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Mucosal Veterinary Vaccines: Comparative Vaccinology

Chapter 61

Douglas C. Hodgins

Department of Pathobiology, Ontario Veterinary College, University of Guelph, Guelph, Ontario, Canada

Lijuan Yuan

Food Animal Health Research Program, Ohio Agricultural Research and Development Center, The Ohio State University, Wooster, Ohio

Viviana Parreño

Institute of Virology, Center of Research in Veterinary Science, National Institute of Agricultural Technology, Buenos Aires, Argentina

Lynette B. Corbeil

Department of Pathology, UCSD Medical Center, University of California at San Diego, San Diego, California

Linda J. Saif

Food Animal Health Research Program, Ohio Agricultural Research and Development Center, The Ohio State University, Wooster, Ohio

In spite of advances in nutrition, genetics, housing, and therapeutics, diseases of the respiratory, reproductive, and enteric tracts of domestic animals and poultry continue to be major causes of morbidity and mortality, with economic losses of billions of dollars a year (Whiteley *et al.*, 1992). Although vaccines have been developed and licensed for prevention of many of these diseases (summarized in **Tables 61.1** to **61.3**), there is a need for improvement in vaccine efficacy. Reasons for low vaccine efficacy include inappropriate antigens in vaccine preparations (*e.g.*, *in vitro* expressed antigens instead of *in vivo* expressed antigens), inappropriate immune responses (*e.g.*, systemic instead of mucosal or Th2-type instead of Th1-type), and inappropriate use of otherwise efficacious vaccines (*e.g.*, vaccination after onset of disease) (Tizard, 2000).

The majority of current poultry vaccines are attenuated agents delivered orally, nasally, or by other mucosal routes, for reasons of ease of administration, economy, and protective efficacy. Until relatively recently, however, few vaccines for domestic mammals have been delivered by mucosal routes. Management practices for mammals differ from those for poultry, and mass vaccination techniques for mucosal delivery have not been pursued as zealously.

Recently, however, a number of attenuated live vaccines for nasal administration have been developed for respiratory tract infections, especially of horses and pets, with use of traditional methodologies. Improved protective efficacy and rapid onset of immunity, in comparison with killed vaccine products, have led to their widespread acceptance. Attenuated live vaccines for enteric diseases have also been marketed, but in many cases efficacy has been disappointing. Better strategies for induction of immunity in the gastrointestinal tract are needed. In contrast, effective parenteral vaccines for the most common diseases of the reproductive tract in veterinary species have been available for years, and there has been little motivation to develop mucosal vaccines.

Many of the diseases of the respiratory and gastrointestinal tracts are most devastating in the neonatal period. For these diseases, active immunization may not provide protection before natural exposure to the pathogen. Maternal vaccination to enhance passive immunity has been widely used in veterinary medicine, especially for

Table 61.1. Commercial Veterinary Vaccines for Diseases of the Gastrointestinal, Respiratory, and Reproductive Tracts of Ruminants

Species	Agent[a]	Diseases	Vaccine Types[b,c]	Comments[d]
Bovine	Bovine coronavirus	Enteritis in neonates, winter dysentery in adults, Respiratory tract infections in feedlot calves	K,L	Maternal vaccination for passive immunity, and/or oral vaccination of newborns
	Bovine herpesvirus-1	Respiratory tract infection, reproductive tract infection	K,L	Also modified live nasal
	Bovine respiratory syncytial virus	Respiratory tract infection	K,L	
	Bovine rotavirus	Neonatal enteritis	K,L	Maternal vaccination for passive immunity and/or oral vaccination of newborns
	Bovine virus, diarrhea virus	"Mucosal disease," respiratory tract infection, abortion	K,L	
	Brucella abortus	Reproductive tract infection, abortion	L	
	Campylobacter fetus subsp. *veneralis*	Reproductive tract infection, abortion	K	
	Dictyocaulus viviparus	Parasitic bronchitis (lungworm)	L	Oral administration of third-stage infective larvae
	Escherichia coli	Neonatal enteritis	K	maternal vaccination for passive immunity
	Haemophilus somnus	Bronchopneumonia, reproductive tract infection, abortion	K	
	Leptospira spp.	Reproductive tract infection, abortion, nephritis	K	
	Mannheimia (Pasteurella) haemolytica	Bronchopneumonia	K,L,S	Some bacterins are prepared from cultures grown under iron-restricted conditions
	Parainfluenza virus-3	Respiratory tract infection	K,L	Some live vaccines given nasally
	Pasteurella multocida	Bronchopneumonia	K,L	
	Salmonella spp.	Enteritis	K	
	Tritrichomonas foetus	Reproductive tract infection, abortion	K	
Ovine	*Campylobacter fetus* spp. fetus *Campylobacter jejuni*	Abortion	K	
	Chlamydia psittaci	Abortion	K	
	Mannheimia haemolytica	Bronchopneumonia	K	Some bacterins are prepared from cultures grown under iron-restricted conditions
	Pasteurella trehalosi	Bronchopneumonia	K	Some bacterins are prepared from cultures grown under iron-restricted conditions
	Toxoplasma gondii	Early embryonic death, abortion	L	Live tachyzoites

[a] Agents and vaccines have been selected as representative of common disease concerns and current commercial vaccines. Tabulation of all mucosal pathogens and all licensed commercial vaccines in all jurisdictions has not been attempted.
[b] K indicates a killed (inactivated) vaccine; L indicates live (attenuated or nonattenuated) vaccine; S indicates a culture supernatant vaccine.
[c] Vaccine data have been collated from manufacturers' product monographs, including monographs published in Entriken (2001) and Glennon and Jeffs (2000).
[d] Vaccines are administered by the intramuscular or subcutaneous routes unless indicated otherwise. Inclusion of a vaccine in the table does not imply efficacy.

Table 61.2. Commercial Veterinary Vaccines for Diseases of the Gastrointestinal, Respiratory, and Reproductive Tracts of Pigs and Horses

Species	Agent[a]	Diseases	Vaccine Types[b,c]	Comments[d]
Porcine	*Actinobacillus pleuropneumoniae*	Pneumonia	K,L	
	Bordetella bronchiseptica	Atrophic rhinitis	K,L	Maternal vaccination for passive immunity, with or without vaccination (nasal or intramuscular) of newborns
	Escherichia coli	Neonatal enteritis	K	Maternal vaccination for passive immunity
	Haemophilus parasuis	Pneumonia	K	
	Swine influenza virus	Respiratory tract infections	K	
	Leptospira spp.	Abortion, stillbirths	K	
	Mycoplasma hyopneumoniae	Enzootic pneumonia	K	
	Porcine parvovirus	Abortion, mummified fetuses, stillbirths	K	
	Pasteurella multocida	Atrophic rhinitis, pneumonia	K	
	Porcine reproductive and respiratory syndrome virus	Abortion, stillbirths, neonatal respiratory tract Infection	K,L	
	Porcine rotavirus	Neonatal enteritis	K,L	Maternal vaccination for passive immunity, with or without vaccination (intraperitoneal, oral, or intramuscular) of newborns
	Pseudorabies virus	Abortion, mummification, stillbirths, Respiratory tract infection	L	
	Salmonella spp.	Enteritis	K,L	Some by oral or nasal routes
	Streptococcus suis	Pneumonia, arthritis, meningitis	K	Maternal vaccination for passive immunity and/or vaccination of newborns
	Transmissible gastroenteritis virus	Enteritis	K,L	Maternal vaccination (some vaccines by oral route) for passive immunity, with or without intraperitoneal or oral vaccination of newborns
Equine	Influenza virus	Respiratory tract infection	K,L	A cold adapted live vaccine is given nasally; an ISCOM vaccine has been marketed since 1987
	Equine herpesvirus-4	Equine viral rhinopneumonitis	K,L	
	Equine rotavirus	Neonatal enteritis	K	Maternal vaccination for passive immunity
	Streptococcus equi	Strangles	E,L	A live vaccine is marketed for nasal use

[a] Agents and vaccines have been selected as representative of common disease concerns and current commercial vaccines. Tabulation of all mucosal pathogens and all licensed commercial vaccines in all jurisdictions has not been attempted.
[b] E indicates an extract or subunit vaccine; K indicates a killed (inactivated) vaccine; L indicates live (attenuated or nonattenuated) vaccine.
[c] Vaccine data have been collated from manufacturers' product monographs, including monographs published in Entriken (2001) and Glennon and Jeffs (2000).
[d] Vaccines are administered by the intramuscular or subcutaneous routes unless indicated otherwise. Inclusion of a vaccine in the table does not imply efficacy.

Table 61.3. Commercial Veterinary Vaccines for Diseases of the Gastrointestinal, Respiratory, and Reproductive Tracts of Dogs, Cats, and Chickens

Species	Agent[a]	Diseases	Vaccine Types	Comments
Canine	*Bordetella bronchiseptica*	Tracheobronchitis ("kennel cough")	K,L,E	Some live vaccines by nasal route, in combination with parainfluenza virus, with or without CAV2
	Canine adenovirus-2	Tracheobronchitis ("kennel cough")	L	In combination with other agents of tracheobronchitis
	Canine parvovirus-2	Enteritis	K,L	Vaccination of young puppies: suppressive effects of maternal antibodies are problematic; some low-passage and/or high-virus-titer vaccines are marketed
	Leptospira spp.	Nephritis	K	
	Parainfluenza virus-2	Tracheobronchitis ("kennel cough")	L	In combination with other agents of tracheobronchitis
Feline	Feline calicivirus	Respiratory tract infections	K,L	Some by nasal or ocular routes
	Chlamydia psittaci	Respiratory tract infection, conjunctivitis	K,L	
	Feline herpesvirus-1	Rhinotracheitis, conjunctivitis, keratitis, stomatitis, abortion, pneumonia	K,L	Some by nasal or ocular routes
Chicken	Infectious bronchitis virus	Respiratory tract infection, reproductive tract infection	K,L	Ocular, aerosol, nasal, or drinking water administration; killed vaccines parenterally after priming with live vaccine by mucosal route
	Eimeria spp.	Coccidiosis (enteritis)	L	Live oocysts on feed or in water in first week of life, up to 8 species of *Eimeria* simultaneously
	Infectious laryngotracheitis virus	Respiratory tract infection	L	Vaccination via cloaca, ocular, nasal, feather follicle, and drinking water
	Avian influenza virus	Respiratory tract infection	L	Recombinant vaccine in fowl pox virus vector
	Mycoplasma spp.	Respiratory tract infection	K,L	Ocular, aerosol
	Newcastle disease virus	Respiratory tract infection, enteritis, neurological disease	K,L	Ocular, aerosol, nasal, drinking water; killed vaccines parenterally after priming with live vaccine by mucosal route
	Pasteurella multocida	Fowl cholera	K,L	Live vaccine is administered in drinking water

[a] Agents and vaccines have been selected as representative of common disease concerns and current commercial vaccines. Tabulation of all mucosal pathogens and all licensed commercial vaccines in all jurisdictions has not been attempted.
[b] E indicates an extract or subunit vaccine; K indicates a killed (inactivated) vaccine; L indicates live (attenuated or nonattenuated) vaccine.
[c] Vaccine data have been collated from manufacturers' product monographs, including monographs published in Entriken (2001) and Glennon and Jeffs (2000).
[d] Vaccines are administered by the intramuscular or subcutaneous routes unless indicated otherwise. Inclusion of a vaccine in the table does not imply efficacy.

control of enteric diseases. Severe practical difficulties arise, however, with diseases such as parvovirus enteritis in puppies, in which a smooth transition must be made from protection by passive maternal antibodies to protection by active immunity, without permitting a window of disease susceptibility to occur in between. This transition is difficult to achieve because induction of active immunity is commonly inhibited by maternal antibodies. Various strategies are used to address this problem, but improved adjuvants and antigen delivery systems would improve the reliability of neonatal immunization.

