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CHAPTER

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Mucosal Vaccine Development for Veterinary and Aquatic Diseases

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I. INTRODUCTION

Vaccines are essential for controlling disease in livestock, companion, and zoo animals and wildlife and for controlling fertility and disease in pest species. Effective mucosal vaccines have myriad advantages over parenteral vaccines that are shared across human and veterinary species. For instance, mucosal vaccines stimulate both the mucosal and systemic immune systems, meaning that mucosal vaccines can reduce pathogen colonization and shedding and thus protect the population or herd against infection. Major challenges for mucosal immunization are to generate effective immunity instead of immunological tolerance as well as overcoming interference from passively acquired maternal antibodies. Oral tolerance is a suppressive mechanism designed to prevent the host immune system from overreacting to innocuous antigens such as those present in feed or commensal flora [1]. Once oral tolerance has been induced, subsequent exposure to that antigen from mucosal or systemic routes will prevent induction of a robust immune response [2]. The vast majority of mucosal vaccines use live attenuated forms of a pathogen that can replicate in the target species, avoiding induction of oral tolerance, but under very rare circumstances may revert back to virulence. Other forms of live vaccines include viruses such as replication-deficient adenovirus [3], canarypox virus carrier vaccines [4], and others that act as a vector to express genes of interest from various pathogens. These carrier viruses have no ability or limited ability to replicate in the immunized species; nor is reversion to a replication competent, highly infectious virus

Mucosal Vaccines
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possible, making them a safer vaccine choice. There are some reports that mucosal delivery of live viral vaccine to the upper respiratory tract may overcome maternal antibody interference [5]. Previous chapters provided detailed analyses of the numerous advantages of mucosal vaccines, and these will not be discussed in depth here. Instead, aspects of mucosal vaccine development that are particularly important for the veterinary field, such as mass delivery, vaccines that differentiate between infection and vaccination (which has important trade implications), and economic considerations, will be discussed. Examples of the numerous commercially available mucosal veterinary vaccines and discussion of novel experimental vaccines will be provided. Vaccine development for administration in veterinary species to protect human health will also be discussed.

A. Considerations for Mucosal Veterinary Vaccine

1. *Mass Delivery*

Mass delivery methods for mucosal immunization, such as administration of the vaccine in drinking water or in feed, in sprays (for avian species, which are ingested at preening), in ovo (for avian species), or for immersions (for aquatic species), allow vaccination of hundreds or thousands of animals over a short period of time. Most important, mass delivery of mucosal vaccines means that each individual animal does not need to be restrained, which can be extremely stressful for animals (especially those that are not routinely handled by humans), and restraint may be potentially hazardous to the person or teams of people administering the vaccines. A major drawback to mass delivery is that a uniform dose per animal is not always given across the population. Other mucosal routes that require restraint are vaccines administered intranasally, by drenching (oral immunization by syringe), or through eyedrop (conjunctival) administration. These delivery methods have the advantage that they promote a mucosal immune response and the dose per animal is easily controlled, but they do require animal restraint, which makes them more expensive to deliver.

2. *Differentiation of Infected From Vaccinated Animals Vaccines*

With today's global economy, trade in animals and in animal products such as meat, eggs, and fur occurs locally, nationally, and internationally. For trade purposes, it is advantageous to be able to distinguish animals that have been infected by disease from animals that have been vaccinated. Vaccines that allow for the immunological differentiation between animals that have been infected and those that have been vaccinated are called DIVA vaccines. DIVA vaccines often lack one or more proteins present in the wild-type microorganism, which can be determined through immune assays. The first-generation DIVA vaccines were developed when it was discovered that pigs vaccinated with the attenuated strains of Aujeszky's disease virus did not develop antibody against select protein epitopes [6], whereas animals infected by wild-type viruses did have these protein-specific antibodies in their serum. These first-generation marker vaccines were soon improved upon by using genetically modified live vaccines that lacked selected glycoproteins [7], and enzyme-linked immune sorbent assays were developed that could determine which pigs were vaccinated because they lacked antibodies against the glycoprotein but had antibodies specific for other PrV proteins [8]. By using these strains as vaccines or genetically engineered live viral vaccines, researchers could track the success of eradication efforts; this resulted in effective eradication programs of the disease in many parts of Europe [8]. Without the option of using DIVA vaccines, producers may choose not to vaccinate so as to avoid the presence of antibodies in the herd that they cannot guarantee are due to the vaccine and not the presence of the disease-causing agent in their herd. At the same time, vaccines made from live viruses, such as

replication-deficient adenovirus that express genes of interest from various pathogens or modified viruses with select genes deleted, are considered genetically modified organisms (GMOs). Animals vaccinated with GMOs may face regulatory hurdles that must be overcome before the products from these animals can be sold. Indeed, some countries are reluctant to license GMO vaccines.

3. *Economics and Trade*

Livestock production is a large-scale business and mucosal vaccination will not be implemented unless the disease causes significant mortality and/or morbidity (such that production metrics are negatively affected). Further, the vaccine will be used only if it is sufficiently affordable such that its use does not significantly reduce profitability. Depending on the disease, how easily it spreads, and how well the infectious agent survives in the environment, culling of a barn rather than mass vaccination may be a more economically feasible option to clear an infection. However, from a welfare point of view, such a practice is highly problematic and often results in public outcry. Moreover, as antimicrobial-resistant pathogens continue to emerge, there is increasing pressure to raise livestock with reduced levels of antibiotics. Vaccines offer the potential of controlling such infections and represent effective alternatives to antibiotics.

Together, adaptation of mucosal vaccines for mass delivery to livestock, DIVA vaccination and its impact on trade considerations, and the cost of vaccine development and implementation all influence whether vaccines will be used in a veterinary setting.

