Vaccines Produced by Conventional Means to Control Major Infectious Diseases of Man and Animals

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The publication by Jenner of "An Inquiry Into the Cause and Effects of Variolae Vaccinae" in 1798 is the first documented evidence for the use of vaccination. In the 190-year period since then, a number of vaccines have been developed to control the infectious diseases of man and animals. This chapter reviews the development of some of these vaccines and their use in controlling such major diseases as diphtheria, rinderpest, Newcastle disease, smallpox, pertussis, yellow fever, rabies, etc. Yet, infectious diseases are still a major problem in the world today, accounting for over 15 million human deaths annually in Africa, Asia, and South America alone (Warren, 1985) and untold numbers of animal deaths worldwide. Newer techniques will be needed to make vaccines safer and more effective and, most importantly, more available throughout the world. Other chapters in this book will describe these techniques, but for the present, it is the vaccines described in this chapter that are the key to disease control and prevention programs.

I. Vaccines for DNA Viruses

A. PARVOVIRIDAE

The most important animal parvoviruses are feline panleukopenia virus, mink enteritis virus, and canine parvovirus, all of which are serologically related, and the porcine parvovirus, which is serologically distinct. The parvovirus that infects mink, Aleutian disease virus, causes persistent infection leading to an unusual immune complex disease. Parvoviruses have been isolated from the feces, serum, and joints of man. The B-19 human parvovirus has been shown to cause Firth disease or erythema infectionsum, a rash on the limbs, with upper respiratory tract symptoms and arthralgia. Human parvoviruses have also been shown to be the principal cause of aplastic crisis in sickle cell anemia patients. Other members of the parvovirus genus that are less important are bovine parvovirus, avian parvovirus, goose parvovirus (which causes hepatitis), minute virus of mice, Kilham rat virus, and the adeno-associated viruses.

1. Feline Panleukopenia Virus

The first parvovirus shown to be a filterable agent was feline panleukopenia virus (Verge and Christoforoni, 1928), which was not characterized until much later (Johnson, 1967a,b; Johnson et al., 1967). The virus affects most members of the family Felidae, causing enteritis and bone marrow hypoplasia that results in severe leukopenia. The virus infects kittens *in utero* and postnatally causes cerebellar hypoplasia and ataxia (Kilham and Margolis, 1966).

Immunization. The first panleukopenia vaccines were produced by infecting susceptible cats and harvesting their tissues. Filtrates from these tissue suspensions were treated with chemical inactivants such as formalin. Two inoculations of this type of vaccine produced long-lasting protection (Leasure et al., 1934; Enders and Hammond, 1940). However, subsequent vaccines, produced in primary feline kidney cell culture and inactivated with formalin, proved safer and more effective (Bittle et al., 1970; Davis et al., 1970). An attenuated live-virus vaccine was also very effective in inducing immunity and protection (Slater and Kucera, 1966). Concern for the wide-scale use of an attenuated live-virus vaccine centers on the premise that shedding may spread the virus to other species, possibly allowing the emergence of pathogenic variants in these species. Attempts to immunize cats orally with attenuated live-virus vaccines have not been successful, but intranasal aerosol application has been effective (Scott and Glauberg, 1975; Schultz et al., 1973).

2. Canine Parvovirus

Three members of the parvovirus family are known to infect dogs: minute virus of canines, a defective canine adeno-associated virus, and the pathogenic canine parvovirus. Canine parvovirus is related to feline panleukopenia virus and may have originated from one of the mammalian parvoviruses. In young dogs, the virus causes leukopenia and severe intestinal disease with necrosis of crypt epithelium in the small intestine. Myocarditis occasionally develops, causing sudden heart failure in young dogs. In 1978 an epizootic of this disease occurred in the United States, causing high mortality (Eugester, 1978).

Immunization. Because of the close relationship between feline panleukopenia virus and canine parvovirus, inactivated feline panleukopenia vaccine initially was used to protect susceptible dog populations (Appel *et al.*, 1979a). Later, inactivated and attenuated vaccines produced with the canine virus proved to be much more effective (Pollack and Carmichael, 1983; Appel *et al.*, 1979b). These parvovirus vaccines have been formulated with other vaccines, including canine distemper and canine adenovirus vaccines, and are administered routinely to young dogs.

3. Porcine Parvovirus

Porcine parvovirus has a distant serologic relationship to other parvoviruses, but it causes disease only in swine. The virus, which is widespread, causes abortions, fetal death, and infertility in sows infected early in gestation. Immune sows reinfected during gestation give birth to normal piglets.

Immunization. Numerous reports have shown the effectiveness of inactivated porcine parvovirus vaccines in swine (Suzuki and Fujsaki, 1976; Mengeling *et al.*, 1979; Wratthal *et al.*, 1984; Fujisaki *et al.*, 1978; Joo and Johnson, 1977). For example, Mengeling *et al.* (1979) demonstrated the value of a vaccine in which the virus was inactivated with acetylethyleneimine.

An attenuated live-virus vaccine has been described (Paul and Mengeling, 1980) in which a porcine isolate was attenuated by 120–165 passages in a swine testicular cell line. This vaccine effectively induced antibodies and protected challenged animals. The vaccine virus did not cross the placenta; however, it did kill fetuses when inoculated *in utero*. The virus was shed in feces, and so could be transmitted to unvaccinated animals.

The inactivated vaccines now used in United States have been

effective in controlling porcine parvovirus infection. Vaccination is recommended for gilts and sows before breeding.

B. ADENOVIRIDAE

Adenoviruses cause significant disease in dogs, foxes, and man, but have also been isolated from cattle, swine, goats, sheep, horses, turkeys, and chickens, where they produce mild infections, mainly associated with the respiratory and intestinal tracts.

There are at least 80 different adenoviruses, 43 of which occur in man. Each adenovirus has a narrow host range.

1. Canine Adenovirus

There are two canine adenoviruses. Canine adenoviruses 1 (CAV-1) causes infectious canine hepatitis, which at one time was widespread, but now has been controlled by vaccination. Infected dogs also develop corneal opacities following this infection, as a result of the formation of immune complexes and uveitis within the anterior chamber of the eye (Carmichael *et al.*, 1975). In foxes, CAV-1 produces a rapidly fatal encephalitis.

Canine adenovirus 2 (CAV-2) causes respiratory disease in dogs, but neither hepatitis nor encephalitis in dogs or foxes. The respiratory disease varies depending on the strain of virus and bacterial superinfection. CAV-2 may be transmitted by aerosol, whereas CAV-1 is spread by other direct means such as contact with urine or saliva from infected animals. CAV-1 and CAV-2 are oncogenic in experimentally infected hamsters (Sarma *et al.*, 1967; Dulcac *et al.*, 1970).

Immunization. Dogs that recover from natural CAV-1 infection are immune for a long period. The first CAV-1 vaccines were produced by formalin inactivation of tissue homogenates from infected dogs.

CAV-1 was first adapted to tissue culture by Cabasso *et al.* (1954) and by Fieldsteel and Emery (1954). The latter modified the virus by serial passage in porcine and canine tissue cultures; the resulting vaccine immunized dogs and did not produce clinical signs of infection except for occasional corneal opacity similar to that caused by natural infection. Dogs immunized with CAV-1 vaccines are also protected against CAV-2 (Appel *et al.*, 1973). The immunity produced by the attenuated live-virus CAV-1 vaccines is long lasting and has drastically reduced the incidence of the canine disease.

Although CAV-2 is closely related to CAV-1, when it is inoculated parenterally into dogs, it does not cause disease, although the virus is shed from the respiratory tract (Appel *et al.*, 1973). Such dogs become

immune to both CAV-1 and CAV-2. An attenuated live-virus vaccine containing CAV-2 is now being widely used in place of older CAV-1 vaccines (Bass *et al.*, 1980); this has resulted in a much lower incidence of corneal opacity in recipients. However, because of the oncogenicity of adenoviruses in other hosts, the respiratory shedding of CAV-2 virus should be a concern.

2. *Human Adenovirus*

In man, adenoviruses mainly produce disease of the respiratory tract which varies in severity depending on the virus and the age of infected individuals. The viruses cause acute pharyngitis in infants and children, pharyngitis and conjunctivitis in children, and acute respiratory disease (ARD) in military recruits and institutionalized young adults. Pneumonia may also occur, expecially following ARD.

Immunization. A vaccine consisting of adenovirus types 3, 4, and 7, grown in monkey kidney cell culture and inactivated with formalin, was introduced in 1958 for use in U.S. military recruits. The vaccine was effective in reducing ARD in this population (Sherwood *et al.*, 1961). However, the vaccine was withdrawn from use in 1963 because of concern for possible oncogenicity of the adenoviruses, and of the SV_{40} virus present in the monkey kidney cell culture.

A subsequent adenovirus, type 4, was passed in human tissue and was, therefore, devoid of SV_{40} genomic material. This virus, encapsulated in enteric-coated capsules, proved to be a safe and effective vaccine (Chanock *et al.*, 1966; Edmonson *et al.*, 1966). An adenovirus 7 vaccine, prepared in the same way and given simultaneously with adenovirus 4 vaccine, was equally effective (Top *et al.*, 1971). The administration of a vaccine with only one of these viruses was not effective in controlling ARD caused by any of several different adenoviruses, but formulation of a vaccine with both viruses proved to be broadly cross-protective and have had a major influence in controlling ARD in U.S. military recruits.

C. HERPESVIRIDAE

Over 70 herpesviruses produce disease in man and animals. These viruses have an affinity for epithelial tissues and nervous system tissues. These tropisms lead to specific disease syndromes involving the respiratory and urogenital tracts, and the central and peripheral nervous systems. The viruses often cause persistent infections, which can be latent and can be reactivated. Herpesvirus disease are generally much more severe in young humans and animals. Some herpes-

viruses, including Epstein–Barr virus (EBV) in humans and Marek's disease virus in fowl, are associated with malignancies.

The presence of specific antibodies may prevent or modify the clinical disease but does not prevent infection. Vaccines have been developed for those herpesviruses causing major diseases in animals; however, despite the seriousness of human herpesvirus diseases, including those caused by herpes simplex virus, EBV virus, cyto-megalovirus, and varicella virus, progress has been slow in developing vaccines for humans. This stems from concern over possible persistence and oncogenicity of vaccine viruses. In the past few years, several attenuated live-virus varicella vaccines have been tested and found safe and efficacious (Takahashi *et al.*, 1974; Asano *et al.*, 1977; Arbeter *et al.*, 1983).

1. Infectious Bovine Rhinotracheitis Virus

Since the initial recognition of infectious bovine rhinotracheitis (IBR) in the early 1950s and the later recognition of another manifestation of the disease, infectious pustular vulvovaginitis, this bovine herpesvirus has been acknowledged as a major problem in livestock. The respiratory disease varies from mild to severe, and herd mortality can be as high as 10% in an acute outbreak. The virus may cause abortions in pregnant cattle, and the genital disease results in a chronic vulvovaginitis but not abortions, apparently due to a lack of viremia.

Immunization. Attenuated live-virus vaccines were developed by serial passage of field isolates in bovine kidney cell cultures. Such vaccine viruses have reduced virulence when administered intranasally and do not produce disease when administered parenterally (Schwarz *et al.*, 1957; York *et al.*, 1957). Vaccine virus does not spread from vaccinated to unvaccinated contact animals. The widescale use of such vaccines has controlled this disease effectively. When administered by the intranasal route (Todd *et al.*, 1971), these vaccines had the advantage of producing more rapid protection, but long-term protection was no greater than with perenterally administered vaccines (McKercher and Crenshaw, 1971). Because of the possibility that the attenuated live-virus vaccines given parenterally might cause abortion, their use has been restricted to nonpregnant animals. A formalin-inactivated vaccine has also been developed, but requires multiple inoculations, and the serum neutralizing (SN) antibody response is low (Matsuaba *et al.*, 1972). However, inactivated vaccines are used especially in dairy cattle because of concern that the attenuated live-virus vaccines may cause abortion.

2. Equine Rhinopneumonitis Virus

Four herpesviruses affect horses: equine rhinopneumonitis virus (EHV-1) causes abortion which may be epizootic, and also, occasionally, rhinopneumonitis; EHV-2 is cytomegalovirus found in buffy coat cells of most horses, but its role in causing disease is unknown; EHV-3 causes equine coital exanthema, a urogenital tract disease; and EHV-4 is the main cause of equine rhinopneumonitis. EHV-1 and -4 are related antigenically, whereas types 2 and 3 are distinct (Sabine *et al.,* 1981; Studdert and Blackney, 1979).

