ILAR Journal, 2015, Vol. 56, No. 1, 63–73

doi: 10.1093/ilar/ilv006
Article

Human Microbiota-Associated Swine: Current Progress and Future Opportunities

Mei Wang and Sharon M. Donovan

Mei Wang, PhD, is a research specialist and Sharon M. Donovan, PhD, RD, is a professor in the Department of Food Science & Human Nutrition, University of Illinois, Urbana, Illinois.

Address correspondence and reprint requests to Dr. Sharon M. Donovan, Department of Food Science & Human Nutrition, University of Illinois, 905 South Goodwin Avenue, 339 Bevier Hall, Urbana, IL 61801 or email sdonovan@illinois.edu.

Abstract

Gnotobiotic (GN) rodent models have provided insight into the contributions of the gut microbiota to host health and preventing disease. However, rodent models are limited by several important physiological and metabolic differences from humans, and many rodent models do not dependably replicate the clinical manifestations of human diseases. Due to the high degree of similarity in anatomy, physiology, immunology and brain growth, the domestic pig (*Sus scrofa*) is considered a clinically relevant model to study factors influencing human gastrointestinal, immune, and brain development. Gnotobiotic piglet models have been developed and shown to recapitulate key aspects of GN rodent models. Human microbiota-associated (HMA) piglets have been established using inocula from infants, children, and adults. The gut microbiota of recipient HMA piglets was more similar to that of the human donor than that of conventionally reared piglets harboring a pig microbiota. Moreover, *Bifidobacterium* and *Bacteroides*, two predominant bacterial groups of infant gut, were successfully established in the HMA piglets. Thus, the HMA pig model has the potential to be a valuable model for investigating how the gut microbiota composition changes in response to environmental factors, such as age, diet, vaccination, antibiotic use and infection. The HMA also represents a robust model for screening the efficacy of pre- and probiotic interventions. Lastly, HMA piglets can be an ideal model with which to elucidate microbe–host interactions in human health and disease due to the similarities to humans in anatomy, physiology, developmental maturity at birth, and the pathophysiology of many human diseases.

Key words: gnotobiotic; gut microbiota; human microbiota-associated; piglet; prebiotics; probiotics

Introduction

The human gut is colonized by a complex microbial community with a population approximately 3 to 10 times greater than the total number of host cells of which the body consists (Björkstén et al. 2001). Germ-free (GF) animal studies have shown that gut microbiota and their hosts do not simply coexist, but rather form a mutualistic relationship (Hooper et al. 2001). It is now clear that the structure and functions of the gut microbiota play a crucial role in human health through its contributions in fermentation of undigested carbohydrates, vitamin biosynthesis, regulation of energy storage, maturation of the immune system, pathogen colonization resistance, and brain development (Douglas-Escobar et al. 2013; Li et al. 2014). Alteration in the composition of the gut microbiota has been associated with digestive tract diseases, including necrotizing enterocolitis (NEC) (Mai et al. 2011; Wang et al. 2009) and inflammatory bowel diseases (IBD) (Aomatsu et al. 2012; Michail et al. 2012; Schwiertz, Jacobi et al. 2010; Walker et al. 2011). Additionally, strong evidence from human studies and animal models links intestinal microbiota dysbiosis with a broad-range of immune, metabolic, and neurodevelopmental disorders (Li et al. 2014), including asthma (Vael et al. 2011), eczema (Gore et al. 2008; Wang et al. 2008), obesity (Karlsson et al. 2012; Ley et al. 2006; Schwiertz, Taras et al. 2010; Turnbaugh et al. 2009), and autism (Kang et al. 2013; Parracho et al. 2005; Wang et al. 2013).

Defining the mechanistic underpinnings whereby the intestinal microbiota influences human health and disease has been hampered by individual variation in host genetics and microbiota and ethical concerns of using invasive procedures in human subjects, particularly infants and children. Animal models, especially GN rodents, have been extensively employed for exploring the cross talk between the host and commensal bacteria (Chow et al. 2010; Gootenberg and Turnbaugh 2011; Leser and Mølbak 2009; Smith et al. 2007). Comparative studies of GF and conventional (CV) mice have demonstrated that the gut microbiota profoundly impacts host biology, ranging from intestinal morphology and motility, mucosal and systemic immunity, to absorptive and metabolic functions (Smith et al. 2007). The term CV refers to an animal or human colonized by the microorganisms normally associated with its particular species. For example, GF mice have shorter ileal villi and crypt, and slower rate of small intestinal cell turnover than CV mice (Smith et al. 2007; Yi and Li 2012). Furthermore, GF animals have fewer and smaller Peyer's patches and mesenteric lymph nodes and greatly reduced fecal IgA than animals raised under specific pathogen-free (SPF) conditions (Honda and Takeda 2009; Macpherson and Harris 2004; Round and Mazmanian 2009). Moreover, gene expression profile of mouse ileal epithelium is altered in the absence of commensal bacteria (Hooper and Gordon 2001).

While GN rodent models have provided insight into host–microbe interactions, rodent models are limited by several important physiological and metabolic differences from humans (Graham and Aman 1987; Heinritz et al. 2013). More importantly, many rodent models do not dependably replicate clinical manifestation observed in human diseases (Lunney 2007). Therefore, more clinically relevant animal models are needed. Nonhuman primates are good models for humans because they share significant physiological, metabolic, biochemical, and genetic similarity with humans; however, expensive housing, long lifespan, and ethical concerns limit their use (Puiman and Stoll 2008; Shen 2010). The domestic pig (*Sus scrofa*) is closely related to the human in terms of anatomy, physiology, and genetics, and is considered the preferred nonprimate model for humans (Dawson 2011; Guilloteau et al. 2010; Meurens et al. 2012; Odle et al. 2014). In addition, the piglet is an excellent model for infectious diseases (Meurens et al. 2012). The goal of this review is to highlight the usefulness and limitations of the CV pig as a model for human gastrointestinal physiology, immunology, and neurodevelopment. In addition, findings of recent studies using GN and human microbiota-associated (HMA) pigs, and future directions with the model will be discussed.

The Piglet as a Model for Human Physiology, Immunology, and Neurodevelopment

Pigs have served as biomedical models for decades. Advantages of the swine model are highlighted in Table 1. Swine have high genome and protein sequence homology with humans, which facilitates understanding of gene–microbiome interactions and the availability of molecular probes and antibodies. For example, when porcine reagents are not available, antibodies and probes directed against human proteins and gene sequences often cross-react with porcine samples (Lunney 2007). From a nutritional perspective, pigs and humans are omnivorous, whereas rodents are granivorous. In terms of the gastrointestinal anatomy and physiology, pigs are also more similar to humans than are rodents (Guilloteau et al. 2010; Odle et al. 2014). Also, both pigs and human are colon fermenters, whereas fermentation take place in the cecum of rodents (Heinritz et al. 2013). Pigs are also immunologically similar to humans. For example, porcine immune responses more closely resemble human responses than mouse responses with >80% of parameters studied, whereas the immune response in mice was more similar to the human in <10% of comparisons (Dawson 2011). Humans and pigs also share similar brain growth and development patterns. The major brain growth spurt of the pig extends from late prenatal to the early postnatal period, resembling that of the human, which is different from other animals including rats (Dobbing and Sands 1979). Additionally, gross anatomical features such as gyral pattern and gray and white matter distribution of the piglet brain are comparable to those of human infants (Conrad et al. 2012). Furthermore, the possiblity of using pigs from the same litter and similar disease progression make the pig an excellent model for human gastrointestinal physiology, immunology, and neural development.

Due to similarities in immune function, pigs are also an outstanding model for infectious diseases and vaccine development (Meurens et al. 2012) and have been used extensively to study infectious diseases relevant to human health, including respiratory (*Bordetella pertussis* [Elahi et al. 2007], cornona virus [Saif 1996], influenza viruses [Khatri et al. 2010], *Mycobacterium tuberculosis* [Gil et al. 2010], *Pseudomonas aeruginosa*, and *Staphylococcus aureus* [Nielsen et al. 2009]) and gastrointestinal pathogens (*Cryptosporidium parvum* [Vítovec and Koudela 1992], *Helicobacter pylori* [Nedrud 1999], hepatitis E virus [Krawczynski et al. 2011], norovirus [Cheetham et al. 2006], and rotavirus [Saif et al. 1996]).