II. EXPLORATION OF COMMERCIAL AND EXPERIMENTAL MUCOSAL VETERINARY VACCINES

Whether an effective vaccine can be generated against an infectious agent requires extensive knowledge of disease pathogenesis and epidemiology across all target species. Livestock (cattle and other ruminants, pigs, avian species such as chickens and turkeys, etc.), companion animals (horses, dogs, cats, rabbits, guinea pigs, etc.), wildlife (deer, bison, koala, etc.), pest species (skunks, raccoon, mice, rats, etc.), zoo animals, and aquatic species have unique economic, social, and immunological characteristics that affect whether a mucosal vaccine will be sought for development and/or whether existing mucosal vaccines will be used.

A. Mucosal Vaccines for Livestock

The growing human population has lead to an increased need for protein from food animals and for animal by-products, which has led to a steady increase in the number of intensive livestock operations worldwide. Such operations can include hundreds or thousands of animals contained in a pasture or barn, and their close proximity to one another can facilitate spread of disease to vulnerable members within the population. Because mucosal vaccination is superior to systemic vaccination in preventing colonization and shedding of pathogens, mucosal vaccination is especially important in controlling infections in livestock. Tremendous strides have been made in veterinary mucosal vaccine development and commercial availability, many of which are listed in Table 48.1.

The following sections provide examples of mucosal veterinary vaccines under development against selected pathogens using animals from the target species (rather than mice or other experimental animals where possible).

1. *Suidae*

A. PORCINE TRANSMISSIBLE GASTROENTERIDITIS AND PORCINE EPIDEMIC DIARRHEA VIRUS

Both transmissible gastroenteritis virus and porcine epidemic diarrhea virus can cause severe

TABLE 48.1 Commercially Available Vaccines for Livestock Species, Companion Animals, Birds, and Wildlife Against Infectious Diseases Listed in the OIE Manual of Infectious Diseases [9] (http://www.oie.int/manual-of-diagnostic-tests-and-vaccines-for-terrestrial-animals/)

Vaccine name	Virus, bacteria, or subunit antigen	Disease	Route
BOVIDAE			
Bovilis IBR	Bovine herpesvirus 1 (BHV1)	Bovine rhinotracheitis	Intranasal
TSV-2	Infectious bovine rhinotracheitis (IBR) virus and parainfluenza3 (PI3) virus	Bovine rhinotracheitis Parainfluenza,	Intranasal
INFORCE 3	BRSV, IBR, and PI3	Bovine respiratory disease	Intranasal
NASALGEN IP	IBR, PI3	Bovine rhinotracheitis Parainfluenza	Intranasal
Once PMH IN	*Mannheimia haemolytica*, *Pasteurella multocida*	*M. haemolytica-P. multocida*	Intranasal
BOVILIS CORONAVIRUS	Bovine coronavirus	Bovine enteric disease	Intranasal
Calf-Guard	Bovine rotavirus and coronavirus	Bovine enteric disease	Oral
Respioval	BRSV, IBR, and PI3	Bovine respiratory disease	Intranasal
EQUINE			
Flu Avert I.N.	Equine influenza virus type H3N8 strain (EIV A/Equine 2/Kentucky/91)	Influenza	Intranasal
PINNACLE I.N.	*Streptococcus equi*	Strangles	Intranasal
SUIDAE			
ProSystem TREC	Rotavirus (two modified live G serotypes 5 and 4 of serogroup A), transmissible gastroenteritis virus, colibacillosis (*Escherichia coli* pilus antigens K88, K99, F41, and 987P), *Clostridium perfringens* type C	Bovine enteric disease, transmissible gastroenteritis, colibacillosis, enterotoxemia	Two oral and one intramuscular
ENTERO VAC	Avirulent live *E. coli* F4 (K88)	Enteritis	Oral administration in drinking water
EDEMA VAC	Avirulent live *E. coli* F18 (K99) vaccines	Edema	Oral administration in drinking water
COLIPROTEC F4	Avirulent live *E. coli* F4 (K88)	Postweaning diarrhea (PWD)	Oral
Enterisol Ileitis	Live attenuated *Lawsonia intracellularis*	Porcine proliferative enteropathy (ileitis)	Oral administration in drinking water
NITRO-SAL FD,	Avirulent live *Salmonella enterica* Cholerasuis	Salmonellosis	Oral administration in drinking water
Argus SC/ST	Avirulent live *S.* Cholerasuis	Salmonellosis	Oral administration in drinking water

(*Continued*)

TABLE 48.1 (Continued)

Vaccine name	Virus, bacteria, or subunit antigen	Disease	Route
Salmonella T/C vaccine	Avirulent live *S*. Cholerasuis	Salmonellosis	Oral administration in drinking water
MAXI/GUARD Nasal Vac	*Bordetella bronchiseptica*	Respiratory disease	Intranasal
Ingelvac ERY-ALC	Avirulent live *Erysipelothrix rhusiopathiae*	Erysipelas	Oral administration in drinking water
Suvaxyn E-ora	Avirulent live *E. rhusiopathiae*	Erysipelas	Oral administration in drinking water
CANINE			
NASAGUARD-B	Avirulent live *B. bronchiseptica*	Canine infectious respiratory disease (CIRD) (kennel cough)	Intranasal
VANGUARD B	Avirulent live *B. bronchiseptica*	Kennel cough	Intranasal
BRONCHI-SHIELD ORAL	Avirulent live *B. bronchiseptica*	Kennel cough	Administered by syringe into the buccal cavity
FELINE			
NOBIVAC Feline-Bb	Avirulent live *B. bronchiseptica*	Diseases caused by *Bordetella bronchiseptica*	Intranasal
WILDLIFE OR PEST ANIMALS			
Raboral V-RG	Live vaccinia virus vaccine encoding the rabies virus glycoprotein	Rabies	Oral in baits
ONRAB	Live adenovirus vector encoding the rabies glycoprotein	Rabies	Oral in Ultralite bait matrix
RABIGEN SAG2	Modified live attenuated rabies virus vaccine	Rabies	Oral in baits
AVIAN			
Burse BLEN-M	Live infectious bursal disease virus (IBV)	Infectious bursal disease	Drinking water
S-706 and SVS 510	Live IBV	Infectious bursal disease	Drinking water and coarse spray
Univax-Plus	ST-12 and 51 A/C4 strains of IBV	Infectious bursal disease	Drinking water
UNIVAX-BD	Live IBV	Infectious bursal disease	In ovo, Drinking water
Bursine-2	Live IDV	Infectious bursal disease	Drinking water
CLONEVAC D-78	Field isolate of IBV	Infectious bursal disease	Coarse spray, drinking water

