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Review

The pig: a model for human infectious diseases

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An animal model to study human infectious diseases should accurately reproduce the various aspects of disease. Domestic pigs (*Sus scrofa domesticus*) are closely related to humans in terms of anatomy, genetics and physiology, and represent an excellent animal model to study various microbial infectious diseases. Indeed, experiments in pigs are much more likely to be predictive of therapeutic treatments in humans than experiments in rodents. In this review, we highlight the numerous advantages of the pig model for infectious disease research and vaccine development and document a few examples of human microbial infectious diseases for which the use of pigs as animal models has contributed to the acquisition of new knowledge to improve both animal and human health.

Pigs as a model for humans

An animal model is established because it is believed to replicate appropriately the condition under investigation and is thought to respond in the same way as humans to the infection. This belief may be based on particular evidence or it may be inferred from the similarities between the animal model and humans. The species used as a model should be easy to handle and available to multiple investigators. Then, it should survive long enough to develop the disease, fit the available animal facilities, be of sufficient size to provide numerous samples, and be multiparous to produce multiple animals for each gestation.

The pig is very similar to humans in terms of anatomy, genetics and physiology. Pigs can vary from miniature to large breeds (Box 1). Choosing the right breed and age allows various surgical and non-surgical procedures typically used in human medicine, including catheterization, heart surgery, valve manipulation, endoscopy and broncho-alveolar lavages. These procedures are particularly difficult or impossible to perform in many animal models including rodents. In terms of genetics, the size and the composition of the porcine genome are comparable to those of humans [1]. Pigs are also remarkably similar to humans in terms of physiology. Both species are omnivorous and their organs generally share common functional features [2,3]. Highlighting these similarities, pig-to-primate organ transplantation models are being used successfully [4].

Three principal types of animal models are usually mentioned: (i) spontaneous, (ii) experimentally induced, and (iii) transgenic. The two more common types are spontaneous and experimentally induced, whereas the third type has only been used extensively in the mouse.

In this review we focus on advantages of the porcine model (Box 2) over the mouse model in the study of infectious diseases and discuss cases where pig models have made an impact.

The immune system of pigs

After the primate and murine immune system, the porcine immune system is probably the best characterized and offers a wide range of established methodologies and tools [5] (Box 3). Similar to other mammals, pigs have a full set of innate and adaptive immune effectors. Although some porcine host defense polypeptides are specific and α-defensins are absent [6], most proteins of the immune system share structural and functional similarities with their human counterparts. The porcine immune system more closely resembles humans for >80% of analyzed parameters, whereas mice were more similar to humans in <10% [7]. Among the main differences between pigs and humans are the inversion of lymph nodes, two types of Peyer's patches (PP), and the transfer of passive immunity from the sow to the piglet, which is principally colostrum- and milk-dependent as a consequence of the epitheliochorial placentation [8]. In humans, in whom placentation is hemochorial, maternal blood comes into direct contact with the fetal chorion and the transfer of passive immunity depends less on mammary gland secretions.

For the most part, all the immune cell populations identified in humans and mice are present in pigs. Many studies have described the porcine cluster of differentiation (CD), the cell-surface proteins that allow the identification and the characterization of the various immune cell populations [9]. Similarly to humans, and in contrast to

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Box 1. Inbred versus outbred pigs

The pig (*Sus scrofa domesticus*) was domesticated more than 9000 years ago in multiple areas including near the Tigris Basin and the Far East, as revealed by archaeological evidence from figurines and bones [73]. Including outbred and inbred breeds, there are currently approximately 730 pig breeds or lines worldwide of which two-thirds reside in China and Europe and over 270 are considered as being endangered or at critical levels [74]. Pigs can vary from miniature breeds to large breeds that can reach up to 350 lbs. In the pork industry, only six outbred breeds are dominant for meat production: Large White (Yorkshire), Landrace, Duroc, Hampshire, Berkshire and Piétrain. Besides these meat production breeds are miniature breeds, which develop adult human-sized organs between 6 and 8 months of age [3]. In the USA the most common are Göttingen, Hanford, Sinclair, Yucatan and Yucatan micro breeds [3]. In pigs, offspring are precocial and can be recovered by Cesarian section and maintained in germfree isolators. This feature makes the porcine species highly suited to study the interactions between pathogens and microbiota. Miniature pigs and outbred farm pigs can be maintained in germfree isolators for 6–8 weeks or longer depending on isolator size [75]. Livestock porcine breeds used in experimental protocols will best mimic animal variation reflective of outbred human populations [72]. This variability is especially relevant in reflecting the full scope of responses to vaccines or therapeutics as would be expected in humans. For minipig outbred stocks such as miniature Göttingen piglets, although there is heterogeneity between animals, the full pedigree can be obtained from the supplier (http://minipigs.com) [72]. Inbred pigs, including three strains of inbred miniature pigs, each homozygous for a different allele of the major histocompatibility locus (MHC), have been developed by selective breeding based on tissue-typing of the offspring of each generation [76]. These inbred histocompatible pigs can be useful in cell-transfer experiments between animals and, as with inbred mice, the extent of inter-animal variation is reduced.

