Chapter 11
The Importance of Animal Models in the Development of Vaccines

Tadele G. Kiros, Benoît Levast, Gaël Auray, Stacy Strom, Jill van Kessel, and Volker Gerdts

Abstract Efficient translation of basic vaccine research into clinical therapies greatly depends upon the availability of appropriate animal models. Testing novel vaccine candidates in animal models is a critical step in the development of modern vaccines. Animal models are being used to assess the quality and quantity of the immune response, to identify the optimal route of delivery and formulation, to determine protection from infection and disease transmission, and to evaluate the safety and toxicity of the vaccine formulation. Animal models help to make the translation from basic research to clinical application, and they often allow prediction of the vaccine potential, which helps in predicting the financial risks for vaccine manufacturers. Choosing an appropriate animal model has become increasingly important for the field, as each model has its own advantages and disadvantages. In this review, the criteria for selecting the right animal model, the advantages and disadvantages of various animal models, as well as the future needs for animal models are being discussed.

Keywords Animal model • Vaccine development • Vaccine delivery • Infectious disease

11.1 Introduction

Animal models are commonly used to assess a variety of immunological parameters including humoral and cell-mediated immunity, onset and duration of immunity, systemic versus mucosal immunity, protection against challenge infection and

T.G. Kiros • B. Levast • G. Auray • S. Strom • J. van Kessel • V. Gerdts (✉)
Vaccine and Infectious Disease Organization-InterVac, and Department of Veterinary Microbiology, University of Saskatchewan, 120 Veterinary Road, Saskatoon S7N 5E3, SK, Canada
e-mail: volker.gerdts@usask.ca

reduction of disease transmission. A plethora of animal models exists, ranging from very small insects to very large livestock species, such as horses or cattle. Animal models are being used to investigate very specific immune mechanisms, such as the trafficking and interaction of effector cells, or they can be used to assess larger aspects of vaccine development, such as the induction of herd immunity or to model the spread of a certain disease within a naïve or vaccinated population. Animal models can range greatly, from transgenic and cloned animals to outbred species; from surgical models that facilitate access to certain immune compartments to "humanized" animals; from neonatal to aging animals; and from gnotobiotic to wild type animals. They can be used to model single infections versus co-infections, chronic diseases and autoimmune disorders, and they can be used to analyze herd immunity following vaccination, transmission amongst infected and non-infected animals, as well as studying transfer of passive immunity via the placenta, colostrum, and milk. Thus, choosing the appropriate animal model is critical for the development of modern, more effective vaccines. However, the use of animals for research also comes with an ethical responsibility to treat the animal in the best possible way, and to avoid suffering or unnecessary pain. Thus, the use of animals in research should be limited to circumstances for which no other model exists and should be monitored through ethics committees involving the public.

11.2   Animal Models for Vaccine Research

Testing vaccines in animal models is a critical step in vaccine development, and often the most critical decision point in the long process of developing and registering a vaccine. Hundreds of different models are available to assess various aspects of the immune response. A plethora of species, strains, and mutants are available for these studies and some of them are reviewed in this review.

Many countries promote replacement and reduction of animal experiments for research as much as possible (Wiles et al. 2006), however, as there is no other method currently available to test the induction of immune responses to vaccination the use of animals remains critical in the development of vaccines. However, choosing the most appropriate animal model is crucial for success of the projects and in the long run to save animals and research money. Most vaccines have been evaluated at one point in small rodents, most likely mice. Mice have the advantage of being readily available at a low cost, they are easy to handle, they have defined genetic backgrounds, and their immune functions are well characterized. Furthermore, an abundance of immunological reagents exists for mice allowing a very detailed analysis of the immune response to vaccines. Fewer reagents are available for other species, which limits the level of detail in the analysis. However, large animal species such as pigs, cows and sheep have the advantage of being physiologically and immuno-logically closer related to man and often are host to the same or closely related pathogens. (Elahi et al. 2007; Gerdts et al. 2001). Moreover, large animal species are predominantly outbred, which is important for the development of vaccines as

a normal distribution for vaccine responders and non-responders can be seen. The genome for most species has been sequenced and annotated (Bishop et al. 2011), or is in the final process of being annotated. A detailed overview of the potential advantages and disadvantages of various species for vaccine research is provided in Table 11.1.

Animal models can be grouped into models used to assess an immune response only, natural disease models, surrogate disease models and surgical or experimental models. These models vary greatly in their scope, their cost and their requirement for special infrastructure.