Limitations of the Conventional Pig Model

Conventional piglets are extensively used for studies of early nutrition on gastrointestinal, immune and neural development (Guilloteau et al. 2010; Odle et al. 2014; Rytych et al. 2012); however, a major limitation of the CV piglet model is that the gut microbiota of piglets differs from that of human infants. Phyla-level gut bacterial composition of mother-fed or formula-fed (FF) 3-month-old infants (Donovan et al. 2012) and 21-day-old piglets (unpublished observations) are compared in Figure 1. While differences between mother-fed or FF neonates of both species can be appreciated, marked differences in the gut microbiota

Table 1 Advantages of the swine model

- Omnivorous – nutritional requirement and physiology similar to human
- High genome and protein sequence similarities with human
- Immune system more closely resembles human
- Brain growth and development patterns similar to human
  - The major brain growth spurt similar to human
  - Gross anatomical features of the brain are comparable to that of human infants
- Body size – allowing various surgical manipulation and collection of adequate quantity of samples.
- Large litter size (10–12 piglets/litter)
- Similar disease progression
  - Metabolic diseases, such as obesity and heart disease
  - Infectious diseases (e.g., influenza viruses, rotavirus, *Helicobacter pylori*, and *Neisseria meningitides* infection)

Conrad et al. (2012); Dobbing and Sands (1979); Lunney (2007); Meurens et al. (2012)

between neonates of the two species exist. For example, Actinobacteria (mainly *Bifidobacterium*) predominates (>50% of 16S rRNA sequences) in both breastfed (BF) and FF infants, whereas little Actinobacteria (<0.2% of 16S rRNA sequences) is detectable in piglets. The predominant phyla in both sow-reared (SR) and FF-fed piglets are Bacteroidetes and Firmicutes, which is more similar to the adult human (Ley et al. 2006). Additionally, both SR and FF piglets have greater microbial diversity than human infants. Establishment of the intestinal microbiota after birth plays a vital role in development of the neonatal gastrointestinal and immune systems (Adlerberth and Wold 2009; Sjögren et al. 2009). Recent data have also shed light on the ability of microbiota to influence brain development and behavior (Collins et al. 2012; Desbonnet et al. 2014; Diaz Heijtz et al. 2011). However, differences in the native gut microbiota between the infant and the piglet complicate direct translation of results from piglets to humans. A solution to this problem is to develop piglets harboring a human gut microbiota.

Germ-Free and Gnotobiotic Pigs

Gnotobiotic animals are animals colonized with known strains of bacteria or microbiota. They are delivered by cesarean section (or sterile hatching of eggs) under aseptic conditions and are raised within sterile isolators and fed sterile water and food in order to control their exposure to microorganisms (Butler 2009; Gustafsson et al. 1957). Germ-free animals are gnotobiotic animals that have been maintained free from microorganisms, including bacteria, fungi, viruses, and parasites throughout their life. Gnotobiotic experiments take advantages of highly controlled, repeatable experimental design, which reduces interindividual variation. As of the writing of this review, over 500 publications have used GN piglets.

Gnotobiotic pigs have been used to study the impact of bacterial colonization on the host, including organ growth, intestinal morphology, physiology, and immune development (Table 2). Relative to CV pigs, GF pigs have smaller thyroid and liver size, but larger spleen, lung, heart, and gall bladder mass at 7 weeks of age (Shurson et al. 1990). Shirkey and colleagues (2006) investigated the effects of colonization of different bacterial species on small intestinal morphology and observed that the relative length of the small intestine (SI) was smaller in GF and mono-associated (MA) piglets than in CV piglets at postnatal day 13. They also showed that GF and MA piglets had lower relative weights of proximal SI regions than that of CV piglets. This is consistent with previous findings reporting that the SI thickness of GF pigs was lower than CV pigs (Shurson et al. 1990). In addition, GF and MA piglets had shorter crypt depths, longer villi height, reduced lamina propria cellularity, and smaller Peyer's patches in their SI compared to their CV counterparts (Shirkey et al. 2006; Willing and Van Kessel 2007). The intestinal microbiota also affects brush border enzyme activities. Aminopeptidase N and lactase phlorizin hydrolase activities were lower in SI enterocytes of CV piglets in comparison with piglets maintained GF or mono-

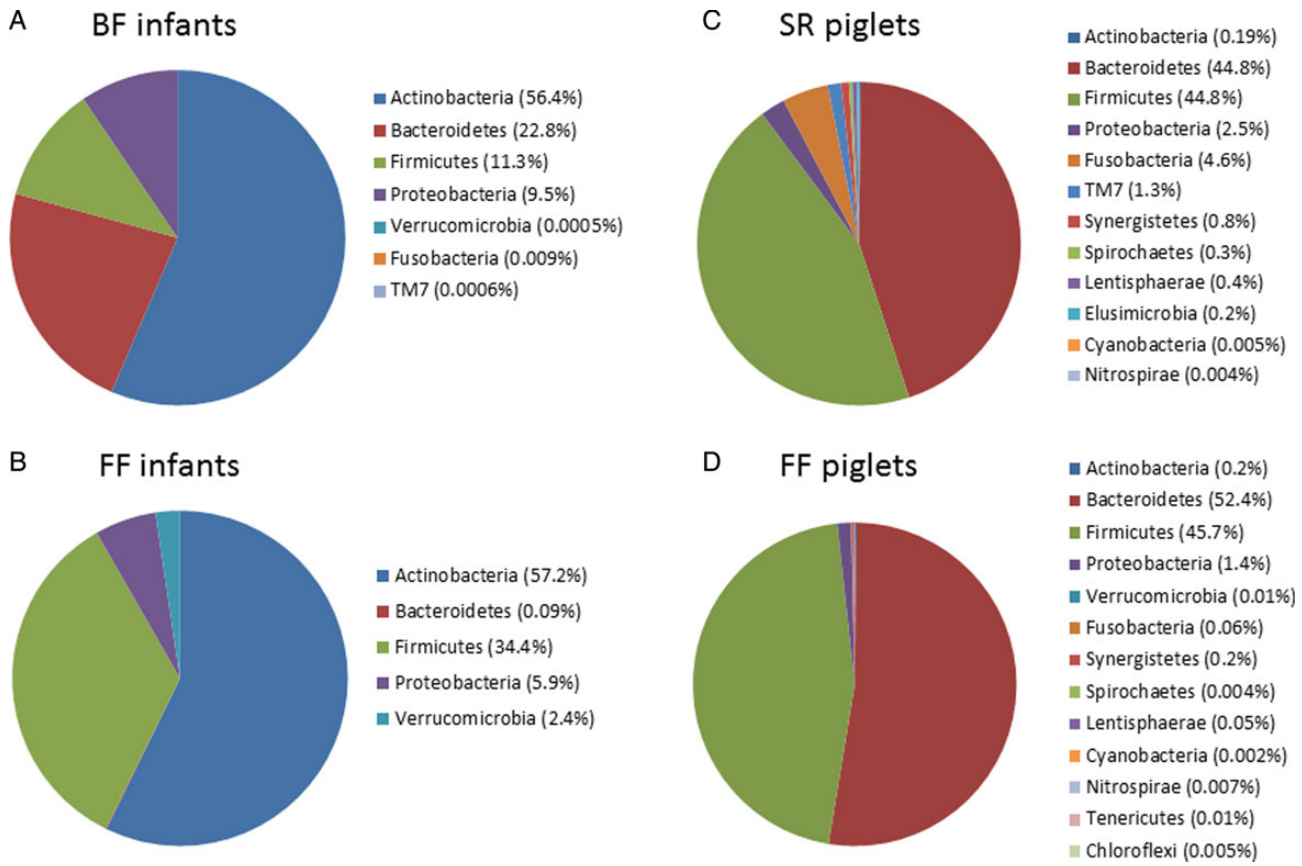

Figure 1 Mean relative abundances of bacterial phyla within fecal microbiota of 3-month-old infants and 21-day-old piglets. Data were obtained by pyrosequencing of V1–V3 regions of bacterial 16S rRNA genes. The numbers in parentheses in the figure legends indicate the percentage of total 16S rRNA sequences. Panel: (A) BF infants at 3 months; (B) FF infants at 3 months; (C) SR piglets at 21 days (D) FF piglets at 21 days. Human infant data are from Chapkin et al. (2010), and piglet data are unpublished. Abbreviations: BF, breast-fed; FF, formula-fed; SR, sow-reared.