Box 2. Advantages of the porcine model

- Availability (most important meat-producing livestock species worldwide)
- Human size, especially the miniature Hanford pig
- Possibility of performing various surgical procedures and of collecting many samples
- Omnivorous (similar for physiology)
- Lifespan (10–20 years)
- Various breeds (541), outbred and inbred
- Early sexual maturity (5–8 months)
- Gestation (114 days)
- Short generation interval (12 months)
- All-season breeding
- Large litter size (10–12 piglets/litter), 24–36 piglets/year
- Well-developed standardization of breeding conditions
- High genome and protein sequence homologies with human counterparts
- Closely resemble humans for >80% of immune parameters analyzed (vs <10% for mice)
- Many tools are available (cell lines, antibodies, ELISA and microarrays)
- Cloning and transgenic technology advances
- Cheaper and ethically more acceptable than primates, which are closer to humans than pigs

rodents [10], pigs have high percentages of neutrophils in the peripheral blood (50–70%). Regarding the differentiation of auxiliary T cells, the Th1/Th2/Th17/Treg paradigm, originally developed in the mouse [11], provides a useful model of directed response to infectious pathogens. Th1 cells secrete interleukin-2 (IL-2) and interferon-γ (IFN-γ), which activate cytotoxic T cells and macrophages, cell types involved in the control of infections by intracellular agents. Th2 polarized cells secrete IL-3, IL-4, IL-5, IL-10 and IL-13, which stimulate antibody production and control infections by extracellular microorganisms. Th17 cells, described more recently, characterized by IL-17 synthesis, are involved mainly in the control of extracellular pathogens and autoimmunity. Treg cells contribute to the induction and the maintenance of peripheral tolerance. Globally functional orthologs for all the cytokines involved in the Th1/Th2/Th17/Treg paradigm and corresponding cells have been described in pigs [12–14]. IL-8, a chemoattractant for neutrophils, has a direct ortholog in pigs, whereas there is no direct homolog in mice [10]. Regarding macrophages and the

Box 3. The porcine toolbox

The publication of the complete version of the pig genome (approximately 2.7–2.9 billion bp for 18 autosomes and the X and Y sex chromosomes) under the auspices of the Swine Genome Sequencing Consortium is imminent [77]. Analysis of porcine sequences has indicated an almost identical gene content to human sequences, although some gene-order differences have been identified [78]. Regarding genomic comparisons between human, murine and porcine sequences, more structural resemblances were shown between human and porcine than between human and murine sequences [1,78]. Porcine genomic sequences and expressed sequences tags are available on Pig Expression Data Explorer (http://pede.dna.affrc.go.jp), NCBI (http://www.ncbi.nlm.nih.gov/projects/mapview) and Ensembl (http://www.ensembl.org) websites. A high-quality bacterial artificial chromosome map of the genome has been assembled and more than 375 000 single nucleotide polymorphisms were identified [79]. Microarray tools are also available for pigs and Agilent Technologies sells a porcine gene expression microarray with 43 803 60-mer oligoprobes and Affymetrix sells a system with 23 937 probe sets for assessing the expression of 23 256 transcripts. Proteomics approaches are also used and the pig proteome database (http://www.peptideatlas.org/) covers ~20 tissues with more than 15 000 peptides. However, mapping of the pig proteome is limited, a although this is progressing rapidly. The progress in porcine immunology is evident from the numerous private companies selling antibodies, ELISA systems and other reagents, although the list of immunologic tools available for swine is less than for humans and mice. Pigs also have the advantage that many antibodies directed against human markers cross-react with porcine targets. Various databases currently exist for porcine immunological tools including antibodies and real-time PCR primers. A database established by Dr H. Dawson (USDA, ARS, Beltsville, USA) can be found under 'Porcine Immunology and Nutrition Database' at http://199.133.11.115/fmi/iwp/cgi?-db=PINdb&-loadframes. Extensive information on swine leukocyte antigens is also available, with 116 SLA allelic genes deposited in the Immune Polymorphism Database (http://edi.ac.uk/ipd/index.htlm). It is now possible to predict cytotoxic T cell epitopes in the NetMHCpan (http://www.cbs.dtu.dk/services/NetMHCpan/) and even information on the crystal structure of SLA-1 [80]. There has been significant recent progress in pig transgenesis [72]. Different transgenesis techniques have been used successfully including pronuclear DNA microinjection, sperm-mediated gene transfer, lentiviral gene transfer, and somatic nuclear transfer [72]. Excellent transgenic porcine models have been developed for the study of human diseases such as Alzheimer's disease, retinitis pigmentosa, cystic fibrosis and diabetes [72]. Further refinements of transgenic techniques such as inducible transgene expression, a *Cre–loxP* system for conditional transgenic modifications, a non-viral episomal expression system and zinc-finger nuclease technology are already available or should become available shortly [72].

nitric oxide (NO) pathway, which are particularly important in the innate response to infectious agents, differences between humans and rodents are reported. For instance, similar to observations in humans, there is no evidence of NO production by porcine macrophages after lipopolysaccharide (LPS) stimulation even with IFN-γ priming [15]. Toll-like receptors (TLR) and dendritic cells (DCs) have a pivotal role in the recognition of microorganisms and in the induction and the control of innate and adaptive immune responses to pathogens. Also with respect to TLR and DC biology, the porcine immune system is functionally more similar to its human counterpart when compared to mice. In pigs and humans, TLR7 and TLR9 are mainly restricted to plasmacytoid DCs, whereas these receptors are also expressed on murine conventional DCs [16]. This has consequences for the use of TLR ligands as stimulatory molecules for vaccines, and transposition of vaccine responses observed in mouse models to humans will be more uncertain than using the porcine model. For example, immunostimulation using CpG as a TLR9-ligand is very efficient in mice but less so in humans and pigs [17]. Other important elements are the extreme resistance of mice to endotoxin shock – as compared to humans and pigs – and the propensity of mice to develop hypothermia in response to endotoxin challenge instead of hyperthermia, which is the most common outcome in humans [10]. These features severely decrease the potential of mice as a model in the study of sepsis, one the principal causes of mortality in the USA [10].

These characteristics emphasize that pigs represent valuable intermediate species to assess knowledge on infectious diseases obtained using rodent models that may have applicability to the study of human infectious diseases.

Anatomic considerations

To be fully workable and to allow human-like clinical monitoring, an animal model should be similar to humans in terms of anatomy and size. The following section highlights some differences and similarities in the main anatomic compartments.