11.2.1   Models to Assess an Immune Response

Models to assess an immune response typically include mice and small rodents, and in most cases are based on the use of specific strains, or knockouts. For example, the linkages between innate and acquired immune response to vaccination can be assessed by using mice that are defective in innate signalling pathways, such as MyD88$^{-/-}$ or TRIF$^{-/-}$ mice. To assess the type of an immune response induced by a specific vaccine Balb/c mice versus C57 black are commonly used, since reagents are available to assess both cytokine secretion and specific antibody isotypes. However, numerous other strains are available to assess the immune response in mice. Other species commonly used include rabbits, rats and guinea pigs. The advantages of these models is the ability to rapidly assess the immune response to a certain antigen and are commonly used for large screen testing of adjuvants, vaccine formulations or for the assessment of the best route of immunization. Specific strains, knockouts, or even humanized animals are being used to assess certain qualities of the immune response including a shift towards T helper (Th) 1, Th2 or Th17 responses, induction of mucosal versus systemic immunity, onset and duration of immunity etc. The one key characteristic though is that these models can't be used to assess protection against infection, and thus are somewhat limited for the development of vaccines.

11.2.2   Surrogate Models

These models are commonly used in preclinical vaccine development and refer to the use of species that only under experimental conditions can be infected with the pathogen of interest. These models are somewhat artificial as often higher infection doses, artificial routes of infection, or lack of clinical symptoms are being used. However, they offer the advantages of working with animals that can be easily housed and handled, are cost-effective or are well defined in terms of the immune system. Most often mice are being used, not only for developmental purposes but also from a regulatory point of view for registering a vaccine product, as it allows

Table 11.1 Advantages and disadvantages of selected animal models for vaccine development

Species	Advantages	Disadvantages
Mouse	Low cost ($20–120 per animal); easy to handle; can be housed in groups in cage systems/microisolators; immune system very well characterized; short breeding cycles; hemo-chorial placentation allowing transfer of some IgG subclasses; abundance of reagents and assays available; genetically well defined and matched; adaptive transfer of immune cells possible; plethora of well-defined transgenic strains available	Small size; access to mucosal surfaces and several immune compartments limited; manipulation of neonates very difficult; short neonatal period; limited routes for vaccine delivery; PRR-expression on dendritic cells differs from humans; short life-span
Rat	Low cost ($60–120 per animal); easy to handle; can be housed in cage systems/microisolators; immune system very well characterized; relatively short breeding cycles; hemo-chorial placentation allowing transfer of some IgG subclasses; reagents available but limited; some MHC-matched strains available	Not as easy to handle as mice; less reagents available; small size; limited access to immune compartments and mucosal surfaces; need to be housed in smaller groups, sometimes individually
Cotton rat	Moderate cost ($80–120); can be housed in cages; excellent model for respiratory infections; reagents for common cytokines and biomarkers available; hemo-chorial placentation allowing transfer of some IgG subclasses; moderate life-span	Require individual housing; difficult to handle; small size, limited access to certain immune compartments; few reagents available
Woodchuck	Excellent model for hepatitis virus infections; hemo-chorial placentation; relatively long life span (up to 20 years in captivity)	Require individual housing; specific needs; difficult to handle; small size; limited access to certain immune compartments; few reagents available
Guinea pig	Moderate cost ($50–150); easy to handle; can be housed in cages; routinely used to produce polyclonal sera	Small size; limited access to immune compartments; few reagents available
Rabbit	Moderate cost ($50–150); easy to handle; can be housed in cages or groups; good serum donors; larger life span; hemo-chorial placentation	Require infrastructure to house larger numbers; fewer reagents available
Ferret	Moderate cost ($150–300); excellent model for respiratory viral infections including influenza; outbred species; access to mucosal compartments	Small size; difficult to handle; requires specific training and infrastructure
Pig	Moderate cost ($50–1,000); large size allows access to mucosal surfaces and various immune compartments; physiologically very similar to humans; epithelio-chorial placenta type; no transfer of antibodies; access to fetus and fetal tissues; genome has been sequenced and is currently being annotated; outbred species; MHC-matched lines available that facilitate adoptive transfer; "minipigs" that are smaller in size and easier to handle; widely accepted model for xenotransplantation; mucosal delivery of vaccines possible; large toolkit available	Requires facilities and training; anatomically reverse lymph nodes; grow very fast; host to endogenous retroviruses.