(*Continued*)

TABLE 48.1 (Continued)

Vaccine name	Virus, bacteria, or subunit antigen	Disease	Route
HVT	FC-126 live strain of turkey herpesvirus (HVT)	Marek's disease	In ovo
SB1	SB1 strain of chicken herpesvirus	Marek's disease	In ovo
VAXXITEK HVT + IBD	Live serotype 3 HVT	Marek's disease	In ovo
Bursal Disease–Marek's Disease Vaccine Serotype 3	ST-14 strain of live bursal disease virus, FC-126 strain of HVT	Infectious bursal disease and Marek's disease	In ovo
VECTORMUNE HVT IBD vaccine	HVT expressing an infectious bursal disease key protective antigen	Marek's disease	In ovo
CEVAC MD HVT	Serotype 3 HVT Marek's disease virus	Marek's disease	In ovo
MD-Vac	Live serotype 3 virus Marek's Disease Vaccine	Marek's disease	In ovo
Poulvac Ovoline CVI	Live serotype 1 Marek's disease virus	Marek's Disease	In ovo
IB-VAC-H	Live Holland strain of IBV	Massachusetts type bronchitis	Coarse spray or drinking water
Bronchitis Vaccine, Mass Type	IBV	Massachusetts type bronchitis	Intraocular, Coarse aerosol spray, Drinking water
MILDVAC-Ma5	Live Ma5 strain of Massachusetts type bronchitis	Massachusetts type bronchitis	Beak-O-Vac or coarse spray, drinking water
NEWHATCH-C2	Live B1 type C2 strain of Newcastle disease virus	Newcastle disease	Coarse spray
Gallivac HB1 Mass	B1 strain of Newcastle disease virus, Massachusetts type IBV	Newcastle disease and Massachusetts type bronchitis	Coarse spray, drinking water
Newcastle-Bronchitis Vaccine	B1 strain of Newcastle disease virus and IBV of the Massachusetts and Connecticut types	Newcastle disease and Massachusetts type bronchitis	Intraocular or coarse spray
AVIPRO ND-IB POLYBANCO	Newcastle disease virus and IBV of the Massachusetts and Connecticut types	Newcastle disease and infectious bronchitis, Massachusetts and Connecticut type bronchitis	Intraocular or drinking water
COMBOVAC-30	Live clone 30 strain of Newcastle disease virus and IBV Massachusetts and Connecticut types	Newcastle disease and Massachusetts and Connecticut types bronchitis	Coarse spray, drinking water
Poulvac ST	Modified-live *Salmonella enterica* Enteritidis *Salmonella Heidelberg*, or *Salmonella enterica* Typhimurium	Salmonellosis	Coarse spray then drinking water

(*Continued*)

TABLE 48.1 (Continued)

Vaccine name	Virus, bacteria, or subunit antigen	Disease	Route
SALMUNE	Live avirulent *S.* Enteritidis, *S. Heidelberg*, or *S.* Typhimurium	Salmonellosis	Coarse spray, drinking water
AVIPRO MEGAN EGG	Live avirulent *S.*Typhimurium	Salmonellosis	Coarse spray
M-NINEVAX-C	Live avirulent M-9 strain of *P. multocida*, Heddleston type 3−4 cross-strains	*P. multocida*, owl cholera in chickens and turkeys	Drinking water
H.E. VAC	Live apathogenic avian adenovirus	Hemorrhagic enteritis	Drinking water
ORALVAX HE	Live turkey avirulent type II avian adenovirus of pheasant origin	Hemorrhagic enteritis	Drinking water
REOGUARD L	Live 1133 strain of avian reovirus	Tenosynovitis	Drinking water
ENTEROVAX	Live avian reovirus (tenosynovitus biotype)	Reovirus induced tenosynovitis (viral arthritis)	Spray or drinking water
IMMUCOX	Live oocysts of *Eimeria* spp.	Coccidiosis	Oral by chicken feed
COCCIVAC-B52	Live oocysts of *Eimeria acervulina*, *Eimeria maxima*, *E. maxima MFP*, *Eimeria mivati*, and *Eimeria tenella*	Coccidiosis	spray cabinet administration
COCCIVAC−D2	*E. tenella*, *E. mivati*, *E. acervulina*, *E. maxima*, *Eimeria brunetti*, and *Eimeria necatrix i*	Coccidiosis	Spray cabinet administration
HATCHPAK COCCI III	Live oocysts of *E. maxima*, *E. acervulina*, and *E. tenella*	Coccidiosis	Coarse spray
INNOVAX-ILT	Live recombinant serotype 3THV with genes from laryngotracheitis virus	Fowl laryngotracheitis and Marek's disease	In ovo
LT-IVAX	Live attenuated fowl laryngotracheitis virus	Fowl laryngotracheitis	Intraocular
LT BLEN	Live fowl laryngotracheitis virus	Fowl laryngotracheitis	Intraocular or drinking water
ART VAX	Live chemically induced mutant of *Bordetella avium*	*B. avium* rhinotracheitis (turkey coryza)	Coarse spray, drinking water
TREMOR BLEN D	Live avian encephalomyelitis virus	Avian encephalomyelitis	Drinking water
AQUATIC SPECIES			
AQUAVAC-ESC	Modified live *Edwardsiella ictaluri* RE-33	Edwardsiellosis	Immersion
Furogen Dip	*Aeromonas salmonicida* bacterin	Furunculosis	Immersion
Ermogen	Formalin-inactivated *Yersinia ruckeri* serotype I (Hagerman strain)	Enteric redmouth disease	Immersion
AquaVac Vibrio Oral	Inactivated *Vibrio anguilarum* 01 and 02a	Vibrosis	Oral