With the exception of the hemiazygos vein, which enters the coronary sinus, whereas in other species it enters the vena precava, the porcine heart is anatomically very close to its human counterpart [2,3]. The coronary artery distribution, the blood supply to the coronary arteries, and the right-sided dominant circulatory system are also similar to that of humans [2,3].

The porcine lungs have two lobes on the left side and four lobes on the right [2,3], whereas humans have three right and two left lobes. On the left they are designated as left cranial and caudal lobes and on the other side as right cranial, right middle, right caudal and right accessory lobes. The right cranial lobe is directly connected to the trachea [2]. As in many mammalian species including pigs, and in contrast to mice, the human lung has extensive interlobular and intralobular connective tissue, which joins the major vessels and the bronchi to the pleural surface [18]. The upper respiratory tracts of pigs and humans are also anatomically similar and, with the exception of primates, the human Waldeyer's ring most closely resembles the anatomical arrangement of the porcine lymphoid tissues in the nasopharynx [19]. Furthermore, pigs possess tonsils, which are absent in mice.

There are some anatomic differences between humans and pigs in the gastrointestinal tract (GIT) [2,3]. Although both species are monogastric, a prominent diverticulum is present in pigs at the level of the pylorus, the sphincter controlling gut access. The porcine small intestine differs in the length of its segments and in the branching of mesenteric vessels. In addition, in pigs there is a continuous ileal PP. The cecum, the ascending and transverse colon, and the proximal portion of the descending colon are arranged in a series of centrifugal and centripetal coils differing structurally from those of humans [2,3].

Porcine kidneys are very similar in size to human kidneys [2,3]. Moreover, pigs have also multirenculate and multipapillate kidneys with true calices.

Pigs are highly relevant for skin studies [3,20]. In contrast to rodent skin, pig skin has analogous gross, microscopic and ultrastructural features to human skin. The epidermal thickness and the dermal: epidermal thickness ratios are comparable. Of particular importance, pig skin is usually hairless and has a fixed subcutaneous layer and dermal hair follicles. These similarities suggest that when confronted with identical stimuli, pig and human skins will respond similarly [20]. However, in a study comparing Bama minipig and human skin, some differences including pigment cell distribution and sweat gland types were observed [20].

The central nervous system and particularly the brain of pigs are also good models for humans given both their size and the anatomic characteristics of the structures [3,21]. Pigs also have white matter-predominant gyrencephalic brains and the development and the blood flow are comparable to those in human.

All these characteristics and the general size of organs make pigs closer to humans than mice are. Consequently, pigs more similarly respond to human pathogens.

The pig as a model to study human microbial infectious diseases

The pig has been used as a model for a number of infectious diseases relevant to human health. These models include both natural disease models, which are based on a porcine pathogen closely related or identical to the human pathogen, and experimental or surrogate infection models, in which a human pathogen under experimental conditions is given to a pig. The third potential model using transgenic animals is not yet represented in the study of human infectious diseases.

Systemic infectious diseases

Examples of systemic infectious diseases include infections with *Staphylococcus aureus*, pseudorabies virus (PrV) and classical swine fever virus (CSFV). Sepsis caused by *S. aureus* constitutes a serious human health concern worldwide [22]. Pigs have been identified as a source of methicillin-resistant *S. aureus*. Reflecting the range of diseases that *S. aureus* can be involved in, infection models for *S. aureus* in pigs include wound infection [23], osteomyelitis [24], pneumonia [25] and sepsis [22]. In 8-week-old pigs, the porcine osteomyelitis model developed lesions with a

similar pattern and presentation to osteomyelitic lesions in pre-pubertal children following hematogenous spread of *S. aureus* [24].

Alphaherpesviruses, such as PrV in pigs and herpes simplex virus 1 (HSV-1) in humans, have coevolved with their hosts. Their interactions with epithelial barriers and the immune system have prompted host barrier adaptations and immunological responses. Because alphaherpesviruses do not circulate in rodents, they cannot be used as homologous models, whereas pigs with PrV are suitable homologous models for studying alphaherpesvirus–host interactions to identify immune evasion pathways and new targets for drugs that block virus replication. Recent research using the pig model [26] demonstrated that the interaction of PrV with the nasal mucosa, the primary site of replication, is very similar to that of HSV-1 [27]. The virus replicates first in epithelial cells, activating cellular serine proteases that drill holes in the basement membrane, thereby allowing the virus to pass through this barrier and spread quickly in the lamina propria and submucosa. Subsequently, alphaherpesviruses spread along axons to the neuronal cell body of sensory neurons. Recently it has been shown that IFN-α promotes a latent stage in both PrV- and HSV-1-infected neurons [28].

CSFV induces a systemic disease in pigs, sharing all characteristics of human viral hemorrhagic fevers. These symptoms include high fever, hypotension, coagulation and hematological disorders including severe thrombocytopenia and generalized lymphoid depletion. An immunological hallmark of CSF and human hemorrhagic fevers is strongly elevated circulating inflammatory cytokine and interferon levels. Interestingly, CSFV and viruses responsible for human hemorrhagic fevers have a strong tropism for infection of antigen-presenting cells (APCs), suggesting an important role for this phenomenon in pathogenesis [29–31]. This is particularly puzzling considering their role in protective immunity. We propose that the porcine CSFV model could help to elucidate the important role of macrophages, DCs and the inflammatory/interferon type I system for development of fatal disease versus protective immunity both in CSFV and human viral hemorrhagic fevers. The knowledge obtained will provide novel options for intervention strategies to treat patients with severe hemorrhagic fevers.

Infectious diseases of the respiratory tract

Pigs have been frequently used as model hosts for respiratory pathogens including *Bordetella pertussis* [32], influenza viruses [33], *Mycobacterium tuberculosis* [34], *Pseudomonas aeruginosa* and *S. aureus* [25].