Table 2 Differences in morphology, physiology, and immunology between gnotobiotic and conventional pigs

Outcome	Remarks	References
Organ growth	GF pigs had a smaller thyroid and liver, but larger spleen, lung, heart, and gall bladder than CV at 7 weeks of age.	Shurson et al. (1990)
Relative SI length & weight	In GF and MA pigs, the relative length of SI was reduced compared with CV at postpartum day 13.	Shirkey et al. (2006)
	Compared to GF and MA, relative weight of proximal SI regions was higher for CV; while higher relative weight in the distal regions was reported in GF.	Shirkey et al. (2006)
	The SI thickness of GF pigs was reduced compared with CV.	Shurson et al. (1990)
SI morphology	Relative to CV pigs, GF & MA pigs had short crypt depths and longer villi height.	Shirkey et al. (2006); Willing and Van Kessel (2007)
	GF and MA pigs had reduced lamina propria cellularity; GF had smallest Peyer's patch, MA intermediate, and CV largest.	Shirkey et al. (2006)
	GF pigs had a slower turnover of SI epithelial cells compared with CV pigs.	Shurson et al. (1990); Willing et al. (2007)
Brush border enzyme activity	Aminopeptidase N and lactase phlorizin hydrolase activities were lower in SI enterocytes of CV pigs in comparison with GF and MA pigs at 14 days of age.	Willing et al. (2009)
Immune development	GF pigs had fewer leukocytes and lower proportion of mature neutrophils in blood at 7 weeks of age.	Shurson et al. (1990)
	Mono-associated GF pigs with *Escherichia coli* strains increased numbers of dendritic and T cells in diffuse lymphoid tissue of the jejunum 2 days post-association.	Haverson et al. (2007)
	Serum immunoglobulin level in piglets colonized with a mixture of defined bacteria was significantly higher than in GF piglets in the first 6 weeks of life.	Butler et al. (2000)
	SI expression of proinflammatory cytokines IL-1β and IL-6 were higher in GF and MA pigs relative to CV at postnatal day 13.	Shirkey et al. (2006)

Abbreviations: CV, conventional; GF, germ-free; MA, mono-associated; SI, small intestine.

associated with nonpathogenic *Escherichia coli* or *Lactobacillus fermentum* at 14 days of age (Willing and Van Kessel 2009). Shorter villus height was observed in CV pig enterocytes (Willing and Van Kessel 2007), thus the lower enzyme activity in CV pigs may be partly explained by reduced cell maturity or mature cell number. In addition, reduced enzyme activity in CV pigs could be due to microbial brush border enzyme deactivation (Willing and Van Kessel 2009).

Several studies have investigated the role of bacterial colonization on the host immune development. Haverson and colleagues (2007) compared the immunological structure of the lamina propria in the jejunum of GF piglets with piglets associated with two strains of commensal *E.coli* between 1 and 4 days of age. By two days after transfaunation, they found that mono-association of GF piglets with *E. coli* increased the numbers of dendritic and T cells in diffuse lymphoid tissue of the jejunum. Additionally, SI expression of proinflammatory cytokines interleukin-1β (IL-1β) and IL-6 were higher in GF and MA piglets compared with CV piglets at postnatal day 13 (Shirkey et al. 2006). Other studies have shown effects on systemic immunity as well; relative to GF piglets, serum immunoglobulin level of piglets colonized with a mixture of defined bacteria was significantly greater on the first 6 weeks of life (Butler et al. 2000).

Gene microarray profiling of the SI epithelium in GF and CV piglets confirmed the essential role of a commensal microbiota for normal development of the host intestinal transcriptome. Genes involved in transcription, cell proliferation and differentiation, nutrient transport and metabolism, xenobiotic metabolism and immune responsiveness were upregulated in GN piglets bearing a microbiota from CV piglets versus GF piglets (Chowdhury et al. 2007).

Despite the fact that GN piglets differ in aspects of gastrointestinal and immune development relative to CV piglets, GN pigs still provide a unique and powerful model for study of enteric diseases that affect both humans and pigs. For example, GN piglets have been used to investigate disease pathogenesis and/or immunity to rotavirus (Saif et al. 1996), enterohemorragic *Escherichia coli* (Brady et al. 2011), *Clostridium difficile* (Steele et al. 2010), and *Shigella dysenteriae* Type I (Jeong et al. 2010) infections, among others (Meurens et al. 2012). Furthermore, beneficial effects of probiotics (Azevedo et al. 2012; Liu et al. 2013) and the application of vaccination (Jeong et al. 2013) have been tested with GN pig infection models.

The Human Microbiota–Associated (HMA) Pig Model and its Current Application

In most GN pig studies, pigs were colonized with single or multiple strains of bacteria. These studies are useful for delineating the physiological functions of specific microbes, but the effects of single or multiple bacteria on the host are not representative of a complex microbiota. Recently, HMA animal models have been developed, both in rodents and pigs. The HMA rodent model has been used to investigate how the gut microbiome is influenced by dietary components and, in turn, influences host health and disease (Gootenberg and Turnbaugh 2011). For example, production of equol from dietary soy isoflavone, the microbial reduction of cholesterol, the effects of a defined diet changes on the gut microbial community structure and functions, and the biogeography and assembly of the gut microbiota have all been studied in HMA rodent models (Gootenberg and Turnbaugh 2011). Additionally, HMA mice have been important for understanding the role of the microbiota in a variety of human diseases (Gootenberg and Turnbaugh 2011), including the biological effects of microbiota obtained from obese adults (Turnbaugh et al. 2006) or children with kwashiorkor (Smith et al. 2013). These studies have definitively proven that the clinical signs and symptoms commonly associated with many human diseases could be recapitulated by transferring the microbiome and, in the case of

kwashiorkor, providing a similar diet (Smith et al. 2013). However, due to the differences in anatomy and physiology between rodents and humans, some important members of human gut microbiota, such as *Bifidobacterium* do not readily colonize the rodent gut (Raibaud et al. 1980). Thus, results obtained from the use of rodent models may be difficult to extrapolate to humans, especially human infants, who are extensively colonized with bifidobacterial species.

Several studies have investigated the possibility of transfaunation of gut microbiota from humans to piglets (Table 3). In the study of Pang and colleagues (2007), piglets delivered by cesarean section were housed in an SPF barrier system and were inoculated orally with a fecal suspension collected from a healthy 10-year-old boy. The culture-independent analysis of the gut microbiota of recipient piglets and the human donor revealed that the microbiota of HMA piglet was more similar to that of human donor than to that of CV piglets. Moreover, *Bifidobacterium* and *Bacteroides*, two predominant bacterial groups of the infant gut, were successfully established in the gastrointestinal tract of piglets. Furthermore, introduction of solid food during the weaning period significantly altered the gut microbiota in HMA piglets; this change in the gut microbiota is similar to that observed in human infants (Table 3).