diarrhea in newborn piglets. A DNA vaccine expressing S proteins from both viruses delivered by attenuated *Salmonella* Typhimurium was constructed as a potential vaccine. and its immunogenicity was assessed [10]. Twenty-one-day-old piglets were orally immunized with the attenuated *S.* Typhimurium with empty DNA vaccine or DNA vaccine expressing the S proteins at a dosage of 1.6×10^{11} CFU per piglet and then booster immunized with 2.0×10^{11} CFU after 2 weeks. Virus-neutralizing S-protein-specific immunoglobulin G (IgG) and secretory immunoglobulin A (SIgA) as well as systemic cellular immune responses (interferon gamma, interleukin 4, and lymphocyte proliferation) was significantly higher in the vaccinated group than in the control and empty DNA vaccine cohorts. These data show that *S.* Typhimurium can be used to carry DNA vaccines and, when delivered orally, may promote a protective immune response.

2. *Caprinae and Ovidae*

A. BRUCELLA OVIS

Ovine epididymitis caused by *Brucella ovis* infection has been reported in the Americas, European countries, Australia, New Zealand, and South Africa. This disease can lead to genital lesions and reduced fertility in rams, placentitis and abortions in ewes, and increased perinatal mortality in lambs [11]. While safer than subcutaneous vaccination, conjunctival vaccination with live *Brucella melitensis* Rev 1 vaccine can causes abortions, is highly virulent, and is not a DIVA vaccine; therefore it is not recommended in countries that are free from *B. melitensis* [12]. Alternatively, conjunctival immunization in rams using a thermoresponsive and mucoadhesive in situ gel composed of poloxamer 407 (P407) and chitosan (Ch) could effectively deliver recombinant BLS-OMP31. (BLS is part of the enzyme lumazine synthase from *Brucella* spp. that is both highly immunogenic and a carrier of foreign peptides and *B. ovis* antigen OMP31 [13].) Serum and preputial, saliva, lacrimal, and nasal secretions showed significant antigen-specific IgG antibody, and the levels remained elevated in serum only for several months. Relative to unvaccinated rams, the rams from the vaccinated cohort showed significant induction of antigen-specific SIgA after the first and second immunization in lacrimal, preputial, or nasal secretions (but not in nasal secretions or in serum), but antibodies levels declined rapidly [14]. Further, conjunctival immunization induced a significant BLS-OMP31-specific hypersensitivity response to intradermal injections relative to the control rams, which indicates induction of cell-mediated immunity. Conjunctival administration of BLS-OMP31-P407-Ch may be a promising alternative to current *B. ovis* immunization strategies.

3. *Bovinae*

A. BOVINE HERPESVIRUS 1

Bovine herpesvirus 1 (BoHV-1) is responsible for infectious bovine rhinotracheitis, infectious pustular vulvovaginitis, conjunctivitis, abortion, encephalomyelitis, and mastitis in cattle. Parenteral BoHV-1 glycoprotein E deleted mutant viral DIVA vaccines used in conjunction with diagnostic testing and targeted culling of animals infected with field strains has led to eradication of this disease in some European countries [15,16]. Additional DIVA mucosal vaccines are under development. For example, a small trial showed that calves vaccinated intranasally with BoHV-1 glycoprotein E deleted mutant virus or BoHV-1 triple mutant virus (BoHV-1 *tmv*), which incorporates mutation for three genes, including glycoprotein E within a single virus, were protected against infectious challenge [17]. While both DIVA vaccines were protective against clinical disease, only BoHV-1 *tmv*-vaccinated calves generated significantly higher virus-neutralizing titers

after challenge relative to the sham controls, and they showed a more rapid cellular immune response onset and a more rapid viral clearance. Although this virus has worldwide distribution, use of marker vaccines will continue to contribute to eradication efforts.

B. HEMORRHAGIC SEPTICEMIA

Water buffalo, cattle, and bison are affected by hemorrhagic septicemia (HS), which is an acute, highly fatal form of pasteurellosis. This economically important bacterial disease affects Asia, Africa, and the Middle East, and sporadic outbreaks occur in Southern Europe. An HS vaccine containing avirulent *Pasteurella multocida* strain B:3,4 (fallow deer strain) has been used in Myanmar to control HS in cattle and water buffaloes [18]. Earlier intranasal vaccines failed to protect against subcutaneous challenge, and the efficacy of this vaccine for primary vaccination of young buffaloes was brought into question [19,20]. However, a later study showed that an intranasal vaccine containing live gdhA-derivative *P. multocida* B:2 that was boosted 2 weeks later was protective against a subcutaneously administered challenge with live wild-type *P. multocida* [21]. Importantly, the vaccine was also effective in protecting in-contact buffalo against a virulent parental strain and has been recommended by the Food and Agriculture Organization of the United Nations as an effective vaccine in Asia.