Pigs play an integral role in the ecology, epidemiology and evolution of the influenza virus. Based on the same subtypes that infect pigs and man (H1N1, H1N2 and H3N2) and the similarities between clinical diseases, pathogenesis and tissue tropism of influenza virus infections in pigs and humans, pigs are ideal experimental animals to study different aspects of influenza infections. In addition, as highlighted by the recent H1N1 pandemic of swine origin, pigs play an important role in the ecology and evolution of the influenza virus. The virus does not circulate in mice and only Mx-deficient mouse lines are susceptible to the virus. In such animals the virus distribution is systemic, often resulting in high mortality, which is not observed in pigs and humans. In humans and swine, virus replication occurs mainly locally in the upper and lower respiratory tract, with similar viral receptors being involved in virus infection and similar immune cell infiltration and local cytokine responses [33,35,36]. The sialic acid receptor distribution in pigs with respect to α2,6-and α2,3-linked galactose is strikingly similar to that in humans. In both species, α2,6 linkages predominate in the upper respiratory tract, whereas α2,3-linked galactose is restricted to the epithelium of the lower respiratory tract. This results in efficient replication of viruses with human-like but not avian-like receptor specificity in the epithelial cells of the upper respiratory tract [35]. This, and similar adaptation processes of the virus polymerase [37,38], may explain the relatively high resistance of both pigs and humans to infection with avian influenza viruses such as the H5N1 subtype. This is in contrast to ferrets, which are highly susceptible to H5N1 infections. For example, the pig has been employed to characterize early local inflammatory cytokine responses in the lung of infected animals. These studies have demonstrated the involvement of strong local proinflammatory cytokines and interferon type I responses that correlate with disease severity [39,40] and the involvement of various T lymphocyte subsets and DCs in pathogenesis [33]. Based on such results it has been proposed that the porcine natural host model may be of value to assess the therapeutic potential of cytokine antagonists for influenza [41] or corticosteroids for respiratory coronaviruses [42] to treat patients with respiratory distress syndrome. In particular, the emergence of the swine-origin H1N1 pandemic virus has recently highlighted the importance of the pig, which is fully susceptible to this virus and shows similar clinical signs. Furthermore, it appears that there is little evolutionary pressure forcing human pandemic H1N1 isolates to adapt to pigs [43]. By contrast, adaptive mutations in key viral genes such as polymerase differ between mouse and pig models [44], putting into question the data obtained in mice because this is an artificial host for influenza virus. Based on such similarities in viral pathogenesis and their immune systems, another potential area in which pigs could be used as a model is in the identification or confirmation of correlates of protection for heterosubtypic protection against influenza virus. The advantage of pigs over the ferret model is the availability of more immunological reagents and methods [5], including extensive information on swine leukocyte antigens.

The experimental model of ventilator-associated pneumonia induced by the inoculation of high concentrations of *P. aeruginosa* and *S. aureus* in mechanically ventilated piglets was very useful in understanding the local and systemic responses to lung infection and for the determination of potential measures of prevention or therapeutic modulation [25].

Other bacterial infections include infections with *Bordetella* spp. For example, *B. pertussis*, a strictly human pathogen, can infect newborn piglets under experimental conditions and induce clinical symptoms and pathology similar to the disease in infants [45]. Compared to the existing mouse model, the pig offers a number of

Box 4. Development of new vaccines using the pig model

Pigs are an ideal model for vaccine research. They are relatively cheap and because of their size can be housed in large groups in standard animal isolation rooms. They do not require special handling facilities, and can be routinely bled and immunized using well-established standard procedures. Vaccines can be administered either intramuscularly, subcutaneously, intradermally, orally or intranasally. In addition, more advanced methods of administration such as needle-free injectors, gene-guns and microneedles have been successfully used in pigs. As described earlier, pigs offer easy access to the various immune compartments including systemic and mucosal lymphoid tissues. Compared to mice, large numbers of immune cells can be isolated from each of these compartments and used for various immune assays. Depending on the age and breed, this can vary significantly. In addition, secretions such as saliva, nasal secretions, urine and feces can be easily collected and assessed for the presence of secretory IgA, IgM and IgG. Furthermore, pigs offer the advantage of the ability to test vaccines in an outbred population, and thus allow a more accurate assessment of the efficacy of potential human vaccines. Pigs have an epitheliochorial placentation, which means that there is no transplacental transfer of antibodies or larger molecules during gestation. Thus, the neonate depends on passively transferred immunity via colostrum and milk, which includes antibodies, proteins and immune cells. Maternal immunization is a common practice in the swine industry, and both colostrum and milk can be collected from the lactating sow. The litter size in domestic pigs is about 10–12 piglets per litter. Interestingly, during the first 7 days, piglets can be exchanged between litters, and vaccines can therefore be tested in the presence or absence of maternal antibodies.

advantages, including access to maternal antibodies in colostrum and milk, and access to mucosal immune compartments. Using the pig model, Elahi *et al.* demonstrated that maternal antibodies play an important role in protection against *B. pertussis* [46]. Thus, the pig model has become a very valuable model in testing of vaccines for human infants [32] (Box 4). Interestingly, *Bordetella parapertussis*, a close relative of *B. pertussis* and a frequent cause of whooping cough in humans, can infect older pigs and has developed strategies to overcome the innate defenses of the pig. Such strategies include higher resistance by *B. parapertussis* than *B. pertussis* to neutralization by porcine β-defensin 1 in the respiratory tract [47].

Infectious diseases of the digestive tract

Pigs have been used to study various human GIT pathogens including *Cryptosporidium parvum* [48], *Helicobacter pylori* [49], hepatitis E virus (HEV) [50], norovirus and rotavirus.