In another study, piglets derived by cesarean section were inoculated with human infant or adult microbiota (Zhang et al. 2013) (Table 3). The piglets were housed in sterile isolators and maintained on infant formula or solid diet for swine. High throughput sequencing of the 16S rRNA V6 region was used to monitor to what extent the transplanted human microbiota changed in piglets over time. When infant stool was transferred, the microbiota composition of the HMA piglets converged toward that of the human donor. In contrast, the microbiota of HMA piglets harboring the adult human microbiota did not converge toward the composition of the donor even 20 days post-inoculation. In a more recent study (Zhang et al. 2014), piglets derived by hysterectomy were inoculated with a suspension of fecal samples obtained from a BF infant between 17 and 23 days postpartum. The piglets were maintained in germ-free isolators and fed sterilized infant formula. Sequencing the V4 region of 16S rRNA genes showed that HMA pigs harbored a microbiota similar to that of the infant donor. Collectively, these studies demonstrate the feasibility of transplantation of a complex human gut microbiota to piglets. Additionally, in comparison with the CV counterparts, the intestinal immunity of HMA piglets is well developed (Che et al. 2009), whereas that of HMA rodents is not (Imaoka et al. 2004). Therefore, the HMA piglet model provides a significantly improved system for research on gut ecology and host–microbe interactions, particularly when the human infant is the population of interest (Pang et al. 2007; Zhang et al. 2013, 2014).

The HMA pig model has been used in several recent publications to study dietary prebiotics and probiotics and for infection models. The first use of HMA piglets was described by Shen and colleagues (2010), who studied the prebiotic activity of short-chain fructo-oligosaccharides (scFOS). The piglets were inoculated with fecal suspension from a 27-year-old man and fed basal diets alone (control) or supplemented with scFOS at 0.5 g/kg body weight daily for 37 days after birth. The composition of the fecal microbiota was monitored by denaturing gradient gel electrophoresis and quantitative polymerase chain reaction (PCR). As demonstrated previously in human trials (Bouhnik et al. 1999; Gibson et al. 1995), supplementation of scGOS increased the abundance of *Bifidobacterium*. The bifidogenic effect of GOS (3 g/L of formula) has also been examined in newborn CV piglets; however, no significant increase in fecal bifidobacteria abundance was detected after 15 days of supplementation. Differences in bifidogenic effects of FOS observed in CV and HMA piglets may partly due to differences in *Bifidobacterium* species composition between HMA and CV piglets. For example, HMA piglets harbor *Bifidobacterium* of human origin, such as *B. longum*, *B. breve*, *B. catenulatum*, and *B. adolescentis*, while *Bifidobacterium* found in the gut of piglets are *B. suis*, *B. globosum*, and *B. pseudolongum* (Harrman and Knol 2005; Heinritz et al. 2013). Previous studies have shown prebiotics stimulate bifidobacteria species differently. For example, a mixture of scGOS and polydextrose in infant formula increased *B. longum* but not *B. catenulatum* counts (Scalabrin et al. 2012). Because of the important role of *Bifidobacterium* in preventing intestinal infection, promoting gut integrity, and modulating the host immune homeostasis (Gibson and Roberfroid 1995), stimulating the growth of gut *Bifidobacterium* is considered as a marker of prebiotic effect (Roberfroid et al. 2010). Therefore, HMA piglets provide a more attractive model than CV piglets for evaluation of potential prebiotics.

Another application of the HMA piglet model is for testing therapeutic interventions, such as probiotics and vaccination on host immune response and gut microbiota. Wen and colleagues (2014) tested dose-dependent effects of *Lactobacillus rhamnosus* GG (LGG) on the immune response to human rotavirus (HRV) vaccination in the HMA pig model. They observed that the human gut microbiota stimulated neonatal immune development, as evidenced by a significant increase in the frequencies of interferon (IFN)-γ producing T cells and a decrease in the frequencies of CD4+CD25-FoxP3+ regulatory T cells (Tregs), and IL-10- or TGF-β-producing Tregs in HRV-vaccinated pigs. Furthermore, the higher dose of LGG (14 doses, up to a 10^9 colony-forming-unit [CFU]/dose), but not the lower dose (9 doses, up to 10^6 CFU/dose), increased the LGG counts in the intestinal contents of HMA pigs and significantly enhanced HRV-specific IFN-γ-producing T cell responses. Moreover, oral supplementation of LGG prevented the changes in gut microbial composition caused by HRV infection (Zhang et al. 2014).

Future Opportunities for the HMA Pig Model

The HMA Pig Model for Studies of Human Gut Microbiome

Because of the important role of human microbiota in the maintenance of health and causation of disease, several international efforts have designed to the study of human microbiota in recent years, including the Human Microbiome Project (HMP) (http://commonfund.nih.gov/hmp/index [Human Microbiome Consortium 2012]) and the Metagenomics of the Human Intestinal Tract (MetaHIT) (www.metahit.eu [Qin et al. 2010]) initiatives. While much progress has been made by describing the composition of the gut microbiome in the human population and linking it to age- and health-related outcomes, much of the data at this point are associative. Furthermore, confounding factors that influence the composition of the gut microbiota are difficult or impossible to control at the present time in human studies. These factors include individual variation in the host genetics and microbiota, current and past environmental exposures, and dietary nutrient composition and caloric load (Gootenberg and Turnbaugh 2011). The HMA pig model provides the ability to minimize many of the confounding variables mentioned above and, as such, will be valuable for studying microbiota composition change due to external factors, such as age, diet, viral infections, vaccination, and antibiotic use on the development of gut microbiota.

Table 3 Studies on the development of human microbiota-associated piglet model

	Pang et al. (2007)	Zhang et al. (2013)			Zhang et al. (2014)
		Experiment 1	Experiment 2	Experiment 3	
Human donors	10-y-old boy ($n = 1$)	Adults ($n = 10$; 50–70 y)	3-mo-old BF baby ($n = 1$)	Adults ($n = 10$; 50–70 y)	17–23 d-old BF infant ($n = 1$)
Fecal inoculation	1 ml of 5% fecal suspension	3 ml of 10% fecal suspension	3 ml of 10% fecal suspension	3 ml of 10% fecal suspension	1 ml of 5% fecal suspension
Age inoculated	d 0–2, 4, 6, 8, and 10	Once at d 8	Once at d 5 or d 30	Once at d 23	d 0–2
Length of study	35 d	28 d	15 or 41 d	36 d	33 d
Housing	SPF system	Sterile isolator	Sterile isolator	Sterile isolator	Sterile isolator
Diet	d 0-18: Piglet formula d 19-35: Infant cereal	Infant formula	Infant formula	d 0-22: Infant formula d 23-36: Solid porcine diet	Sterile formula
Analytical methods	ERIC-PCR, TTGE, qPCR	Illumina HiSeq	Illumina HiSeq	Illumina HiSeq	Illumina MiSeq
Major findings					
	• GN microbiota more closely resembled donor than other humans or CV pigs. • Microbial succession was similar to humans.	• ↓ diversity • Large UniFrac distance in microbiota between donor and HMA pigs	• ↓ Proterobacteria • ↑ Firmicutes • Maintain diversity • Microbiota converged toward human donor	• ↓ diversity • Large UniFrac distance in microbiota between donor and HMA pigs.	• GN pigs carried a microbiota similar to the human donor's microbiota
Conclusions	Transplantation of gut microbiota from human to piglets is feasible.	The pig intestine can be colonized with human fecal microbiota to generate a realistic model of human GI tract.			Human gut microbiota could be transplanted to and colonize GN pigs.

Abbreviations: BF, breast-fed; CV, conventional; d, day; ERIC-PCR, enterobacterial repetitive intergenic consensus sequence-PCR; GI, gastrointestinal; GN, gnotobiotic; HMA, human microbiota-associated, qPCR, quantitative PCR; SPF, specific pathogen free; TTGE, temperature gradient gel electrophoresis; y, year.

The HMA Pig Model for Investigating the Role of Microbiota on Normal Development

Establishment of the gut microbiota after birth plays an important role in stimulating the development of the neonatal gastrointestinal, immune and neural systems. Studies in GF animals have shown that colonization of the commensal microbiota is required for normal intestinal epithelial cell proliferation and migration, and maintenance of villus morphology (Shirkey et al. 2006; Willing and Van Kessel 2007, 2009). GF animals do not develop normal lymph node architecture and have a reduced antibody production (Macpherson and Harris 2004; Round and Mazmanian 2009). Evidence for the role of gut microbiota in neural development is intriguing, and mechanistic data is rapidly emerging. Diaz Heijtz and colleagues (2011) investigated the impact of colonization of gut microbiota on the mammalian brain development and behavior and reported that GF mice displayed increased motor activity and reduced anxiety compared to SPF mice with a normal gut microbiota. Additionally, GF mice exposed to gut microbiota early in life showed characteristics similar to SPF mice, including reduced expression of synaptophysin and PSD-95, two proteins that are specifically involved in synaptogenesis pathways (Diaz Heijtz et al. 2011).