C. BOVINE VIRAL DIARRHEA VIRUS

Calves do not receive maternal antibodies in utero; instead, they receive antibodies through colostrum in the neonatal period. However, while maternal antibodies are critically required to protect the vulnerable neonate against infectious diseases, circulating maternal antibodies interfere with the neonate's ability to develop its own immunity (referred to as maternal interference). Because colostrum is composed mainly of IgG1 (which is not transported across the mucosal epithelium of the upper respiratory tract) and although IgA does cross the mucosa, dimeric IgA makes up only 10% of antibodies in colostrum, it was suggested that the upper respiratory tract may not be affected by maternal interference. To test this hypothesis, cows were vaccinated with modified live bovine viral diarrhea virus (BVDV) vaccine composed of BHV-1, BVDV-1, BVDV-2, PI-3, and BRSV antigens. Calves were shown to have high circulating maternally derived IgG antibodies serum but extremely low titers of maternally derived IgG in nasal secretions [22]. Maternally derived IgA in nasal secretions were present but at much lower levels than in serum. Calves (3–8 days old) either were not vaccinated against BVDV or received one or two (day 0 and day 35) immunizations by the intranasal route. Within 5–7 days after birth, maternally derived IgA in nasal secretions were not detected. Calf-derived (i.e., endogenous) BVDV1- and BVDV-2-specific IgA production was detected within 10 days after vaccination. A secondary intranasal vaccination after 5 weeks induced a strong memory antibody response with sustained IgA levels in nasal secretions. Collectively, these studies demonstrated that the mucosal immune system in newborn calves is functional and responsive to vaccination without being affected by maternal interference.

4. Avian Species

Chicken, turkey, duck, and other avian barns and houses are populated by very large numbers of birds for egg production or for production of meat. Standard laying houses are reported to hold from 100,000 to 500,000 hens, and broiler houses routinely house 20,000 birds. With these numbers, it is not surprising that the industry has actively sought vaccines that could be administered by mucosal routes rather than by parenteral routes, which generally rely on injection with needles. The majority of

mucosal avian vaccines are live viruses administered by eyedropper into the eye (intraocular or conjunctival), orally into the drinking water, as a coarse spray whereby birds consume the vaccine during preening, and through the in ovo route. In-feed oral vaccination and spray cabinet (intranasal) routes are also used for some commercial vaccines. In ovo injection has become widely used as a means to deliver precise, uniform doses with the capacity to inject up to 60,000 eggs per hour. In ovo immunization has the added benefit that this method avoids stress to chicks, is sanitary, and has an earlier exposure time than any other immunization method.

A. AVIAN INFLUENZA

Many experimental mucosal avian vaccines are under development to combat avian influenza. It was reported that an intranasally delivered bioadhesive liposome using tremella or xanthan gum and containing the experimental inactivate avian H5N3 virus as a model antigen elicited high mucosal SIgA and serum IgG in chickens [23]. Even the low pathogenic strains such as H9N2 avian influenza virus (AIV) can affect the economic success of commercial poultry industry by causing mild respiratory disease and decreased egg production. Immunizations for multiple forms of avian influenza are under way as experimental vaccines. For instance, *Lactobacillus plantarum* NC8 strain was engineered to express select peptides from H9N2 AIV. Both oral and intranasal vaccination of 3-week-old white leghorn layer chickens succeeded in inducing immunity, but the intranasal route induced stronger immunity and showed less body weight loss, lung virus titers, and pathology after challenge with the H9N2 virus [24]. These nontraditional mucosal vaccine delivery platforms showed that they may be good choices for commercial avian influenza vaccine development.

B. Companion Animals

1. Leporidae

A. RABBIT HEMMORRHAGIC DISEASE

Rabbit hemorrhagic disease (RHD) is a lethal disease of adult rabbits caused by rabbit calicivirus [25]. Oral immunization of rabbits with recombinant vaccinia virus [26] or recombinant myxoma virus [27] coding for VP60, the major structural protein of RHD virus (RHDV) induced protection against challenge with virulent RHDV. However, little horizontal transfer was achieved. Other researchers showed that oral immunization with VP60 protein expressed in transgenic potatoes generated partial protection against viral challenge [28].

Rabbits have been used to investigate whether the uterus is a suitable mucosal vaccination site. An experimental vaccine consisting of ovalbumin (OVA), recombinant truncated glycoprotein 1 from bovine herpes virus, and a fusion protein of porcine parvovirus VP2 and bacterial thioredoxin (rVP2−TrX) was formulated with poly I:C, host defense peptide and polyphosphazene as adjuvants. Surgery was performed to isolate each uterine horn, and this triple antigen−triple adjuvant vaccine was injected into the lumen of the uterine horns (referred to as intrauterine immunization) [29]. Significant induction of OVA and tGD-specific serum IgG and IgA was observed over time in intrauterine-immunized animals. Uterine, lung, and vaginal tissues obtained 1 month after the single immunization showed significant OVA-specific IgG and IgA response relative to sham treatment. Significantly increased tGD-specific and rVP2-TrX antigen-specific IgG titers (but not IgA titers) were observed in lung, vagina, and uterine tissue relative to controls. The results indicate that a subunit vaccine formulated with appropriate adjuvants can trigger both systemic and mucosal immunity when administered into the uterine lumen.

