tested for germ tube formation by inoculation into RPMI 1640 medium (Gibco catalog no. 11875-085). Yeasts were identified by PCR of genomic DNA using primers ITS4 (5’-TCCTCCGCTTATTGATATGC-3’) and ITS5 (5’-GGA AGTAAAAATCTGACAAAGG-3’) that amplify the internal transcribed spacer region of the nuclear-encoded rRNA genes (White et al., 1990). DNA sequences were aligned against the non-redundant nucleotide database using BLAST. Candida albicans isolates were analysed using multilocus sequence typing (MLST) as described previously (Bougnoux et al., 2003; Wrobel et al., 2008). Oral and rectal swabs were also collected from normally healthy pigs on the University of Illinois Veterinary Research Farm (VRF). Thirty-four pigs were sampled from a herd of c. 200. Fourteen pigs were from the nursery unit (15–40 lbs), 10 were growers (40–100 lbs) and 10 were from the finishing unit (100–280 lbs). Swabs were plated on Sab + Cml and yeasts were identified as described above. Animal experiments were conducted with the approval of the University of Illinois Institutional Animal Care and Use Committee.

Farrowing and artificial rearing of piglets

VRF piglets are an outbred population that includes a mix of the Yorkshire, Landrace and Duroc breeds. Piglets were farrowed normally and housed with the sow in farrowing crates to ensure intake of colostrum. On day 4, piglets were moved to an artificial rearing environment. Piglets were handled minimally; they were not subjected to any of the normal processing of VRF piglets that typically includes ear notching, removal of needle teeth, tail docking, iron dextran injection (100 mg; Aspen Veterinary Resources), penicillin G injection (300 000 U; Butler Schein) and castration of males. Approximately equal numbers of male and female piglets were used in our studies.

Piglets were moved to 4 × 4-ft raised pens constructed from painted steel hog panels, lined with solid plastic sheeting to prevent animal-to-animal contact. Pens had plastic slotted flooring over a flush pan. All material was easily disinfected. Piglets had 24-h access to water via a nipple-triggered drinking cup. Rooms were ventilated to outdoor air by a variable-speed, 12-in exhaust fan. Room temperature was controlled by use of a room heater or an air conditioning unit. Heat lamps were lowered over each pen, when additional heat was required. Housing met or exceeded the standards and recommendations of the National Research Council (2011).

Piglets were fed Ralco-Birthright Acidified Baby Pig Milk Replacer (Ralco Nutrition, Inc.; five cups per gallon of water; 360 mL kg−1 body weight, up to 2000 mL daily). In the initial study, milk replacer was provided hourly using a timer and pump system. The pig’s bowl, stock bottles and tubing were washed and bleached daily, and always returned to the same pig’s feeding system to prevent cross-contamination between animals. Subsequent work showed that milk replacer can be provided in a plastic baby pig waterer (see Miller Little Giant item number 291-25). Half of the total daily volume of milk replacer was provided twice daily. Plastic waterers were washed and bleached daily, then returned to the same pen. Research workers wore clean coveralls and boots. Gloves were changed between handling animals and materials in different pens. A bleach-filled boot wash outside each room was used to control spread of C. albicans in the animal facility.

Inoculation of piglets with C. albicans and assessment of colonization

Piglets and their environment were confirmed C. albicans-free before each study. Oral swabs sampled liberally over all oral surfaces. For rectal swabs, the swab tip was inserted into the pig’s rectum and withdrawn, often covered in fecal material. Environmental swabs were wet in the piglet’s drinking cup, then rubbed thoroughly across all pen surfaces. Swabs were plated on Sab + Cml as described above.

After 1 day of acclimation, pigs were inoculated orally with 10⁹ C. albicans cells. One of three strains was used:

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SC5314 (the most commonly studied isolate, for which the genome sequence is known; Jones et al., 2004), 216 (an oral isolate from a normally healthy human; Wrobel et al., 2008) or 3317 (collected from the oral cavity of a VDL necropsy pig evaluated for lameness; see above). Candida albicans strains were taken from −80 °C storage and streaked onto YPD plates (L⁻¹: 10 g yeast extract, 20 g peptone, 20 g glucose and 20 g Bacto agar). Plates were incubated at 37 °C for 24 h then stored at 4 °C for ≤1 week. An isolated, representative colony was inoculated into 20 mL YPD and incubated for 16 h at 30 °C with shaking (200 r.p.m.). Yeasts were collected by centrifugation, washed twice in Dulbecco’s phosphate-buffered saline without calcium or magnesium (DPBS) and counted using a hemocytometer. The yeast inoculum was resuspended in 1 mL DPBS. Piglets readily drank the inoculum or DPBS control from a needleless tuberculin syringe.

Oral, rectal and environmental swabs were collected 8 h following inoculation, and once every 24 h thereafter. Swabs were plated on Sab + Cml. Plates were incubated for 24 h at 37 °C and C. albicans colonies counted. A seven-category scale was used to report the plating results: 0 (no growth), 1 (1–25 colonies), 2 (26–99 colonies), 3 (100–499 colonies), 4 (500–1000 colonies), 5 (>1000 colonies, with distinct colony growth still visible) and 6 (lawn of growth). Piglets were weighed daily to monitor growth.