Studies of gastrointestinal, immune, and neural development often require tissue collection from the gastrointestinal tract, immune organs, and brain; however, due to ethical concerns and the limitation of invasive procedure, collecting tissue samples from human subjects is extremely difficult or impossible. Therefore, clinically relevant animal models are needed. Because of the high degree of similarity in anatomy, physiology, immunology, and brain growth and development patterns between pigs and humans, piglets are considered an ideal model for research on gastrointestinal, immune, and brain development. Previous studies have shown environmental factors, such as diet and the use of antibiotics, pre- and probiotics, modify the composition of gut microbiota (Li et al. 2014). Germ-free piglets colonized with human intestinal communities provide a tool for examining the environmental factors on the establishment of gut microbiota and how the resultant microbiota impacts the development of gastrointestinal, immune, and neural systems.

The HMA Pig Model for Delineating Mechanisms of Microbiota-Associated Diseases

As previously discussed, symbiotic host–microbiota interactions play a key role in maintaining homeostasis. Shifts in the bacterial composition of the human gut microbiota have been associated with several human disorders. Table 4 summarizes association between gut microbiota change and microbiota-associated diseases. Much of the information regarding the role of gut microbiota in human diseases comes from cross-sectional studies in which microbial community structures are altered in subjects with disease compared to healthy controls. However, it remains unclear whether changes in gut microbiota composition are the cause or the consequence of the diseases. Studies designed to access a causative role for the gut microbiota are critically needed. Understanding dysbiosis in human subjects is challenging because of the extraordinary complexity of the gut ecosystem and the tremendous variability in microbiota between healthy individuals (Gill et al. 2006). HMA pigs provide an excellent model for isolating microbiota as an environmental factor in disease models. For example, fecal samples could be collected from lean and obese humans or individuals suffering from microbiota-associated diseases, such as IBD and NEC, and healthy controls and then used to colonize GF pigs. Using HMA pigs, together with metabolomics, metaproteomics, host gene expression profiling, and metatrascriptomics, we may be able to delineate the role of gut microbiota in diseases at the cellular and molecular level. Using HMA piglets to identify potential biomarkers of microbiota-associated diseases through the use of metabolomics and metaproteomics has implications for development of diagnostic and therapeutic strategies for both infectious and

Table 4 Selected disorders associated with gut microbiota alteration

Disorders	Alteration in gut microbiota	References
Asthma	↑ *Clostridum difficile*	Penders et al. (2007)
	↑ *Bacteroides fragilis* subgroup and *Clostridium* subcluster XIVa	Vael et al. (2011)
Eczema	↓ bacterial diversity	Wang et al. (2008)
	↑ *Bifidobacterium pseudocatenulatum*, *Esherichia coli* and *Clostridium difficile*	Penders et al. (2007);Gore et al. (2008)
Necrotizing enterocolitis	↑ γ-proteobacteria and ↓ Firmicutes	Mai et al. (2011)
	↑ *Citrobacter*- and *Enterococcus*-like sequences	Mshvildadze et al. (2010)
	↓ bacterial diversity, ↑ γ-proteobacteria	Wang et al. (2009)
Inflammatory bowel diseases	↑ γ -proteobacteria, ↓ clostridia in ulcerative colitis	Michail et al. (2012)
	↓ Clostridia,e.g. *Faecalibacterium*, *Bacteroides* and bifidobacteria	Aomatsu et al. (2012);Schwiertz et al. (2010a)
	↓ bacterial diversity	Ott et al. (2004); Walker et al. (2011); Michail et al. (2012)
	↓ Firmicutes and ↑ Bacteroidetes	Walker et al. (2011)
Obesity	↓ Bacteroidetes and ↑ Firmicutes	Ley et al. (2006)
	↑ *Enterobacteriaceae,* ↓*Desulfovibrio* and *Akkermansia muciniphila*-like bacteria	Karlsson et al. (2012)
	↓ bacterial diversity, ↓ proportion of Bacteroidetes, ↑ proportion of Actinobacteria	Turnbaugh et al. (2009)
	↑ Bacteroidetes, ↓ Methanobrevibacter	Schwiertz et al. (2010b)
Autism spectrum disorders	↑ incidence of the *Clostridium histolyticum* group	Parracho et al. (2005)
	↑ *Sutterella* spp. and *Ruminococcus torques*	Wang et al. (2013)
	↓ bacterial diversity and ↓ *Prevotella*, *Coprococcus* and unclassified *Veillonellaceae*	Kang et al. (2013)

noninfectious conditions. However, a current limitation is the completeness of bioinformatics repositories for metabolomics and proteomics.

The HMA Pig Model for Studies of Gut Microbiota-Targeted Therapies

Pre-and probiotics have been studied in recent decades as a way to modulate gut microbial composition and functions (Ducatelle et al. 2014). Prebiotics are defined as "a selectively fermented ingredient that results in specific changes, in the composition and/or activity of the gastrointestinal microbiota, thus, conferring benefit(s) upon host health" (Roberfroid et al. 2010, p. S2). Prebiotics have the potential to stimulate the growth of beneficial bacteria, such as *Bifidobacterium* and *Lactobacillus*. Probiotics are "live microorganisms which when consumed in adequate amounts, confer a health benefit on the host" (FAO/WHO 2001, p. 2). *Bifidobacterium* and *Lactobacillus* are the most commonly used probiotics (Walsh et al. 2014). Other bacterial genera such as *Akkermansia* and *Faecalibacterium* have also been reported as potential probiotics (Thomas et al. 2014). Pre- and/or probiotic intervention has been used successfully for promoting health and prevention or treatment of some microbiota-associated disorders, such as eczema, IBD, NEC, and obesity in human studies (Kadooka et al. 2010; Li et al. 2014); however, the mechanisms underlying the beneficial effects of pre- or probiotics remain incompletely understood. Understanding the impact of pre- or probiotics on the gut microbiota and host requires carefully controlled studies in which potential confounding variables such as host genotype, diet, and environmental exposure can be controlled. Gnotobiotic animals can be reared under well-controlled conditions, representing one way to constrain some of these variables. Recently, GN mice harboring a mixture of 15 species of human gut microbiota were studied prior to and after gavage with five fermented milk strains (McNulty et al. 2011). The results revealed only a minimal change in the composition of the microbiota, whereas significant changes in the expression of microbiome-encoded enzymes in numerous metabolic pathways, especially the pathways related to carbohydrate metabolism, were observed (McNulty et al. 2011). Compared to rodents, pigs colonized with human microbiota are more similar to humans in anatomy, physiology, microbiota, and genetics, providing a more attractive model for elucidating molecular bases of pre- and probiotic action.

Conclusions

Emerging studies have demonstrated the feasibility of generating and maintaining GN and HMA piglets for relatively long periods of time. These models represent robust systems in which to dissect the intricacies underlying host–microbe relationships essential for maintaining health and preventing disease. To date, studies have not yet exploited HMA piglets as recipients of microbiota associated with specific diseases, as has been effectively exploited in GN rodent models. The HMA piglet is the optimal model for preclinical screening of novel pre-, pro-, and symbiotic preparations and elucidating the impact of these preparations on microbiota composition and host responses.

Acknowledgments

This work was supported in part by Hatch funds (Project ILLU-971-346) distributed by the Division of Nutritional Sciences at the University of Illinois through its Vision 20/20 program.

References

Adlerberth I, Wold AE. 2009. Establishment of the gut microbiota in Western infants. Acta Paediatr 98:229–238.

Aomatsu T, Imaeda H, Fujimoto T, Takahashi K, Yoden A, Tamai H, Fujiyama Y, Andoh A. 2012. Terminal restriction fragment length polymorphism analysis of the gut microbiota profiles of pediatric patients with inflammatory bowel disease. Digestion 86:129–135.