Immunization. The first vaccine for equine rhinopneumonitis was developed by Doll and Bryans (1963), who adapted an EHV-1 isolate to suckling hamsters. This vaccine produced a mild disease, but induced protective immunity against the more serious respiratory disease and abortion that occurs in older animals. However, this vaccine sometimes caused abortions, and the virus was known to spread from vaccinated to unvaccinated horses.

A more attenuated strain of EHV-1 has been used widely and is considered to be reasonably effective (Mayr, 1970). This strain was attenuated by passage in hamsters and in pig kidney cell culture before adaptation to an equine cell line (Gerber *et al.,* 1977). This vaccine induces low levels of SN antibodies, and protects against respiratory disease but not against abortion.

A formalin-inactivated vaccine, emulsified in an oil adjuvant, has also been found safe and effective in preventing respiratory disease. This vaccine, in controlled field trials, lowered the abortion rate significantly (Bryans, 1980; Bryans and Allen, 1981). The attenuated live-virus and the inactivated vaccines contain only EHV-1, but apparently there is enough cross-protection induced by repeated vaccinations to protect against the EHV-4 respiratory disease.

3. Pseudorabies Virus

This herpesvirus is unusual in that it occurs naturally in many species—cattle, sheep, goats, swine, dogs, cats, rats, and mice. It produces a fatal disease in all of these species except adult swine, in which the disease is mild. In each species except swine the primary sign is intense puritis resulting in the animal biting the affected area. Infection rapidly spreads to the central nervous system, leading to paralysis and death. In adult swine, the signs of infection are mild, usually of a respiratory nature, but abortions follow in approximately 50% of pregnant sows. In young pigs, especially neonates, the infection may be fatal.

Immunization. Since the disease is prevalent only in swine, this is the only animal for which a vaccine has been developed. In examining a virulent strain of pseudorabies virus, Bartha and Kojnok (1963) found two plaque sizes. The small plaque size variant, called K, occurred naturally and had reduced virulence for rabbits. Further studies with this strain passaged in chick embryo or calf testicle cell culture produced a safe vaccine for swine. One dose induced partial immunity and a second dose yielded good immune responses in all recipients.

McFerran and Dow (1975) adapted the K strain to Vero cells and showed that one dose of a vaccine prepared from this passage material was protective. Pigs vaccinated with this vaccine did not shed the virus. Although the vaccine did not prevent infection, it prevented clinical disease. This attenuated live-virus vaccine is used widely.

4. Feline Rhinotracheitis Virus

Feline rhinotracheititis virus, which was first isolated in 1957, produces a widespread respiratory disease in cats (Crandell and Maurer, 1958). The virus may also cause fetal death.

Immunization. An attenuated live-virus vaccine was developed by passage of a field isolate in feline kidney cells (Bittle and Rubic, 1975). The vaccine is given parenterally and is safe and effective (Scott, 1977). Low levels of SN antibodies persist for at least 6 months. Vaccinated cats are resistant to intranasal instillation of virulent virus; they may be reinfected, but do not develop clinical disease (Bittle and Rubic, 1976; Kahn *et al.*, 1975). The vaccine has controlled this important respiratory pathogen and, when combined with a feline calicivirus vaccine, has drastically reduced the incidence of respiratory disease in this species.

5. Avian Infectious Laryngotracheitis Virus (LTV)

This herpesvirus is highly contagious and causes lesions in the larynx, trachea, and bronchi of infected fowl. The infection causes the formation of an exudate that produces the characteristic respiratory distress and rattling in severely affected birds. Birds that recover from this disease are immune for a long period, but may remain as carriers and a source of virus for reinfection of flocks.

Immunization. The earliest method of immunization was developed by Beaudette and Hudson (1933), who applied virulent virus from tracheal exudate to the mucosa of the bursa of Fabricius and the cloaca with a stiff brush. This produced a local infection and a solid systemic immunity. The use of fully virulent virus caused occasional outbreaks

of disease, particularly when the scarification was not properly done or insufficient virus was present, and birds did not become immune. The virus was propagated on the chorioallantoic membrane of embryonated eggs by Burnet (1934), and this became a source of vaccine material. Other methods of vaccination involved intranasal instillation (Benton *et al.*, 1958) and feeding in drinking water (Zamberg *et al.*, 1971). Attenuated strains of LTV have been developed by serial passage in cell culture (Gelenczei and Marty, 1964) and by feather follicle passage (Molgard and Cavett, 1947). Attenuated strains isolated from outbreaks or selected from passage are now used in preference to virulent virus.

6. Marek's Disease Virus

Marek's disease virus causes lymphoproliferative disease in chickens, occurring in three forms: neural, ocular, and visceral (the latter mainly in young birds) (Sevoian and Chamberlain, 1962; Biggs and Payne, 1963). Sevoian was the first to provide evidence that Marek's tumors were caused by a virus and were transmissible. The virus has been established as a gamma herpesvirus (Churchill and Biggs, 1967; Nazerian and Burmeister, 1968; Soloman *et. al.*, 1968).

Immunization. Fatalities from Marek's disease caused major economic losses in the poultry industry until a vaccine was developed for its control. This was accomplished by Churchill *et al.* (1969), who attenuated by serial passage a virus isolated from chickens that is parenterally administered at 1 day of age. Thereafter, Okazaki *et al.* (1970) selected a herpesvirus from turkeys (HVT) that was relatively avirulent in chickens, and Zander *et al.* (1972) and Schat and Calnek (1978), selected a natural avirulent strain from chickens.

These three vaccines are effective, but the HVT strain has been more widely used because it can be obtained from infected cells and can be lyophilized (Calnek *et al.*, 1970). The vaccines are given parenterally to chicks at hatching and produce good protection (80–100%) against virulent virus challenge (Purchase *et al.*, 1972).

D. POXVIRIDAE

Viruses of the family Poxviridae infect most domestic animals and man. From the standpoint of immunoprophylaxis, the most important poxviruses are: *Orthopoxvirus*, smallpox (variola), mousepox (ectromelia); *Avipoxvirus*, fowlpox, pigeonpox; *Capripoxvirus*, sheeppox, goatpox; *Leporipoxvirus*, myxomatosis virus; *Parapoxvirus*, contagious pustular dermatitis virus. All these poxviruses cause serious disease in

their primary host species and some may infect other species. Each of the poxviruses causes characteristic vesicular lesions on the skin and mucous membranes, with the exception of myxomatosis virus which produces hyperplastic lesions in the form of myxomas and fibromas.

Ectromelia (mousepox) is caused by a virus closely related to vaccinia virus and produces a serious disease of laboratory mice. Vaccination with vaccinia apparently will reduce the morbidity and mortality of mousepox in a colony, but it will not prevent infection and may act to maintain a silent reservoir of virus (Buller and Wallace, 1985).

Sheeppox is one of the most serious pox diseases, occurring in Europe, the Middle East, and Africa, but it is controlled by vaccination (Aygun, 1955; Sabban, 1955). Goatpox occurs mainly in the Middle East and Africa; a goatpox vaccine has been reported to also immunize against sheeppox (Rafyi and Ramyar, 1959).

Contageous pustular dermatitis virus is unrelated to sheeppox, but causes a pox-like disease in young lambs in which vesicles form around the skin of lips, nostrils, and eyes. Boughton and Hardy (1935) showed that animals could be protected by scarification with dried contageous pustullar dermatitis virus material similar to that use with vaccinia.

Myxomatosis virus causes a fatal disease of domestic rabbits and may be spread by direct contact or by blood meals of insects such as mosquitos and fleas (Myers *et. al.,* 1954). McKercher and Saito (1964) developed a safe and efficacious attenuated live-virus vaccine by passage of the virus in rabbit kidney cell culture.

1. Smallpox (Variola) Virus

This virus, once the cause of epidemics that decimated entire cities, has now been eradicated. The control was brought about by world-wide vaccination and isolation of infected persons. Another factor in the control of smallpox was that variola virus had no other host except man.

Immunization. Material from lesions of smallpox-infected individuals had been used for centuries to infect susceptible persons so they would develop a mild form of the disease and become resistant. This variolation was dangerous but much safer than natural exposure to the smallpox virus. Jenner (1798) practiced an improved form of this method by using cowpox virus (vaccinia) to inoculate susceptible persons, as described in Chapter 6.

Most vaccinia vaccines were produced by scarifying the skin of a calf with infected material and harvesting the lymph from the crusted lesions as aseptically as possible. This was stored in 40% glycerol to

stabilize the virus and preservatives were added to destroy bacteria. Sheep and rabbits were also used similarly for vaccine production. Vaccinia virus was also adapted to grow in embryonated eggs (Goodpasture *et al.*, 1935). Vaccinia virus is very stable, can be produced at a low cost, and is simple to administer. These factors played a major role in allowing the wide-scale use of vaccinia for the eradication of smallpox.

2. *Fowlpox Virus*

Fowlpox virus occurs mainly in chickens and produces pox lesions on the wattles, comb, mouth, nostrils, and eyes. The disease is spread mainly by direct contact with infected birds and blood-sucking parasites such as mosquitoes. Although it is a fairly resistant virus, it is not otherwise very transmissible.

Immunization.   Fowlpox vaccine was originally made by scarifying cockrel combs with virulent virus and harvesting the exudate. Johnson (1929) demonstrated that dried exudate would produce immunity when scarified in the wing web or applied to the thigh skin free of feathers. Fowlpox virus was later cultivated on the chorio allontoic membrane by Goodpasture *et al.* (1931) and used as a source of vaccine material. Later the virus was adapted to tissue culture. An attenuated live-virus fowlpox vaccine produced in cell culture may be used in 1-day-old chicks (Siccardi, 1975). Another vaccine, an attenuated strain (HP-1) developed by Mayr *et al.* (1976), is given orally to 5-day-old chicks, then repeated after 3 to 4 months.

E. HEPADNAVIRIDAE

Hepadnaviridae is a new-found family of viruses containing hepatitis B virus of man as well as three similar but distinct viruses that infect woodchucks, beechey ground squirrels, and Pekin ducks. These viruses have many of the same ultrastructural, molecular, and biological features and their surface antigens cross-react to a small, variable degree. The host range appears to be specific for each virus. Hepatitis B infects man and certain higher primates, including the chimpanzee and gibbon. Infection with these hepadnaviruses results in subacute hepatitis, which often becomes progressive and chronic.

Hepatitis B Virus

The most important of these viruses is hepatitis B virus, which produces a chronic disease in man (Blumberg *et al.*, 1967; Prince, 1968). Hepatitis B virus is transmitted by blood, saliva, and semen, but

also from mother to offspring, the latter route accounting for as much as one-third of persistent infections. The disease is usually self-limiting, but in 5–10% of patients the infection becomes chronic, with the virus persisting in a carrier state. There are over 200 million chronic carriers of this virus worldwide. A late sequela in chronic carriers is hepatocellular carcinoma. It is estimated that 40–60% of malignancies in Africa are the result of hepatitis B-induced onco-genesis.

Immunization. The development of a vaccine for hepatitis B was hampered by the difficulty of growing the virus in cell culture. Krugman *et al.* (1970) was the first to show that hepatitis B virus-infected serum could be heat-inactivated and retain its antigenicity. They also showed that this inactivated serum given parenterally could protect subjects exposed to virulent hepatitis B virus. This led to the use of plasma from infected but healthy virus carriers as the source of antigen. Carriers produce large quantities of the hepatitis B virus, along with its outer coat protein. By purifying and inactivating the coat protein, a safe and effective vaccine was developed (Hilleman *et al.*, 1978). The coat protein, naturally formed into 22-nm particles, was purified by ammonium sulfate concentration, isopycnic banding, rate zonal separation and enzymatic digestion. The purified protein parti-cles were then inactivated with 1:4000 formalin. The particles induce good levels of protective antibody when given in a series of three injections (Symuness *et al.*, 1980). However, the high cost of this vaccine has limited its use. Newer vaccines produced by recombinant DNA methods are now being used, as described in other chapters.