Gnotobiotic (Gn) piglets have been used to study *Helicobacter* infections [49]. The low neutrophil responses associated with inflammation induced in infected piglets mimic responses of *H. pylori*-infected pediatric patients, and piglets have been extremely useful in demonstrating that virulence factors such as urease and motility are required for productive infection [49]. Although non-human primates are the most common animal model used to study HEV infection, pigs – a natural reservoir for HEV genotypes 3 and 4 – have been successfully used to elucidate the structural and functional relationship of HEV genes and to understand the mechanism of HEV replication [50].

Human noroviruses (HuNoVs) are a major cause of foodborne gastroenteritis worldwide [51]. NoV infections have been identified in multiple species including swine [52]. On the basis of sequence or antigenic similarities between human and swine NoVs [52] and epidemiologic studies [53], there are mounting concerns regarding potential interspecies and zoonotic transmission of NoVs. Owing to the lack of a cell culture system for HuNoVs, little is known about NoV replication strategies and no vaccines, antivirals or therapies are available for HuNoVs. Humans show different genetic susceptibilities to NoV infection, depending on their histoblood group antigen (HBGA) phenotype and the NoV strain [54]. Pigs share HBGAs related to those of humans on their epithelial cells [55], with similar tissue distributions and expression patterns [56]. As in humans, the HBGA type of pigs influences their susceptibility to HuNoV strains that bind to the corresponding HBGAs [56].

Rotavirus (RV) is a leading cause of childhood diarrhea, but current attenuated human RV (AttHRV) oral vaccines fail in impoverished countries where diarrhea mortality is highest [57]. Gn pigs are the only animal model susceptible to human (HRV) diarrhea, so studies of neonatal Gn pigs have enhanced our understanding of HRV pathogenesis, immunity and vaccine strategies [58]. Neonatal pigs resemble infants in several ways [58,59]. As outbred animals they are more representative of human population heterogeneity. Similar to infants, they are immunocompetent at birth, but immunologically immature [60]. As in humans, secretory immunoglobulin A (sIgA) is dominant in the intestine, milk and mucosal secretions [61]. Light chain repertoire and usage in porcine B cells are similar to those observed in humans [62]. HRV-infected Gn pigs exhibit diarrhea, anorexia, dehydration, viremia and intestinal lesions mimicking those in children, in contrast to the lack of RV diarrhea or lesions seen in widely used adult mouse models [58,63]. RV diarrhea leads to upregulated proinflammatory cytokines in the blood of infected children and HRV-infected neonatal Gn pigs, which correlate with diarrhea severity [58,63]. Acute HRV infection in children is associated with higher expression of TLR 2, 3, 4, 7 and 8 on blood mononuclear cells, which play a role in pathogenesis and immunity [64]. Likewise, RV-infected Gn pigs have increased numbers of TLR3-expressing APCs in blood and spleen [65]. These findings document the importance of similar immune mediators in the pathogenesis of HRV disease in children and in Gn pigs.

AttHRV oral vaccines are a focus for controlling HRV gastroenteritis, but for unexplained reasons vaccine efficacy in developing countries is substantially lower than in developed countries [57]. Studies of HRV in Gn pigs were based on using virulent (Vir) HRV (Gn pig-passaged infant stool) and the corresponding AttHRV (cell-cultured) Wa strain (G1P1A [8]) (the same serotype as the licensed monovalent HRV vaccine), the most common RV G and P serotypes associated with HRV gastroenteritis worldwide [58]. The following immune parameters in VirHRV-challenged pigs were significantly correlated with protection: intestinal and blood IgA HRV-specific antibody-secreting cells (ASCs) and antibodies and frequencies of IFN-γ-producing $CD4^+$ and $CD8^+$ T cells in the gut [58,66]. Thus, high protection rates are associated with IgA antibodies and balanced Th1/Th2 responses.

Identification of serum IgA antibodies or ASC as a correlate for gut or fecal IgA antibodies and protection strongly parallels findings for HRV-infected infants, making the piglet model highly relevant for understanding HRV pathogenesis and for vaccine testing. Other major advances in rotavirus research emanating from Gn pig studies include: (i) cell culture propagation of the first HRV after passage and amplification in Gn pigs, (ii) delineation of the independent roles of two RV outer capsid proteins (VP4 and VP7) in cross-protection, and (iii) recognition of the potential role for a nonstructural protein (NSP4) in RV virulence [58,66,67].

Integumentary and mucosal infectious diseases

Although pigs have a very similar integumentary system and eyes to humans, the pig model has not been used extensively as a model to study human skin and eye infectious diseases. However, the pig model has been used to study contact-lens-induced *Acanthamoeba keratitis* [68]. Using adult Yucatan micropigs, He *et al.* observed lesions similar to those identified in humans, including dense white ring-like infiltrates, stroma edema and keratitis precipitates [68]. The lesions and their chronic nature and the anatomical similarity of the pig to the human eye will make the porcine model a valuable addition for investigating the cell biology of *A. keratitis*, the host immune response, and potential therapeutics.

Pigs aged 13 weeks have also been tested as a large animal model for female genital infection with *Chlamydia trachomatis*, a strict pathogen of oculogenital epithelial cells [69]. Vanrompay *et al.* demonstrated that serovar E strains Bour and 468 could ascend in the porcine genital tract [69]. Bacteria replicated in the superficial epithelial cervical and uterine layers, the most common target for *C. trachomatis*. Moreover, pigs mounted an inflammatory response and produced specific antibodies against the pathogen, making pigs an attractive model in which to study the pathology, pathogenesis and immune response to *C. trachomatis* genital infection [69].