Animal		
Sheep, goat	Moderate cost ($100–300); easy to handle; large size allows access to mucosal surfaces and various immune compartments; epithelio-chorial placenta type; no transfer of antibodies; access to fetus and fetal tissues; long neonatal period; functional mucosal immune system at birth; mucosal delivery of vaccines easily possible; outbred species	Requires special facilities and training; seasonal breeders, lambs available max three times a year; only 1–2 lambs per ewe
Cattle	Large size allows access to mucosal surfaces and various immune compartments; physiologically very similar to humans; epithelio-chorial placenta type; no transfer of antibodies; access to fetus and fetal tissues; mucosal immune system fully functional at birth; access to various immune compartments including colostrums and milk; long neonatal period; genome has been sequenced at annotated; reagents for the most common cytokines, isotypes and biomarkers available; mucosal delivery of vaccines; outbred species	Moderate cost ($500–2,000); requires special facilities and training; long breeding cycle, only 1–2 offspring per year
Horse	Epithelio-chorial placenta type; long-life span; excellent model for the elderly; access to a variety of immune compartments including the mucosal surfaces; long neonatal period; mucosal immune system fully functional at birth; outbred species	High cost ($500–3,000); need for special facilities and training
Dog	Moderate cost ($150–450); longer-life span; access to immune compartment and mucosal sites; endothelial-chorial placenta type; outbred species	Need for special facilities and training; not as easy to handle in larger groups; few reagents available; immune system not fully developed at birth
Cat	Moderate cost ($100–250); longer-life span; access to immune compartment and mucosal sites; endothelial-chorial placenta type, outbred species	Need for special facilities and training; not as easy to handle in larger groups; few reagents available; immune system not fully developed at birth
Non-human primate	Very costly ($1,000–20,000); physiologically very similar to humans (depending on species); easy access to mucosal sites and many immune compartments; immune functions well defined; outbred species; hemo-chorial placenta type	Very costly ($1,000–20,000); need for special facilities and training; immune system develops post partum

MHC major histocompatibility complex, *PRR* pattern recognition receptor

screening of large numbers of candidate vaccines in a rapid and efficient way and in most cases is more cost effective. In particular the ability to specifically knock out individual genes has helped in the understanding of very specific immune functions and the ability to adoptively transfer immune cells from one animal to another is another major advantage of using mice as surrogate model. More recently, the creation of "humanized" mice, which are generated by the transfer of human stem cells into fetal animals, has further enhanced the potential of surrogate models for vaccine development (Macchiarini et al. 2005; Shultz et al. 2007). However, the use of other species as surrogate models is becoming more and more popular. For example, cotton rats are widely accepted as an excellent model for respiratory viruses, and ferrets are being used to model *Influenza virus* infections. Guinea pigs and domestic pigs can be used for tuberculosis research, and pigs are being used for a number of pathogens including *Enteromoeba histolytica* (Girard-Misguich et al. 2011), *Chlamydia trichomatis* and *Hendra virus* (Meurens et al. 2012). We recently developed a novel model for pertussis in newborn piglets (Elahi et al. 2005). This model resembles the disease in human much closer and allows the assessment of both vaccine induced immune responses as well as study of the interaction between the bacterium *Bordetella pertussis* and the host (Polewicz et al. 2011). Interestingly, pigs are natural host to *B. bronchiseptica*, and thus many of the results can be directly translated into the development of veterinary vaccines (Elahi et al. 2007). Thus, the use of surrogate models has many advantages over models that are being used to assess the immune response to vaccination only. Surrogate models can be used to understand the role of various aspects of the immune responses including innate and acquired immunity, mucosal versus systemic immunity as well as trafficking of effector cells from one immune compartment to another, but offer the major advantage that these findings can be correlated with protection against experimental challenge infection.

11.2.3   Natural Disease Models

These models are based on a specific pathogen and its natural host and have the advantage of resembling the interaction between host and pathogen within the appropriate biological context. Thus, natural models can be used to analyze various aspects of the immune response to immunization and infection including the role of virulence factors during invasion, penetration and toxicity, as well as the host's immune response to the pathogen. Natural disease models include many large animal species, which has proven to be a very successful strategy for developing vaccines against both human and animal diseases (Table 11.1). For example, the use of large animal models has helped in the development of vaccines against several important infectious diseases including *Herpes simplex virus* (HSV) infections, *Escherichia coli*, *Rota-* and *Coronavirus*, *Respiratory syncytial virus* (RSV), *Influenza* and *West Nile virus* (WNV), to name a few (Baron and Coombes 2007; Hall and Khromykh 2004; Osterrieder et al. 2006; Potter et al. 2004; Rouse and Kaistha 2006). An important advantage of large animal models is the ability to use the natural route

of challenge and therefore obtain more relevant correlates of immune-mediated protection. In addition, using large animal models one can find high- and low-responders, which then can be further characterized using genome, proteome and kinome analysis (Jalal et al. 2009; Wilkie and Mallard 1999).