C. Wildlife

1. *Koala*

A. CHLAMYDIA PECORUM

Many wild koalas in Australia are known to have *Chlamydia pecorum*, which causes debilitating ocular and urogenital infections in koalas with clinical signs that include conjunctivitis and infertility. A single-dose anti-*C. pecorum* vaccine formulated to contain three major outer-membrane proteins (MOMPs) or polymorphic membrane proteins (PMPs) (an antigenic membrane bound surface-exposed adhesion protein that is important for attachment to the cell membrane [30]) and a 1:2:1 ratio with PCEP poly[di(sodium carboxylatoethylphenoxy)phosphazene], immune defense regulatory peptide (IDR1002), and poly I:C was tested in wild koalas. Although the vaccine was administered subcutaneously, anti-MOMP IgA increased 10- to 100-fold at ocular and upper genital tract (UGT) sites in 50% and 40% of the koalas, respectively. The PmpG vaccine also triggered a 10- to 100-fold increase post vaccine IgA antibodies at the UGT or the ocular sites in 40% and 50% of koalas, respectively, which suggests that the vaccines elicited a mucosal response in at least some of the koalas. The cohort vaccinated with MOMP vaccine showed decreased chlamydia loads with no new occurrence of infection, but the other vaccination group and the control group showed increased loads with incidences of new infections, suggesting that the MOMP vaccine may be superior [31]. Further development must be undertaken to improve vaccine uptake to more members of the population, but these results suggest that a parenteral vaccine may succeed in promoting mucosal responses when properly formulated.

2. *Prairie Dogs*

A. PLAGUE

Sylvatic plague caused by *Yersinia pestis* and carried in fleas can significantly affect the population dynamics of prairie dogs (*Cynomys* spp.). In turn, reduced prairie dog numbers can affect the population dynamics of ferrets, burrowing owls, and several canine and avian predators. Administration of insecticides can control the fleas and reduce transmission of *Y. pestis*, but there is evidence that the fleas can develop resistance [32]. A vaccine that can be used as an alternative to the use of insecticides is actively sought. The orthopoxvirus raccoonpox (RCN) was genetically modified to express two protective *Y. pestis* antigens (designated RCN-F1/V307) and mixed with bait for oral vaccination of prairie dogs in a lab setting. Sixty percent of prairie dogs that consumed bait containing RCN-F1/307 and were then challenged at 270 days post-vaccination survived, which was a significantly higher percentage than that in the placebo group [33]. Rates of survival were improved if two oral baits were consumed months apart.

3. *Multispecies*

A. RABIES

Rabies virus, a member of the Rhabdoviridae family, causes neuroinvasive rabies disease in many wild animals, including bats, possums, raccoons, skunks, foxes, coyotes, groundhogs, wolves, and monkeys. It can also infect companion animals such as dogs, cats, rabbits, and horses. It is spread through saliva and can be transmitted through bites and scratches. Symptoms include fever, violent movements, uncontrolled excitement, fear of water, aggressive behavior, and death. Most human cases of rabies come from contact with an infected domestic dog [34].

Raboral V-RG is an oral vaccine composed of a live vaccinia virus encoding the rabies virus glycoprotein [35]. It is encased in a packet with fish meal and set out as bait for raccoons, foxes, coyotes, and the like; the packet can have a flavor coating to attract target species [36]. An alternative oral vaccine in Canada is ONRAB, a

live adenovirus vector encoding the rabies glycoprotein that is administered as an oral vaccine in Ultralite bait matrix [37]. A comparative study showed that when Raboral V-RG or ONRAB was distributed by aircraft at a density of 75 baits/km^2 and sera from raccoons and skunks were collected 5–7 weeks later, skunks showed no significant difference in the proportion of antibody-positive animals, regardless of the vaccine used [38]. In contrast, the proportion of antibody-positive raccoons was significantly higher in the ONRAB-baited areas than in the RABORAL V-RG-baited areas, suggesting that ONRAB may be a better choice to vaccinate more species.

D. Mucosal Aquatic Vaccines

The aquaculture industry is growing faster than any other farmed animal industry in the world, with an increase of more than 10% between 2011 and 2016 to 70 million tons, and an increasingly high proportion of high-quality protein used to feed the world's growing population comes from aquaculture. As with any farming industry, the risk of infectious diseases increases as the density of animals increases, and excellent fish health management practices, such as controlling stocking densities, maintaining adequate oxygen levels and water quality, and reducing pathogen loads, are critical to control disease.

Improved understanding of mucosal immunity in farmed fish and crustaceans may lead to the development of cost-effective mucosal vaccines. It is estimated that there are 25,000 fish species in the world, and they are extremely diverse to accommodate living in warm or cold climates, in fresh or salt water, and in depths at high or low pressure [39]. Teleosts (bony fish) lack bone marrow; instead, their B lymphocytes mature within the kidney [40]. Mucosa-associated lymphoid tissue in teleost fish is composed of skin-associated lymphoid tissue, gill-associated lymphoid tissue, and a diffuse gut-associated lymphoid tissue [40]. Antibody-producing cells have been identified in the cutaneous dermis and mucus, which may indicate a "mucosal" immune system in fish [41]. It is not yet clear whether fish B and T cells home back to mucosal sites upon mucosal infection after immunization [42]. For instance, oral or anal immunization of carp with formalin-killed *Vibrio anguillarum* followed by a booster immunization by the same route resulted in slightly enhanced antigen-specific Ig titers detected in skin mucus and bile. Serum antibody titers were elevated after anal intubation but not in response to oral immunization [43]. How the route of immunization affects immunity in fish warrants further study.

Fish body temperature takes on the local temperature, which can have a significant impact on the metabolism and rate of growth of fish as well as on their immune system. For instance, development of antibodies in fish adapted to low temperatures ($<15^{\circ}$C) may require at least 4–6 weeks, whereas the time period to develop antibodies may be a few weeks in fish adapted to warmer temperatures. This time frame suggests that even if fish have a functional immune system, they may not develop immunity in a timely manner to protect them from infection. The major causative agents of infectious diseases in finfish aquaculture include bacteria, viruses, parasites, and fungi. Infectious agents can infect fish at some developmental stages and not at others. Although some fish may have a functional immune system in the larva or fry stage, others may not, which means that proper biosecurity rather than vaccination may be critical to protecting them against infection. Farmed fish that are routinely vaccinated include Atlantic salmon (*Salmo salar*), rainbow trout (*Onchorhynchus mykiss*), and Atlantic cod (*Gadus morhua*). With effective vaccine development, there has been a decline in the use of antibiotics along with improved health and increased growth of the fish.