Although progress is being made in disease prevention in veterinary species, ever-changing management practices (*e.g.*, earlier weaning of piglets, larger dairy operations) appear to be generating new patterns of disease, requiring new control strategies. The emergence of new pathogens (*e.g.*, porcine reproductive and respiratory syndrome virus [PRRSV], porcine circovirus-2) also presents new challenges for vaccine research before some of the older challenges have been met. In this chapter, we focus on mucosal veterinary vaccines and vaccine concepts related to selected pathogens associated with economically important respiratory, reproductive, and enteric diseases, with an emphasis on livestock species. Our intent is to highlight progress, to review existing and future vaccination strategies, and to acknowledge the unique contributions of this research to our understanding of mucosal vaccines and immunology.

RESPIRATORY VACCINES

Respiratory tract infections are a major cause of morbidity and mortality among farm animals, poultry, and pets (Tables 61.1 to 61.3). In many cases, disease conditions are intensified by current management practices such as the mixing of recently weaned, stressed beef calves from multiple sources in auction barns. Certain disease conditions, such as atrophic rhinitis of pigs, result from the interplay of several pathogens, and multiple agents must be represented in vaccines. Some respiratory pathogens such as *Mycoplasma hyopneumoniae* in young pigs are causing new patterns of disease as management practices change (*e.g.*, weaning at an earlier age), requiring changes in vaccine strategies. Other pathogens such as PRRSV have only recently emerged, and improved vaccines await advances in understanding of the agent and of disease pathogenesis. A discussion of respiratory vaccines for even the major pathogens of veterinary species is beyond the scope of the present review. This section therefore focuses on selected pathogens of cattle and horses to illustrate general principles.

Bovine respiratory vaccines

Introduction and background

Respiratory tract disease has been a major problem for beef, veal, and dairy producers for decades. From the perspectives of both economic and animal welfare considerations, efficacious and practical means to reduce the burden of disease are long overdue. Most attention has focused on pneumonic pasteurellosis, commonly termed "shipping fever," in recently weaned beef calves soon after entry to feedlots. Economic losses to the North American feedlot industry alone have been estimated at nearly $1 billion per year (Whiteley *et al.*, 1992). This does not include losses due to enzootic pneumonia in veal, dairy, or beef calves before weaning. Both bacteria and viruses have been implicated in the disease processes, and vaccines have been marketed for over 80 years (Mosier *et al.*, 1989). We have summarized the agents associated with respiratory disease in cattle and the vaccine preparations marketed for their control (Table 61.1). *Mannheimia haemolytica* is discussed in detail as an example of past, current, and future vaccine approaches.

Mannheimia haemolytica

M. haemolytica, classified until recently as *Pasteurella haemolytica* (Angen *et al.*, 1999), is a gram-negative, facultatively anaerobic bacterium. In experimental studies, it causes a fibrinous pneumonia comparable to that seen in cattle dying of pneumonic pasteurellosis (Friend *et al.*, 1977; Shoo, 1989), and it is considered the most important bacterial pathogen in bovine pneumonias (Mosier, 1997). The first 7 decades of vaccine history have been reviewed by Mosier *et al.* (1989). As a single agent, this bacterium induces pneumonia in experimental studies, but most field cases of pneumonia involve predisposing factors of stress (sudden shifts in weather conditions, change of feed, weaning, and commingling with other cattle) and interactions with viral pathogens (reviewed by Yates [1982] and by Hodgins *et al.* [2002]). To be effective, vaccination strategies against respiratory disease in feedlot cattle will therefore need to induce immunity to multiple pathogens.

M. haemolytica can be cultured intermittently from the nasopharynx of healthy cattle and is considered part of the normal flora (Babiuk and Acres, 1984; Frank, 1984). Under ill-defined conditions of stress, *M. haemolytica* serotype A1 populations increase rapidly in the nasopharynx (Frank and Smith, 1983). Invasion of the lower respiratory tract, facilitated in many cases by viral effects on clearance mechanisms of the tracheal and bronchial mucosa, leads to vascular damage, exudation of fibrin, and necrosis of lung parenchymal tissue (lesions reviewed by Rehmtulla and Thomson [1981]). Morbidity rates of 15% to 45% and mortality rates of 1% to 5% are common among weaned calves arriving at feedlots (Kelly and Janzen, 1986). Antibiotics are used extensively in treatment and prevention (Mechor *et al.*, 1988; Hoar *et al.*, 1998).

The earliest vaccines for "shipping fever" were bacterins of doubtful efficacy (Miller *et al.*, 1927) or causing detrimental effects (Farley, 1932; Friend *et al.*, 1977). The identification and characterization of a heat-labile exotoxin (leukotoxin) cytolytic for ruminant leukocytes, produced by *M. haemolytica* during logarithmic growth (Shewen and Wilkie, 1985), initiated an intensive search for other protective antigens of *M. haemolytica* that continues today. Other antigens suggested as playing a role in disease pathogenesis and of poten-

tial value in vaccine formulation include the capsular polysaccharide of *M. haemolytica* A1 (Brogden *et al*., 1995), a sialoglycoprotease (Abdullah *et al*., 1992; Lee *et al*., 1994), a neuroaminidase (Straus and Purdy, 1994), outer membrane proteins (Gatewood *et al*., 1994), fimbriae (Morck *et al*., 1987, 1989), and iron-regulated proteins (Morck *et al*., 1991; Potter *et al*., 1999).

Most of the *M. haemolytica* vaccines now available commercially consist of culture supernatants or cell extracts (with or without leukotoxin) and are licensed for use by the intramuscular or subcutaneous routes. Significant protection in experimental challenge studies has been reported for a number of these products (Conlon *et al*., 1991, 1995; Confer and Fulton, 1995). Investigations of vaccine efficacy are conducted almost exclusively in ruminants because the leukotoxin, the best documented of its virulence factors, is toxic only to ruminant leukocytes (Kaehler *et al*., 1980; Shewen and Wilkie, 1982). Efficacy in field trials can be considerably lower than in controlled experiments because of suboptimal timing of vaccination, variation in exposure to pathogens other than *M. haemolytica*, and variation in immunity to *M. haemolytica* in nonvaccinated control calves (Thorlakson *et al*., 1990).

In general, the proportion of IgG to IgA in respiratory tract secretions increases from the upper respiratory tract to the lower respiratory tract. It is unclear, however, what proportion of IgG detected in the bovine lung results from local secretion and how much is serum derived (Butler, 1983). The protection mediated by parenteral vaccination with nonreplicating vaccines suggests that serum antibodies have at least limited access to the lung compartment.

A number of attempts have been made to develop live *M. haemolytica* vaccines for parenteral (intradermal or intramuscular) administration. Efficacy studies have yielded variable results (Smith *et al*., 1985; Purdy *et al*., 1986; Mosier *et al*., 1998). This approach has limitations for use in feedlot calves because the use of antibiotics prevents adequate replication of the live vaccine.

A major challenge in developing effective vaccines against *M. haemolytica* is to adapt vaccines to existing management practices or to adapt management practices to feasible vaccines. There are limited opportunities to vaccinate beef calves raised on open-range land before weaning. Conventional two-dose vaccination, with primary vaccination weeks before weaning, is labor intensive and increases stress for the calves. Delayed vaccination, with a single dose of vaccine at the time of feedlot arrival, can induce anamnestic responses in calves that have been primed by natural exposure (Conlon *et al*., 1995), but it does not allow sufficient time for naïve (highly susceptible) calves to respond immunologically. One approach with merit is to vaccinate calves at about 3 months of age when they are being handled for branding (Harland *et al*., 1992), followed by a booster dose at weaning (6 to 8 months of age).

Oral vaccination. Recent studies have shown that transgenic clover expressing a truncated form of the leukotoxin of *M. haemolytica* is a potential oral vaccine (Lee *et al.,* 2001). Oral administration of dried transgenic plant material would be a low-labor, low-stress method of vaccination. It is doubtful whether plant-expressed antigens survive passage through the rumen (first stomach) in ruminant species, but the authors hypothesize that repeated exposure of the tonsils to antigen during rumination will induce mucosal immune responses. Immunogenicity trials of the recombinant plant antigen in calves have not been reported.

Others have reported that live, genetically modified *M. haemolytica* can be administered to recently weaned calves as a top dressing over pelleted feed (Briggs and Tatum, 1999). It is hypothesized that the vaccine strain of *M. haemolytica* could colonize the nasopharynx (without causing pneumonic disease) and induce mucosal immune responses. Mortality of high-risk feedlot calves was reduced from 16% to 0%. Significant increases in IgA antibodies were noted in nasal secretions by 3 days postvaccination. While these results are promising, continued studies are needed to confirm a lack of virulence under varied feedlot conditions. Environmental considerations, such as the potential for contamination of run-off water in feedlots vaccinating thousands of cattle, could also block licensing.

Another approach has involved microencapsulation of nonreplicating antigens for oral immunization of ruminants (Bowersock *et al.,* 1994a). The anatomy of ruminant digestive tracts, with prolonged passage times for materials lingering in the rumen, complicates vaccination by the oral route. Incorporation of microencapsulated antigen into macroparticles (5 mm in diameter) allows antigen to bypass the rumen (Bowersock *et al*., 1999) and pass promptly into the reticulum (second stomach). An oral vaccine containing microencapsulated antigens of *M. haemolytica* has been shown to induce partial protection (Bowersock *et al*., 1994b).

Nasal vaccination. Preliminary work by Rebelatto *et al.* (2001) indicates that vaccination by the nasal route using alginate microencapsulated antigen may be useful in cattle. Nasal (but not oral) vaccination led to high levels of IgG antibodies in serum and nasal secretions.