Azevedo MS, Zhang W, Wen K, Gonzalez AM, Saif LJ, Yousef AE, Yuan L. 2012. *Lactobacillus acidophilus* and *Lactobacillus reuteri* modulate cytokine responses in gnotobiotic pigs infected with human rotavirus. Benef Microbes 3:33–42.

Björkstén B, Sepp E, Julge K, Voor T, Mikelsaar M. 2001. Allergy development and the intestinal microflora during the first year of life. J Allergy Clin Immunol 108:516–520.

Bouhnik Y, Vahedi K, Achour L, Attar A, Salfati J, Pochart P, Marteau P, Flourie B, Bornet F, Rambaud JC. 1999. Short-chain fructooligosaccharides administration dose-dependently increases fecal bifidobacteria in healthy humans. J Nutr 429:113–116.

Brady MJ, Radhakrishnan P, Liu H, Magoun L, Murphy KC, Mukherjee J, Donohue-Rolfe A, Tzipori S, Leong JM. 2011. Enhanced actin pedestal formation by enterohemorrhagic Escherichia coli O157:H7 adapted to the mammalian host. Front Microbiol 2:226. doi:10.3389/fmicb.2011.00226.

Butler JE. 2009. Isolator and other neonatal piglet models in developmental immunology and identification of virulence factors. Anim Health Res Rev 10:35–52.

Butler JE, Sun J, Weber P, Navarro P, Francis D. 2000. Antibody repertoire development in fetal and newborn piglets, III. Colonization of the gastrointestinal tract selectively diversifies the preimmune repertoire in mucosal lymphoid tissues. Immunology 100:119–130.

Chapkin RS, Zhao C, Ivanov I, Davidson LA, Goldsby JS, Lupton JR, Mathai RA, Monaco MH, Rai D, Russell WM, Donovan SM, Dougherty ER. 2010. Non-invasive stool-based detection of infant gastrointestinal development using gene expression profiles from exfoliated epithelial cells. Am J Physiol GI Liver Physiol 298:G582–G589.

Che C, Pang X, Hua X, Zhang B, Shen J, Zhu J, Wei H, Sun L, Chen P, Cui L, Zhao L, Yang Q. 2009. Effects of human fecal flora on intestinal morphology and mucosal immunity in human flora-associated piglet. Scand J Immunol 69:223–233.

Cheetham S, Souza M, Meulia T, Grimes S, Han MG, Saif LJ. 2006. Pathogenesis of a genogroup II human norovirus in gnotobiotic pigs. J Virol 280:10372–10381.

Chow J, Lee SM, Shen Y, Khosravi A, Mazmanian SK. 2010. Host-bacterial symbiosis in health and disease. Adv Immunol 107:243–274.

Chowdhury SR, King DE, Willing BP, Band MR, Beever JE, Lane AB, Loor JJ, Marini JC, Rund LA, Schook LB, Van Kessel AG, Gaskins HR. 2007. Transcriptome profiling of the small intestinal epithelium in germfree versus conventional piglets. BMC Genomics 8:215.

Collins SM, Surette M, Bercik P. 2012. The interplay between the intestinal microbiota and the brain. Nat Rev Microbiol 10:735–742.

Conrad MS, Dilger RN, Johnson RW. 2012. Brain growth of the domestic pig (*Sus scrofa*) from 2 to 24 weeks of age: a longitudinal MRI study. Dev Neurosci 34:291–298.

Dawson H. 2011. A comparative assessment of the pig, mouse, and human genomes: structural and functional analysis of genes involved in immunity and inflammation. In:

McAnulty PA, DayanGanderup ADN-C, Hastings KL, eds. The Minipig in Biomedical Research. London: CRC Press, Taylor & Francis Group. p. 321–341.

Desbonnet L, Clarke G, Shanahan F, Dinan TG, Cryan JF. 2014. Microbiota is essential for social development in the mouse. Mol Psychiatry 219:146–148.

Diaz Heijtz R, Wang S, Anuar F, Qian Y, Björkholm B, Samuelsson A, Hibberd ML, Forssberg H, Pettersson S. 2011. Normal gut microbiota modulates brain development and behavior. Proc Natl Acad Sci USA 108:3047–3052.

Dobbing J, Sands J. 1979. Comparative aspects of the brain growth spurt. Early Hum Dev 3:79–83.

Donovan SM, Wang M, Li M, Friedberg I, Schwartz SL, Chapkin RS. 2012. Host-microbe interactions in the neonatal intestine: Role of human milk oligosaccharides. Adv Nutr 3:450S–455S.

Douglas-Escobar M, Elliott E, Neu J. 2013. Effect of intestinal microbial ecology on the developing brain. JAMA Pediatr 167:374–379.

Ducatelle R, Eeckhaut V, Haesebrouck F, Van Immerseel F. 2014. A review on prebiotics and probiotics for the control of dysbiosis: present status and future perspectives. Animal 22:1–6. [Epub ahead of print]

Elahi S, Holmstrom J, Gerdts V. 2007. The benefits of using diverse animal models for studying pertussis. Trends Microbiol 15:462–468.

FAO/WHO (Food and Agriculture Organization/World Health Organization). 2001. Probiotics in food: health and nutritional properties and guidelines for evaluation. Report of a Joint FAO/WHO Expert Consultation on Evaluation of Health and Nutritional Properties of Probiotics in Food including Powder Milk with Live Lactic Acid Bacteria, Cordoba, Argentina, 1–4 October 2001.

Gibson GR, Beatty ER, Wang X, Cummings JH. 1995. Selective stimulation of bifidobacteria in the human colon by oligofructose and inulin. Gastroenterology 108:975–982.

Gibson GR, Roberfroid MB. 1995. Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. J Nutr 125:1401–1412.

Gil O, Díaz I, Vilaplana C, Tapia G, Díaz J, Fort M, Cáceres N, Pinto S, Caylà J, Corner L, Domingo M, Cardona PJ. 2010. Granuloma encapsulation is a key factor for containing tuberculosis infection in minipigs. PLoS One 5:e10030. doi:10.1371/journal.pone.0010030.

Gill SR, Pop M, Deboy RT, Eckburg PB, Turnbaugh PJ, Samuel BS, Gordon JI, Relman DA, Fraser-Liggett CM, Nelson KE. 2006. Metagenomic analysis of the human distal gut microbiome. Science 312:1355–1359.

Gootenberg DB, Turnbaugh PJ. 2011. Companion animals symposium: humanized animal models of the microbiome. J Anim Sci 89:1531–1537.

Gore C, Munro K, Lay C, Bibiloni R, Morris J, Woodcock A, Custovic A, Tannock GW. 2008. *Bifidobacterium pseudocatenulatum* is associated with atopic eczema: a nested case-control study investigating the fecal microbiota of infants. J Allergy Clin Immunol 121:135–140.

Graham H, Aman P. 1987. The pig as a model in dietary fibre digestion studies. Scand J Gastroenterol Suppl 129:55–61.

Guilloteau P, Zabielski R, Hammon HM, Metges CC. 2010. Nutritional programming of gastrointestinal tract development. Is the pig a good model for man? Nutr Res Rev 23:4–22.

Gustafsson B, Kahlson G, Rosengren E. 1957. Biogenesis of histamine studied by its distribution and urinary excretion in germ free reared and not germ free rats fed a histamine free diet. Acta Physiol Scand 41:217–228.

Haarman M, Knol J. 2005. Quantitative real-time PCR assays to identify and quantify fecal bifidobacterium species in infants receiving a prebiotic infant formula. Appl Environ Microbiol 71:2318–2324.

Haverson K, Rehakova Z, Sinkora J, Sver L, Bailey M. 2007. Immune development in jejunal mucosa after colonization with selected commensal gut bacteria: a study in germ-free pigs. Vet Immunol Immunopathol 119:243–253.

Heinritz SN, Mosenthin R, Weiss E. 2013. Use of pigs as a potential model for research into dietary modulation of the human gut microbiota. Nutr Res Rev 26:191–209.