II. Vaccines for RNA Viruses

A. Picornaviridae
  1. Foot-and-Mouth Disease (FMD) Virus
  2. Poliomyelitis Virus
  3. Avian Encephalomyelitis (AE) Virus

B. Caliciviridae
  Feline Calcivirus

C. Reoviridae
  1. Reoviruses
    Avian Reovirus
  2. Rotaviruses
    a. Bovine Rotavirus
    b. Porcine Rotavirus

  3. Orbiviruses
    a. Bluetongue Virus
    b. African Horse Sickness Virus

D. Birnaviridae
  Infectious Bursal Disease (IBD) Virus

E. Togaviridae
  1. *Alphavirus*
  2. *Pestivirus*
    a. Hog Cholera (HC) Virus
    b. Bovine Virus Diarrhea (BVD) Virus
  3. *Rubivirus*
    Rubella Virus
  4. *Arterivirus*
    Equine Arteritis Virus

F. Flaviviridae (Yellow Fever Virus)

G. Orthomyxoviridae
   1. Human Influenza Virus
   2. Equine Influenza Virus

H. Paramyxoviridae
   1. Parainfluenza Virus
      a. Sendai Virus
      b. Canine Parainfluenza Virus
      c. Bovine Parainfluenza Virus
   2. Mumps Virus
   3. Newcastle Disease Virus
   4. Measles Virus
   5. Canine Distemper Virus

   6. Rinderpest Virus
   7. Bovine Respiratory Syncytial Virus
      (RSV)

I. Rhabdoviridae
   Rabies Virus

J. Retroviridae
   Feline Leukemia Virus (FeLV)

K. Coronaviridae
   1. Avian Infectious Bronchitis Virus
      (IBV)
   2. Transmissible Gastroenteritis
      Virus (TGE)

A. PICORNAVIRIDAE

The four genera in the family Picornaviridae are: *Aphthovirus, Rhinovirus, Enterovirus,* and *Cardiovirus.* Viruses in the first three genera cause important diseases in domestic animals and man, whereas viruses in the fourth infect rodents. The picornaviruses in general have a primary affinity for superficial tissues especially of the digestive and respiratory tracts. Viruses in the first three genera also have an ability to mutate, thus yielding many serotypes.

Rhinoviruses cause clinical disease in man, horses, and cattle. No vaccines have been developed for the infections of humans because of the multiplicity of viral serotypes. The number of serotypes in horses (three) and cattle (two) is fewer; nevertheless, no vaccines are available.

Over 100 enteroviruses exist; of these, vaccines have been developed only for poliomyelitis viruses, avian encephalomyelitis, and duck hepatitis viruses. Other viruses in this group either are of low pathogenicity or the number of serotypes is so large as to preclude the development of vaccines. The exception is human hepatitis A virus, which causes a serious disease and has one serotype; the development of both inactivated virus and attenuated live-virus vaccines is in progress (Hilleman *et al.,* 1982; Provost *et al.,* 1983).

1. Foot-and-Mouth Disease (FMD) Virus

FMD was the first animal disease shown to be caused by a virus (Loeffler and Frosch, 1898). FMD viruses cause one of the most economically important diseases of animals and its control is critical to the world's supply of animal protein. The viruses are widespread and occur in many cattle producing regions of the world. The viruses also

affect other cloven-footed animals including sheep, swine, and goats. FMD virus infection produces vesicles on oral mucous membranes, including the tongue, gums, and dental pads, but also on the skin including the interdigital spaces and teats. These vesicles on the mucous membranes coalesce and erupt, leaving large denuded areas. The mucous membrane and skin lesions can incapacitate an animal for weeks, thus severely disrupting its productivity. The viruses are highly contagious and persist for long periods in infected animals. Animals that recover from natural infection are immune for approximately one year. Vallee and Carre (1922) showed that there was more than one FMD virus; seven serotypes with over 60 subtypes have now been identified, making the development of effective vaccines difficult.

Immunization. The first FMD vaccine for cattle was reported in 1925 and consisted of a formalinized emulsion of vesicular epithelium (Vallee *et al.,* 1925). A similar but improved version called the Schmidt–Waldmann vaccine followed and contained vesicle material from the tongue epithelium of infected cattle. This material was treated with formalin and used with aluminum hydroxide (Schmidt, 1936; Waldmann and Klobe, 1938). Another advance was described by Frenkel (1947), who infected superficial layers of bovine tongue epithelium in culture and inactivated the newly replicated virus with formalin to produce a more uniform product. Although this method is used today in some areas of the world, most FMD vaccines are now produced by growing the virus in baby hampster kidney cells (Mac-Pherson and Stoker, 1952; Mowat and Chapman, 1962; Capstick *et al.,* 1962).

The imines replaced formalin as an inactivant in most FMD vaccines after Brown *et al.* (1963) showed that viral inactivation was more complete, and safer vaccines could be produced by this process. All inactivated FMD vaccines contain more than one serotype, including the serotypes most common in the area in which the vaccines are to be used. Although inactivated vaccines that are produced and used properly can effectively lead to the control FMD, their stability could be improved, thereby lowering their cost. This is discussed further in the chapter by Brown.

Attenuated live-virus FMD vaccines have been developed (Henderson, 1978) but are not used because of the fear that the virus might persist in animals and in meat and milk products from animals (Hyslop, 1966-1967).

2. Poliomyelitis Virus

There are three polio viruses and minor variants of each. The viruses infect man by entry into the upper alimentary tract, infecting cells,

and spreading via the blood to the central nervous system, producing neuronal destruction in the medulla and spinal cord. The degree of paralysis that follows infection depends on such factors as virus strain and virus tropism. The vast majority of persons infected with wild polioviruses show no apparent clinical disease. Paralysis occurs only in an estimated 1% of infected individuals; polio 1 virus is responsible for at least 85% of cases.

Immunization. Early attempts to develop inactivated poliovirus vaccines were hampered by not knowing that there are three distinct viruses. The differentiation of the three viruses by Bodian (1949) and Kessel and Pait (1949) was a major step toward controlling the disease. Enders *et al.* (1949) found that poliovirus would grow in extraneural tissues of human origin and thus laid the groundwork for the development of poliovirus vaccines. The first vaccine (Salk) contained all three polio viruses grown in monkey kidney cell culture and inactivated with formalin (Salk *et al.,* 1954). This vaccine, introduced in 1953, reduced the incidence of paralytic poliomyelitis 80–90% where it was used; however, multiple doses were required and intestinal tract infection was not prevented, thus allowing the virus to continue to spread.

There was an intense effort in the 1950s to develop an attenuated live-virus vaccine that could be administered orally, and could protect the intestinal tract, thus breaking the chain of transmission. Koprowski, Sabin, and Cox each developed vaccine strains of reduced neurovirulence that underwent extensive laboratory and field studies (Koprowski *et al.,* 1956; Sabin, 1955; Cox *et al.,* 1959). The strains developed by Sabin were finally licensed in the United States; they produced rapid immunity as well as protection of the intestinal tract while preventing spread to unvaccinated, susceptible persons in contact with vaccinees. This improved the overall level of immunity in communities. With the widescale use of oral poliomyelitis vaccine, the incidence of paralytic disease in the United States has dropped to less than 10 cases per year. The occasional reaction to the attenuated vaccine is discussed in the chapter by Hogle and Filman.

3. Avian Encephalomyelitis (AE) Virus

Avian encephalomyelitis was first discribed and shown to have a viral etiology by Jones (1932, 1934). The virus is widespread and affects young chickens (1–3 weeks old). Characteristic clinical signs are ataxia and tremors of the head and neck. Extensive neuronal degeneration occurs in the anterior horn of the cord and in the medulla and pons. The virus may affect older laying birds, causing a drop in egg production.

Immunization. Flocks that have survived an outbreak or subclinical infection during the growing period are resistant to further infection (Schaaf and Lamoreux, 1955). Moreover, infected chickens 16–20 weeks of age undergo only mild disease, providing an opportunity for vaccination (Schaff, 1958).

Calnek and Taylor (1960) successfully immunized immature birds with an attentuated live-virus vaccine delivered in drinking water. A number of vaccines have been developed including the 1143 strain (Calnek, 1961), the NSW-1 strain (Westbury and Senkovic, 1978), the Philips Duphar strain (Folkers *et al.*, 1976), and a strain grown in chicken pancreas cell culture (Miyamae, 1978). Inactivated AE virus vaccines have been developed for use in susceptible breeding flocks that are in production (Schaaf, 1959; Calnek and Taylor, 1960; Butterfield *et al.*, 1961; MacLeod, 1965).

B. CALICIVIRIDAE

Two viruses in the family Caliciviridae cause significant disease in animals, vesicular exanthema virus in swine and sea lions and feline calicivirus. Caliciviruses have also been isolated from humans, calves, reptiles, nonhuman primates, birds, dogs, and fish, but do not produce significant disease in these animals.

Vesicular exanthema virus caused a disease in swine closely resembling FMD (Traum, 1933). Eradication of this disease followed the discovery that the main source of contagion was uncooked infected meat in garbage fed to swine, and the consequent enforcement of garbage cooking laws.

Feline Calicivirus

Fastier (1957) first isolated a calicivirus from a domestic cat and showed that it produced an upper respiratory tract infection. A large number of viral isolates, with different neutralization patterns, were made from clinically ill cats (Crandell *et al.*, 1960; Bittle *et al.*, 1960). These serotypes were later shown to have a common antigen and are now considered a single serotype (Povey and Ingersoll, 1975). This virus is widespread, having been isolated from cats in many countries. The virus produces a disease that is generally mild, but, if allowed to progress, the lesions may extend from the upper respiratory tract into the lung causing pneumonia and death (Kahn and Gillespie, 1971).

Immunization. Cats that recover from natural infection and have neutralizing antibodies can be reinfected, but they do not have recurrent clinical disease. An attenuated live-virus calicivirus vaccine has been prepared by serial passage at low temperature; this

vaccine virus produces only mild clinical signs in recipients (Bittle and Rubic, 1976). This attenuated live-virus vaccine is administered parenterally; it induces high levels of neutralizing antibody and protects vaccinated cats challenged intranasally with both homologous and heterologous strains (Kahn *et al.*, 1975; Scott, 1977). Immunity from vaccination persists for at least 6 months and probably longer. The calicivirus is combined with feline rhinotracheitis and feline panleukopenia to make a multivalent vaccine that is routinely used in domestic cats (Bittle and Rubic, 1975). Inactivated vaccines have also been licensed and are used in multivalent vaccines.

C. REOVIRIDAE

The family Reoviridae is divided into three genera: *Reovirus, Rotavirus,* and *Orbivirus.* Infections cause by member viruses are common in mammals and birds.

1. Reoviruses

Reoviruses are commonly isolated from dogs, cats, sheep, cattle, horses, mice, rats, rabbits, birds, and man. Only in birds is the disease serious enough to warrant control with vaccines. The reoviruses are commonly found in sewage, and the mode of transmission is thought to be the fecal–oral route.

Avian Reovirus. In chickens and turkeys, reoviruses produce a widespread disease called viral arthritis (tenosynovitis). This disease of the synovial membranes, tendon sheaths, and myocardium was first recognized by Dalton (1967) and by Olsen and Solomon (1968). Viral arthritis occurs primarily in meat-producing birds, and in acutely affected flocks there is a high rate of condemnation. There are at least five avian reovirus serotypes, but they are antigenically unrelated to the mammalian reoviruses.

Immunization. Vaccination of breeding stock is an effective way to control viral arthritis. Van der Heide *et al.* (1976) used an attenuated live-virus vaccine and Cessi and Lombardini (1975) used an inactivated vaccine in laying hens to protect chicks with maternal antibody. This eliminated transmission and protected susceptible day-old chicks.

2. Rotaviruses

Rotaviruses produce acute gastroenteritis in many species, especially in newborns, including newborn calves, foals, lambs, piglets, puppies, monkeys, and humans. The clinical signs are similar in all species; in each there is acute diarrhea followed by dehydration and

rapid loss of weight. Secondary bacterial infection may exaggerate the symptoms and also cause pneumonia. The viruses infect epithelial cells of villi, causing desquamation and loss of absorptive function, resulting in diarrhea, rapid dehydration, and emaciation. Secretory antibody is very important in protecting the epithelial surface of the small intestine (Snodgrass and Wells, 1976); antibody contained in colostrum is protective when in high titer.

   a. Bovine Rotavirus.   Bovine rotovirus causes a rapidly spreading disease in neonatal calves (Mebus, 1969). The antigenic relationship of the bovine rotavirus and other rotaviruses isolated from children, calves, piglets, mice, and foals is very close (Woode *et al.,* 1976).