DNA-based vaccine strategies. Immunization of large animals with plasmid vaccines has proven to be more difficult than for mice (Braun *et al*., 1999; Babiuk *et al*., 2002). Doses of up to 1000 μg have been administered to cattle in attempts to optimize immune responses (Babiuk, *et al*., 1999). Although long-lived immune responses can be induced (Braun *et al*., 1999), antibody responses develop slowly (in comparison with those induced by protein-based vaccines), and revaccination may be necessary for detectable responses (Braun *et al*., 1999). These attributes discourage the use of plasmid DNA technology by itself for vaccination against *M. haemolytica*, since frequency of vaccination and labor costs are key issues. Further advances in plasmid design and delivery (Babiuk *et al*., 2002) or combination of plasmid vaccines with other vaccination approaches may make DNA vaccines feasible for use in cattle. Unmethylated CpG oligodeoxynucleotides have been investigated as potential adjuvants for veterinary vaccines (Rankin *et al*., 2001) and may find a role in conventional protein-based vaccines or plasmid DNA vaccines.

Equine respiratory vaccines

Introduction and background

Respiratory tract disease affects virtually every aspect of equine husbandry, including working, pleasure, and race horses. Considerable efforts are expended to prevent epizootics of respiratory disease in stables, fairs, shows, and race tracks. We have summarized the major respiratory pathogens of horses and the vaccine preparations marketed for their control (Table 61.2). Equine influenza virus is discussed in detail as an example of past, current, and future vaccine approaches.

Equine influenza virus

Equine influenza virus causes epizootics of upper and lower respiratory tract disease almost worldwide. Infection can occur in horses of all ages, but epidemics often involve younger animals (van Maanen and Cullinane, 2002). Clinical signs include high fever, a persistent dry cough, nasal discharge, anorexia, and depression (Ardans, 1999). Secondary bacterial pneumonia may complicate the clinical picture (Timoney, 1996).

Equine influenza viruses are classified as type A influenza. Antigenic differences in the hemagglutinin (H) and neuraminidase (N) glycoproteins define the two recognized subtypes, A/equine/1 (H7N7) and A/equine/2 (H3N8) (Wilson, 1993). The A/equine/1 subtype has not been associated with outbreaks of equine influenza since 1980 (Timoney, 1996; van Maanen and Cullinane, 2002). Two lineages of A/equine/2, American and European, have been identified (Yates and Mumford, 2000). Multiple virus strains are included in vaccines since protection against heterologous strains is incomplete (Yates and Mumford, 2000). Antigenic drift is sufficient to require regular reappraisal of strains included in vaccines (Mumford and Wood, 1993).

Natural infection induces IgA antibodies in nasal secretions, IgGa and IgGb antibodies in serum (Hannant *et al.*, 1989; Nelson *et al.*, 1998), and circulating cytotoxic T lymphocytes (Hannant and Mumford, 1989). Protection against reinfection persists for at least a year (Hannant *et al.*, 1988). Vaccination with inactivated virus vaccines induces serum IgG (T) antibodies without detectable IgA in nasal secretions (Nelson *et al.*, 1998) and without cytotoxic T cell activity (van Maanen and Cullinane, 2002). Two or three doses of vaccine are typically administered in the primary series, with booster doses administered at least once a year thereafter. More frequent vaccination is advised for horses at high risk of infection (Wilson, 1993). Protection is typically incomplete and of limited duration (Morley *et al.*, 1999). Improved adjuvants can enhance the level and duration of antibody responses to inactivated virus vaccines (Mumford *et al.*, 1994c; van Maanen and Cullinane, 2002). Suppressive effects of maternal antibodies on responses to inactivated vaccines have led to recommendations not to vaccinate foals before 6 months of age (van Oirschot *et al.*, 1991; van Maanen and Cullinane, 2002).

Immune stimulating complex (ISCOM) vaccines. A subunit equine influenza vaccine based on the ISCOM adjuvant technology has been licensed and marketed in Europe since 1987 (Newmark, 1988). Antibody responses to ISCOM-based vaccines typically are of higher titer and are more persistent than those stimulated by conventional inactivated vaccines (Mumford *et al.*, 1994a; Brugmann *et al.*, 1997). Protection has been demonstrated against experimental challenge, 15 months after a three-dose vaccination series (Mumford *et al.*, 1994b). Protection may be due in part to the ability of ISCOM-adjuvanted vaccines to induce cytotoxic T lymphocytes (CTLs) (Morein *et al.*, 1999). Although ISCOM-based vaccines can induce IgA antibody responses following nasal administration (Hu *et al.*, 2001), the commercial influenza ISCOM vaccine is administered parenterally.

Nasal vaccination. Nasal administration of inactivated equine influenza with cholera toxin B subunit has been reported to induce mucosal IgA antibodies and protection against experimental challenge (Hannant *et al.*, 1998). Recently, a cold-adapted, temperature-sensitive live vaccine for nasal administration was commercialized (Chambers *et al.*, 2001). Protection against experimental challenge was demonstrated 6 months after a single vaccination (Townsend *et al.*, 2001). This is a notable improvement in efficacy and practicality over conventional killed vaccines.

Plasmid DNA vaccines. Experimental plasmid vaccines encoding the hemagglutinin gene of equine influenza have been examined in horses. Three doses of plasmid administered to the skin and mucosal sites (tongue, conjunctiva, and third eyelid) induced protection against clinical disease and partial protection against viral shedding. Protection against clinical disease was reduced if plasmid was administered only to the skin (Lunn *et al.*, 1999).

Pox virus vectored vaccines. Both canarypox (ALVAC strain) and attenuated vaccinia virus (NYVAC strain) have been used as vaccine vectors for expression of hemagglutinins of A1 and A2 subtypes of equine influenza virus. Intramuscular vaccination induced serum antibodies and partial protection was achieved following natural exposure to A2 influenza virus (Taylor *et al.*, 1992).

Future needs

For some respiratory pathogens (*e.g.*, *M. hyopneumoniae* in pigs), the critical antigens associated with protective immune responses have not been identified. For other pathogens (*e.g.*, PRRSV) there is also a need to identify the appropriate immune response (Th1 or Th2 type) needed for protection. For some complex disease conditions (*e.g.*, pneumonic pasteurellosis in cattle) there is continuing uncertainty about whether all of the relevant contributing pathogens have been identified. Although many parenteral vaccines are efficacious in reducing lower respiratory tract disease, there is a need to investigate whether induction of mucosal immunity in the upper airways, in combination with systemic immunity, can further reduce infection rates, transmission of pathogens, and economic losses. Finally, there is a need to devise and implement changes in management procedures to reduce

disease exposure (by nonimmunological methods) and to optimize immune interventions by improving the timing of vaccinations.

VACCINES FOR GENITAL INFECTIONS

Vaccines to prevent reproductive tract disease have received much emphasis in veterinary medicine. This is especially true of food-producing animals because reproductive failure is an economic problem. Although vaccines to prevent reproduction are of interest for abandoned pets or deer in areas of overpopulation, this section deals only with vaccines designed to prevent infectious disease of the reproductive mucosa. We have outlined and summarized pathogens associated with reproductive tract disease in veterinary species and the vaccine preparations marketed for their control (Tables 61.1 to 61.3).

Infections causing adverse pregnancy outcome can be classified by route of infection: hematogenous or ascending. Several hematogenous infections have a predilection for the gravid uterus, resulting in early or late abortions (Corbeil *et al.*, 2001). These include leptospirosis, chlamydial infection, and brucellosis in several animal species, *Haemophilus somnus* infection in cattle and sheep, and *Neospora caninum* infection in cattle. Although vaccines are available for leptospirosis and hemophilosis, the vaccine for brucellosis, which has been available since the 1940s, is the prototype. Several *Brucella* species cause abortion or epididymitis/orchitis in the primary host (*B. abortus* in cattle, *B. suis* in swine, *B. melitensis* in goats, *B. ovis* in sheep, *B. canis* in dogs, and *B. marinum* [or *B. delphini*] in marine mammals). Infection may be acquired via the gut mucosa or the conjunctiva/upper respiratory mucosa, and the infection localizes in the reticuloendothelial system and endometrium/placenta by systemic spread. Thus, systemic vaccines are effective. A modified live *B. abortus* vaccine, along with a "test and slaughter" eradication program, has been successful in controlling bovine brucellosis in North America. The modified live vaccine (*B. abortus* strain 19) is very effective in stimulating cell-mediated immunity (CMI), which is critical for protection against this facultative intracellular pathogen. Strain 19 now has been largely replaced by the new attenuated strain RB51, which does not stimulate an antibody response known to interfere with diagnostic assays. There is considerable information on mechanisms of immunity to brucellosis, but since the focus of this volume is mucosal immunity, no more will be said on hematogenous infection of the genital tract.

Ascending, local infections of the reproductive tract are usually transmitted sexually. The two best examples of vaccines for sexually transmitted infections of animals are *Campylobacter fetus* subsp. *venerealis* (formerly *Vibrio fetus* subsp. *venerealis*) and *Tritrichomonas foetus*. Both are host-specific, bovine sexually transmitted disease (STD) pathogens that infect only the reproductive mucosa. Both are extracellular pathogens that do not invade the mucosa of the reproductive tract but may be found in the placenta and fetus. The localized nature of these infections and transmission limited to coitus suggest that mucosal immunity must be important. Because a vaccine has been available for *C. fetus* subsp. *venerealis* for several decades and its use has controlled the disease in developed countries, that vaccine will be described first.

Vibriosis

Vibriosis (or campylobacteriosis) is a chronic bacterial genital infection with no overt clinical signs other than reproductive failure (Corbeil *et al.*, 1981). After months of infection, the uterus is cleared first, followed by the vagina. Convalescent immunity is partially protective for a limited time. Antibody is effective in protection against this extracellular pathogen, as demonstrated by systemic passive immunization (Berg *et al.*, 1979). The antibody response to infection is primarily IgA in the vagina and IgG in the uterus (Corbeil *et al.*, 1981). Systemic immunization with a whole cell vaccine results in both IgG1 and IgG2 antibody responses to surface antigen in serum, uterine, and vaginal secretions (Corbeil *et al.*, 1981). This response prevents infection and can rapidly clear infected cows (Corbeil and Winter, 1978). That is, the vaccine can be used prophylactically and therapeutically. Immunization is efficacious even though surface antigenic variation occurs in the face of a local immune response (Corbeil and Winter, 1978). Presumably, immune clearance occurs when the dynamic interaction between protection and evasion is shifted in favor of the host. This appears to occur earlier when the response is primarily IgG than when IgA predominates (Corbeil *et al.*, 1981). This may be related to the ability of the IgG antibodies to mediate opsonization and intracellular killing of the bacterium, an ability that IgA antibodies lack (Corbeil and Winter, 1978). Although this work was done many years ago, it sets a precedent for systemic immunization for prophylaxis and therapy for reproductive mucosal infections.