Two independent experiments were conducted. In the first, C. albicans isolates were tested using one pig per strain. A DPBS-inoculated pig was maintained in the same room to control for unintentional C. albicans transfer. Pigs were monitored for 21 days, including 15 days on milk replacer and 6 days on solid feed (pelleted Diet 111 from the University of Illinois Department of Animal Sciences feed mill; per ton: 625.2 lbs corn, 500 lbs whey, 404.8 lbs soybean meal, 200 lbs lactose, 150 lbs applein, 60 lbs fat, 22.8 lbs lime, 12.6 lbs dicalcium phosphate, 8 lbs zinc oxide, 7 lbs swine trace minerals, 4 lbs Vitamix ADEK, 2.8 lbs dL-methionine, 2 lbs iodized salt and 0.8 lbs lysine). In the second experiment, C. albicans isolates were tested using two pigs per strain. Pigs were housed individually, with complete experimental replicates in separate rooms, and monitored for 13 (1st group) or 14 days (2nd group) while fed milk replacer. An uninoculated control pig was placed in each room. Because duplicate animals were used for each treatment, results from the second experiment are featured here. However, results were reproducible across both experiments.

Pigs were anesthetized with an intramuscular injection of telazol/ketamine/xylazine (4.4 mg kg⁻¹), then euthanized by intracardiac overdose of sodium pentobarbital. Oral and rectal swabs were plated on Sab + Cml. Tissue sections were collected from the esophagus, stomach, duodenum, proximal jejunum, mid jejunum, distal jejunum, ileum, cecum, spiral colon and large colon. Candida albicans associated with the GIT mucosa was evaluated by collecting scrapings from the lumen of each tissue using the edge of a sterile glass microscope slide. A portion of the mucosa was weighed and homogenized in DPBS. Serial 10-fold dilutions were plated on Sab + Cml and incubated for 24 h at 37 °C to count CFU. Another portion of the mucosa was baked overnight in a vacuum oven at 80 °C. The ratio of dry weight to wet weight was calculated, then multiplied by the mass of the original mucosal sample that was homogenized and plated. This final dry weight equivalent was used as a divisor to calculate CFU g⁻¹ dry weight. Reported results were rounded to the nearest order of magnitude.

In addition to the piglets housed in the artificial rearing environment, a piglet of similar age was selected from the VRF farrowing unit and necropsied as a control. All samples from this animal were negative for C. albicans (data not shown).

Results

Yeasts isolated from pigs

To determine how readily C. albicans could be isolated from pigs, swabs were collected from pigs that were submitted to the VDL, and also from pigs at the VRF (see Materials and methods). VDL submissions provided the means to sample pigs from a variety of local sources. While some pigs were submitted because of infectious disease (enteritis, pneumonia), others were normally healthy (used for teaching purposes) or evaluated for conditions such as lameness. Eight of the nine VDL pigs were yeast-positive as detected by our culture technique; C. albicans was isolated from four of them. There was no association between C. albicans isolation and pig health. Candida tropicalis (two pigs), Trichosporon asahii (one) and Candida catenulata (one) were also isolated from the C. albicans-positive pigs. Issatchenka orientalis (two pigs), C. catenulata (one), C. tropicalis (two) and Candida rugosa (one) were isolated from the C. albicans-negative pigs. MLST analysis of the C. albicans pig isolates showed that all were from clade 8, and clustered closely with strains commonly found in central Illinois wildlife (Wrobel et al., 2008). One isolate had an identical diploid sequence type (DST 785) with a deer isolate (strain DA047). In all four C. albicans pig strains, genotype designations for the ACC1, ADP1, MPI1, SYA1 and ZWF1b loci were identical (7, 6, 3, 6 and 37 respectively), matching eight of the 11 wildlife isolates described by Wrobel et al. (2008).
The ease of identifying C. albicans-positive pigs from the VDL contrasted sharply with results from VRF pigs. Of 34 randomly selected VRF pigs, only nine were yeast-positive (three nursery pigs, two growers and four finishers). Importantly, none of the pigs was positive for C. albicans. A single yeast species each was isolated from eight of the nine pigs, including C. catenulata (three pigs), Trichosporon montevideense (two) and Candida glabrata, C. tropicalis, and Debaryomyces hansenii (one pig each). One pig had two different yeasts: C. catenulata and D. hansenii.

Stable C. albicans colonization of piglets

Lack of C. albicans isolation from VRF pigs but frequent isolation among pigs from other farms suggested that pigs may be colonized naturally with C. albicans, as long as an encounter occurs. We hypothesized that the C. albicans-negative status of the VRF pigs was due to biosecurity practices that prevent direct or indirect contact of pigs with wildlife and/or their feces. To test this hypothesis, normally farrowed VRF piglets were removed from the sow at 4 days, housed individually in an artificial rearing environment and fed commercial sow milk replacer. Following acclimation, pigs were fed a C. albicans inoculum to test

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**Fig. 1.** Pig growth (increase in kg body weight) over the study timeline. Purple = strain SC514; red = strain 216; blue = uninoculated control; green = strain 3317.