   Immunization.   An attenuated live-virus vaccine developed by Mebus *et al.* (1976) is administered in two doses to cows prior to calving. This is meant to stimulate colostral antibody, which is passed on to the nursing calves. This vaccine has also been combined with an attenuated live-virus coronavirus vaccine and entero-toxigenic *E. coli* vaccine to prevent calf scours.

   b. Porcine Rotavirus.   Leece *et al.* (1976) isolated a rotavirus from piglets with fatal diarrhea. Additional reports of this disease showed that it was widespread and warranted the development of a vaccine for its control.

   Immunization.   Early attempts to immunize pigs by oral administration of a bovine attenuated live-virus rotavirus vaccine were unsuccessful (Leece and King, 1979). Presently an attenuated porcine rotavirus vaccine containing two serotypes, A^1 and A^2, is licensed in the United States and is being used in combination with a transmissible gastroenteritis (TGE) vaccine. The vaccines are administered to pregnant sows by both parenteral and oral routes. At least two doses of vaccine are given orally, 5 and 3 weeks before farrowing, and one dose is given parenterally 1 week before farrowing. This induces antiviral colostral antibody for the protection of suckling piglets.

3. Orbiviruses

   The member viruses of this genus replicate in arthropods as well as in vertebrates. The most important viruses are the bluetongue viruses, and African horse sickness viruses. Colorado tick fever virus, the only virus in this genus that infects man, is transmitted by the bite of infected ticks. The disease is usually benign, and infection produces long-lasting immunity.

   a. Bluetongue Virus.   Bluetongue viruses infect ruminants and are transmitted by *Culicoides* gnats. The most serious disease is in sheep, which develop fever, depression, oral lesions, pneumonia, and lame-

ness. Mortality can be high, especially in lambs. Ewes infected early in gestation may produce lambs with hydrocephalus and other congenital deformities. Although cattle rarely have clinical bluetongue disease, *in utero* transmission can occur, resulting in congenital deformities.

Of the 24 distinct bluetongue viruses, 5 occur in the United States. Infection with one bluetongue virus confers resistance to reinfection with that same virus for several months, but cross-protection against infection with other viruses is minimal.

Immunization. A multivalent attenuated live-virus vaccine developed in South Africa by serial passage of several different viruses in sheep proved difficult to standardize. A more uniform vaccine was later developed by Alexander *et al.* (1947); it contained strains of at least five viruses, attenuated by passage in chick embryos, and gave broad protection against the multiple viruses seen in the field. A similar vaccine was developed by McKercher *et al.* (1953), who isolated bluetongue virus in the United States and also attenuated a strain of serotype 10 by serial passage in chick embryos (McKercher *et al.,* 1957). Recently McConnell and Livingston (1982) have been attempting to incorporate more bluetongue virus strains into multivalent attenuated live-virus vaccines.

Inactivated vaccines for bluetongue would have the advantage of greater safety than attenuated live-virus vaccines. They would eliminate the possible chance of reversion to virulence, and the chance of vaccine-associated abortion and birth defects. Such vaccines are under development (Stott *et al.,* 1979).

b. African Horse Sickness Virus. African horse sickness virus causes an acute disease in equine animals in Africa, the Middle East, and Asia. It was shown to be caused by a virus (McFadyean, 1900) and has been more thoroughly characterized by Breeze *et al.* (1969). The viruses are transmitted to horses by *Culicoides* species and affect principally the vasculature of the respiratory tract causing edema of the lungs, head, and neck. The viruses also cause cardiac lesions.

Immunization. Some animals that recover from natural infection may be reinfected, so immunity is not permanent. A spleen pulp vaccine inactivated with formalin was made by DuToit *et al.* (1933) and administered in multiple doses. Later, an attenuated live-virus vaccine was developed by serial intracerebral passage of a field isolate in mice (Alexander and DuToit, 1934). However, because there are nine African horse sickness viruses, it has been necessary to adapt each to mice and to combine them in a polyvalent vaccine. Such vaccine has been effective in protecting horses.

D. BIRNAVIRIDAE

Infectious Bursal Disease (IBD) Virus

The virus that causes IBD was first isolated by Winterfield and Hitchner (1962) using embroyonated eggs. It causes a disease of chickens in commercial poultry-producing areas. The virus affects mainly young birds 3–6 weeks of age, with clinical signs of diarrhea and dehydration. The lesions arise in lymphoid tissues such as the bursa of Fabricius, thymus, and spleen, producing immunosupression with the associated opportunistic infections.

Immunization. Both attenuated live-virus and inactivated vaccines have been developed to control IBD. The vaccines are used mainly for immunizing breeder flocks to confer passive immunity through the yolk sac of the egg. Maternal antibody protects chicks for 1–3 weeks. If breeder flocks are boosted with oil–adjuvant-inactivated vaccines, maternal antibody may last longer. There are several types of attenuated live-virus vaccines with varying degrees of virulence. These vaccines are administered in water, etc., to chicks from 1 day to 2–3 weeks of age in broiler-breeder flocks, followed by vaccination with an inactivated product at approximately 16–18 weeks of age (Lukert and Hitchner, 1984).

E. TOGAVIRIDAE

The four genera in the family Togaviraidae are: *Alphavirus, Pestivirus, Rubivirus,* and *Arterivirus.* Each of these genera contain important pathogens.

1. Alphavirus

The *Alphavirus* genera include:

1. Western equine encephalomyelitis (WEE) virus
2. Eastern equine encephalomyelitis (EEE)
3. Venezuelan equine encephalomyelitis (VEE) virus

All cause encephalitis in horses and humans. In horses, the mortality rate of EEE in over 80%, and that from WEE is about 20–30%. The main mode of transmission is by culicine mosquitoes; however, VEE has been transmitted from horse to horse by contact with body fluids.

Immunization. Infections with togaviruses produce viremia, long-term humoral antibody responses, and immunity. Early vaccines were made from formalin-inactivated infected animal brain tissue. The

cultivation of both WEE and EEE in the chick embryo by Higbee and Howitt (1935) made possible the development of successful inactivated vaccines (Beard *et al.*, 1938). More recent vaccines for WEE and EEE are produced in tissue culture. An attenuated live-virus VEE vaccine, first developed for humans, is also used for horses in endemic areas (Berge *et al.*, 1961; McKinney *et al.*, 1963). The vaccine virus is grown in primary chick embryo cell cultures; it induces long-lasting immunity. An inactivated VEE vaccine has also been developed and is combined with WEE and EEE vaccines in a trivalent formulation.

2. Pestivirus

a. Hog Cholera (HC) Virus.   Hog cholera virus and bovine virus diarrhea (BVD) virus antigenically are closely related pestiviruses, but are specific in the diseases they cause in swine and cattle, respectively.

HC virus produces an acute febrile disease marked by multiple hemorrhages, necrosis, and infarcts in internal organs. Lethargy, vomiting, and encephalomyelitis are seen in a high percentage of infected animals during an outbreak and mortality is high.

Immunization.   Passive protection with convalescent swine serum from swine has been used effectively for short-term control of outbreaks for many years (Dorset *et al.*, 1908). Antiserum and either virulent or partially attenuated virus strains were also used to establish active immunity. Although there is only one antigenic type of HC virus, variant biotypes have arisen that are more difficult to protect against with standard vaccines. The presence of neutralizing antibody correlated well with protection.

An inactivated virus vaccine prepared from defibrinated swine blood taken during the acute phase of the disease and treated with crystal violet or phenol was safe, but required multiple doses (McBryde and Cole, 1936).

Attenuation of HC virus was first accomplished by passage in rabbits (Baker, 1946; Koprowski *et al.*, 1946). Tissue culture attenuated live-virus vaccines eventually replaced the rabbit vaccine; the latter produced a rapid and long-lasting immunity.

A large number of different attenuated live-virus HC vaccines with different characteristics have been used over the years, but residual pathogenicity, shedding, and spread of vaccine viruses have remained problems. A vaccine containing BVD virus was tested in swine, based on evidence that this virus could block replication of HC virus (Sheffy *et al.*, 1961). However, the BVD vaccine did not protect against all strains of HC virus (Tamoglia *et al.*, 1966).

Formerly, control of HC was difficult because HC virus persists in infected meat scraps fed to swine in uncooked garbage. However, since 1969 no vaccines have been used in the United States. By controlling the transport of swine and cooking all garbage used as feed, the disease has been eradicated from the United States, and several European countries.

b. *Bovine Virus Diarrhea (BVD) Virus.* BVD virus causes a widespread disease of cattle, especially in young stock. Clinical signs, which vary in severity, include scouring, ulcerations of the oral cavity, and abortion. Young animals that recover often remain stunted and unproductive for long periods. BVD viral strains differ in their cytopathic effects in tissue culture; cattle infected with noncytopathogenic strains during the first 3 months of gestation can transmit the virus to the fetus, which may be born viremic and immunologically tolerant. Later exposure to cytopathogenic strains, naturally or by vaccination, can cause offspring to develop the more severe form of the disease (Bolin *et al.,* 1985).

Immunization. A cytopathogenic strain of virus isolated from a calf and designated Oregon (C24V) strain (Gillespie *et al.,* 1960) became less pathogenic for calves after 32 passages in bovine kidney tissue culture (Coggins *et al.,* 1961). This has been the standard vaccine strain and has been used widely for many years. For cattle never exposed to BVD antigen, this vaccine strain is safe and effective; however, persistently infected cattle may react strongly to vaccination with the cytopathic strain of BVD virus, causing a mucosal disease syndrome. It is important to identify and eliminate persistently infected cattle from herds.

3. Rubivirus

Rubella Virus. In man, rubella virus causes a generally mild exanthematous disease, with malaise and respiratory symptoms. Complications include arthritis, thrombocytopenia purpura, and encephalitis. Gregg's observation (1941) that rubella virus produces fetal abnormalities if infection occurs early in pregnancy emphasized the destructive effects of this virus and the need to develop a means to protect against infection.

Immunization. Natural exposure to rubella virus evokes nasopharyngeal antibody, which is important in preventing reinfection. Antibody, especially IgG antibody in mother's plasma, is important in preventing fetal infection.

Two groups isolated rubella virus in tissue culture (Parkman *et al.,* 1962; Weller and Nova, 1962), allowing the first attempts to develop

vaccines. Both inactivated and attenuated live-virus vaccines were tried before the latter evolved as superior products. The attenuated live-virus vaccines were developed using different cell culture systems, including monkey kidney (Parkman *et al.*, 1966), duck embryo (Buynak, 1968), rabbit kidney (Peetermans and Huygelen, 1967), canine kidney (Musser and Hilsabeck, 1969), and human diploid cells (Plotkin, 1967). The vaccines now being used are more than 95% effective in inducing protective levels of antibody that persist for at least 9 years. The annual incidence of rubella in the United States has dropped from 56,000 reported cases in 1970 to less than 700 in 1985.

4. Arterivirus

Equine Arteritis Virus.   Equine arteritis virus was first isolated by Doll *et al.* (1957). The disease caused by this virus is characterized by edema of limbs, stiffness, and swelling in the tissues surrounding the eye, and abortion.

Immunization.   Horses that recover from infection develop longlasting immunity. An effective attenuated live-virus vaccine was developed by serial passage of Bucyrus field strain virus in primary equine and rabbit kidney cell culture and an equine dermal cell line (Doll *et al.*, 1968; Wilson *et al.*, 1962; McCollum, 1986). The vaccine has been shown in challenge trials to protect recipients for as long as 24 months (McCollum, 1986); it does not cause any clinical manifestations and it is not spread to susceptible horses in contact with vaccinated recipients.

F. FLAVIVIRIDAE (YELLOW FEVER VIRUS)

Yellow fever virus causes acute hepatitis and hemorrhagic fever in man, characterized by jaundice, shock, and renal damage. Transmission is by mosquitoes belonging to the *Aedes* genus throughout tropical areas of South America and Africa. The virus is maintained in a transmission cycle between mosquitoes and monkeys, with man being infected when he enters a territory in which the monkey–mosquito cycle exists.

Immunization.   An attenuated live-virus yellow fever vaccine was developed by passage of the virulent Asibi strain in mouse brain and cell culture until it had lost its pathogenicity for monkeys and man (Theiler, 1951). The vaccine virus, termed 17D, is propagated in embryonated eggs. The vaccine, given as a single dose, is extremely safe and efficacious, providing immunity for at least 10 years.