Currently, there are three main forms of vaccination for aquatic species: immersion, oral delivery, and injection.

1. Immersion Vaccines

For immersion immunization, gills are likely the main site of antigen entry, but uptake by the skin, lateral line, and gut have also been suggested and may, in fact, contribute to induction of mucosal immunity [44]. Immersion vaccines are effective for a number of bacterial pathogens, and they are practical, cheap, and easy to batch-administer, especially to small fish. A disadvantage to this vaccination route is that it requires large amounts of vaccine, and levels of protection and duration of immunity may vary across vaccines.

An experimental vaccine for immersion of catfish 10–30 days posthatch with modified live *Edwardsiella ictaluri* vaccine was shown to produce a protective immune response against *Enteric septicemia* [45]. Other researchers showed that introducing several small lesions in the skin and then immersing the fish in a vaccine suspension containing formalin-killed *Streptococcus iniae* produced a protective immune response against these bacteria, and they suggest that the response was equal in effectiveness to that produced by intraperitoneal injection [46].

2. Oral and Intranasal Vaccines

Oral delivery can be accomplished with fish of any age. It is relatively cheap and non-labor-intensive, it is not stressful for the fish, and it is the only option to deliver vaccine to fish in the seawater growth stage. Disadvantages include the fact that large quantities of the antigen are required, it is impossible to ensure equal distribution among the farmed animals, and the duration of immunity is generally less than that observed with injection or immersion. Oral vaccines can be made to adhere to finished feed. The challenge is to maintain antigen stability in countries with high heat and humidity as well as in the high-acid environment of the stomach once consumed. Bioencapsulation has been used, wherein feed was incubated in a vaccine suspension prior to feeding the fry. Different encapsulation techniques, including formulation with liposomes [47] or alginate beads [48], have been used to protect the antigens from the destructive environment in the gut.

As with any animal, oral administration may lead to induction of tolerance, which may be compounded by the young age of the animal and repeated low-dose administration. Some researchers believe that oral vaccines may be more suited to act only for booster immunization to avoid induction of oral tolerance. However, studies have shown that primary oral vaccination of salmon in the seawater growth stage with an oral salmonid rickettsial septicemia vaccine formulated with a bioadhesive cationic polysaccharide protected the salmon against a lethal pathogen challenge [49]. Other researchers showed that oral vaccination with a DNA vaccine, wherein the vector expressing a gene from infectious pancreatic necrosis virus encapsulated in alginate microspheres, protected salmonid fish against infectious challenge [50]. Oral vaccination of rainbow trout with an experimental vaccine bacterin of *Yersinia ruckeri* O1 failed to protect against enteric redmouth disease (yersiniosis), but the same dose administered anally was protective against infectious challenge. These data suggest that the oral vaccine needs to be protected from degradation in the stomach to be effective [51].

Rainbow trout (*O. mykiss*) vaccinated intranasally as early as 24 days posthatch with a live attenuated infectious hematopoietic necrosis virus vaccine, killed enteric red mouth bacterin, or saline. Upon challenge with the respective pathogen 28 days later, vaccinated groups were significantly more protected than their age-matched mock control groups [52]. These data suggest that the intranasal route may be amenable for vaccine targeting if it becomes adapted for mass vaccination.

3. *Injected Vaccine*

Many farmed fish are vaccinated by an intraperitoneal injection, a route that is very labor intensive. This method can also be stressful for the fish and must be performed on fish of sufficient size, which means that vaccination of fry is difficult. No commercial mucosal vaccines are available against viruses that infect fish, so these vaccines are administered by injection.

With the rise in aquaculture comes an increased need to protect the livestock against infectious diseases. Increased efforts to elucidate the immunology of the target species of fish and pathogenesis of the parasite, virus, or bacteria that target them will undoubtedly lead to development of new vaccines suitable for mass delivery.

E. Immunocontraceptive Vaccines

Population management has different considerations, depending on whether the species of interest are wildlife, pests, companion animals, or zoo animals. Generally, for all but pest species, an ideal immunocontraceptive would be reversible, safe, long-lasting, and cost-effective. While in some species, reduction in sexual or aggressive behavior may be a beneficial side effect of contraception, some species may require such behavior to maintain the herd hierarchy. Therefore species-specific needs should be a consideration before vaccination [53].

For decades, two reliable parenteral immunocontraceptives against gonadotropin-releasing hormone (GnRH; also known as luteinizing hormone releasing hormone) or zona pellucida proteins (ZP) have been used to reversibly control fertility in many animal species. Antibodies generated against GnRH neutralize this pituitary hormone, which in turn inhibits steroidogenesis and gametogenesis in male and female mammals. Porcine zona pellucida vaccines prepared by using ZP isolated from pig ovaries or recombinant ZP antigen (SpayVac from ImmunoVaccine Technologies, Canada) are one of the most studied immunocontraceptive vaccines in wildlife. Anti-ZP antibodies bound to sperm impede binding and penetration of the ovum, and anti-ZP antibodies interfere with follicle development in some species [54,55].