Vaccine efficacy also varies dramatically when immunizing the very young or the elderly (Lambert et al. 2005; Lang et al. 2011; Moxon and Siegrist 2011). Natural disease models including *Parvovirus*, *E. coli* and *Rotavirus* infections in pigs and calves have been used to establish the concept of maternal vaccination as an effective strategy to reduce the risk of infection in the neonate. These studies identified vaccine strategies to optimize the passive transfer of maternal immunity to the newborn and determined the duration of protection following passive transfer of maternal antibodies (Dobrescu and Huygelen 1976; Kohara et al. 1997; McNulty and Logan 1987; Mostl and Burki 1988). As a result, this concept has been introduced into human medicine and several vaccines are now available for immunization of pregnant mothers, and additional candidates are being considered by several countries in the world (Blanchard-Rohner and Siegrist 2011; Edwards 2003; Poehling et al. 2011).

Another major advantage of natural disease models is the ability to study co-infections between two or more pathogens. There is increasing evidence in the literature that co-infections substantially contribute to the establishment of disease, and in many case are responsible for severe complication and even lethal disease outcomes. This is the case for many viral infections as these are typically followed by a secondary bacterial infection. However, it is also believed to be the case for two viral infections, such as *Hepatitis B* and *C virus* (Rodriguez-Inigo et al. 2005), or others. Several co-infection models are well established in large animals including models for respiratory infections in cattle such as combinations of *Respiratory bovine coronaviruses* (RBCV)/*Pasteurella haemolytica* (Storz et al. 2000), *Bovine herpes virus 1* (BHV-1)/*Mannheimia hemolytica* model (Yates 1982), *Bovine virus diarrhea virus* (BVDV)/*Mycoplasma bovis* (Prysliak et al. 2011) to name a few. Other examples include a *Porcine reproductive and respiratory syndrome virus* (PRRSV)/*Streptococcus suis* model in pigs (Xu et al. 2010). Thus, using natural disease models has the advantage of being able to study the effect of multifactorial or co-infections in the same host.

11.2.4   Surgical Models

They have been used to explore various aspects of vaccine formulation and delivery, including the route of administration, targeting to specific receptors and the induction of mucosal versus systemic immunity. Surgical models allow access to specific immune compartments such as the intestine, lymph nodes or skin tissues. For example, we developed an intestinal gut-loop model in large animals (Gerdts et al. 2001), that can be used to assess the potential of oral vaccines *in vivo*. Following the original concept of Thiery-Vella loops (Yardley et al. 1978), this model is based on the surgical creation of independent intestinal segments that can remain within the animal for

more than 6 months without altered blood or lymph support (Gerdts et al. 2001). After a certain period of time, the segments can be collected and the immune responses in each segment in Peyer's patch, lamina propria and intestinal epithelium assessed (Meurens et al. 2009). The major advantage of this model is the fact that the loops are independent from each other and thus allow the assessment of multiple immune responses to different vaccine formulations within the same animal. This model is now available in a number of species including calves, sheep, pigs and even chicken (Aich et al. 2007). Other surgical models include cannulation of blood vessels or even lymphatics, which allows for the collection of large numbers of specific immune cells (Yen et al. 2006). For example, pseudoafferent lymph which is especially rich in dendritic cells can be collected after removal of the lymph nodes and subsequent stenosis of afferent and efferent lymphatics (Rothel et al. 1998). Other examples of surgical models include the insertion of catheters or pumps for vaccine release at very specific sites, slow release over time or even placement of a bolus to analyze a depot effect.

11.2.5 Experimental Models

Animal models are also being used to assess specific issue such as vaccine delivery, topical application or safety and toxicity of vaccine formulations, or individual components thereof. In most cases, this is required by regulatory authorities, which often require the use of at least two species to show safety, in most cases small rodents. However, large animal models have been recognized as useful models. For example, the physiology of the skin is very similar between humans and pigs, which make the pig a good model for studying intracutaneous or topical delivery of vaccines, as well as assessing the safety of novel vaccine formulations.