Honda K, Takeda K. 2009. Regulatory mechanisms of immune responses to intestinal bacteria. Mucosal Immunol 2: 187–196.

Hooper LV, Gordon JI. 2001. Commensal host-bacterial relationships in the gut. Science 292:1115–1118.

Hooper LV, Wong MH, Thelin A, Hansson L, Falk PG, Gordon JI. 2001. Molecular analysis of commensal host-microbial relationships in the intestine. Science 291:881–884.

Human Microbiome Project Consortium. 2012. Structure, function and diversity of the healthy human microbiome. Nature 486:207–214.

Imaoka A, Setoyama H, Takagi A, Matsumoto S, Umesaki Y. 2004. Improvement of human faecal flora-associated mouse model for evaluation of the functional foods. J Appl Microbiol 96:656–663.

Jeong KI, Venkatesan MM, Barnoy S, Tzipori S. 2013. Evaluation of virulent and live *Shigella sonnei* vaccine candidates in a gnotobiotic piglet model. Vaccine 31:4039–4046.

Jeong KI, Zhang Q, Nunnari J, Tzipori S. 2010. A piglet model of acute gastroenteritis induced by *Shigella dysenteriae* Type 1. J Infect Dis 201:903–911.

Kadooka Y, Sato M, Imaizumi K, Ogawa A, Ikuyama K, Akai Y, Okano M, Kagoshima M, Tsuchida T. 2010. Regulation of abdominal adiposity by probiotics (*Lactobacillus gasseri* SBT2055) in adults with obese tendencies in a randomized controlled trial. Eur J Clin Nutr 64:636–643.

Kang DW, Park JG, Ilhan ZE, Wallstrom G, Labaer J, Adams JB, Krajmalnik-Brown R. 2013. Reduced incidence of Prevotella and other fermenters in intestinal microflora of autistic children. PLoS One 8:e68322.

Karlsson CL, Onnerfält J, Xu J, Molin G, Ahrné S, Thorngren-Jerneck K. 2012. The microbiota of the gut in preschool children with normal and excessive body weight. Obesity 20:2257–2261.

Khatri M, Dwivedi V, Krakowka S, Manickam C, Ali A, Wang L, Qin Z, Renukaradhya GJ, Lee CW. 2010. Swine influenza H1N1 virus induces acute inflammatory immune responses in pig lungs: a potential animal model for human H1N1 influenza virus. J Virol 84:11210–11218.

Krawczynski K, Meng XJ, Rybczynska J. 2011. Pathogenetic elements of hepatitis E and animal models of HEV infection. Virus Res 161:78–83.

Leser TD, Mølbak L. 2009. Better living through microbial action: the benefits of the mammalian gastrointestinal microbiota on the host. Environ Microbiol 11:2194–2206.

Ley RE, Turnbaugh PJ, Klein S, Gordon JI. 2006. Microbial ecology: human gut microbes associated with obesity. Nature 444:1022–1023.

Li M, Wang M, Donovan SM. 2014. Early development of the gut microbiome and immune-mediated childhood disorders. Semin Reprod Med 32:74–86.

Liu F, Li G, Wen K, Wu S, Zhang Y, Bui T, Yang X, Kocher J, Sun J, Jortner B, Yuan L. 2013. *Lactobacillus rhamnosus* GG on

rotavirus-induced injury of ileal epithelium in gnotobiotic pigs. J Pediatr Gastroenterol Nutr 57:750–758.

Lunney JK. 2007. Advances in swine biomedical model genomics. Int J Biol Sci 3:179–184.

Macpherson AJ, Harris NL. 2004. Interactions between commensal intestinal bacteria and the immune system. Nat Rev Immunol 4:478–485.

Mai V, Young CM, Ukhanova M, Wang X, Sun Y, Casella G, Theriaque D, Li N, Sharma R, Hudak M, Neu J. 2011. Fecal microbiota in premature infants prior to necrotizing enterocolitis. PLoS ONE 6:e20647. doi:10.1371/journal.pone.0020647.

Meurens F, Summerfield A, Nauwynck H, Saif L, Gerdts V. 2012. The pig: a model for human infectious diseases. Trends Microbiol 20:50–57.

Michail S, Durbin M, Turner D, Griffiths AM, Mack DR, Hyams J, Leleiko N, Kenche H, Stolfi A, Wine E. 2012. Alterations in the gut microbiome of children with severe ulcerative colitis. Inflamm Bowel Dis 18:1799–1808.

Mshvildadze M, Neu J, Shuster J, Theriaque D, Li N, Mai V. 2010. Intestinal microbial ecology in premature infants assessed with non-culture-based techniques. J Pediatr 156:20–25.

Nedrud JG. 1999. Animal models for gastric Helicobacter immunology and vaccine studies. FEMS Immunol Med Microbiol 24:243–250.

Nielsen OL, Iburg T, Aalbaek B, Leifsson PS, Agerholm JS, Heegaard P, Boye M, Simon S, Jensen KB, Christensen S, Melsen K, Bak AK, Backman ER, Jørgensen MH, Groegler DK, Jensen AL, Kjelgaard-Hansen M, Jensen HE. 2009. A pig model of acute *Staphylococcus aureus* induced pyemia. Acta Vet Scand 51:14. doi:10.1186/1751-0147-51-14.

Odle J, Lin X, Jacobi SK, Kim SW, Stahl CH. 2014. The suckling piglet as an agrimedical model for the study of pediatric nutrition and metabolism. Annu Rev Anim Biosci 2:419–444.

Ott SJ, Musfeldt M, Wenderoth DF, Hampe J, Brant O, Fölsch UR, Timmis KN, Schreiber S. 2004. Reduction in diversity of the colonic mucosa associated bacterial microflora in patients with active inflammatory bowel disease. Gut 53:685–693.

Pang X, Hua X, Yang Q, Ding D, Che C, Cui L, Jia W, Bucheli P, Zhao L. 2007. Inter-species transplantation of gut microbiota from human to pigs. ISME J 1:156–162.

Parracho HM, Bingham MO, Gibson GR, McCartney AL. 2005. Differences between the gut microflora of children with autistic spectrum disorders and that of healthy children. J Med Microbiol 54(Pt 10):987–991.

Penders J, Thijs C, van den Brandt PA, Kummeling I, Snijders B, Stelma F, Adams H, van Ree R, Stobberingh EE. 2007. Gut microbiota composition and development of atopic manifestations in infancy: the KOALA Birth Cohort Study. Gut 56:661–667.

Puiman P, Stoll B. 2008. Animal models to study neonatal nutrition in humans. Curr Opin Clin Nutr Metab Care 11:601–606.

Qin J, Li R, Raes J, Arumugam M, Burgdorf KS, Manichanh C, Nielsen T, Pons N, Levenez F, Yamada T, Mende DR, Li J, Xu J, Li S, Li D, Cao J, Wang B, Liang H, Zheng H, Xie Y, Tap J, Lepage P, Bertalan M, Batto JM, Hansen T, Le Paslier D, Linneberg A, Nielsen HB, Pelletier E, Renault P, Sicheritz-Ponten T, Turner K, Zhu H, Yu C, Li S, Jian M, Zhou Y, Li Y, Zhang X, Li S, Qin N, Yang H, Wang J, Brunak S, Doré J, Guarner F, Kristiansen K, Pedersen O, Parkhill J, Weissenbach J; MetaHIT Consortium, Bork P, Ehrlich SD, Wang J. 2010. A human gut microbial gene catalogue established by metagenomic sequencing. Nature 464:59–65.

Raibaud P, Ducluzeau R, Dubos F, Hudault S, Bewa H, Muller MC. 1980. Implantation of bacteria from the digestive tract of man and various animals into gnotobiotic mice. Am J Clin Nutr 33 (Suppl 11):2440–2447.