Trichomoniasis

Trichomoniasis is a similar chronic genital mucosal infection of cattle. It is caused by the protozoan *T. foetus* and results in pregnancy loss. *T. vaginalis* causes a human STD also associated with adverse pregnancy outcome. Thus, bovine trichomoniasis serves as a model for immune prevention of a human reproductive mucosal infection. Because of the economic significance of bovine trichomoniasis and because no chemotherapy is used owing to toxicity, investigations have focused on immunoprophylaxis and therapy. Like *C. fetus* subsp. *venerealis, T. foetus* colonizes the vaginal and uterine or preputial surfaces for months. In fact, mature bulls are often infected for life, whereas young bulls may clear the infection with time. This is probably related to innate immunity. Trichomonads are anaerobic parasites and are found deep in uterine glands and epithelial crypts of the penis and prepuce (Rhyan *et al.*, 1999), where the oxygen tension is probably lowest. In order to elucidate protective acquired immune responses, monoclonal antibodies (mAbs) with putative protective functions were chosen for immunoaffinity purification of a highly glycosylated surface antigen (Corbeil *et al.*, 2001).

Analysis of many isolates of *T. foetus* indicated that the two mAbs recognized different epitopes of the same antigen, which was conserved in all isolates tested. This glycosylated surface antigen was later shown to be a lipophosphoglycan (LPG)/protein complex. Systemic immunization with the immunoaffinity-purified surface antigen, followed by vaginal challenge with *T. foetus*, resulted in statistically significantly earlier clearance of the parasite from vaccinated animals than from controls (Corbeil *et al.*, 2001). Even more important, clearance of immunized animals most often occurred before 7 weeks of infection. Mucosal convalescent immunity cleared controls much later. Others showed that significant inflammation accompanied by reproductive failure did not occur until after 7 weeks of infection, so the vaccine should protect against fetal loss (Parsonsen *et al.*, 1976). Analysis of antibody responses demonstrated predominantly IgG1 responses in the serum and IgA plus IgG1 antibodies in secretions.

This raised several questions. First, why is the systemic response skewed toward IgG1 (a Th2-type response in cattle) and not IgG2 (a Th1-type response)? This question is under investigation. Second, which is more protective: IgG or IgA antibody? And how can that Ig class be enriched to enhance protection? To address the latter questions, preliminary studies were done in mice to determine the best routes and adjuvants to enrich for IgG or IgA in genital secretions (Corbeil *et al.*, 2001). Subcutaneous priming with the immunoaffinity purified surface antigen (called TF1.17 antigen) in Quil A adjuvant and subcutaneous boosting with whole cells enriched for IgG anti-TF1.17 antibodies, whereas subcutaneous priming and vaginal boosting greatly enriched for IgA antibodies in genital secretions. When cattle immunized by these two methods were challenged vaginally with *T. foetus*, those with predominantly IgA or predominantly IgG1 anti-TF1.17 antibodies in genital secretions were equally protected. Later studies with similar nasal immunizations showed that stimulation of the common mucosal immune system yielded results similar to those of vaginal immunization (Corbeil *et al.*, 2001). This raised the question of inductive sites for local immune responses in the genital tract.

Others have suggested that the genital tract is not an inductive site because M cells and mucosa-associated lymphoreticular tissue (MALT) are not present. This is true of cattle as well as mice and humans. However, even though control cows did not have histologically demonstrable MALT in the uterus and vagina, cows experimentally infected with *T. foetus* did (Corbeil *et al.*, 2001). Similar lymphoid nodules and follicles under a modified epithelium were detected in preputial and penial surfaces of bulls infected with *T. foetus* (Rhyan *et al.*, 1999). Immunostaining of parallel sections with mAb to TF1.17 antigen indicated uptake of antigen by epithelial cells and large macrophage or dendritic type cells under the basement membrane near the lymphoid follicles (Rhyan *et al.*, 1999). Similar antigen uptake has been detected in the infected female uterine and vaginal mucosa (unpublished data, Rhyan, J.C., BonDurant, R.H., and Corbeil, L.B.). Thus, even though the parasite is noninvasive, released TF1.17 antigen appears to be taken up by epithelial cells. Rat uterine epithelial cells can present antigen to Th cells (Wira and Rossol, 1995). Also, macrophage/dendritic type cells positive for antigen should be antigen-presenting cells (APCs). Detection of IgG1 and IgA antibodies to TF1.17 antigen in genital secretions of infected animals (Rhyan *et al.*, 1999) and cows (Corbeil *et al.*, 2001) and the histologic demonstration of follicles and putative APCs suggest that inductive sites in the genital tract are formed in response to antigen.

Like *C. fetus* subsp. *venerealis*, *T. foetus* has mechanisms for evasion of immune responses. These include coating of the surface with Ig nonspecifically (Corbeil *et al.*, 1991), epitope variation (Ikeda *et al.*, 1993), and cleavage of IgG1, IgG2, and complement component 3 by extracellular cysteine proteinase (Talbot *et al.*, 1991; Kania *et al.*, 2001). However, as with *C. fetus*, it is clear that the dynamic interaction between host and parasite can be made to favor the host by systemic or mucosal immunization. The usefulness of whole cell vaccines in preventing *T. foetus* in cows has been demonstrated in clinical trials (Kvasnicka *et al.*, 1992). Earlier, Clark *et al.* (1984) demonstrated efficacious immunization of bulls with whole *T. foetus* cells or crude membrane glycoproteins that probably contained TF1.17 antigen. First-generation whole cell vaccines are now commercially available for prevention of trichomoniasis in cows.

Summary

The above studies with *C. fetus* subsp. *venerealis* and *T. foetus* show that:

- STDs can be prevented or even cured by systemic vaccination of both males and females.
- At least for one STD, IgG and IgA of the same antigen specificity are equally protective at the mucosal surface.
- Inductive sites are formed in the mucosa of infected male and female genital tracts, even with noninvasive pathogens.
- Strong and appropriate immune responses will clear microbial infection from the genital tract even when the microbe has multiple immune evasive mechanisms.
- Protection against two STDs has been demonstrated in the natural outbred host (cattle) and thus has advantages over murine models of STD vaccines. In the latter, the human pathogen is usually inoculated into the abnormal murine host and the disease does not mimic the human infection. Furthermore, although inbred mice provide a homogenous experimental model, they do not represent the variation in immune responses seen in the human population. The work on bovine vibriosis and trichomoniasis demonstrates protection under field conditions for two STDs that cause adverse pregnancy outcome in an outbred host. This is an encouraging precedent for control of human STDs and related adverse pregnancy outcomes.

Future needs

Future needs include identification of the protective antigens for most STDs. For antibody-mediated protection of the

genital mucosa, several questions have not yet been addressed. As alluded to earlier, it is not clear how IgG crosses the mucosal epithelium into the secretions since it lacks secretory component and a polymeric Ig receptor-mediated type of transport. To our knowledge, the role of IgE in the genital tract has been largely unstudied. Last, manipulating genital immune responses to enhance Th1- or Th2-type responses is an unexplored research area. This should be important in protection against intracellular or extracellular pathogens. The use of DNA or recombinant vaccines including genes for appropriate cytokines may be an approach to meet this need.

ENTERIC VACCINES

Enteric disease is a major cause of mortality and morbidity in animals. Agents causing diarrhea in animals include viruses (*e.g.*, adenoviruses, pestiviruses, caliciviruses, coronaviruses, parvoviruses, rotaviruses, toroviruses), bacteria (*e.g.*, *Campylobacter* spp., *Clostridium* spp., diarrheagenic *Escherichia coli*, *Salmonella* spp., *Yersinia* spp.), and parasites (*e.g.*, *Coccidia* spp., *Cryptosporidium parvum*). These infections occur most commonly in suckling animals or in poultry less than 3 weeks of age but may also be common postweaning or in susceptible seronegative or stressed adult animals (Saif and Jackwood, 1990). We have summarized veterinary vaccines marketed for prevention and control of diseases caused by mucosal pathogens (Tables 61.1 to 61.3). Because attachment, adhesion, colonization, replication, and invasion by enteric pathogens are largely localized to the gastrointestinal tract, immune effectors on intestinal surfaces, *e.g.*, secretory (S)-IgA antibodies, cytokines, and cytotoxic NK and T cells, play critical roles in protective immunity. Enteric pathogens have different characteristics related to their intestinal tropism and replication, requiring different vaccination strategies.

Enteric viruses have predilections for replication in distinct vertical and longitudinal regions of the small intestine or the large intestine (Saif, 1999a). They cause diarrhea of variable severity via mechanisms that differ from those of enteric bacteria, most of which cause secretory diarrhea mediated by enterotoxins (Bertschinger, 1999). Enteric viruses produce cytolytic infections of enterocytes, leading to varying degrees of villus loss and fusion, resulting in reduced absorptive capacity in the small intestine and malabsorptive, maldigestive diarrhea. A rotavirus nonstructural protein, NSP4, reportedly functions as a viral enterotoxin and plays a role in the pathogenesis of rotavirus diarrhea, according to the results of studies of mice (Estes *et al.,* 2001). The role of NSP4 in rotavirus diarrhea in other species has not been confirmed.

The enteric nervous system has been recognized as a critical component in regulating fluid secretion in the normal gut and a key element in the pathophysiology of rotavirus diarrhea in mice (Lundgren *et al.,* 2000). Neural reflex pathways increase fluid secretion by enterocytes in response to infection with rotavirus and other enteric pathogens (reviewed by Jones and Blikslager [2002]).

Enteropathogenic viruses can be divided into three types according to their preferred site of replication in the intestine (reviewed by Saif, 1999a). Although viruses such as hepatitis viruses and picornaviruses, including human polioviruses and enteroviruses, are shed in feces, they do not replicate in enterocytes and they cause systemic and not gastrointestinal infections. Poliovirus vaccines (oral and parenteral) are often cited as quintessential enteric viral vaccines, but they induce systemic neutralizing antibodies that prevent extraintestinal viral spread and paralytic polio. The applicability of poliovirus as a model for immunity to localized enteric infections is thus limited. Type I viruses infect small intestinal villous enterocytes via the luminal surface and include canine coronavirus, porcine transmissible gastroenteritis coronavirus (TGEV), rotavirus, astrovirus, and calicivirus. TGEV infects and destroys the absorptive enterocytes of the entire villi throughout the small intestine, causing pronounced villous atrophy and often fatal diarrhea. Rotavirus and astrovirus infections are more restricted within the villi and occur mainly in the mid to distal small intestine, causing less severe villous atrophy and diarrhea. Enteric caliciviruses infect villous enterocytes of the proximal small intestine, inducing moderate villous atrophy and diarrhea. Type II viruses such as adenoviruses, bovine coronaviruses, toroviruses, and porcine epidemic diarrhea coronavirus (PEDV) infect villous and crypt enterocytes in the distal small intestine and the large intestine, inducing moderate to severe villous atrophy, crypt aplasia, and diarrhea. Type III viruses such as enteric parvoviruses infect crypt enterocytes basolaterally, causing crypt aplasia, severe villous atrophy, mucosal collapse, and severe hemorrhagic and often fatal diarrhea (Saif, 1999a). Thus, parvovirus infections can be prevented by systemic immunity, whereas prevention of localized enteric virus infections (types I and II) relies largely on intestinal immunity.