Infectious diseases of the nervous system

The pig model has also been used to study infections of the human nervous system. Pathogens causing nervous system pathology include, for example, *Neisseria meningitidis* [70], Nipah virus (NiV) [71], PrV, and some coronaviruses. The pig model of human meningococcal sepsis simulates central aspects of the human disease, including cardiovascular parameters such as cardiac index and mean arterial pressure, vascular leakage, cytokine release and changes in hematological and coagulation parameters. The model, using young pigs, allows investigations from the disease onset, corresponding to the pre-hospital stage in humans [70]. Because meningococcal sepsis is a rare pathology, it has been difficult to assess treatment effects in controlled trials. The porcine model allows studies from onset of disease and could be used for evaluating new therapies [70]. Disease pathogenesis associated with NiV has been systematically studied in pigs, which can be naturally infected [71]. After infection with NiV the majority of young pigs typically develop mild clinical signs such as increased body temperature and mild respiratory difficulties [71]. However, after oronasal or subcutaneous inoculations, severe neurological signs can occur, as generally observed in humans [71]. The depletion and the necrosis reported in infected lymphoid tissues indicate that the virus replicates in lymphoid cells [71]. This observation was confirmed by *in vitro* infection of porcine peripheral blood mononuclear cells. The virus tropism and the secondary bacterial infections observed in pigs raise the possibility that NiV induces immunosuppression.

Box 5. Future research aims

- Development of new tools to assess porcine immune responses
- Continued progress in porcine transgenesis because transgenic pigs are readily applicable to the study of infectious diseases
- Publication of a complete annotated porcine genome sequence (imminent)
- Development of new porcine models for the study of diseases such as shigellosis for which the current respiratory mouse model does not mirror human intestinal disease
- Take advantage of the huge porcine genetic diversity: various breeds respond differently to infection
- Development of new inbred miniature breeds
- Use of porcine models should progress as a consequence of higher restrictions on the use of alternative animal models, such as monkeys or dogs, for ethical reasons
- The pig should increasingly become the 'official' large-animal model
- Increased acceptance of the pig as a valuable model in the scientific community
- More experimental facilities available to house pigs

Concluding remarks

Although not exhaustive, the list of human pathogens for which porcine models have been used documents the contribution and the advantages of pigs in the study of human infectious diseases. Considering the comparable size and similarities in the skin, respiratory and digestive tracts, and the immune system, the pig offers an attractive intermediate animal model for testing of both novel antigen-delivery platforms for skin and mucosal antigen administration and immunostimulants before moving to expensive primate models or to clinical trials. Furthermore, with the development of porcine transgenesis [72], greater possibilities to induce or modulate infectious diseases should be forthcoming. Over the next few years there is no doubt that the pig model will be increasingly accepted as the alternative large animal model to the well-established mouse model (Box 5).

References

1 Hart, E.A. *et al.* (2007) Lessons learned from the initial sequencing of the pig genome: comparative analysis of an 8 Mb region of pig chromosome 17. *Genome Biol.* 8, R168
2 Dyce, K.M. *et al.* (1987) *Textbook of Veterinary Anatomy*, WB Saunders
3 Swindle, M.M. *et al.* (2011) Swine as models in biomedical research and toxicology testing. *Vet. Pathol.* DOI: 10.1177/0300985811402846
4 Ibrahim, Z. *et al.* (2006) Selected physiologic compatibilities and incompatibilities between human and porcine organ systems. *Xenotransplantation* 13, 488–499
5 Summerfield, A. (2009) Special issue on porcine immunology: an introduction from the guest editor. *Dev. Comp. Immunol.* 33, 265–266
6 Sang, Y. *et al.* (2009) Antimicrobial host defense peptides in an arteriviral infection: differential peptide expression and virus inactivation. *Viral Immunol.* 22, 235–242
7 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 *The Minipig in Biomedical Research* (McAnulty, P.A., ed.), CRC Press, Taylor & Francis Group, pp. 321–341