11.3 Choosing the Best Animal Model

The ethical use of animals in vaccine research requires that we only choose animals that resemble the disease as closely as possible or that will help to address very specific issues. This should be considered every time an animal experiment is planned. Three examples of considerations for choosing an appropriate animal model are provided below.

11.3.1 Induction of Both Mucosal and Systemic Immunity

The vast majority of pathogens enter via the mucosal surfaces. The induction of both systemic and mucosal immunity, therefore, is an important goal of future vaccines, and models are required to assess whether future vaccines effectively

induce mucosal immunity (Gerdts et al. 2006). Not every animal model is well suited for the assessment of mucosal immune responses, as the size of the animal itself and that of the oral and respiratory tract predetermines the accessibility of the mucosal tract, the volume of injection, and the actual route of immunization. For example, intranasal vaccination in mice is often associated with inhalation and ingestion of vaccine antigens, which makes it difficult to discriminate between intranasal, oral and intrapulmonary vaccination. In contrast, larger animal models can be used for the controlled delivery of vaccines to the nasal passages and provide easier access to the mucosal surfaces themselves and mucosal compartments in (Gerdts et al. 2006, 2007). For example, sufficient quantities of intraepithelial lymphocytes and lamina propria lymphocytes can be isolated from the mucosal surfaces of pigs, sheep and cattle, without having to compromise on the number of immune cells or having to pool cells from different compartments (Gerdts et al. 2001). Indeed, the nasal passages of sheep and cattle more closely resemble that of humans, and display similar patterns of development (Hein and Griebel 2003; Mutwiri et al. 2002). In these species the mucosal immune system develops well before birth, which stands in clear contrast to mice, in which the mucosal immune system only develops after birth. As mentioned above, intestinal models have been developed that allow controlled vaccine delivery to specific mucosal sites including the intestine and which can be used to evaluate mucosal vaccine delivery technologies and adjuvants (Gerdts et al. 2001; Mutwiri et al. 2005).

11.3.2   Immunization of Neonates

Neonates are amongst the most susceptible to infectious diseases and millions of infants and young children die every year due to infection with infectious pathogens. This is due to a number of factors including the challenges associated with a developing immune system, an inability to respond to glycoconjugate vaccines, limited access to vaccines, as well as the absence of vaccines for devastating diseases such as RSV and others (PrabhuDas et al. 2011). Vaccine research specifically for neonates, however, is currently hampered by the absence of good animal models to study the induction of immune responses and immune memory in the context of a neonatal immune system. For example, the neonatal period in mice is much shorter than in man, which makes the use of mice for developing neonatal vaccines highly problematic. A number of large animal models may be more representative of immune system ontogeny in humans (Elahi et al. 2007). For example, using a fetal lamb model we were able to show that oral immunization with a DNA vaccine was highly effective in fetuses and induced strong mucosal and systemic immune responses, as well as long-term memory in the developing immune system (Gerdts et al. 2000, 2002). Large animal models may be much more appropriate for evaluating vaccine immune responses in the neonate and addressing questions regarding possible interactions between vaccines and maternal antibodies (Polewicz et al. 2011). For example, novel vaccine formulations including adjuvants have to

Table 11.2 Routes of delivery and animal models

Delivery route	Administration	Advantages	Disadvantages	Common animal models
Oral	Feed, water, intragastric tube, gut-loop model	Induction of mucosal immunity, easy to administer	Uptake via the mucosa remains difficult, requires special delivery systems	Most species including mice, rats, poultry, ferrets, pigs, sheep, cattle, dogs, and fish
Occular	Droplets, spray	Induction of mucosal immunity	Delivery difficult in most species	Poultry, fish
Intranasal	Spray, droplets	Induction of mucosal immunity	Problematic in smaller animals; not practical in some live-stock species	Mice, rats, dogs, pigs, sheep, cattle, horses
Intravaginal	Suspension; emulsion	Mucosal immune response, local	Not practical in many species	Mice, guinea pigs, pigs, cattle
Topical	Patches, crème	Systemic immune response	Not practical in many species	Mice, pigs, cattle
Intradermal	Patch, micro-needles, gene-gun, electroporation, laser	Systemic immune response; good uptake by APC	Not always practical	Mice, pigs, cattle
Intramuscular	Syringe, needle, injector, gene-gun	Systemic immune response	Local site reactions, may affect carcass quality	Most species
Intraperitoneal	Syringe, needle, injector	Systemic immune response	Risk of injecting into intestine	Mice, fish

APC antigen presenting cell

be specifically tailored to the neonatal immune system, as recently demonstrated by combining three novel immune modulators into one adjuvant platform. This platform, consisting of host defense peptides, polyphosphazenes and CpG oligodeoxynucle- otides, proved highly effective after a single immunization in both neonatal and adult mice when combined with pertussis (Gracia et al. 2011) and RSV antigens (Kovacs- Nolan et al. 2009). Other combination adjuvants are currently under development (Mutwiri et al. 2011).