The overwhelming majority of immunocontraceptive vaccines are delivered parenterally, but some experimental research has focused on delivery by mucosal routes. Delivery of an effective mucosal vaccine (i.e., not relying on injections or darting) for wildlife or zoo animals would be ideal for administration, as it would be less stressful to the animal and present less risk to the person administering the vaccine. Experimental work in rabbits showed that rabbit ZP glycoprotein B delivered by infection with myxoma virus resulted in infertility in 25% of female rabbits [56]. Brushtail possums (*Trichosurus vulpecula*) were vaccinated with bacterial ghosts (BGs) expressing ZP protein introduced through oral, intranasal/conjunctival, parenteral, and intraduodenal routes. Anti-ZP antibodies were detected in the serum and the ovarian follicular fluid after intranasal/conjunctival immunization [57]. Intraduodenal, but not oral administration of the vaccine, elicited significant systemic immune responses, indicating that protection of BG vaccines from degradation by gastric acidity would enhance the effectiveness of orally delivered vaccines. Superovulation and artificial insemination was used to assess the effect of the immunization with BG-delivered ZP. Immunization by the nasal/conjunctival route resulted in induced antibody-mediated and cell-mediated immune responses, and significantly fewer eggs were fertilized in immunized possum females [58]. Field trials will need to be performed to determine whether mucosal immunization with BG containing possum ZP antigens is suitable for fertility control of wild possum populations.

1. *Efficacy, Safety, and Economic Feasibility of Immunocontraceptive Vaccines*

To be adopted for use, mucosal immunocontraceptive vaccines must be effective in the target animals and have limited or no effect on nontarget animals which may include humans who consume the meat, eggs, or milk from the targeted species. For zoo animals, such as captive African and Asian elephants, altering sex hormone levels in male or females may be advantageous in that they reduce aggression during musth season, but the alterations can also interfere with dominance hierarchy in a herd, which may not be advantageous [59,60]. Further, use of oral bait delivery systems should, if possible, be designed in such a way as to reduce consumption by unintended target species.

The economic practicality of vaccine development such as costs associated with manufacturing and licensing as well as costs associated with treatment, including labor, equipment, and population dynamics, will all determine whether controlling fertility with a vaccine is an effective means to control a population. For instance, research shows that an annual control campaign using baits to sterilize female foxes would reduce the red fox population density by about 30%, but an annual campaign of poisoning would reduce fox density by about 80%. Vaccination would, of course, be the better choice when animal welfare issues are taken into consideration, although it is a less effective means to control population growth [61]. Whether a population can be controlled by immunocontraception or culling depends on the species and the availability of an effective mucosal vaccine and mucosal delivery system.

F. Mucosal Vaccines to Improve Fertility

In addition to contraception, immunization against select targets may be used to improve fertility. Active immunization of cows against inhibin, a protein whose major action is negative feedback regulation of pituitary follicle-stimulating hormone (FSH) secretion, via the subcutaneous route neutralized endogenous inhibin levels, which resulted in increased FSH secretions during the estrous cycle. The immunized cows had a greater number of follicular waves and a greater number of follicles during the estrous cycle, which could be used as a potential source of oocytes for use in in vitro fertilization and embryo transfer programs [62]. Advances have been made with mucosally delivered inhibin vaccines. In buffalo, nasal immunization with a DNA vaccine coding for inhibin and delivered by attenuated *Salmonella* Cholerasuis has been shown to improve follicle development and fertility [63]. However, the buffalo in this trial underwent estrous synchronization, which would not be feasible in the wild and will have to be investigated further to establish its feasibility as a fertility vaccine. While immunization against select targets may affect fertility, care should be taken that targeting a natural protein may affect other pathways that are important for the animal's health. Further, should ovulation rates be affected, it must be established that an increased number of dams do not suffer from complications associated with multiple offspring per parturition before it is known that the vaccine is safe to use.

III. VETERINARY VACCINES AND ONE HEALTH

Veterinary vaccines can also be used to reduce food-borne illness by targeting bacteria that cause no illness or only mild illness in animals, but that can be harmful to humans upon consumption. For example, chicks orally immunized on the first day of life then boosted orally or via the intramuscular route at 6 and 16 weeks of age with a novel attenuated *Salmonella* Enteridis vaccine candidate, showed significantly higher plasma IgG and intestinal SIgA levels as compared to those in the control group

[64]. The lymphocyte proliferation response and $CD45^+$ $CD3^+$ T cell number in the peripheral blood of the vaccinated groups were significantly increased. When the birds were challenged intravenously with the virulent *S. enteritidis* strain in the 24th week, the egg contamination rates were significantly reduced in both vaccinated groups relative to the controls, but total protection was not achieved. These results indicate that this vaccine may reduce incidences of egg contamination and therefore reduce the risk of human salmonellosis.

While rare, Shiga toxin-producing *Escherichia coli* O157:H7 (STEC O157) can have serious consequences in the young and in the aged human population, including hemorrhagic colitis, renal failure, and death. Cattle are widely recognized as an important reservoir of STEC O157 for human exposure, making contaminated beef a potential source of food-borne infection. Parenteral vaccination with a combination of antigens associated with type III secretion system-mediated adherence results in significantly reduced shedding in orally infected animals [65]. As yet, no mucosal vaccines have been developed that significantly reduce colonization in cattle, potentially because of poor cross-protection across STEC strains.

Veterinary mucosal vaccines that protect humans from food-borne infectious diseases have tremendous One Health implications. We anticipate that the number of these vaccines will continue to grow in the future.

IV. CONCLUDING REMARKS

As the examples in this chapter demonstrate, mucosal vaccines are part of routine immunization practices in veterinary medicine and have been for many years. Research is underway to further improve those vaccines, be it through the use of novel adjuvants, better delivery systems, or effective targeting to the site of uptake at mucosal surfaces. However, it is important to note that most of these vaccines are extremely cost-effective, at pennies per dose, and are used as part of mass vaccination in poultry and fish. Thus one would hope that human vaccine manufacturers and regulators recognize the benefits and potential this technology can offer and start to develop mucosal vaccines for humans at a cost-effective price. While some vaccines are already available for mucosal administration in humans, mucosal vaccination has, unfortunately, not yet become part of routine immunization practices in humans.

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