8 Salmon, H. *et al.* (2009) Humoral and cellular factors of maternal immunity in swine. *Dev. Comp. Immunol.* 33, 384–393
9 Piriou-Guzylack, L. and Salmon, H. (2008) Membrane markers of the immune cells in swine: an update. *Vet. Res.* 39, 54
10 Fairbairn, L. *et al.* (2011) The mononuclear phagocyte system of the pig as a model for understanding human innate immunity and disease. *J. Leukoc. Biol.* 6, 855–871
11 Mosmann, T.R. and Coffman, R.L. (1989) TH1 and TH2 cells: different patterns of lymphokine secretion lead to different functional properties. *Annu. Rev. Immunol.* 7, 145–173
12 Kaser, T. *et al.* (2011) Porcine regulatory T cells: Mechanisms and T-cell targets of suppression. *Dev. Comp. Immunol.* 35, 1166–1172
13 Kiros, T.G. *et al.* (2011) Induction, regulation and physiological role of IL-17 secreting helper T-cells isolated from PBMC, thymus, and lung lymphocytes of young pigs. *Vet. Immunol. Immunopathol.* 144, 448–454
14 Murtaugh, M.P. *et al.* (2009) Species specialization in cytokine biology: is interleukin-4 central to the T(H)1-T(H)2 paradigm in swine? *Dev. Comp. Immunol.* 33, 344–352
15 Pampusch, M.S. *et al.* (1998) Inducible nitric oxide synthase expression in porcine immune cells. *Vet. Immunol. Immunopathol.* 61, 279–289
16 Summerfield, A. and McCullough, K.C. (2009) The porcine dendritic cell family. *Dev. Comp. Immunol.* 33, 299–309
17 Mutwiri, G. *et al.* (2009) Approaches to enhancing immune responses stimulated by CpG oligodeoxynucleotides. *Adv. Drug Deliv. Rev.* 61, 226–232
18 McLaughlin, R.F. *et al.* (1961) A study of the subgross pulmonary anatomy of various mammals. *Am. J. Anat.* 108, 149–165
19 Horter, D.C. *et al.* (2003) A review of porcine tonsils in immunity and disease. *Anim. Health Res. Rev.* 4, 143–155
20 Liu, Y. *et al.* (2010) Light microscopic, electron microscopic, and immunohistochemical comparison of Bama minipig (*Sus scrofa domestica*) and human skin. *Comp. Med.* 60, 142–148
21 Lind, N.M. *et al.* (2007) The use of pigs in neuroscience: modeling brain disorders. *Neurosci. Biobehav. Rev.* 31, 728–751
22 Nielsen, O.L. *et al.* (2009) A pig model of acute *Staphylococcus aureus* induced pyemia. *Acta Vet. Scand.* 51, 14
23 Svedman, P. *et al.* (1989) Staphylococcal wound infection in the pig: part I. Course. *Ann. Plast. Surg.* 23, 212–218
24 Jensen, H.E. *et al.* (2010) A non-traumatic *Staphylococcus aureus* osteomyelitis model in pigs. *In Vivo* 24, 257–264
25 Luna, C.M. *et al.* (2009) Animal models of ventilator-associated pneumonia. *Eur. Respir. J.* 33, 182–188
26 Glorieux, S. *et al.* (2011) A trypsin-like serine protease is involved in pseudorabies virus invasion through the basement membrane barrier of porcine nasal respiratory mucosa. *Vet. Res.* 42, 58
27 Glorieux, S. *et al.* (2011) Herpes simplex virus type 1 penetrates the basement membrane in human nasal respiratory mucosa. *PLoS ONE* 6, e22160
28 De Regge, N. *et al.* (2010) Interferon alpha induces establishment of alphaherpesvirus latency in sensory neurons *in vitro*. *PLoS ONE* 5, e13076
29 Geisbert, T.W. and Jahrling, P.B. (2004) Exotic emerging viral diseases: progress and challenges. *Nat. Med.* 10, S110–S121
30 McCullough, K.C. *et al.* (2009) Dendritic cells – at the front-line of pathogen attack. *Vet. Immunol. Immunopathol.* 128, 7–15
31 Summerfield, A. *et al.* (2000) Pathogenesis of granulocytopenia and bone marrow atrophy during classical swine fever involves apoptosis and necrosis of uninfected cells. *Virology* 272, 50–60
32 Elahi, S. *et al.* (2007) The benefits of using diverse animal models for studying pertussis. *Trends Microbiol.* 15, 462–468
33 Khatri, M. *et al.* (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
34 Gil, O. *et al.* (2010) Granuloma encapsulation is a key factor for containing tuberculosis infection in minipigs. *PLoS ONE* 5, e10030
35 Van Poucke, S.G. *et al.* (2010) Replication of avian, human and swine influenza viruses in porcine respiratory explants and association with sialic acid distribution. *Virol. J.* 7, 38
36 Van Reeth, K. *et al.* (1998) Bronchoalveolar interferon-alpha, tumor necrosis factor-alpha, interleukin-1, and inflammation during acute influenza in pigs: a possible model for humans? *J. Infect. Dis.* 177, 1076–1079
37 Manzoor, R. *et al.* (2009) PB2 protein of a highly pathogenic avian influenza virus strain A/chicken/Yamaguchi/7/2004 (H5N1) determines its replication potential in pigs. *J. Virol.* 83, 1572–1578
38 Mehle, A. and Doudna, J.A. (2009) Adaptive strategies of the influenza virus polymerase for replication in humans. *Proc. Natl. Acad. Sci. U.S.A.* 106, 21312–21316
39 Van Reeth, K. *et al.* (2002) Correlations between lung proinflammatory cytokine levels, virus replication, and disease after swine influenza virus challenge of vaccination-immune pigs. *Viral. Immunol.* 15, 583–594
40 Van Reeth, K. *et al.* (2002) *In vivo* studies on cytokine involvement during acute viral respiratory disease of swine: troublesome but rewarding. *Vet. Immunol. Immunopathol.* 87, 161–168
41 Barbe, F. *et al.* (2011) Cytokines and acute phase proteins associated with acute swine influenza infection in pigs. *Vet. J.* 187, 48–53
42 Jung, K. *et al.* (2007) Altered pathogenesis of porcine respiratory coronavirus in pigs due to immunosuppressive effects of dexamethasone: implications for corticosteroid use in treatment of severe acute respiratory syndrome coronavirus. *J. Virol.* 81, 13681–13693
43 Ma, W. *et al.* (2011) 2009 pandemic H1N1 virus causes disease and upregulation of genes related to inflammatory and immune response, cell death and lipid metabolism in pigs. *J. Virol.* 85, 11626–11637
44 Ma, W. *et al.* (2011) Pathogenicity of swine influenza viruses possessing an avian or swine-origin PB2 polymerase gene evaluated in mouse and pig models. *Virology* 410, 1–6
45 Elahi, S. *et al.* (2005) Infection of newborn piglets with *Bordetella pertussis*: a new model for pertussis. *Infect. Immun.* 73, 3636–3645
46 Elahi, S. *et al.* (2006) Maternal immunity provides protection against pertussis in newborn piglets. *Infect. Immun.* 74, 2619–2627
47 Elahi, S. *et al.* (2008) Infection with *Bordetella parapertussis* but not *Bordetella pertussis* causes pertussis-like disease in older pigs. *J. Infect. Dis.* 198, 384–392
48 Argenzio, R.A. *et al.* (1990) Villous atrophy, crypt hyperplasia, cellular infiltration, and impaired glucose-Na absorption in enteric cryptosporidiosis of pigs. *Gastroenterology* 98, 1129–1140
49 Nedrud, J.G. (1999) Animal models for gastric *Helicobacter* immunology and vaccine studies. *FEMS Immunol. Med. Microbiol.* 24, 243–250
50 Krawczynski, K. *et al.* (2011) Pathogenetic elements of hepatitis E and animal models of HEV infection. *Virus Res.* 161, 78–83
51 Green, K.Y. (2007) Caliciviridae: the Noroviruses. In *Fields Virology* (5th edn) (Knipe, D.M. and Howley, P.M., eds), Lippincott Williams & Wilkins, pp. 949–979
52 Wang, Q.H. *et al.* (2005) Porcine noroviruses related to human noroviruses. *Emerg. Infect. Dis.* 11, 1874–1881
53 Mattison, K. *et al.* (2007) Human noroviruses in swine and cattle. *Emerg. Infect. Dis.* 13, 1184–1188
54 Le Pendu, J. *et al.* (2006) Mendelian resistance to human norovirus infections. *Semin. Immunol.* 18, 375–386
55 Balanzino, L.E. *et al.* (1994) Differential interaction of *Escherichia coli* heat-labile toxin and cholera toxin with pig intestinal brush border glycoproteins depending on their ABH and related blood group antigenic determinants. *Infect. Immun.* 62, 1460–1464
56 Cheetham, S. *et al.* (2007) Binding patterns of human norovirus-like particles to buccal and intestinal tissues of gnotobiotic pigs in relation to A/H histo-blood group antigen expression. *J. Virol.* 81, 3535–3544
57 Madhi, S.A. *et al.* (2010) Effect of human rotavirus vaccine on severe diarrhea in African infants. *N. Engl. J. Med.* 362, 289–298
58 Saif, L.J. *et al.* (1996) The gnotobiotic piglet as a model for studies of disease pathogenesis and immunity to human rotaviruses. *Arch. Virol. Suppl.* 12, 153–161
59 Butler, J.E. *et al.* (2009) The piglet as a model for B cell and immune system development. *Vet. Immunol. Immunopathol.* 128, 147–170
60 Hammerberg, C. *et al.* (1989) Immunodeficiency in young pigs. *Am. J. Vet. Res.* 50, 868–874
61 Wagstrom, E.A. *et al.* (2000) Immune components in porcine mammary secretions. *Viral Immunol.* 13, 383–397
62 Butler, J.E. *et al.* (2004) Antibody repertoire development in fetal and neonatal pigs. VII. Characterization of the preimmune kappa light chain repertoire. *J. Immunol.* 173, 6794–6805