11.3.3   Novel Routes of Delivery and Devices

An area of rapid development in vaccine research is the area of vaccine delivery. Both human and animal vaccines are moving away from needles, either because of the risk of broken needles in meat products or because of the low compliance rate in young children and infants. Interestingly, the recent pandemic has revealed that even in adults, the injection via needle is becoming less accepted by the public. Thus, novel strategies for vaccine delivery are required, using needle-free injectors, intradermal patches or topical applications. Appropriate animal models are required that firstly resemble the skin physiology in humans, secondly allow testing of injec- tors and that at the same time allow delivery of the vaccine under real conditions (Table 11.2). Both pigs and cows have been frequently used to assess such novel vaccine technologies, and allow intradermal application of even larger volumes of vaccine (van Drunen Littel-van den Hurk et al. 2006). Needle-free devices such as electroporation (van Drunen Littel-van den Hurk and Hannaman 2010) have been shown to be highly effective in cattle and pigs (van Drunen Littel-van den Hurk et al. 2008, 2010) and are currently developed for practical application. Other devices, such as needle-free injectors have been successfully tested in pigs.

11.4   Conclusions

Animal models are critical for the development of vaccines. They are required to determine the quality and quantity of an immune response to vaccination, they are required for assessing the safety and toxicity of vaccine formulations, they are used to determine the efficacy of the vaccine in providing protection against challenge infection, and they are often used to assess the potential of preventing disease trans- mission within a specific population. Thus, selecting the most appropriate animal model for the specific needs of the research project is critical, and rather than being driven by low cost and ease of handling, researchers should look for models that closely resemble the target species and thus produce results that could be quickly translated into real products. In the long term, large amounts of money, time and resources can be saved that way.

References

Aich P, Wilson HL, Kaushik RS, Potter AA, Babiuk LA, Griebel P (2007) Comparative analysis of innate immune responses following infection of newborn calves with bovine rotavirus and bovine coronavirus. J Gen Virol 88:2749–2761

Baron C, Coombes B (2007) Targeting bacterial secretion systems: benefits of disarmament in the microcosm. Infect Disord Drug Targets 7:19–27

Bishop SC, Lunney JK, Pinard-van der Laan MH MH, Gay CG (2011) Report from the second international symposium on animal genomics for animal health: critical needs, challenges and potential solutions. BMC Proc 5(Suppl 4):S1

Blanchard-Rohner G, Siegrist CA (2011) Vaccination during pregnancy to protect infants against influenza: why and why not? Vaccine 29:7542–7550

Dobrescu L, Huygelen C (1976) Protection of piglets against neonatal E. coli enteritis by immunization of the sow with a vaccine containing heat-labile enterotoxin (LT) I. Protection against experimentally induced diarrhoea. Zentralb Veterinarmed B 23:79–88

Edwards KM (2003) Pertussis: an important target for maternal immunization. Vaccine 21:3483–3486

Elahi S, Brownlie R, Korzeniowski J, Buchanan R, O'Connor B, Peppler MS, Halperin SA, Lee SF, Babiuk LA, Gerdts V (2005) Infection of newborn piglets with Bordetella pertussis: a new model for pertussis. Infect Immun 73:3636–3645

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

Gerdts V, Babiuk LA, van Drunen Littel-van den H, Griebel PJ (2000) Fetal immunization by a DNA vaccine delivered into the oral cavity. Nat Med 6:929–932

Gerdts V, Uwiera RR, Mutwiri GK, Wilson DJ, Bowersock T, Kidane A, Babiuk LA, Griebel PJ (2001) Multiple intestinal 'loops' provide an in vivo model to analyse multiple mucosal immune responses. J Immunol Methods 256:19–33

Gerdts V, Snider M, Brownlie R, Babiuk LA, Griebel PJ (2002) Oral DNA vaccination in utero induces mucosal immunity and immune memory in the neonate. J Immunol 168:1877–1885

Gerdts V, Mutwiri GK, Tikoo SK, Babiuk LA (2006) Mucosal delivery of vaccines in domestic animals. Vet Res 37:487–510