G. Orthomyxoviridae

The family Orthomyxovirus comprises the influenza viruses, the cause of acute, highly contagious respiratory disease in man, horses, swine, and birds. Two structural viral proteins (NF and M) divide this family into three distinct genera: A, B, and C.

The viruses, especially influenza A virus, undergo genetic reassortment, which allows variant viruses to emerge. Two viral proteins, the hemagglutinin and neuraminidase, both located on the surface of virions, are important in inducing immunity.

Vaccines have been widely used for controlling influenza with reasonably good success. Since immunity is more closely related to local secretory IgA antibody than to serum antibody, it is difficult to stimulate and maintain protection with the presently used inactivated vaccines. With human infections, the type A viruses undergo occasional antigenic shifts and drifts, after which the antigens in the vaccine may not be representative of those viruses found in the field. This potential antigenic variability, as well as animal reservoirs for this virus, are responsible for the pandemics associated with this virus. As an example, the acute respiratory disease of swine caused by a type A strain of virus was first recognized in 1918 during the human influenza epidemic and is believed to have been transmitted from swine from humans. Both swine and humans are susceptible to the swine virus. However, the disease in swine occurs sporadically and has not been enough of a problem to warrant the use of vaccines in that species.

1. Human Influenza Virus

Influenza is a respiratory infection with systemic manifestations that include fever, chills, muscular aches, etc. The severity of the disease depends on the virus strain and the susceptibility of the population.

Persons that have recovered from an influenza infection are usually immune to rechallenge with the homologous virus. However, the change of a few amino acids in hemagglutinin may give rise to antigenic drift and reinfection of populations.

Immunization. Because of the epidemic threat of influenza viruses, careful surveillance for new strains is carried out in many parts of the world. The strains that public health officials predict will be the cause of the next winter's epidemics are then scheduled for vaccine production. For vaccine production, virus is grown in the allantoic cavity of embryonated eggs and is purified and concentrated by zonal centri-

fugation. The virus is inactivated with formalin, β-propiolactone, or irradiation. The quantity of viral antigen per dose is standardized before use. The vaccine usually contains several type A viruses and a type B virus. These inactivated whole virus vaccines produce protective levels of antibody in approximately 85% of primed recipients and in 60% of the unprimed recipients. Antibody levels are maintained for approximately 1 year in primed individuals. Attenuated live-virus influenza vaccines have been used extensively in the USSR with varying results. Problems of adverse reactions, inconsistent potency, and questionable appropriateness of strains make it difficult to evaluate the effectiveness of these vaccines.

More recent attempts to develop attenuated live-virus vaccines involve genetic reassortment, a method that offers considerable promise (reviewed by Wright and Karzon, 1987).

2. Equine Influenza Virus

Influenza in horses resembles the disease in man and swine. The two type A influenza viruses of importance in the horse are: A/Equi 1/Prague/56 type 1 and A/Equi 2/Miami/63 type 2. The disease spreads rapidly through susceptible horses, and those that recover are protected for only a short time. Recovery from infection with one virus type does not provide immunity against the other.

Immunization. Vaccines for equine influenza are produced in essentially the same manner as human influenza vaccines. Formal-inactivated vaccines contain both equine type 1 and 2 viruses and one of several adjuvants, as described by Bryans *et al.* (1973). Vaccines of this type are widely used and effectively control equine influenza.

H. PARAMYXOVIRIDAE

The family Paramyxoviridae contains several viruses that cause significant disease in animals. The family is composed of three genera that include the following viruses for which vaccines have been developed.

Paramyxovirus   (parainfluenza virus, mumps virus, and Newcastle's
     disease virus)
Morbillivirus   (measles virus, canine distemper virus, and rinderpest
     virus)
Pneumovirus   (bovine respiratory syncytial virus)

These viruses are transmitted by the respiratory route and are antigenically rather stable.

1. *Parainfluenza Virus*

Parainfluenza viruses infect humans, rodents, swine, dogs, and cattle. These viruses, by themselves, cause mild upper respiratory tract disease, but when combined with other viral and bacterial pathogens may cause a more severe syndrome. Parainfluenza types 1, 2, 3, and 4a/4b infect humans, especially young children. Type 3 is considered the most pathogenic, causing a bronchitis and pneumonitis.

Vaccines for parainfluenza of rodents (Sendai virus infection), dogs (canine parainfluenza), and cattle (bovine parainfluenza) have been developed. There is no licensed parainfluenza vaccine for man.

a. Sendai Virus.   Sendai virus, first isolated during attempts to recover human respiratory viruses in mice (Kuroya *et al.*, 1953), is a parainfluenza type 1 virus that causes respiratory disease in mice, rats, hamsters, and swine. The disease occurs either in an acute-short duration form or a chronic-persistent, clinically inapparent form. Spread is by either direct contact or by aerosol. In mouse colonies, this disease is difficult to control because the virus is so highly infective.

Immunization.   Formalin-inactivated vaccines have been effective in controlling the disease in mice and rats (Fukumi and Takeuchi, 1975; Eaton *et al.*, 1982; Tsukui *et al.*, 1982). Additionally, a temperature-sensitive mutant strain of Sendai virus has been used as an aerosol-delivered vaccine in mice. It suppresses virus replication, but the vaccine virus spreads throughout the colony and makes it difficult to monitor for wild virus strains (Kimura *et al.*, 1979).

b. Canine Parainfluenza Virus.   Outbreaks of mild respiratory disease in laboratory dogs have been attributed to parainfluenza type 2 virus (Binn *et al.*, 1968; Crandell *et al.*, 1968). When other respiratory agents such as mycoplasma and *Bordetella bronchiseptica* were given intranasally after exposure to this parainfluenza virus, more severe respiratory signs occurred (Appel and Percy, 1970). This encouraged efforts to develop a vaccine for canine parainfluenza virus.

Immunization.   An attenuated live-virus parainfluenza vaccine has been shown to protect dogs against aerosol challenge with virulent virus (Emery *et al.*, 1976). The vaccine produces no untoward effects and has been combined with canine distemper and canine adenovirus vaccines in multivalent formulations.

c. Bovine Parainfluenza Virus.   A parainfluenza type 3 virus isolated from cattle can also cause mild respiratory disease (Reisinger *et al.*, 1959). The virus, when combined with other respiratory pathogens including *Pasteurella* and infectious bovine rhinotracheitis virus, causes the severe pneumonia syndrome, called "shipping fever."

Immunization. An attenuated live-virus parainfluenza type 3 vaccine administered parenterally induces good levels of antibodies and affords protection against experimental challenge (Mohanty and Lillie, 1964; Thorsen *et al.*, 1969). This vaccine has been combined with infectious bovine rhinotracheitis virus and bovine virus diarrhea vaccines in multivalent formulations. An inactivated vaccine, requiring two inoculations, induces high hemagglutination-inhibition titers and lessens the severity of the disease in cattle challenged with the same virus (Gale *et al.*, 1963).

2. Mumps Virus

Mumps virus causes an acute infection in man with parotitis as the main clinical manifestation, although the central nervous system and other organs including the testes and ovaries can be affected. Mumps virus has a limited host range; in addition to man, only certain monkey species and laboratory rodents can be infected.

Immunization. Recovery from natural infection with mumps virus confers long-term immunity. Early experiments with formalin-inactivated virus derived from infected parotid glands of monkeys showed that monkeys and humans could be immunized (Enders *et al.*, 1945; Stokes *et al.*, 1946). Habel (1946) found that chick embryo grown virus could be inactivated with ultraviolet light or formalin and would induce protection in monkeys. A similar vaccine was later shown to induce protection in man (Habel, 1951; Henle *et al.*, 1951). Poor antibody responses to multiple inoculations of this type of vaccine encouraged the search for a more effective vaccine.

A mumps virus strain (Jeryl Lynn) has been attenuated by passage in chick embryos (Weibel *et al.*, 1980). The vaccine is immunogenic in 93–98% of subjects, and neutralizing antibodies persist for at least 10 years. The annual incidence of mumps in the United States has been reduced from 160,000 cases in 1968 to less then 3,000 in 1985 by application of this vaccine. As presently used, mumps vaccine is combined with measles and rubella vaccines in a pediatric formulation called MMR.

3. Newcastle Disease Virus

Newcastle disease is one of the most serious widespread diseases affecting poultry. The disease was first described by Kranevelt (1926) and shortly thereafter by Doyle (1927), who named it after the area in England where an outbreak occurred and showed that its cause was a filterable virus. The disease has several forms causing mainly respiratory, enteric, and central nervous system manifestations. The morbid-

ity and mortality vary depending on the virus strain. Burnet (1942) described the hemagglutinating property of the virus, which has been very helpful in its quantitation and immunodiagnosis.

Immunization. A number of attenuated live-virus vaccines have been developed which are widely used to control the disease. The B1 strain (Hitchner and Johnson, 1948), the LaSota strain (Winterfield *et al.*, 1957), and the F strain (Asplin, 1952) are used to immunize birds of all ages by different routes, including by addition to drinking water and by spraying.

Vaccines containing inactivated virus do not produce long-lasting immunity but may be used in certain situations when only short-term immunity is needed, such as when boosting immunity is needed in laying flocks. Increasing the antigen content and using oil–emulsion adjuvants improves the quality of these inactivated vaccines (Stone *et al.*, 1980; Zanella and Marchi, 1982).

4. Measles Virus

Measles is a highly contagious disease of humans, occurring mostly in children, causing exanthemata and sometimes more serious manifestations including encephalomyelitis. The virus has two principal immunogens, the hemagglutinin and the fusion protein (Norrby *et al.*, 1975). The immunity produced by natural infection is long lasting.

Immunization. The growth of measles virus in chick embryo fibroblasts by Enders and Peebles (1954) paved the way for the development of vaccines. A formalin-inactivated measles virus vaccine was shown to induce partial immunity. However, some vaccinated children later exposed to measles virus, either naturally or as attenuated live-virus measles vaccine, developed atypical measles (atypical rashes, edema of hands and feet, and respiratory disease). It was later found that formalin-inactivated vaccine failed to stimulate antibody to the fusion protein: consequently the virus could spread from cell to cell, causing the atypical manifestations of disease (Norrby *et al.*, 1975).

An attenuated live-virus vaccine was developed from the *Edmonston* strain of measles virus by passage first in human cell culture, then in the amnionic sac of embryonated hen's eggs, and finally in chick embryo cells (Milovanovic *et al.*, 1957). This vaccine (Enders Passage Level B) was effective in inducing immunity but produced some adverse effects (Katz and Enders, 1959; Stokes *et al.*, 1962). Further attenuation by growth at lower temperature yielded an equally effective vaccine that produced fewer side effects (Schwarz, 1964; Hilleman *et al.*, 1968). Attenuated live-virus measles vaccine has been combined with mumps and rubella vaccines. Measles vaccine usage

has reduced the incidence of measles in the United States from 440,000 cases in 1960 to 3,000 in 1985.

5. *Canine Distemper Virus*

Canine distemper virus affects most carnivores, causing respiratory, gastrointestinal, and central nervous system disease. The mortality rate in dogs is about 20%. Dunkin and Laidlaw (1926) first described the disease in detail and confirmed the viral etiology proposed earlier by Carre (1905).

Immunization. Laidlaw and Dunkin (1926, 1928a,b) prepared a vaccine by treating virus derived from spleens of infected dogs with formalin. Initial administration of this vaccine, followed 2 weeks later with a small dose of virulent virus, usually produced only a mild disease with solid immunity. This approach was replaced with inactivated virus vaccines, given in multiple doses, which served as the main means of controlling the disease from 1930 to 1950. Green (1945) serially passaged canine distemper virus in ferrets and produced the first attenuated live-virus vaccine; however, this vaccine caused disease in some dogs.

The adaptation of canine distemper virus to the chorioallantoic membrane of embryonated eggs by Haig (1948) was a major step in developing an attenuated live-virus vaccine. Cabasso and Cox (1949) applied this method and after 28 passages showed that the virus lost virulence for ferrets but retained its immunizing property for dogs. Rockborn (1958) adapted a strain of canine distemper virus to cell culture, and this method is now widely used to produce attenuated live-virus vaccines.

6. *Rinderpest Virus*

Rinderpest is an acute highly contagious disease of ruminants characterized by erosions and necrosis of the intestinal mucosa. The disease is epizootic in parts of Africa and Asia, causing great losses of cattle and buffalo.