63 Ward, L.A. *et al.* (1996) Pathogenesis of an attenuated and a virulent strain of group A human rotavirus in neonatal gnotobiotic pigs. *J. Gen. Virol.* 77, 1431–1441
64 Xu, J. *et al.* (2007) Expression of Toll-like receptors in mononuclear cells from children with acute rotavirus diarrhea. *Zhonghua Shi Yan He Lin Chuang Bing Du Xue Za Zhi* 21, 38–40
65 Wen, K. *et al.* (2009) Toll-like receptor and innate cytokine responses induced by lactobacilli colonization and human rotavirus infection in gnotobiotic pigs. *Vet. Immunol. Immunopathol.* 127, 304–315
66 Yuan, L. *et al.* (2008) Virus-specific intestinal IFN-gamma producing T cell responses induced by human rotavirus infection and vaccines are correlated with protection against rotavirus diarrhea in gnotobiotic pigs. *Vaccine* 26, 3322–3331
67 Hoshino, Y. *et al.* (1988) Infection immunity of piglets to either VP3 or VP7 outer capsid protein confers resistance to challenge with a virulent rotavirus bearing the corresponding antigen. *J. Virol.* 62, 744–748
68 He, Y.G. *et al.* (1992) A pig model of *Acanthamoeba keratitis*: transmission via contaminated contact lenses. *Invest. Ophthalmol. Vis. Sci.* 33, 126–133
69 Vanrompay, D. *et al.* (2005) Specific-pathogen-free pigs as an animal model for studying *Chlamydia trachomatis* genital infection. *Infect. Immun.* 73, 8317–8321
70 Nielsen, E.W. *et al.* (2009) A new dynamic porcine model of meningococcal shock. *Shock* 32, 302–309
71 Weingartl, H.M. *et al.* (2009) Animal models of henipavirus infection: a review. *Vet. J.* 181, 211–220
72 Aigner, B. *et al.* (2010) Transgenic pigs as models for translational biomedical research. *J. Mol. Med.* 88, 653–664
73 Larson, G. *et al.* (2005) Worldwide phylogeography of wild boar reveals multiple centers of pig domestication. *Science* 307, 1618–1621
74 Chen, K. *et al.* (2007) Genetic resources, genome mapping and evolutionary genomics of the pig (*Sus scrofa*). *Int. J. Biol. Sci.* 3, 153–165
75 Butler, J.E. and Sinkora, M. (2007) The isolator piglet: a model for studying the development of adaptive immunity. *Immunol. Res.* 39, 33–51
76 Sachs, D.H. *et al.* (1976) Transplantation in miniature swine. I. Fixation of the major histocompatibility complex. *Transplantation* 22, 559–567
77 Archibald, A.L. *et al.* (2010) Pig genome sequence – analysis and publication strategy. *BMC Genomics* 11, 438
78 Sun, H.F. *et al.* (1999) Human chromosome 3 and pig chromosome 13 show complete synteny conservation but extensive gene-order differences. *Cytogenet. Cell Genet.* 85, 273–278
79 Ramos, A.M. *et al.* (2009) Design of a high density SNP genotyping assay in the pig using SNPs identified and characterized by next generation sequencing technology. *PLoS ONE* 4, e6524
80 Zhang, N. *et al.* (2011) Crystal structure of swine MHC class I SLA-1*0401 and identification of 2009-pandemic swine-origin influenza A H1N1 virus (S-OIV) CTL epitope-peptides. *J. Virol.* 85, 11709–11724