Gerdts V, Littel-van den Hurk SD, Griebel PJ, Babiuk LA (2007) Use of animal models in the development of human vaccines. Future Microbiol 2:667–675

Girard-Misguich F, Cognie J, Delgado-Ortega M, Berthon P, Rossignol C, Larcher T, Melo S, Bruel T, Guibon R, Cherel Y, Sarradin P, Salmon H, Guillen N, Meurens F (2011) Towards the establishment of a porcine model to study human amebiasis. PLoS One 6:e28795

Gracia A, Polewicz M, Halperin SA, Hancock RE, Potter AA, Babiuk LA, Gerdts V (2011) Antibody responses in adult and neonatal Balb/c mice to immunization with novel Bordetella pertussis vaccine formulations. Vaccine 29:1595–1604

Hall RA, Khromykh AA (2004) West Nile virus vaccines. Expert Opin Biol Ther 4:1295–1305

Hein WR, Griebel PJ (2003) A road less travelled: large animal models in immunological research. Nat Rev Immunol 3:79–84

Jalal S, Arsenault R, Potter AA, Babiuk LA, Griebel PJ, Napper S (2009) Genome to kinome: species-specific peptide arrays for kinome analysis. Sci Signal 2:pl1

Kohara J, Hirai T, Mori K, Ishizaki H, Tsunemitsu H (1997) Enhancement of passive immunity with maternal vaccine against newborn calf diarrhea. J Vet Med Sci 59:1023–1025

Kovacs-Nolan J, Latimer L, Landi A, Jenssen H, Hancock RE, Babiuk LA, van Drunen Littel-van den Hurk S (2009) The novel adjuvant combination of CpG ODN, indolicidin and polyphosphazene induces potent antibody- and cell-mediated immune responses in mice. Vaccine 27:2055–2064

Lambert PH, Liu M, Siegrist CA (2005) Can successful vaccines teach us how to induce efficient protective immune responses? Nat Med 11:S54–S62

Lang PO, Govind S, Mitchell WA, Siegrist CA, Aspinall R (2011) Vaccine effectiveness in older individuals: what has been learned from the influenza-vaccine experience. Ageing Res Rev 10:389–395

Macchiarini F, Manz MG, Palucka AK, Shultz LD (2005) Humanized mice: are we there yet? J Exp Med 202:1307–1311

McNulty MS, Logan EF (1987) Effect of vaccination of the dam on rotavirus infection in young calves. Vet Rec 120:250–252

Meurens F, Berri M, Auray G, Melo S, Levast B, Virlogeux-Payant I, Chevaleyre C, Gerdts V, Salmon H (2009) Early immune response following Salmonella enterica subspecies enterica serovar Typhimurium infection in porcine jejunal gut loops. Vet Res 40:5

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

Mostl K, Burki F (1988) Incidence of diarrhoea and of rotavirus- and coronavirus-shedding in calves, whose dams had been vaccinated with an experimental oil-adjuvanted vaccine containing bovine rotavirus and bovine coronavirus. Zentralb Veterinarmed B 35:186–196

Moxon ER, Siegrist CA (2011) The next decade of vaccines: societal and scientific challenges. Lancet 378:348–359

Mutwiri G, Bowersock T, Kidane A, Sanchez M, Gerdts V, Babiuk LA, Griebel P (2002) Induction of mucosal immune responses following enteric immunization with antigen delivered in alginate microspheres. Vet Immunol Immunopathol 87:269–276

Mutwiri G, Bowersock TL, Babiuk LA (2005) Microparticles for oral delivery of vaccines. Expert Opin Drug Deliv 2:791–806

Mutwiri G, Gerdts V, van Drunen Littel-van den Hurk S, Auray G, Eng N, Garlapati S, Babiuk LA, Potter A (2011) Combination adjuvants: the next generation of adjuvants? Expert Rev Vaccines 10:95–107

Osterrieder N, Kamil JP, Schumacher D, Tischer BK, Trapp S (2006) Marek's disease virus: from miasma to model. Nat Rev Microbiol 4:283–294

Poehling KA, Szilagyi PG, Staat MA, Snively BM, Payne DC, Bridges CB, Chu SY, Light LS, Prill MM, Finelli L, Griffin MR, Edwards KM (2011) Impact of maternal immunization on influenza hospitalizations in infants. Am J Obstet Gynecol 204:S141–S148

Polewicz M, Gracia A, Buchanan R, Strom S, Halperin SA, Potter AA, Babiuk LA, Gerdts V (2011) Influence of maternal antibodies on active pertussis toxoid immunization of neonatal mice and piglets. Vaccine 29:7718–7726

