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. Andrits 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 gut microbiota 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. 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. albicans* 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 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 (20 g peptone and 20 g Bacto agar) with chloramphenicol at the University of Illinois and sampled to detect yeasts.

**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:
The second experiment are featured here. However, results cate animals were used for each treatment, results from related control pig was placed in each room. Because dupli-
14 days (2nd group) while fed milk replacer. An uninocu-
in separate rooms, and monitored for 13 (1st group) or housed individually, with complete experimental replicates
lates were tested using two pigs per strain. Pigs were
ADEK, 2.8 lbs DL-methionine, 2 lbs iodized salt and
60 lbs zinc oxide, 7 lbs swine trace minerals, 4 lbs Vitamix
604.8 lbs soybean meal, 200 lbs lactose, 150 lbs apprentice,
60 lbs fat, 22.8 lbs lime, 12.6 lbs dicalcium phosphate,
8 lbs zinc oxide, 7 lbs swine trace minerals, 4 lbs Vitamix
ADEX, 2.8 lbs DL-methionine, 2 lbs iodized salt and
0.8 lbs lysine). In the second experiment, C. albi-
cans 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 uninocu-
ated control pig was placed in each room. Because duplic-
ate 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 euthana-
ized 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 jeju-
num, 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 por-
tion 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 calcu-
de CFU g⁻¹ dry weight. Reported results were rounded
to the nearest order of magnitude.

In addition to the piglets housed in the artificial rear-
ing 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 sub-
mited 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 dis-
ease (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 asahiii (one) and Can-
dida catenulata (one) were also isolated from the C. albi-
cans-positive pigs. Issatchenka orientalis (two pigs),
C. catenulata (one), C. tropicalis (two) and Candida rug-
osa (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 des-
ignations for the ACC1, ADP1, MPI1, SYA1 and ZWF1b
loci were identical (7, 6, 3, 6 and 37 respectively), match-
ing 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...
the stability of *C. albicans* colonization and its effect on pig growth and health. Pigs were assigned randomly to one of four experimental groups: an uninoculated control, or inoculation with one of three *C. albicans* strains.

The mean total change in piglet body weight over the course of the study (Δkg) was 5.0 kg (Fig. 1). There was no significant difference between Δkg values for control and *C. albicans*-inoculated piglets (*P* = 0.10 using the Generalized Linear Model with Tukey’s mean separation). Colony counts from plating oral, rectal and environmental swabs showed that *C. albicans* passed through the piglets within 24 h (Fig. 2). After that time, *C. albicans* was detected readily from rectal swabs of most animals, but at a colony count considerably lower than observed for the oral swabs. Oral swab counts remained high throughout the course of the study, with greater numbers recorded for strains 216 and 3317 (both strains of oral origin) compared with SC5314, a human bloodstream isolate from a disseminated candidiasis patient (Gillum *et al.*, 1984). *Candida albicans* in the environment was low, but detectable over the course of the experiment. In all instances, swabs from the uninoculated control piglets were *C. albicans*-negative.

A veterinary pathologist conducted a necropsy of each animal. Gross 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 nonintensive 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.
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 (Segalès 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 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.

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**References**