Immunization. Koch in 1897 developed one of the first means of immunizing cattle against rinderpest by administering bile from infected cattle. Animals that survived were permanently immune. Formalin- and chloroform-inactivated vaccines were developed using tissues from infected animals. These vaccines were safe, but required two or three doses and protection lasted less than a year (Walker *et al.,* 1946). Rinderpest virus has been adapted to several foreign hosts, including goats (Daubney, 1949) and rabbits (Nakamura *et al.,* 1938) and has been attenuated by passage in these animals. Tissues of these

animals have been used to produce vaccines in many countries. However, on continued passage, the seed strains tend to lose their immunogenicity, and vaccines become contaminated with adventitious agents from the foreign host.

The Kabete strain of rinderpest virus was adapted to grow on the chorioallantoic membrane of embryonated eggs, becoming attenuated for cattle after 19 passages (Shope *et al.*, 1946). This vaccine and another containing a lapinized strain of virus adapted to embryonated eggs have been used widely in Africa (Nakamura and Miyamoto, 1953). More recently, the Kabete strain was adapted to bovine kidney cell culture and after 70 passages became avirulent for cattle. This vaccine strain is safe and efficacious in most cattle breeds (Plowright and Ferris, 1962). Protection persists for at least 4 years. Over 150 million doses of this vaccine have been used in Africa, with good success (Maurer, 1980).

7. *Bovine Respiratory Syncytial Virus (RSV)*

Bovine RSV produces a rhinitis and catarrhal bronchiolitis in cattle (Mohanty *et al.*, 1975; Jacobs and Edington, 1975; Paccaud and Jacquier, 1970). The virus appears to be widespread having been isolated in Europe, the United States, and Asia. Human and bovine virus are related antigenically (Paccaud and Jacquier, 1970); however, the cattle virus is not known to infect man.

Immunization.   Nasal secretory antibody is protective but the disease may occur in the presence of serum antibody. An inactivated bovine RSV vaccine is combined with vaccines for infectious bovine rhinotracheitis, bovine virus diarrhea, and bovine parainfluenza components in a multivalent formulation. The efficacy of the RSV component in this formulation is unclear.

I. RHABDOVIRIDAE

Of the viruses in the family Rhabdoviridae that cause disease in man and domestic animals, the most important is rabies virus. Others include vesicular stomatitis viruses (VSV) and bovine emphemeral fever virus (BEFV). VSV occurs sporadically and epizootically, affecting horses, cattle and swine in the United States. There is a formalin-inactivated vaccine (Gearhart *et al.*, 1987), but it is used rarely. BEFV is an arthropod-transmitted disease of cattle occurring mainly in Africa, but also in Asia and Australia; it is controlled by immunization with attenuated live-virus vaccines (Van der Westhuizen, 1967; Inaba *et al.*, 1973; Spradbrow, 1977; Theodoridis *et al.*, 1973).

Rabies Virus

Rabies is an infection of the central nervous system; the disease can occur in most mammals and is usually fatal. There are only a few documented cases of human survivors. After isolating the virus, Pasteur (1881) developed a vaccine for its control. Historically rabies virus has been considered as a single serotype. But now shared antigens have been found in other viruses in Africa of which two, Mokola and Duvenhage, may be associated with human disease (Shope *et al.,* 1970). Mokola virus causes a rabies-like disease in dogs and cats in Zimbabwe (Foggin, 1983).

Immunization. Protection against rabies correlates with SN antibody, which can be assessed by a number of tests. Pasteur's classical vaccine, developed from infected spinal cord tissue dried at room tempreture for 3–14 days, was given in a series of 21–28 inoculations beginning with material dried the longest and progressing through material dried for only 3 days (Pasteur 1881). Even though the last inoculum was virulent enough to cause rabies, the earlier inoculations conferred sufficient immunity to protect the recipients. This method of producing a vaccine was successful in most instances but caused the disease occasionally and was eventually replaced by chemically inactivated vaccines prepared from infected brain tissue. Although effective, these vaccines gave rise to undesirable side-effects because they contained a myelin-related encephalitogen present in brains from mature animals. Substitution of brain tissue from immature animals such as suckling mice, rats, and rabbits with their lesser myelin antigenic content greatly reduced these post-vaccination reactions.

The adaptation of the Flury strain of rabies virus to growth in chick embryos led to the development of attenuated live-virus vaccines produced in this tissue (Leach and Johnson, 1940; Koprowski and Cox, 1948; Koprowski and Black, 1950, 1954).

The growth of rabies virus in tissue culture has further improved rabies vaccines (Kissling, 1958; Cabasso *et al.,* 1965; Emery *et al.,* 1968; Brown *et al.,* 1967; Fenje, 1960; Abelseth, 1964). Yet, despite their benefits, the attenuated rabies vaccines occasionally caused rabies, particularly in cats. Therefore, suckling mouse brain and tissue culture again became the substrates of choice to produce inactivated rabies virus vaccines for animals. In humans the requirement for a safe substrate is more exacting than in animals. For this reason, duck embryos proved better than brain tissue to produce rabies virus (Peck *et al.,* 1956). After inactivation with β-propriolactone (BPL), this virus

was an improved product for humans, although the allergenic effects from duck embryo tissue still present a problem. Therefore the adaptation of the PM rabies virus strain to human diploid cells and inactivation with BPL (Wiktor *et al.,* 1964) was a further improvement. This vaccine is less reactive and more effective for pre- and post-exposure use in humans than any other yet made (Bahmanyar *et al.,* 1976). A similar vaccine produced in BHK cells is also beneficial in animals.

J. RETROVIRIDAE

Retroviruses have an RNA genome, a portion of which encodes the unique enzyme reverse transcriptase. This enzyme imparts to retroviruses the ability to make RNA-directed DNA copies of their genome, which can then act as a transposable element and can be integrated into the host cell DNA. Thus, once a cell is infected, it may escape immune surveillance and destruction and the host animal may be infected for life. The retroviruses thus constitute a considerable challenge to traditional vaccine approaches, as discussed further in the chapters by Arlinghaus and by Nathanson and Gonzalez-Scarano.

Many retroviruses infect mammalian species, from mouse to man. Most notable are the C-type retroviruses, including the primate, murine, and feline leukemia viruses, as well as human T-cell leukemia viruses types I and II; the B-type retroviruses, particularly mouse mammary tumor virus; and the lentiviruses, including caprine infectious anemia virus, Visna virus, equine infectious anemia virus, feline immunodeficiency virus, bovine immunodeficiency virus, and human immunodeficiency viruses (HIV-1 and HIV-2).

In recent years, as HIV has become a major threat, massive efforts have been directed to developing an efficacious vaccine. So far, all attempts have met with failure. In fact, there are only two retrovirus vaccines that have been proven effective: a formalin-inactivated whole virus preparation of the primate SAIDS type D retrovirus, which is capable of protecting monkeys from a lethal challenge (Marx *et al.,* 1986), and the commercially available vaccine for feline leukemia.

Feline Leukemia Virus (FeLV)

FeLV commonly infects cats in urban areas, usually by the oral–nasal route. Kittens under 6 months of age are particularly susceptible. About 1% of infected cats develop persistent anemia from which myeloproliferative disease and hypoplastic anemia may follow. The

immunosuppression caused by FeLV infection may predispose to severe chronic opportunistic infections. Because cats that develop neutralizing antibody are usually immune to infection, vaccines have been developed and tested with that goal in mind.

Immunization. The problem in developing a vaccine for feline leukemia was to find immunogens that could be used without exposing animals to oncogenic materials. Early studies with inactivated whole virus were unsuccessful (Yohn *et al.,* 1976). Although attenuated live-virus vaccines induce sufficiently high levels of neutralizing antibodies to be protective (Jarrett *et al.,* 1974; Pedersen *et al.,* 1979), their oncogenic potential makes them unacceptable. Efforts to develop vaccines containing only viral proteins, such as envelope protein, have had variable results. However, cultivation of FeLV in FL 74-transformed cells, followed by treatment to release viral and cell proteins, yields a vaccine that stimulates antibodies to both viral and cell membrane components. A commercial vaccine using this method of antigen production has been approved for use in the United States; it is based on studies done by Olsen and Lewis (1981). Subsequently, the efficacy of this vaccine has been disputed (Pederson and Ott, 1985).

K. CORONAVIRIDAE

Viruses in the family Coronaviridae cause important diseases including avian infectious bronchitis, transmissible gastroenteritis of swine (TGE), feline infectious peritonitis (FIP), and human coronavirus infections. Other coronaviruses may cause disease in calves, dogs, mice, rats, turkeys, horses, and parrots, but the diseases are of less importance. Coronavirus diseases usually follow a similar pattern, except for FIP. FIP is a chronic debilitating disease manifested as fibrinous peritonitis and pleuritis. The infection may be inapparent, but is fatal in a small proportion of infected cats. The immune response to FIP virus seems to mediate the disease; the immune response is not protective and antibody levels are higher in diseased animals. Immune complexes have also been demonstrated in renal glomeruli of cats with FIP.

Bovine coronavirus causes acute diarrheal disease in neonatal calves (Mebus *et al.,* 1973). An attenuated live-virus vaccine is being used in combination with an attenuated live-virus rotavirus vaccine to control calf diarrhea. The vaccine is administered to pregnant cows near the end of gestation and stimulates colostral antibodies that offer protection to nursing calves.

With the exception of avian infectious bronchitis, most coronavirus

infections have been difficult to control with vaccines. Perhaps this is because primary lesions are in mucous membranes of the respiratory and gastrointestinal tracts, sequestered from immune reactivity. Coronaviruses produce about 15% of common colds in man, second only to rhinoviruses. There are two groups of human coronaviruses that are antigenically distinct.

1. Avian Infectious Bronchitis Virus (IBV)

IBV is a highly contagious respiratory infection of young chickens. The virus may also infect older birds, causing a decrease in egg production. The disease was first shown to be caused by a virus by Bushnell and Brandley (1933). Beaudette and Hudson (1937) propagated the virus in chick embryos, making possible the quantification of the virus and the means for attenuation. There are a number of serotypes of IBV, making the development of an effective vaccine difficult.

Immunization. Immunity following natural infection may last up to 1 year, depending on the serotype and the severity of challenge. Van Roekel *et al.* (1950) first developed an immunization procedure; he used a field strain of virus to infect 7- to 15-week-old birds before they start to lay, inoculating a few of the birds and allowing infection to spread naturally through the flock. Today, there are a number of attenuated live-virus vaccines licensed in the United States. There is good protection (90–100%) against homologous virus strains and about 40% against heterologous strains (Hofstad, 1981). Reduced pathogenicity may be associated with reduced immunogenicity, so a balance must be maintained. The attenuated live-virus vaccines are administered by the usual labor-saving devices of spraying, dusting, or placing the vaccine in drinking water. The wide-scale use of IBV vaccines has significantly reduced the economic loss caused by this disease.

2. Transmissible Gastroenteritis Virus (TGE)

TGE is an often fatal disease of pigs under 2 weeks of age. The main lesion is enteritis, resulting in malabsorption, diarrhea, and dehydration. TGE virus is serologically related to FIP virus, but the diseases have entirely different characteristics. There is one serotype of TGE virus and one serotype of FIP virus.

High levels of maternal TGE antibody in sows' colostrum protect piglets if fed continuously. Immunity of this type has been produced by feeding sows tissues containing virulent TGE virus several weeks prior to gestation. The effects of this virus are relatively mild in older animals.

Immunization. Attenuated live-virus vaccines administered parenterally to pregnant swine in the latter part of the gestation period produce colostral antibodies. Apparently, in sows previously exposed to TGE, this vaccine produces sufficient immune responsiveness to be of value. The vaccine is also used orally in pigs 1–3 days old to induce local immunity. The effectiveness of this use of the vaccine has not been thoroughly demonstrated.

III. Vaccines for Gram-Positive Bacteria

A. *Corynebacterium*
  1. *C. pseudotuberculosis*
  2. *C. diphtheriae*

B. *Bacillus*

C. *Erysipelothrix*

D. *Clostridium*
  1. *C. perfringens*
  2. *C. tetani*

  3. *C. novyi*
  4. *C. chauvoei*
  5. *C. septicum*

E. *Mycobacterium*

F. *Streptococcus*
  1. *S. agalactiae*
  2. *S. pneumoniae*

A. *Corynebacterium*

The genus *Corynebacterium* is a heterogeneous grouping with its species placed together largely on the basis of similar cell wall components (Goodfellow and Minnikin, 1981). These species share a basic cell wall chemistry (Barksdale, 1981) of which the mycolic acids (Silva and Ioneda, 1977), especially trehalose dimycolate, are frequently used as potent adjuvants in immunization protocols.