Potter AA, Klashinsky S, Li Y, Frey E, Townsend H, Rogan D, Erickson G, Hinkley S, Klopfenstein T, Moxley RA, Smith DR, Finlay BB (2004) Decreased shedding of Escherichia coli O157:H7 by cattle following vaccination with type III secreted proteins. Vaccine 22:362–369

PrabhuDas M, Adkins B, Gans H, King C, Levy O, Ramilo O, Siegrist CA (2011) Challenges in infant immunity: implications for responses to infection and vaccines. Nat Immunol 12:189–194

Prysliak T, van der Merwe J, Lawman Z, Wilson D, Townsend H, van Drunen Littel-van den Hurk S, Perez-Casal J (2011) Respiratory disease caused by Mycoplasma bovis is enhanced by exposure to bovine herpes virus 1 (BHV-1) and not to bovine viral diarrhea virus (BVDV) type 2. Can Vet J 52:1195–1202

Rodriguez-Inigo E, Bartolome J, Ortiz-Movilla N, Platero C, Lopez-Alcorocho JM, Pardo M, Castillo I, Carreno V (2005) Hepatitis C virus (HCV) and hepatitis B virus (HBV) can coinfect the same hepatocyte in the liver of patients with chronic HCV and occult HBV infection. J Virol 79:15578–15581

Rothel JS, Corner LA, Lightowlers MW, Seow HF, McWaters P, Entrican G, Wood PR (1998) Antibody and cytokine responses in efferent lymph following vaccination with different adjuvants. Vet Immunol Immunopathol 63:167–183

Rouse BT, Kaistha SD (2006) A tale of 2 alpha-herpesviruses: lessons for vaccinologists. Clin Infect Dis 42:810–817

Shultz LD, Ishikawa F, Greiner DL (2007) Humanized mice in translational biomedical research. Nat Rev Immunol 7:118–130

Storz J, Lin X, Purdy CW, Chouljenko VN, Kousoulas KG, Enright FM, Gilmore WC, Briggs RE, Loan RW (2000) Coronavirus and Pasteurella infections in bovine shipping fever pneumonia and Evans' criteria for causation. J Clin Microbiol 38:3291–3298

van Drunen Littel-van den Hurk S, Hannaman D (2010) Electroporation for DNA immunization: clinical application. Expert Rev Vaccines 9:503–517

van Drunen Littel-van den Hurk S, Babiuk S, Babiuk LA (2006) Needle-free delivery of veterinary DNA vaccines. Methods Mol Med 127:91–105

van Drunen Littel-van den Hurk S, Luxembourg A, Ellefsen B, Wilson D, Ubach A, Hannaman D, van den Hurk JV (2008) Electroporation-based DNA transfer enhances gene expression and immune responses to DNA vaccines in cattle. Vaccine 26:5503–5509

van Drunen Littel-van den Hurk S, Lawman Z, Wilson D, Luxembourg A, Ellefsen B, van den Hurk JV, Hannaman D (2010) Electroporation enhances immune responses and protection induced by a bovine viral diarrhea virus DNA vaccine in newborn calves with maternal antibodies. Vaccine 28:6445–6454

Wiles S, Hanage WP, Frankel G, Robertson B (2006) Modelling infectious disease - time to think outside the box? Nat Rev Microbiol 4:307–312

Wilkie B, Mallard B (1999) Selection for high immune response: an alternative approach to animal health maintenance? Vet Immunol Immunopathol 72:231–235

Xu M, Wang S, Li L, Lei L, Liu Y, Shi W, Wu J, Rong F, Sun G, Xiang H, Cai X (2010) Secondary infection with Streptococcus suis serotype 7 increases the virulence of highly pathogenic porcine reproductive and respiratory syndrome virus in pigs. Virol J 7:184

Yardley JH, Keren DF, Hamilton SR, Brown GD (1978) Local (immunoglobulin A) immune response by the intestine to cholera toxin and its partial suppression with combined systemic and intra-intestinal immunization. Infect Immun 19:589–597

Yates WD (1982) A review of infectious bovine rhinotracheitis, shipping fever pneumonia and viral-bacterial synergism in respiratory disease of cattle. Can J Comp Med 46:225–263

Yen HH, Scheerlinck JP, Gekas S, Sutton P (2006) A sheep cannulation model for evaluation of nasal vaccine delivery. Methods 38:117–123