The enteropathogenicity of bacteria is determined by their virulence factors, including adhesion factors (fimbriae or pili) and enterotoxins; therefore, bacterial vaccines generally need to prevent attachment and toxin action within the intestine (Bertschinger, 1999).

In the following sections, we review vaccine strategies for types I, II, and III enteric infections. TGEV and rotavirus vaccines in pigs will be reviewed to illustrate findings concerning domestic outbred animals instead of inbred laboratory rodent models.

Vaccines to induce passive and active immunity in neonates to type I enteric viruses infecting villous enterocytes

Passive immunity

Prevention of localized intestinal infections requires the presence of sufficient levels of antibodies at the site of pathogen attachment, replication, and invasion (gut lumen). The need to provide neonatal immunity against enteric pathogens prompted studies of maternal vaccines to enhance passive lactogenic immunity (maternal antibodies in colostrum and

milk). The notion that live oral vaccines could mimic natural routes of infection and were preferable to conventional inactivated parenteral vaccines for stimulating protective lactogenic immunity (Bohl *et al.,* 1972; Saif *et al.*, 1972) was put forth over 30 years ago. Studies showed that in TGEV-seronegative sows, only oral immunization with virulent TGEV induced high rates of protection in suckling neonates. Oral immunization with live TGEV induced high titers of S-IgA antibodies in colostrum and milk, whereas systemic immunization induced mainly IgG antibodies. The ability of sows to transmit a high degree of passive lactogenic immunity to their suckling progeny was more closely associated with S-IgA than IgG TGEV antibodies. Bohl and Saif were the first to elaborate the concept of a gut–mammary gland–S-IgA immunologic axis, and these pioneering studies formed part of the basic tenet for a common mucosal immune system. Oral immunization of pregnant sows with attenuated TGEV and rotavirus vaccines is still used to induce passive immunity (Table 61.2). However, it was subsequently shown that in seropositive, naturally infected pregnant swine or cattle, lactogenic antibodies (S-IgA in swine and IgG1 in cattle) could also be enhanced by parenteral vaccination with live or appropriate killed or subunit vaccines (reviewed by Saif and Wesley, 1999; Saif and Fernandez, 1996). These observations concerning vaccine effects in TGEV-seronegative versus -seropositive animals explain some of the vaccine inconsistencies seen in field studies. Killed and modified live vaccines administered orally or parenterally to the mother are used extensively to increase lactogenic immunity in livestock and will be discussed further in the passive immunity section.

Active immunity

Rotavirus is a major cause of dehydrating diarrhea in young livestock, infants, and poultry (Saif and Fernandez, 1996). Multiple rotavirus serogroups (A, B, C, and E) based upon common inner-capsid VP6 antigens and multiple G (VP7, glycoprotein) and P (VP4, protease-sensitive) serotypes based on neutralizing epitopes on VP7 and VP4 capsid proteins of group A rotaviruses have been detected in humans, sheep, swine, cattle, horses, and poultry (Kapikian *et al.,* 2001). Characteristics of rotaviruses applicable to human and animal rotaviruses are reviewed in Chapter 49 on viral gastroenteritis vaccines. Among the distinct rotavirus serogroups and serotypes, cross-protection is minimal or nonexistent. The antigenic divergence among different sero/genotypes of rotaviruses (and enteric caliciviruses) presents a challenge for design of vaccines capable of inducing heterotypic protection.

Commercial modified live and killed rotavirus vaccines for rotavirus diarrhea in livestock and poultry are limited to group A rotaviruses and a group C rotavirus for pigs (Saif and Fernandez, 1996). The first oral rotavirus vaccine for calves was developed in 1972 (1 year prior to the discovery of human rotavirus) with use of a cell-culture-adapted neonatal calf diarrhea rotavirus (NCDV) strain (Mebus *et al.,* 1972). Although a significant reduction in morbidity and mortality was observed in a field trial among vaccinated calves in the majority of herds (in comparison with previous years), subsequent field studies revealed variable efficacy. Experimental studies suggested that maternal antibodies interfered with live vaccine replication and suppressed development of active immunity (Saif and Fernandez, 1996).

The neonatal gnotobiotic pig model of rotavirus infection and disease has been used to study correlates of active protective immunity and to evaluate approaches to improve the immunogenicity and protective efficacy of rotavirus vaccines for nearly 2 decades (Saif *et al.,* 1996; Saif *et al.,* 1997; Yuan and Saif, 2002). Gnotobiotic pigs are free of maternal antibodies (placental transfer of Ig does not occur in swine), but they are immunocompetent at birth. They are maintained aseptically and free of exposure to extraneous rotaviruses, ensuring that exposure to a single pathogen can be analyzed. Initial studies were conducted to mimic natural rotavirus infection (Bohl *et al.,* 1984) and to examine immune correlates of protection (Saif *et al.,* 1997). Gnotobiotic pigs orally inoculated with virulent or attenuated porcine rotaviruses or human rotavirus (HRV) were completely protected from homotypic but not heterotypic (distinct P and G type) rotavirus challenge (Hoshino *et al.,* 1988; Saif *et al.,* 1997). Pigs inoculated with virulent HRV developed significantly higher numbers of virus-specific IgA and IgG antibody-forming cells (AFCs) and memory B cells and higher lymphocyte proliferative responses in the intestinal lamina propria than did pigs inoculated with attenuated HRV (Ward *et al.,* 1996b; Yuan *et al.,* 1996). Pigs inoculated with two or three doses of attenuated HRV were moderately protected against virus shedding and diarrhea after homotypic challenge, similar to results of clinical trials of oral attenuated rotavirus vaccines in infants (Bresee *et al.,* 1999). The magnitude of the intestinal IgA AFC and lymphocyte proliferative responses correlated with the level of protection induced (Ward *et al.,* 1996b; Yuan *et al.,* 1996).

Immunogenicity and protective efficacy of various rotavirus vaccine formulations (attenuated replicating virus, inactivated virus, and recombinant baculovirus-expressed virus-like particles [VLPs]), administration routes, and adjuvants also have been evaluated in the gnotobiotic pig model (Saif *et al.,* 1997; Yuan and Saif, 2002). Inactivated oral or intramuscular HRV vaccines failed to protect against virulent HRV challenge, despite high IgG antibody responses induced in serum and systemic lymphoid tissues by the intramuscular vaccine. Rotavirus subunit vaccines consisting of double-layered VLPs composed of rotavirus inner capsid proteins VP2 and VP6 (2/6-VLPs) administered nasally or orally with mutant heat-labile toxin of *E. coli* (mLT) or ISCOMs as adjuvants (Iosef *et al.,* 2002; Yuan and Saif, 2002) induced IgG AFC responses in systemic lymphoid tissues and low or no IgA AFC responses in intestinal lymphoid tissues and also failed to mediate protection. The failure of intramuscularly administered inactivated HRV vaccines demonstrates that protective immunity against rotavirus requires the induction of IgA antibodies in the intestine,

since systemic IgG antibodies alone were not effective. The failure of nasal or oral 2/6-VLP vaccines suggests that protective immunity to rotavirus diarrhea in neonatal pigs requires the presence of intestinal IgA neutralizing antibodies to the outer capsid rotavirus proteins VP4 and VP7.

However, when 2/6-VLPs adjuvanted with MLT or ISCOM were used as nasal or oral booster doses in pigs orally primed with attenuated HRV, the protective efficacy increased significantly, and the highest numbers of intestinal IgA AFC and serum and intestinal IgA antibody titers were induced by this sequential prime/boost regimen (AttHRV/2/6-VLP), among all the vaccines tested in the gnotobiotic pig model (Iosef *et al.,* 2002; Yuan and Saif, 2002). An interesting finding was that priming with two doses of 2/6-VLP followed by live attenuated HRV was ineffective for inducing IgA antibodies or protection. Thus, the use of a replicating vaccine to prime lymphocytes in the major inductive site (gut-associated lymphoreticular tissue, or GALT), followed by boosting with a nonreplicating vaccine at a second mucosal inductive site (nasopharyngeal-associated lymphoreticular tissue, or NALT), was a highly effective approach to stimulating the mucosal immune system and to inducing active protective immunity against infection and diarrhea.

Using the TGEV model for evaluation of active protection against diarrhea in pigs, researchers also revealed new information about compartmentalization in the common mucosal immune system and its impact on mucosal vaccine strategies and protection (VanCott *et al.,* 1993, 1994). The natural occurrence of a deletion mutant of TGEV with exclusive respiratory tropism, referred to as porcine respiratory coronavirus (PRCV), provided a unique opportunity to study AFC responses and protective immunity to two antigenically related porcine coronaviruses with enteric (TGEV) versus respiratory (PRCV) tropism. The investigators showed that oral immunization of pigs with TGEV induced high numbers of IgA AFCs in the intestine and provided complete protection against TGEV challenge, whereas nasal immunization of pigs with PRCV induced mainly systemic immune responses (IgG AFCs) and provided only partial protection against TGEV challenge. Thus, the nasal PRCV alone failed to elicit sufficient intestinal IgA AFCs to provide full protection against the enteric pathogen, TGEV. Findings from this study, in addition to the studies of rotavirus vaccines, suggest that use of multiple mucosal inductive sites in a prime/boost vaccination regimen may be an effective approach to overcoming the compartmentalization in the common mucosal immune system.

Vaccines to induce active immunity in neonates to type III enteric viruses infecting crypt enterocytes

Canine parvovirus (CPV) infects crypt enterocytes, causing hemorrhagic gastroenteritis in pups (Bridger, 1990). Since CPV is likely disseminated to the basolateral surface of crypts by the hematogenous route, serum neutralizing antibodies (derived maternally or actively produced) are protective against the disease. In 1982, it was demonstrated that pups with hemagglutination inhibition (HI) serum antibody titers of >1:80 were immune to oronasal CPV type 2 challenge (Pollock and Carmichael, 1982). CPV is highly stable in the environment, and pups became susceptible to infection as soon as maternal antibodies declined to HI titers of 1:64 to 1:80. A maternal HI antibody titer as low as 1:20 severely affected the efficacy of a live CPV vaccine, however (Carmichael *et al.,* 1983).