**Fig. 2.** Heatmap depiction of Candida albicans colonies grown from swabs of each pig's oral cavity, rectum and environment over the study timeline. A scale of 0–6 (defined in the text; colored scale bar shown) described the number of CFU grown from each swab on an agar plate. Darker squares indicate higher CFU counts, while beige squares indicate a negative result. Candida albicans strain and individual pig numbers are shown at the right of the figure. Control pigs were not inoculated with C. albicans and remained negative throughout the experiment. Because group (1) pigs were necropsied 1 day later than group (2) pigs, an additional observation was available (day 14). White squares are used as placeholders for the group (2) pigs.
with the mucosal surface. Examination of CFU g⁻¹ mucosal dry weight from each animal revealed yeast cells in the lumen or associated loosely with the mucosal surface. Examination of the entire oro-GIT revealed no evidence of hyperkeratosis, edema, swelling, hemorrhage, erosion or ulceration, consistent with the conclusion that C. albicans was part of the pig commensal microbiota. Limited microscopic examination of GIT tissue revealed yeast cells in the lumen or associated loosely with the mucosal surface. Examination of CFU g⁻¹ mucosal dry weight showed C. albicans colonization up to 10⁷ CFU g⁻¹ in the esophagus (Fig. 3). In general, CFU g⁻¹ values decreased in the lower GIT. Overall, necropsy data paralleled swab data. For example, pig 3317 (1) had few CFU from rectal swabs and necropsy specimens from the lower GIT. Candida albicans was detected throughout the GIT in inoculated animals, but not in the negative controls, where colony counts were zero. Candida albicans colonies recovered from each animal were tested using MLST and verified that the recovered strains were the same as the inoculation strain. These data demonstrated that simply feeding C. albicans to a normally farrowed, untreated piglet resulted in stable colonization with the C. albicans strain of choice.

Discussion

Our culture-based survey of pigs from various farms established that C. albicans can exist as a commensal of normally healthy swine. Identification of a local source of C. albicans-negative pigs suggested their utility in creating a model of C. albicans commensalism. This model demonstrated stable colonization without the need for antimicrobial treatment that is a hallmark of the most commonly used murine model. The pig model allows C. albicans-host interactions to be studied in the presence of the normal GIT microbiota. While the cost of experimental pigs is higher than mice, the piglet studies can be conducted in a non-intensive environment, without requirements for specialized equipment or skills. These features suggest that the model is easily adaptable for use by other investigators.

The yeasts recovered from pigs in our study are among those documented in domestic swine. Urubschurov et al. (2008) identified 17 cultivatable yeasts from the GIT of piglets reared on both a commercial and research farm in Germany. All of the yeasts we identified were on their list, with the exception of C. rugosa and D. hansenii, suggesting broad commonalities between GIT yeasts in swine. Importantly, none of the German pigs had C. albicans, supporting our hypothesis that the C. albicans status of a herd is largely the result of management practices.

The literature documents the presence of C. albicans in commercial swine herds, even as the cause of disease. Zlotowski et al. (2006) described necropsy findings from two pigs infected with porcine circovirus 2 (PCV2) that causes postweaning multisystemic wasting syndrome.

![Heatmap depiction of CFU g⁻¹ mucosal dry weight from each pig](https://academic.oup.com/femsle/article-abstract/357/1/10/2684474/bys)
Each pig had oral or esophageal mucocutaneous lesions from which C. albicans was isolated. PCV2 infection is associated with compromised immune function (Lee et al., 2010), characterized by decreased counts of circulating B and T lymphocytes and depletion of lymphocytes in lymphoid tissues (Segalés et al., 2001; Darwich et al., 2002). These results suggest further intriguing parallels between the pig model and human disease: lymphocyte depletion and immunosuppression caused by HIV infection frequently leads to oro-esophageal candidiasis, which is an initial hallmark of AIDS (Powderly et al., 1999).

An unexpected finding of our work is the large number of C. albicans CFU associated with the esophageal mucosa. Cole et al. (1996) noted that the esophagus is the most common site for human gastrointestinal candidiasis, and the gastroesophageal junction is the most frequently colonized site in the human GIT. These results provide even stronger connections between our piglet model and humans, and suggest the potential for C. albicans esophageal growth to serve as an inoculum that continually seeds the lower GIT.

Future efforts will further evaluate the utility of our piglet model for studying C. albicans–host interactions in the context of colonization and disease. Immunosuppressive treatments could be tested for their effect on extraintestinal C. albicans translocation. The normal microbiota could be evaluated for its role in protection against disseminated disease. The model may prove useful for testing potential treatments or nutritional interventions that could reduce C. albicans colonization.

Acknowledgements
We thank Dennis Birkey and Ted Shearer of the Veterinary Research Farm for their support of the study. We thank Dennis French for assistance with inoculating pigs with C. albicans. We thank Dimitri Kashtanov, Max Van Tassell and Dexter Chen for their assistance with processing tissue samples. The University of Illinois Campus Research Board provided funding for this work.

References