Two corynebacteria—*C. pyogenes* and *C. pseudotuberculosis*—are important in veterinary medicine. The former is frequently associated with ruminate suppurative conditions and abscesses, but it rarely affects man. Infections with this organism are sporadic, because it is an opportunist that gains entry through wounds and abrasions. It may also be seen as a secondary invader in devitalized tissues; e.g., vaccination site abscesses. The efficacy of vaccines, toxoids, and antisera against *C. pyogenes* is equivocal; little is known about immunity to the bacterium.

1. Corynebacterium pseudotuberculosis

Corynebacterium pseudotuberculosis causes caseous lymphadenitis of goats and sheep. It is recognized as a worldwide problem and a serious cause of economic loss to the goat industry (Burrell, 1981; Ashfaq and Campbell, 1980). As with *C. pyogenes,* the responsible bacterium, *C. pseudotuberculosis,* is primarily an opportunist entering wounds or abrasions, where it causes local inflammation before settling in the regional lymph nodes.

Immunization. Cell-mediated immunity is necessary for acquired resistance and protection against *C. pseudotuberculosis* (Jolly, 1965; Tashjian and Campbell, 1983; Irwin and Knight, 1975). Killed and autogenous vaccines and a toxoid vaccine have been used in attempts to immunize against the bacterium (Cameron, 1972; Brogden *et al.,* 1984; Nairn *et al.,* 1977; Burrell, 1978; Anderson and Nairn, 1984; Brown *et al.,* 1986); however no one vaccine has proven highly efficacious.

2. Corynebacterium diphtheriae

Diphtheria, characterized by the formation of a tightly adherent pseudomembrane on the pharyngeal mucous membranes of the throat and trachea, is a highly contagious disease of man caused by the bacterium *C. diphtheriae.* The bacterium can also be isolated from the pharyngeal mucosa of normal individuals. The organism produces a lethal protein exotoxin (Gill and Pappenheimer, 1971; Collier and Kandel, 1971).

Immunization. Successful immunization against *C. diphtheriae* actually protects against the diphtherial exotoxin. Because diphtheria toxin is produced in high yield by the Park-Williams Number 8 strain (PW8), PW8 is used to make diphtherial toxoid for vaccines. As a source of toxin it is rendered nontoxic by incubation with formalin under alkaline conditions. The product's retention of antigenicity, enabling it to induce antitoxin antibodies, makes it an excellent pediatric vaccine. It is commonly utilized in combination with antigens from *C. tetani* and *B. pertussis.*

B. Bacillus

The most important species of the *Bacillus* genus, *B. anthracis,* is the organism responsible for the disease anthrax in both man and animals. Anthrax was the first bacterial disease ever to be reported, being described by Davaine in 1863. Koch in 1876 reproduced the disease via animal inoculation and in 1881 Pasteur successfully vaccinated

against anthrax. In animals, natural infection usually occurs by ingestion of spores that germinate in the mucosa of the esophagus or the intestinal tract. Herbivorous animals, especially cattle, horses, sheep, and goats, are highly susceptible to the disease, usually the result of grazing in infected pastures or consuming infected foods.

In man, anthrax is manifested in three forms: cutaneous (malignant carbuncle), pulmonary (woolsorter's disease), and gastrointestinal with cutaneous being the most common. Death results from the combined effects of an extracellular toxic protein complex (Vodkin and Leppla, 1983) comprised of three components: edema factor, protective antigen, and lethal factor (Leppla, 1982; Stephen, 1981). Effective vaccines require all three components.

Immunization. The attenuated Pasteur vaccine has been supplanted in veterinary medicine by stable spore vaccines, carbo-zoo vaccine, or Stern vaccine (Jackson *et al.,* 1957) prepared from avirulent, nonencapsulated variants of *B. anthracis.* The viable bacterial spores are suspended in 10% saponin. Immunity is attributed to the development of antibodies to the toxins released from growing bacteria. Vaccines of killed bacteria provide little immunity, since no bacterial toxins are produced; hence, no antitoxin antibodies are generated. Purified protective antigen (complex toxin) is both antigenic and immunogenic and has been used as a vaccine for humans. It is prepared by aluminum potassium sulfate precipitation of sterile *B. anthracis* culture filtrates and has proven highly efficacious.

C. *Erysipelothrix*

Erysipelothrix is found in soil, water, and decaying vegetative material and carcasses. The major species of interest is *E. rhusiopathiae,* which has 20 serotypes (Norrung, 1979). The bacterium, most notable for causing swine erysipelas, is capable of invading the tissues of both man and animal. Fatally affected animals develop welt-like, discolored cutaneous lesions, and numerous hemorrhagic lesions in thoracic and abominal viscera; chronic debilitating arthritis predominates in surviving animals.

Immunization. The principal vaccines used to control erysipelas are formalin-killed, alum-adsorbed, whole-cell culture vaccines. These combinations of soluble bacterial glycoprotein and whole killed bacterial cells are usually produced from strains of serotype 2, which possess highly antigenic soluble cell wall glycoproteins. Animals immunized with cell-free culture fluids develop agglutinins to the whole bacteria (White and Verway, 1970). Such vaccines are highly effective in controlling swine erysipelas.

D. *Clostridium*

The pathogenic clostridia invade both man and many animal species of veterinary interest, in which they cause such diseases as tetanus (*C. tetani*), gas gangrene (*C. perfringens, C. septicum, C. oedematous*), botulism (*C. botulinum*), enterotoxemia, and dysentery (*C. perfringens*). The clostridia are widely distributed in soil and water and are common inhabitants of the intestinal tracts of animals and humans. Additionally, the bacteria can often be isolated from infected wounds. Vaccination is not routinely practiced against all clostridial organisms, notably *C. botulinum*. The toxins of *C. botulinum,* which exert their effects upon the nervous system (Schantz and Sugiyama, 1974), are as potent as those of *C. tetani*. The lethal dose of the toxin, however, is less than that required to induce an antibody response.

1. *Clostridium perfringens*

Clostridium perfringens has five serotypes, A–E, classified according to the production of lethal exotoxins. Types A and C are pathogenic for man, whereas all five serotypes can affect animals (see Table I).

Immunization. The exotoxins of *C. perfringens* are antigenic proteins that can be detoxified for use in vaccines. The existence of common capsular antigens, which elicit cross-reactions between the serotypes, demonstrates the considerable heterogeneity of this group. Ewes and lambs are frequently vaccinated against *C. perfringens* enterotoxemias. Effective vaccines employ type-specific alum-

TABLE I

DISEASES CAUSED BY AND THE MAJOR SOLUBLE ANTIGENS OF *C. perfringens*
SEROTYPES A, B, C, D, AND E

Type	Host	Disease	Toxins			
A	Man Sheep Horses	Gas gangrene, food poisoning Yellow lamb disease	α			
B	Sheep	Lamb dysentery	$\alpha,$	$\beta,$	ε	
C	Man Sheep Cattle Piglets	Enteritis necroticans "Struck," toxemia Hemorrhagic enteritis	$\alpha,$	$\beta,$	$\gamma,$	σ
D	Sheep	Enterotoxemia	$\alpha,$	$\sigma,$	ε	
E	Sheep, cattle	Enterotoxemia	$\alpha,$	ι		

precipitation or formalinized toxoids (Smith and Matsuoka, 1959; Kennedy *et al.*, 1977).

2. *Clostridium tetani*

Clostridium tetani elaborates potent neurotropic exotoxins (tetanospasmin and tetanolysin) that may be lethal for susceptible species such as man, horses, mules, swine, cattle, and sheep. Birds are not naturally susceptible to the bacterium. Tetanus toxin is one of the most poisonous toxins known. It acts only on the nervous system and its effect characteristically causes spastic paralysis and generalized convulsions.

Immunization. Protective antitoxin blood levels are obtained by immunizing both humans and susceptible animals with alum-precipitated or absorbed tetanus toxoid (Chodnik *et al.*, 1959). Ramon and Lemetayer (1931) first introduced the concept of active immunization against tetanus when they used formalinized tetanus toxoid precipitated with alum to vaccinate horses. *Clostridium tetani* vaccines are very effective at inducing long-lasting immunity in both man and domestic animal species. A serious, often fatal disease has been successfully controlled with these vaccines.

3. *Clostridium novyi*

Clostridium novyi possesses four antigenic types, A, B, C, and D; type A is the most common clinical pathogen. Types A and B are responsible for gas gangrene both in man and animals (Elder and Miles, 1957). In areas where sheep simultaneously carry a heavy liver-fluke infestation, exposure to *C. novyi* is often associated wtih hepatic necrosis and subcutaneous edema. Migrating flukes produce foci of hepatic necrosis suitable for the germination of spores and the subsequent elaboration of lethal toxins (Williams, 1962).

Immunization. Effective vaccinations for *C. novyi* in animals employ chemically inactivated, detoxified, and adjuvanted suspensions of alum-precipitated formalinized whole broth cultures.

4. *Clostridium chauvoei*

Clostridium chauvoei, which is the etiologic agent for the disease "blackleg" and is pathogenic for animals only, occurs primarily in ruminant species. Protective antigens and toxins with hemolytic and necrotizing activity are formed in susceptible animals (Jayaraman *et al.*, 1962). The necrotizing toxin may effect fatal toxemia with degenerative foci of myonecrosis.

Immunization. Immunity to *C. chauvoei* can be produced via

vaccination with its alum-precipitated formalinized cultures (Chandler and Gulasekhuram, 1970).

5. *Clostridium septicum*

Clostridium septicum, in contrast to *C. chauvoei,* is pathogenic for both man and animal. In man, it is associated with gas gangrene and in affected animals, primarily ruminants, it is the agent most closely identified with the diseases malignant edema and braxy. The organism produces four lethal necrotizing, hemolyzing toxins that cause an increase in capillary permeability and myonecrosis.

Immunization. Immunity to *C. septicum* is induced with injection of formalinized bacterial cultures. The antitoxin provides homologous protection and additionally protects against *C. chauvoei.* Animals are often vaccinated with mixtures of Clostridial species; i.e., *novyi, chauvoei, septicum, perfringens,* and *sordelli* in one combination vaccine. These are highly efficacious vaccines and are routinely used in veterinary medicine.

E. *Mycobacterium*

Although infections with *Mycobacterium tuberculosis* primarily occur through inhalation of the tubercle bacillus, ingestion of large numbers of the bacilli in contaminated milk or infectious sputum can readily produce disease in susceptible species. The bacterium is pathogenic in man, but can also cause disease in monkeys, pigs, and occasionally in cattle, dogs, and parrots. The disease may be asymptomatic or produce severe, debilitating pulmonary lesions. If infection is not restricted by the immune system, the disease may be fatal (Comstock, 1982; Bloom and Godal, 1983). Since bacteriocidal mechanisms of the normal macrophage prevent *M. tuberculosis* from multiplying intercellularly (Goren, 1977; Goren *et al.,* 1976), protective immunity depends on cell-mediated immunity (Lagrange, 1984).

Mycobacterium bovis, closely related to *M. tuberculosis,* and *M. avium* causes disease primarily in cattle and birds. They can, however, be contagious to man, sheep, and pigs.

Immunization. Immunoprophylaxis for tuberculosis is based on vaccination with an attenuated, relatively avirulent strain of *M. bovis* that does not produce lesions. The strain is known as BCG or the bacillus of Calmette and Guérin (1924, 1926). Worldwide, this is one of the most widely used human vaccines, as it has proven efficacious in controlling a severe disease. Additionally, BCG has been used for nonspecific enhancement of resistance against tumors and other infec-

tions. The cell wall of *M. tuberculosis* is a potent immunostimulant when used in Freund's adjuvant.

F. *Streptococcus*

Although streptococci may be normal inhabitants of the gastrointestinal tract, they may also be pathogenic for both man and animals. On the basis of characteristic cell wall components, the streptococci are traditionally divided into Lancefield groupings (Lancefield, 1934).