Others have compared the immunogenicity and protective efficacy of commercial vaccines and concluded that substantial differences existed in their ability to immunize and protect pups with maternal antibodies (Larson and Schultz 1997). During the past 4 decades of CPV vaccine development, modified live viruses have proved to be superior to inactivated intramuscular vaccines (Appel, 1999). A study by Pratelli *et al.,* (2000) showed that a modified-live variant CPV-2b vaccine elicited protective immunity in 100% of pups whose maternal antibody titers were 1:10 to 1:40 and even in 60% of pups with antibody titers of 1:320.

Synthetic peptides (Casal *et al.,* 1995), DNA plasmids expressing VP1 (Jiang *et al.,* 1998), recombinant VLPs formed by baculovirus-expressed VP2 (Casal, 1999), and chimeric plant viruses expressing VP2 peptide (Langeveld *et al.,* 2001) have been evaluated in dogs or mice without maternal antibodies and have demonstrated good immunogenicity and/or protective efficacy. Further efficacy tests in pups in the presence of maternal antibodies are needed to assess their commercial potential.

Vaccines to induce immunity against enteric bacterial infections in neonates

Oral vaccines for induction of active immunity against bacterial diarrhea are not commonly used in livestock, although *E. coli* diarrhea is an important problem in postweaning pigs. Fimbrial vaccines are routinely administered parenterally to pregnant cattle, sheep, and swine to protect their suckling neonates against enterotoxigenic *E. coli* (ETEC) infections (Moon and Bunn, 1993). Such vaccines are practical and effective because (1) fimbriae are required for the adhesion-colonization of bacteria early in the pathogenesis of the disease; (2) most fatal ETEC infections in farm animals occur in the neonatal period; and (3) more than 90% of the ETEC strains in farm animals belong to a small family of fimbrial antigen types. Moreover, the vaccine strategy used to induce lactogenic immunity, parenteral vaccination of field-exposed seropositive mothers, is the same as that shown to be effective for parenteral application of rotavirus vaccines in rotavirus-seropositive mothers (Saif and Fernandez, 1996; Saif and Jackwood, 1990).

Studies of live oral enteric vaccines in animals have clarified the mechanisms of induction of protective immunity against enteric disease and contributed to our understanding of the common mucosal immune system. However, commercial live oral vaccines have often shown inadequate or inconsistent efficacy under field conditions (Saif and Jackwood, 1990). Major obstacles to improved efficacy of oral vaccines include maternal antibodies in the intestine of neonates (mainly colostrum and milk antibodies), which

interfere with live vaccine replication; inability of attenuated vaccine strains to adequately infect or stimulate S-IgA antibodies in the intestine (*i.e.*, less immunogenic than virulent field strains); use of inappropriate or unstable antigens for subunit vaccines; lack of oral delivery vehicles or mucosal adjuvants for subunit vaccines; and infection by pathogens prior to vaccination.

Differences in veterinary species and mouse models

Studies of adult mice and rabbits have shown that inactivated rotavirus vaccines and various formulations of VLPs (VP2/6, VP2/6/7, VP2/4/6/7), administered via intramuscular, oral, or nasal routes with or without adjuvants, induce complete or significant partial protection against rotavirus infection (Crawford *et al.,* 1999; O'Neal *et al.,* 1998; Siadat-Pajouh and Cai, 2001). However, only protection against infection, not against diarrhea, can be assessed in adult mouse or rabbit models, as these species are susceptible to rotavirus-induced diarrhea only during the first 2 weeks of life (Ciarlet *et al.,* 1998; Ward *et al.,* 1990). Studies in pigs indicate that protection rates against rotavirus diarrhea upon challenge correlate with the magnitude of IgA AFC and memory B cell responses in intestinal lymphoid tissues but not with such responses in systemic lymphoid tissues (Yuan and Saif, 2002). Thus, the capacity to induce sufficient levels of intestinal IgA AFCs or antibodies and sufficient memory B cell responses appears to be critical for the efficacy of rotavirus vaccines in large animals and likely in human infants (Coulson *et al.,* 1992).

The relative importance of B cells versus $CD4^+$ and $CD8^+$ T cells in protective immunity to rotavirus has been extensively studied in adult mice because inbred mouse strains, antibodies to CD4 and CD8 T cells, and gene knockout mice are available to facilitate such studies (Franco and Greenberg, 2000; McNeal *et al.,* 2002). In these studies neither $CD4^+$/$CD8^+$ T cells nor antibodies were essential for induction of protective immunity to rotavirus infection in adult mice, but usually one of these effectors (T or B cells) was necessary for elimination of primary rotavirus infection. The redundant nature of the immune responses to rotavirus in mice, the multiple immunologic and possibly nonimmunologic pathways to resolve rotavirus infections (Franco and Greenberg, 2000), the age factor (adult models) and host differences in the pathogenesis of rotavirus infection in mice and pigs (Conner and Ramig, 1997; Saif *et al.*, 1997), and the use of highly inbred mouse strains contribute to the discrepancies seen between the adult mouse and neonatal gnotobiotic pig models.

Future directions

To develop more effective vaccines against enteric pathogens, improved methods are needed to induce high levels of intestinal IgA antibodies against the appropriate microbial antigens. Vaccines should also induce heterotypic protection, active immunity in the presence of maternal antibodies, and if possible long-lasting immunological memory against enteric pathogens. In nature, such memory is presumably maintained by frequent boosting by repeated environmental exposure to these common and stable enteric pathogens, most of which are endemic in animal and human populations. Novel vaccines (*e.g.*, transgenic plants), adjuvants (*e.g.*, MLT, ISCOM, CpG-oligodeoxynucleotides, 1α, 25-dihydroxyvitamin D3), and vaccine delivery systems (*e.g.*, recombinant plant or animal viruses, bacterial vectors, and microparticles) should be explored and evaluated in relevant animal models.

A recent study showed that intramuscular immunization with *E. coli* F4 fimbriae reduced $F4^+$-*E. coli* excretion in feces of suckling pigs upon challenge. Addition of 1α, $25(OH)_2D3$ (a Th2 modulating adjuvant) reduced shedding significantly and was associated with secondary IgA antibody responses postchallenge. Addition of CpG (a Th1 modulating adjuvant) reduced diarrhea and was associated with enhanced lymphocyte proliferative responses (Van der Stede *et al.,* 2003).

A newly developed coronavirus expression system (Alonso *et al.,* 2002; Enjuanes *et al.,* 2001) has potential for use as a virus vector for delivery of mucosal vaccines. Since coronaviruses infect the mucosal surfaces of the respiratory and intestinal tracts, these vaccines can be targeted to mucosal inductive sites. Nonpathogenic coronaviruses infecting many species of interest are available for development of expression systems. The tissue and species tropism of coronaviruses may be manipulated by engineering the S gene, which is the tropism determinant, to target specific tissues in their individual hosts (Enjuanes *et al.,* 2001).

Attenuated *S. typhimurium* strains expressing heterologous antigens have been widely evaluated, mostly in mice, as vector vaccines for human mucosal pathogens (reviewed by Fooks, 2000). Recombinant *S. typhimurium* has been used as an antigen delivery system for oral immunization of chicks against the coccidian parasite *Eimeria tenella* (Pogonka *et al.,* 2003). Recombinant *Salmonella* vaccines have also been used to express antigens of TGEV and of bovine enterotoxigenic *E. coli* (ETEC) K99 fimbriae (Chen and Schifferli, 2001; Ascon *et al.,* 1998).

Transgenic plants expressing recombinant proteins from enteropathogens may provide inexpensive edible vaccines for induction of intestinal immunity. Plants expressing antigens of enteric viruses, such as the TGEV S protein (Tuboly *et al.,* 2000) or rotavirus NSP4-cholera toxin B and A2 subunit fusion protein (Yu and Langridge, 2001), induce antibody responses following parenteral immunization. Further research is necessary to improve immune responses and immunological memory following ingestion of transgenic plant vaccines.

PASSIVE IMMUNITY

The passive transfer of maternal immunity provides essential protection in newborn mammals. Although the neonatal immune system is competent to mount primary immune responses against antigens of many infectious agents, developing both humoral and cell-mediated immunity, in many cases primary (active) immune responses do not develop

quickly enough to prevent disease and death. Maternal immunologic assistance thus can provide a critical (though temporary) aid to survival for neonates.

The enhancement of passive immunity through vaccination of the mother has been a successful disease prevention strategy in domesticated animals. Vaccinated mothers develop higher levels of specific antibodies in colostrum and milk and thereby increase levels of immunity in their offspring (Glezen, 2001; Saif and Fernandez, 1996; Tizard, 2000). Passive immunity can also be enhanced by oral administration of immune milk or heterologous antibody preparations (*e.g.*, chicken egg yolk IgY, [Ikemori, 1992; Kuroki, 1994] or monoclonal antibodies) or by parenteral administration of hyperimmune plasma (Becu *et al.*, 1997).

Unfortunately, passive antibodies often interfere with active immunization of young animals and birds. Various vaccination strategies have been developed to minimize the suppressive effects of maternal antibodies, but improved adjuvants and antigen delivery systems are needed to facilitate efficient and predictable induction of active immunity in the presence of maternal antibodies. This section will address past, current, and future approaches and considerations for using passive immunity in veterinary species.

Transfer of maternal immunity

The transfer of systemic passive immunity from the mother to her offspring can occur prenatally, via the placenta or yolk sac, or postnatally via ingestion of colostrum and milk, depending upon the species. The main Ig isotype transferred in most species is IgG. Mechanisms of transport of Ig from dam to offspring are described elsewhere in this volume. In mice and rats, transplacental transfer of Ig occurs in combination with prolonged (16 and 21 days, respectively) postnatal transfer by means of colostrum and milk (Pastoret, 1998). In dogs and cats, transfer of IgG occurs by a combination of prenatal and postnatal mechanisms, with 5% to 10% of total transfer occurring before birth (Tizard, 2000). In ruminants, horses, and pigs, offspring are born virtually agammaglobulinemic, and transmission of Ig occurs only via colostrum for a limited time after birth (Pastoret, 1998; Wagstrom *et al.*, 2000). After the transition from production of colostrum to milk, Ig are no longer absorbed from the intestines and act only locally.

Immunoglobulin absorption in neonates of large domestic species is facilitated by the presence of protease inhibitors in the colostrum (Westrom *et al.*, 1982), and its efficiency declines rapidly after birth, with maximal absorption occurring in the first 4 hours. The cessation of absorption of intact macromolecules is termed "gut closure" and occurs at different ages in different species. In calves and pigs closure normally occurs by 24 to 36 hours after birth.