Roberfroid M, Gibson GR, Hoyles L, McCartney AL, Rastall R, Rowland I, Wolvers D, Watzl B, Szajewska H, Stahl B, Guarner F, Respondek F, Whelan K, Coxam V, Davicco MJ, Léotoing L, Wittrant Y, Delzenne NM, Cani PD, Neyrinck AM, Meheust A. 2010. Prebiotic effects: Metabolic and health benefits. Br J Nutr 104(Suppl 2):S1–63.

Round JL, Mazmanian SK. 2009. The gut microbiota shapes intestinal immune responses during health and disease. Nat Rev Immunol 9:313–323.

Rytych JL, Elmore MR, Burton MD, Conrad MS, Donovan SM, Dilger RN, Johnson RW. 2012. Early life iron deficiency impairs spatial cognition in neonatal piglets. J Nutr 142:2050–2056.

Saif LJ. 1996. Mucosal immunity: an overview and studies of enteric and respiratory coronavirus infections in a swine model of enteric disease. Vet Immunol Immunopathol 54:163–169.

Saif LJ, Ward LA, Yuan L, Rosen BI, To TL. 1996. The gnotobiotic piglet as a model for studies of disease pathogenesis and immunity to human rotaviruses. Arch Virol Suppl 12:153–161.

Scalabrin DM, Mitmesser SH, Welling GW, Harris CL, Marunycz JD, Walker DC, Bos NA, Tölkkö S, Salminen S, Vanderhoof JA. 2012. New prebiotic blend of polydextrose and galacto-oligosaccharides has a bifidogenic effect in young infants. J Pediatr Gastroenterol Nutr 54:343–352.

Schwiertz A, Jacobi M, Frick JS, Richter M, Rusch K, Köhler H. 2010a. Microbiota in pediatric inflammatory bowel disease. J Pediatr 157:240–244.

Schwiertz A, Taras D, Schäfer K, Beijer S, Bos NA, Donus C, Hardt PD. 2010b. Microbiota and SCFA in lean and overweight healthy subjects. Obesity 18:190–195.

Shen YT. 2010. Primate models for cardiovascular drug research and development. Curr Opin Investig Drugs 11:1025–1029.

Shen J, Zhang B, Wei H, Che C, Ding D, Hua X, Bucheli P, Wang L, Li Y, Pang X, Zhao L. 2010. Assessment of the modulating effects of fructo-oligosaccharides on fecal microbiota using human flora-associated piglets. Arch Microbiol 192:959–968.

Shirkey TW, Siggers RH, Goldade BG, Marshall JK, Drew MD, Laarveld B, Van Kessel AG. 2006. Effects of commensal bacteria on intestinal morphology and expression of proinflammatory cytokines in the gnotobiotic pig. Exp Biol Med 231:1333–1345.

Shurson GC, Ku PK, Waxler GL, Yokoyama MT, Miller ER. 1990. Physiological relationships between microbiological status and dietary copper levels in the pig. J Anim Sci 68:1061–1071.

Sjögren YM, Tomicic S, Lundberg A, Böttcher MF, Björkstén B, Sverremark-Ekström E, Jenmalm MC. 2009. Influence of early gut microbiota on the maturation of childhood mucosal and systemic immune responses. Clin Exp Allergy 39:1842–1851.

Smith K, McCoy KD, Macpherson AJ. 2007. Use of axenic animals in studying the adaptation of mammals to their commensal intestinal microbiota. Semin Immunol 19:59–69.

Smith MI, Yatsunenko T, Manary MJ, Trehan I, Mkakosya R, Cheng J, Kau AL, Rich SS, Concannon P, Mychaleckyj JC, Liu J, Houpt E, Li JV, Holmes E, Nicholson J, Knights D, Ursell LK, Knight R, Gordon JI. 2013. Gut microbiomes of Malawian twin pairs discordant for kwashiorkor. Science 339:548–554.

Steele J, Feng H, Parry N, Tzipori S. 2010. Piglet models of acute or chronic *Clostridium difficile* illness. J Infect Dis 201:428–434.

Thomas LV, Ockhuizen T, Suzuki K. 2014. Exploring the influence of the gut microbiota and probiotics on health: a symposium report. Br J Nutr 112(Suppl 1):S1–18.

Turnbaugh PJ, Hamady M, Yatsunenko T, Cantarel BL, Duncan A, Ley RE, Sogin ML, Jones WJ, Roe BA, Affourtit JP, Egholm M, Henrissat B, Heath AC, Knight R, Gordon JI. 2009. A core gut microbiome in obese and lean twins. Nature 457:480–484.

Turnbaugh PJ, Ley RE, Mahowald MA, Magrini V, Mardis ER, Gordon JI. 2006. An obesity-associated gut microbiome with increased capacity for energy harvest. Nature 444:1027–1031.

Vael C, Vanheirstraeten L, Desager KN, Goossens H. 2011. Denaturing gradient gel electrophoresis of neonatal intestinal microbiota in relation to the development of asthma. BMC Microbiol 11:68. doi:10.1186/1471-2180-11-68.

Vítovec J, Koudela B. 1992. Pathogenesis of intestinal cryptosporidiosis in conventional and gnotobiotic piglets. Vet Parasitol 43:25–36.

Walker AW, Sanderson JD, Churcher C, Parkes GC, Hudspith BN, Rayment N, Brostoff J, Parkhill J, Dougan G, Petrovska L. 2011. High-throughput clone library analysis of the mucosa-associated microbiota reveals dysbiosis and differences between inflamed and non-inflamed regions of the intestine in inflammatory bowel disease. BMC Microbiol 11:7. doi:10.1186/1471-2180-11-7.

Walsh CJ, Guinane CM, O'Toole PW, Cotter PD. 2014. Beneficial modulation of the gut microbiota. FEBS Lett 588:4120–4130.

Wang L, Christophersen CT, Sorich MJ, Gerber JP, Angley MT, Conlon MA. 2013. Increased abundance of *Sutterella* spp. and *Ruminococcus torques* in feces of children with autism spectrum disorder. Mol Autism 4:42. doi:10.1186/2040-2392-4-42.

Wang M, Karlsson C, Olsson C, Adlerberth I, Wold AE, Strachan DP, Martricardi PM, Aberg N, Perkin MR, Tripodi S, Coates AR, Hesselmar B, Saalman R, Molin G, Ahrné S. 2008. Reduced diversity in the early fecal microbiota of infants with atopic eczema. J Allergy Clin Immunol 121:129–134.

Wang Y, Hoenig JD, Malin KJ, Qamar S, Petrof EO, Sun J, Antonopoulos DA, Chang EB, Claud EC. 2009. 16S rRNA gene-based analysis of fecal microbiota from preterm infants with and without necrotizing enterocolitis. ISME J 3:944–954.

Wen K, Tin C, Wang H, Yang X, Li G, Giri-Rachman E, Kocher J, Bui T, Clark-Deener S, Yuan L. 2014. Probiotic *Lactobacillus rhamnosus* GG enhanced Th1 cellular immunity, but did not affect antibody responses in a human gut microbiota transplanted neonatal gnotobiotic pig model. PLoS One 9:e94504. doi:10.1371/journal.pone.0094504.

Willing BP, Van Kessel AG. 2007. Enterocyte proliferation and apoptosis in the caudal small intestine is influenced by the composition of colonizing commensal bacteria in the neonatal gnotobiotic pig. J Anim Sci 85:3256–3266.

Willing BP, Van Kessel AG. 2009. Intestinal microbiota differentially affect brush border enzyme activity and gene expression in the neonatal gnotobiotic pig. J Anim Physiol Anim Nutr (Berl) 93:586–595.

Yi P, Li L. 2012. The germfree murine animal: an important animal model for research on the relationship between gut microbiota and the host. Vet Microbiol 157:1–7.

Zhang H, Wang H, Shepherd M, Wen K, Li G, Yang X, Kocher J, Giri-Rachman E, Dickerman A, Settlage R, Yuan L. 2014. Probiotics and virulent human rotavirus modulate the transplanted human gut microbiota in gnotobiotic pigs. Gut Pathog 6:39. doi:10.1186/s13099-014-0039-8.

Zhang Q, Widmer G, Tzipori S. 2013. A pig model of the human gastrointestinal tract. Gut Microbes 4:193–200.