Failure of passive transfer (FPT) in domestic large animals

Absorption of colostral Ig can be highly effective, supplying the newborn with serum antibodies at similar levels to those in the mother's circulation. Failure of passive transfer is a common problem, however, in newborn calves and foals (Besser and Gay, 1994; Tyler-McGowan and Hodgson, 1997). Failure of passive transfer may occur because of the production of low quantities of colostrum, production of colostrum with inadequate levels of maternal antibodies (e.g., with mastitis or agalactia), ingestion of low quantities of colostrum, or inefficient absorption in the gut (Quigley and Drewry, 1998). Colostral supplements and replacers (Arthington *et al.*, 2000; Quigley and Drewry, 1998) as well as plasma products have been developed commercially to address this problem, with variable success. Since transfer of maternal antibodies in farm animals is dependent upon ingestion of colostrum, the benefits of vaccination of the dam for enhancing passive immunity are lost if absorption of colostral Ig is inefficient (Hodgins and Shewen, 1994).

Mechanisms of clearance of passively acquired IgG

The half-life of Ig varies considerably among species of domestic animals. Recent findings suggesting that the FcRn is involved in homeostasis of serum levels of IgG in general do not preclude distinct mechanisms functioning in neonates. Studies conducted by Besser *et al.* (1987, 1988b) indicate that the main route of clearance of passively acquired IgG1 in calves is transfer from the serum to the intestine. Approximately 70% of passively acquired IgG1 is eliminated by this route. If titers of passive circulating antibodies are high enough, the transfer of antibodies from the circulation to the intestinal lumen is sufficiently efficient to mediate at least short-term partial protection against rotavirus diarrhea (Besser *et al.*, 1988a). The same mechanism may be functional in piglets (reviewed by Saif and Wesley, 1999; Parreño *et al.*, 1999; Ward *et al.*, 1996a). The persistence of titers of circulating maternal antibodies is generally considered in the design of vaccination strategies for young animals because of suppressive effects of maternal antibodies on active immune responses. However, it is not always necessary to wait for titers of maternal antibodies to decline to the limit of detection before vaccination (Hodgins and Shewen, 1998), and in maternally immune piglets, induction of memory can occur even in the presence of detectable antibodies (Boersema *et al.*, 1998).

Passive immunity in the respiratory tract

Experiments in colostrum-deprived lambs (Jones *et al.*, 1989) and calves (Mosier *et al.*, 1995) have demonstrated the ability of parenterally administered immune antisera of appropriate specificity and high titer to mediate protection following experimental challenge with *M. haemolytica*. Although antisera prepared against *M. haemolytica* continue to be marketed in some countries for prevention of pneumonia in calves, lack of documentation of the specificity and antibody titer of these products makes their value questionable. Parenteral administration of hyperimmune plasma raised against *R. equi* has been shown to protect against pneumonia in young foals in experimental (Hooper-McGrevy *et al.*, 2001) and field studies (Becu *et al.*, 1997). Hyperimmune plasma is available commercially for prophylactic use in foals.

Prepartum vaccination of beef cows (Van Donkersgoed *et al.*, 1995) and dairy cows (Hodgins and Shewen, 1994; Hodgins and Shewen, 1996) has been demonstrated to increase titers of antibody to *M. haemolytica* in their colostrum and in the serum of their calves. Virtually all adult cattle have serum antibodies to *M. haemolytica*; vaccination therefore serves to trigger anamnestic responses.

Passive immunity in the gastrointestinal tract

Rodents have been a popular model for the study of passive protection by milk antibodies. However, rats and mice actively transport IgG from the gut into the circulation during the first 2 weeks of life. Thus, antibodies in ingested milk contribute to both local and systemic immunity in rodents, in contrast to the strictly local effects occurring in humans and most domestic animals. In pigs, horses, dogs, and cats, IgG is the most abundant Ig in colostrum, but IgA predominates in milk. Parenteral vaccination, by enhancing serum IgG antibody titers, contributes to IgG antibodies in colostrum but has limited effects on IgA antibodies in milk. Although Saif and Bohl (1983) and Salmon (1995) observed IgA antibody responses in sow's milk after the administration of live viruses into the mammary gland, this is not the usual mechanism for induction of IgA antibodies to enteric pathogens in milk of seronegative animals. Rather, in swine, Bohl and Saif (Bohl *et al.,* 1972; Saif *et al.,* 1972) showed that IgA antibodies in milk with specificity for enteric pathogens appear after sufficient antigenic stimulation (TGEV infection) of the intestine. On the basis of these observations, they first proposed trafficking of IgA lymphoblasts from the gut to the mammary gland in monogastrics. This proposed gut–mammary–S-IgA immunologic axis provided part of the initial basis for the concept of a common mucosal immune system. The trafficking of gut-origin IgA lymphoblasts to the mammary gland was confirmed experimentally in mice (Roux *et al.*, 1977) and subsequently verified in swine (Salmon *et al.,* 1984). In contrast, Sheldrake and Husband (1985) found little evidence of a gut–mammary gland axis in ruminants. Instead, in ruminants, IgG1 is the main isotype in both colostrum and milk (Butler, 1983), and it is selectively transported from serum primarily prepartum, but also postpartum, with a marked decrease in serum IgG1 prepartum (Butler, 1983). An IgG1-specific Fc receptor, probably the FcRn (Kacskovics *et al.*, 2000), expressed on the basolateral surface of mammary gland alveolar epithelial cells is responsible for this transport. It is interesting that both the expression of this IgG1 receptor in ruminants and the trafficking of IgA lymphoblasts to the mammary gland in monogastrics are regulated by pregnancy hormones (prolactin, estrogen, and progesterone) (Weisz-Carrington *et al.*, 1978; Barrington *et al.*, 2000).

Milk antibodies provide passive protection to the neonatal intestinal tract by immune exclusion, preventing the attachment of viruses, bacteria, and parasites, and by neutralizing enterotoxins, virulence factors, and viruses. S-IgA antibodies, presumably because of their resistance to cleavage by digestive enzymes and their higher levels in milk, appear to be more efficient in mediating protection in the gut of pigs and other monogastrics (Saif and Jackwood, 1990; Saif and Fernandez, 1996), but high persisting levels of passive IgG antibodies are also protective (reviewed in Saif and Wesley 1999; Ward *et al.*, 1996a; Parreño *et al.*, 1999). In ruminants, IgG1 antibodies, also relatively resistant to proteolytic enzymes (Brock *et al.*, 1977) and predominant in milk, may supplant the role of S-IgA.

Numerous vaccines are marketed for vaccination of cows and sows to provide lactogenic immunity to rotavirus, coronavirus, and *E. coli* in suckling offspring (Table 61.2). Vaccine efficacy has been variable and is influenced by numerous factors related to the host, the vaccine, and management. A discussion of key concepts follows, in which coronavirus and rotavirus enteric vaccines in swine and cattle serve as examples (reviewed by Saif and Jackwood, 1990; Saif and Fernandez, 1996; Saif and Wesley, 1999).

Ideally, suckling animals become subclinically infected with enteric pathogens while receiving adequate passive antibodies to prevent disease, and they develop active immunity (or are primed; Boersema *et al.*, 1998) to prevent subsequent diarrhea. This balance between passive immunity and disease has been disrupted in intensive animal production systems by exposing animals to high pathogen doses in confined, contaminated environments. In addition, earlier weaning practices, with feeding of supplements or milk replacers, curtail or dilute milk antibodies. Consequently, without maternal vaccines, antibody titers to enteric pathogens usually decrease to unprotective levels in milk.

Maternal enteric vaccines are commonly used in two populations of pregnant animals. To control epidemic infections (such as epidemic TGEV), they are targeted for use in naïve, seronegative animals to induce primary immune responses. To control endemic infections (such as with rotavirus and *E. coli*), booster vaccines are used in seropositive, field-exposed animals to stimulate anamnestic responses. Vaccine strategies for the latter vaccines generally have been more successful than for the former, reflecting a greater success in designing vaccines to boost rather than to prime for mucosal immunity to provide lactogenic immunity against enteric pathogens. To date, only virulent TGEV given to pregnant sows effectively stimulates high levels of IgA antibodies in milk and passive protection (reviewed by Saif and Jackwood, 1990; Saif and Wesley, 1999). Use of oral highly attenuated TGEV vaccines (safe for piglets but which replicate poorly in sows) induces lower IgA milk antibody titers and low or variable efficacy in the field (Moxley and Olsen, 1989). Parenteral killed TGEV vaccines induce only low milk IgG antibody titers and usually the lowest protection rates. Attempts to develop maternal TGEV recombinant subunit vaccines based on the surface TGEV spike (S) protein that induces neutralizing antibodies or live vector vaccines expressing the S protein have also been of limited success in TGEV-seronegative swine (reviewed by Saif and Wesley, 1999). However, prime/boost strategies such as intramuscular administration of TGEV S protein following oral/nasal priming with attenuated TGEV have shown promise as a means of enhancing milk IgA antibody titers (Park

et al., 1998). Epidemic TGEV outbreaks have also declined following the appearance of a respiratory variant of TGEV. This variant induces TGEV-neutralizing antibodies in milk and at least moderate protection against TGEV after repeated respiratory infections in sows, raising unresolved questions about bronchus-associated lymphoid tissue (BALT)–mammary gland lymphocyte trafficking (reviewed in Saif and Wesley, 1999).

Whereas TGEV is associated with both epidemic and endemic infections in swine, rotavirus and *E. coli* infections are endemic in swine and cattle. Booster vaccination strategies are required to enhance lactogenic immunity to such endemic enteric pathogens because antibody titers in milk decline dramatically during lactation. Although studies are limited, parenteral vaccination of TGEV- or rotavirus-seropositive (field-exposed) pregnant sows with attenuated vaccines effectively boosted both S-IgA and IgG antibodies in milk (Saif and Jackwood, 1990; Saif and Fernandez, 1996; Saif and Wesley, 1999). These findings concur with reports of increased breast milk IgA antibodies in women endemically exposed to cholera and parenterally boosted with a cholera vaccine (Svennerholm *et al.*, 1977). The finding that parenteral boosting is effective in increasing IgA antibodies in mucosal secretions of animals orally primed with a live pathogen is consistent with observations that after intestinal replication of rotavirus in pigs, IgA memory B cells initially reside in the ileal Peyer's patches but are subsequently also present in substantial numbers in spleen but not bone marrow (Yuan *et al.*, 2001). Thus, systemic stimulation of such IgA memory B cells by parenteral booster vaccines could yield IgA antibodies in serum for transport to mucosal secretions via the polymeric Ig receptor. These observations have led to mucosal prime/boost strategies for human and animal rotavirus vaccines that are currently being tested in gnotobiotic pigs (Yuan and Saif, 2002).

Under field conditions, antibodies to endemic intestinal pathogens are also common in bovine colostrum and milk, but without the boosting effect of highly immunogenic vaccines, antibody titers are often too low to protect calves (Besser and Gay, 1994; Saif *et al.*, 1983; Saif and Fernandez, 1996; Saif and Jackwood, 1990). Thus, vaccines are marketed for prepartum vaccination of cows against rotavirus, coronavirus, and *E. coli* to enhance passive immunity in their calves (Table 61.1), but the field efficacy of these viral vaccines has been questioned (Waltner-Toews *et al.*, 1985). A number of important variables may account for vaccine failures. These include vaccine titer and dose, inactivating agent, virus strain, adjuvant, inoculation route, and parity of the mother (reviewed by Saif and Fernandez, 1996). Because colostrum and milk of ruminants contain mainly serum-derived IgG1, parenteral (intramuscular, subcutaneous, or intramammary) inoculation of rotavirus seropositive cows with optimal live, inactivated, or subunit (VP 2/4/6/7 VLP) rotavirus vaccines effectively boosts both IgG1 and virus-neutralizing antibody titers in serum and milk.

A positive correlation has been shown between serum titers of rotavirus-neutralizing antibodies in neonatal beef calves and resistance to rotavirus diarrhea (Kohara and Tsunemitsu, 2000). Vaccination of pregnant dairy cows with modified live ($\geq 10^7$ plaque-forming units), binary ethyleneamine (but not β-propiolactone) inactivated rotavirus in incomplete Freund's adjuvant (IFA) (but not AlOH adjuvant) or recombinant 2/4/6/7 VLPs in IFA significantly increased titers of IgG1 and virus-neutralizing antibody to rotavirus in colostrum and milk (Saif *et al.*, 1983; Saif and Fernandez, 1996; Kim *et al.*, 2002). These colostral supplements mediated passive protection in calves against experimental oral rotavirus challenge (reviewed by Saif and Fernandez, 1996; Saif *et al.*, 1983).

Prepartum vaccination of cows and sows with bacterins prepared from enteropathogenic *E. coli* for prevention of diarrhea in their offspring is also commonly practiced (Table 61.2). Under modern farming practices, dairy and veal calves rarely are fed whole milk from their dams for more than 1 or 2 days. Thus, vaccine efficacy is based on antibodies absorbed from colostrum or retained temporarily in the gut, rather than on a continuing supply of immune milk. Besser *et al.* (1988a) demonstrated that transfer of passive IgG1 antibodies from the serum to the intestine in calves could mediate this short-term protection. Piglets, in contrast, continue to receive immune milk until weaning at 2 to 3 weeks of age. The importance of a continuous supply of passive antibodies for protection against TGEV has been demonstrated experimentally (Saif and Wesley, 1999).

Numerous commercial Ig preparations with antibody activity against specific enteric pathogens have been marketed over the past several decades. Products intended for prevention of *E. coli* enteritis in calves include dried bovine colostrum and whey, hyperimmune sera raised in horses, and mouse monoclonal antibodies to the K99 (F5) antigen of *E. coli*. These products are administered orally in the first 12 hours of life to prevent adhesion of enteropathogenic *E. coli*. Bovine Ig products containing antibodies to antigens of *E. coli* pathogenic for neonatal pigs have also been marketed for oral use in piglets. Orally administered bovine colostral whey containing rotavirus antibodies also passively protected piglets (in the absence of circulating antibodies) against rotavirus in a dose-dependent manner but did not interfere with induction of active serum antibody responses (Schaller *et al.*, 1992).

Oral administration of chicken egg yolk antibodies

Immunization of chickens shows promise as an efficient method of producing polyclonal antibodies for passive protection. Specific antibodies of the IgY isotype are induced by vaccination and are concentrated in egg yolk. Laying hens can produce about 20 g of IgY per year. Yolk antibodies with virus neutralizing activity provide partial protection against rotavirus (Kuroki *et al.*, 1994) and coronavirus diarrhea (Ikemori *et al.*, 1997). Yolk antibodies have also provided protection against enterotoxigenic *E. coli* in calves (Ikemori *et al.*, 1992) and piglets (Yokoyama *et al.*, 1997). Protective effects of yolk antibodies are dependent on antibody titers in the oral preparations (Marquardt, 2000). Thus, development

of better means to protect yolk antibodies from digestive processes will improve both the efficacy and the economic viability of yolk antibodies for clinical applications (Mine and Kovacs-Nolan, 2002).

Induction of active immunity in the presence of maternal antibodies

For many diseases of newborns and neonates, passive immunity is the only practical means of providing timely protection. Unfortunately, it is well documented that maternal antibodies can suppress active immune responses following vaccination. This effect has been observed with both live and nonreplicating vaccines and for both systemic and mucosal immune responses (Siegrist *et al.*, 1998; Parreño *et al.*, 1999). Antibody responses especially are affected; recent evidence suggests that T-lymphocyte responses may not be suppressed (Siegrist *et al.*, 1998). Titers of maternal antibodies are maximal for most species of interest in the first week of life and then decline gradually over the next few months, but variability of titers among individuals is high. With many vaccines, a "window of disease susceptibility" of variable duration occurs when titers of maternal antibodies are too low to mediate protection but too high to permit effective vaccination.

A number of strategies are used to cope with this problem. Some veterinary vaccines for cattle are sold with the disclaimer that "animals vaccinated before 6 months of age should receive a booster dose of vaccine at 6 months of age." This provides little solace for the many diseases of cattle occurring in the first weeks or months of life. A common strategy for vaccines of dogs and cats is to administer a series of doses of vaccine from an early age (at which only a few individuals will be responsive) and to continue vaccinating until an age at which virtually all can respond to vaccination. This strategy has economic disadvantages for the pet owner. Some manufacturers produce low-passage, high-virus-titer vaccines especially for use in situations where high titers of maternal antibodies and high pathogen exposure are anticipated. This is similar to a strategy once (but no longer) approved by the World Health Organization for vaccination of children in developing countries against measles (Gellin and Katz, 1994). Preliminary evidence suggests that incorporation of vaccine antigens in highly structured ISCOMs or nasal application of vaccines can enhance immune responses in the presence of maternal antibodies (Van Binnendijk *et al.*, 1997; Brockmeier *et al.*, 1997).

Future needs

Maternal vaccination to enhance passive immunity is already widely used in veterinary medicine. Some of these vaccines, especially vaccines against enteric viruses, have limited efficacy; new approaches are needed to enhance immunogenicity in an economically viable manner. Commercial products have already been developed as supplements for newborns that have received inadequate amounts or quality of colostrum. Although some of these products contain guaranteed minimum titers of antibodies against specific organisms, there is a need to expand this quality control to additional common pathogens of neonates.

There is an urgent need for development of adjuvants and delivery systems capable of reliably inducing active immunity in neonates in spite of the presence of maternal antibodies. The ability to provide continuity of immune protection from birth, by combining passive immunity with active immunization, would have a major impact on neonatal morbidity and mortality in animals and humans.

CONCLUSIONS

Research on mucosal veterinary vaccines has contributed new concepts to the field of mucosal immunity. Investigations of pathogen–host interactions in outbred animals have illustrated the complexity of these interactions and have encouraged rethinking of established paradigms. Early studies of an enteric coronavirus infection of swine (TGEV) led to the concept of the gut–mammary gland–S-IgA immunologic axis and provided part of the basic tenet for a common mucosal immune system (Bohl *et al.*, 1972; Saif *et al.*, 1972). Later studies of TGEV and a deletion mutant of TGEV with respiratory tropism (PRCV) revealed that functional compartmentalization exists within the common mucosal immune system whereby nasal inoculation of pigs with PRCV failed to elicit sufficient intestinal IgA antibody responses to fully protect against the enteric pathogen TGEV (Van Cott *et al.*, 1993, 1994). Subsequent studies have explored new prime/boost mucosal immunization strategies to elicit intestinal immunity to the enteric pathogen rotavirus in naïve pigs (Saif, 1999b; Yuan and Saif, 2002). In these studies, only oral priming with attenuated virus led to successful nasal booster responses with use of nonreplicating (VLP) vaccines combined with mucosal adjuvants such as ISCOM or mLT. Thus, use of a replicating vaccine to prime lymphocytes at a major mucosal inductive site (GALT), followed by boosting with a nonreplicating vaccine at a second inductive site (NALT), effectively stimulated intestinal IgA antibodies and induced active protection against rotavirus diarrhea.

Although there is progress in developing safe and effective nonreplicating vaccines to boost mucosal immune responses, including the use of parenteral booster vaccines in field-exposed animals (Saif and Fernandez, 1996), there is still a need to develop effective, safe vaccines to prime for mucosal immunity. Mucosal adjuvants (mLT, ISCOM, CpG, cytokines [Rankin *et al.*, 2001]) and new delivery systems (replicating vectors, microparticles [Bowersock *et al.*, 1994a]) have shown promise in animal studies reviewed in this chapter. However, their economical production and their final evaluation under field conditions, including in the presence of maternal antibodies (as relevant), are needed.

Considerable research effort has been devoted to development of vaccines for respiratory diseases of domestic animals. In some instances attenuated organisms delivered by mucosal routes have demonstrated improved efficacy over nonreplicating antigens given by systemic routes. For many

respiratory diseases, however, further progress in development of mucosal vaccines will have to await advances in understanding of disease pathogenesis and identification of protective antigens. In contrast, studies of ascending infections of the reproductive tract in cattle have demonstrated the efficacy of systemic vaccination to clear established infections and highlight the possibility of therapeutic vaccines.

Finally, it is important to realize that there are species differences to consider in designing vaccines to elicit mucosal immunity. For example, the primary Ig in mammary secretions of ruminants is IgG1, which is actively transported to the mammary gland from serum and provides effective passive immunity to the nursing offspring against enteric pathogens. Thus, parenteral immunization of the mother is effective in stimulating passive immunity in ruminants against enteric pathogens. In contrast, in monogastrics, IgA predominates in milk and IgA lymphoblasts that traffic to the mammary gland originate in the intestine. Therefore, oral vaccines in monogastrics may provide a more effective vaccine strategy to induce IgA antibodies in milk against enteric pathogens (Saif and Fernandez, 1996). If the aforementioned vaccine concepts and ones reviewed in this chapter are applied with new and effective mucosal adjuvants, delivery systems, and bioengineered vectors expressing the appropriate microbial antigens, it is likely that a new generation of veterinary vaccines will emerge to better cope with existing and emerging mucosal pathogens.

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