1. *Streptococcus agalactiae*

Streptococcus agalactiae, a streptococcus group B organism, causes severe mastitis in the bovine species and has been identified as a major cause of serious neonatal infections in man (Eickoff *et al.,* 1964). Capsular antigens form the four major type-specific antigens (Ia, Ib, II, and III), with type III organisms being most commonly associated with neonatal meningitis. In infants, early-onset disease occurs within the first days of life and is characterized by sepsis and pneumonia. The mortality rate is high. Late-onset disease occurs around 1 month of age and is characterized by meningitis (Einstein *et al.,* 1982).

Immunization. There is a direct correlation between the absence of maternal IgG antibody to type III antigen and the incidence of neonatal infection. Thus, susceptibility to the bacterium is related to the absence of significant levels of maternal serum antibody being transferred transplacentally to the fetus. Current vaccine developments are directed toward maternal immunization with type III antigen (Einstein *et al.,* 1982).

2. *Streptococcus pneumoniae*

Streptococcus pneumoniae, the etiologic agent of pneumococcal pneumonia in human infants and adults, may cause septicemia, meningitis, and inner ear infections. Aerosol transmission of the bacterium, often in association with viral upper respiratory infections, is the major mode of transmitting *S. pneumoniae* infections. *Streptococcus pneumoniae* possesses a capsular polysaccharide capable of deterring phagocytosis, thus enhancing the virulence of the bacterium. Of over 80 types of the bacterium identified, 10 serotypes are most frequently associated with the disease.

Immunization. A polyvalent pneumococcal vaccine prepared from soluble purified capsular polysaccharides of the 23 most predominant *S. pneumoniae* serotypes has proven effective in adults. The capsular polysaccharides are well-tolerated and highly immunogenic; signifi-

cant rises in protective serum antibody titers are achieved following vaccination (Kasper *et al.*, 1982). However, vaccination of infants has not proven beneficial, because they develop no higher antibody titers to the bacteria than do unvaccinated infants (Ginsburg, 1986).

IV. Vaccines for Gram-Negative Bacteria

A. Entobacteria
    1. *Escherichia coli*
    2. *Salmonella* spp.
    3. *Yersinia pestis*

B. *Pasteurella*
    1. *P. multocida*
    2. *P. haemolytica*

C. *Hemophilus*
    1. *H. pleuropneumoniae*
    2. *H. somnus*

D. *Bordetella*
    1. *B. pertussis*
    2. *B. bronchiseptica*

E. *Brucella*
    1. *B. abortus*
    2. *B. melitensis*
    3. *B. ovis*

F. *Campylobacter*
    1. *C. fetus venerealis*
    2. *C. fetus intestinalis*

G. *Vibrio*
    V. cholera

H. *Leptospira* spp.

I. *Neisseria*
    N. meningitidis

J. *Rickettsia*

A. ENTEROBACTERIA

Many members of this family are indigenous to the gastrointestinal tract with the fecal–oral route, often the most important mode of transmission in animals. Established carriers may intermittently shed bacteria.

1. Escherichia coli

Enterotoxigenic pathogenic strains of *Escherichia coli* may cause severe, potentially fatal, diarrheal disease in both man and domestic species, particularly neonatal cattle and swine. The capsular (K) antigens are cell-surface proteins and/or polysaccharides associated with virulence. The K88 antigen mediates adhesion to the microvillus of intestinal epithelial cells; production and release of enterotoxin follow (Bywater, 1970; Lonroth *et al.*, 1979). *Escherichia coli* neonatal enteritis of newborn calves is also a serotype-specific disease (Myers

and Guinee, 1976). All important colostral antibodies in both swine and cattle are anti-K antibodies (usually K99) (Myers and Guinee, 1976; Moon *et al.*, 1978).

Immunization. Vaccination of gilts, sows, heifers, and cows with vaccines prepared from the K88 or other pilus-associated antigens has reduced morbidity and mortality from *E. coli* nec natal enteritis of newborn piglets and calves (Nagy *et al.*, 1978; Rutter, 1975; Kohler *et al.*, 1975; Childrow and Porter, 1979; Myers and Guinee, 1976). To prepare the porcine vaccines, bacterial strains specific for the herds to be vaccinated are used to immunize animals 3 weeks prior to parturition, thereby generating specific, protective colostral antibodies. Recombinant DNA technology, discussed in the chapter by Collett has introduced the potential to construct *E. coli* vaccine strains that would afford considerably better protection than those currently available.

2. Salmonella spp.

Salmonella species are a major cause of invasive enteric infections in humans and domestic animals, with domestic poultry constituting the largest reservoir of *Salmonella* organisms in nature. Normally, infection occurs through the oral route. *Salmonella* is a facultative intracellular pathogen; therefore, cell-mediated immunity is more important than humoral immunity in resistance to the disease, salmonellosis (Fields *et al.*, 1986a,b; Dougan *et al.*, 1987; Woolcock, 1973).

Salmonella typhi, the only *Salmonella* species that has a capsular polysaccharide (Vi antigen), is the etiologic agent of typhoid fever, a serious and common disease in underdeveloped areas (Edelman and Levine, 1986). This pathogen infects humans only; there is no suitable animal model for typhoid fever.

Immunization.   Few vaccines have been developed for *Salmonella*, and most are of low efficacy with undesirable side-effects. Live vaccines are more effective than killed ones in promoting better immunity (Levine *et al.*, 1983; Dougan *et al.*, 1987; Roantree, 1967). With respect to *S. typhi*, vaccines containing the inactivated bacteria offer only limited and transient protection with undesirable side-effects (Levine, *et al.*, 1983). The attenuated strain of *S. typhi*, Ty-21a, requires multiple doses to achieve 60–70% protection (Hirschel *et al.*, 1985). Consequently, typhoid fever has not been controlled by immunization, although the Vi antigen has recently been hailed as the agent of a preventative vaccine (Robbins and Robbins, 1984) without adverse side-effects (Acharya *et al.*, 1987).

3. Yersinia pestis

Yersinia pestis is the etiologic agent of plague or "black death" in man, a highly fatal disease with fever and purulent lymphadenopathy. Although not a disease of domestic animals, rats, ground squirrels, and other rodents may be affected. The bacterium is spread by the rat flea, *Xenopsylla cheopis,* from rat to rat and from rat to man.

Immunization.   The most widely used vaccine for the prevention of *Y. pestis* infection is Haffkine's vaccine, first developed in 1897. This vaccine is prepared from heat and phenol-killed virulent cultures. Formalin-killed virulent bacteria are also successful, as are living avirulent strains (Grasset, 1946). The is no evidence that any vaccine protects against pneumonic plague, the most contagious and fatal form of the disease. Furthermore, vaccine protection is only recommended for plague research workers. Disease control is primarily dependent upon eradication of rodent carriers of *Y. pestis.*

B. *Pasteurella*

Pasteurella multocida and *P. haemolytica* are common commensals of the mucous membranes of the respiratory tract and oropharynx of healthy cattle, sheep, swine, dogs, and cats. When the bacteria multiply unchecked, they can penetrate the oral and/or respiratory mucosa, where they quickly grow and overpower the host's defense systems.

1. *Pasteurella multocida*

Pasteur first described this bacterium as the etiologic agent of fowl cholera; it is also associated with bovine pneumonia, swine plague, and an epizootic hemorrhagic septicemia in ungulates. The bacterium's heat-stable antigens have been used as serologic indicators in the gel diffusion precipitin test to define its 16 serotypes (Brogden *et al.,* 1978).

Immunization. Pasteur's successful bacterial vaccine to fowl cholera, and the first vaccine ever used, consisted of avirulent cultures of the *P. multocida* attenuated by prolonged growth on artificial medium. Killed *P. multocida* vaccines are prepared from virulent immunogenic strains of the bacterium. The organisms are suspended in formalinized saline, incorporated into an adjuvant, and injected subcutaneously (Heddleston *et al.,* 1974). These vaccines induce substantial immunity to fowl cholera. Additionally, live vaccines for oral administration have been developed for use in the poultry

industry (Dougherty *et al.*, 1955; Heddleston *et al.*, 1974; Olson, 1977; Bierer and Derieux, 1972).

Pasteurella multocida is usually mixed with modified live or killed bovine rhinotracheitis virus, parainfluenza 3 virus, bovine viral diarrhea virus, and *P. hemolytica* bacteria in combination vaccines to protect against *Pasteurella* pneumonia in cattle.

2. *Pasteurella haemolytica*

Bovine pneumonic pasteurellosis (shipping fever) is a severe fibrinous pneumonia of feedlot cattle usually associated with biotype A, serotype 1 infections with this organism.

Immunization.   Administration of either killed or live vaccines has been of limited efficacy in controlling shipping fever. Partial protection from experimental disease follows immunization of cattle with either live *P. haemolytica* by aerosol or parenteral routes (Panciera *et al.*, 1984; Confer *et al.*, 1984) or lyophilized *P. haemolytica* vaccines consisting of chemically altered, streptomycin-dependent, or modified live organisms given intramuscularly or intradermally (Confer *et al.*, 1986). Humoral antibody responses appear to correlate strongly with protection against experimental disease (Confer *et al.*, 1985; McKinney *et al.*, 1985). For example, purified *P. haemolytica* lipopolysaccharide stimulates specific antibody formation and has protected calves challenged with the bacterium from developing the disease (Hilwig *et al.*, 1985).

C. *Hemophilus*

These obligate parasites, restricted to respiratory and pharyngeal mucous membranes, cause important diseases in porcine (*H. pleuropneumonia, H. suis,* and *H. parasuis*), equine (*H. equigenitalis*), bovine (*H. somnus*), and avian (*H. gallinarum, H. paragallinarum*) hosts. Most *Hemophilus* species require two factors, hemin (X) and nicotinamide adenine dinucleotide (V), for growth.

Antigens associated with protection and virulence have been described for *H. paragallinarum* (Yamamoto, 1984), the etiologic agent of infectious coryza in chickens. Birds that have recovered from natural infection possess varying degrees of immunity to re-exposure (Page *et al.*, 1963); immunity is serotype-specific. Adjuvanted vaccines containing multiple bacterial serotypes are prepared from chicken embryos or formalinized bacterial broth cultures and are effective vaccines in preventing infectious coryza in chickens.

1. Hemophilus pleuropneumoniae

This bacterium is the etiologic agent of the porcine disease, contagious pleuropneumonia, which is characterized by severe multifocal, necrotizing pneumonia with venous thrombosis and associated serofibrinous pleuritis (Didier *et al.*, 1984). The disease is of considerable economic importance to the swine industry, being most prevalent in situations where swine are raised under intensive management conditions. *Hemophilus pleuropneumoniae* possesses major and minor antigens that are both common and serotype specific (Gunnarson *et al.*, 1978; Gunnarson, 1979; Mittal *et al.*, 1982). Since high antibody titer apparently provides little protection from the disease, cell-mediated immunity may be important in protection from infection (Rapp and Ross, 1984; Rosendal *et al.*, 1981).

Immunization. No adequate immunoprophylaxis against contagious pleuropneumonia is currently available, although many vaccines have been tried (Nielsen, 1976; Henry and Marstellar, 1982; Christensen, 1982; Masson *et al.*, 1982). Prior infection with one serotype provides protection from heterologous serotypes (Nielsen, 1976). The bacterial strains used in vaccines are serotype specific and, while not preventing the disease, can reduce its severity (Christensen, 1982).

2. Hemophilus somnus

Hemophilus somnus is the cause of infectious meningoencephalitis, a disease with low morbidity but high mortality in cattle. Whole or sonicated bacterial cells and bacterial protein are immunogenic (Noyer *et al.*, 1976) and efficacious bacterins foster protective immunity in calves (Williams *et al.*, 1978). The bacterins of *H. somnus*, adjuvanted with aluminum hydroxide, are prepared from highly immunogenic strains of the bacterium and grown in serum-free media for use as vaccines.

D. Bordetella

The species in this genera, *B. pertussis, B. bronchiseptica,* and *B. parapertussis,* can be either parasites or, as in swine and dogs, common inhabitants of the upper respiratory tract. These small, serologically related bacilli produce a dermonecrotic toxin. Infection is by aerosol transmission with bacteria adherent to tracheal cilia (Bemis *et al.*, 1977). Local, not serum, antibody concentration is important in clearance of the infection.

1. Bordetella pertussis

The etiologic agent of whooping cough, *B. pertussis,* produces two distinct hemagglutinins, leukocytosis-promoting factor-hemagglutinin (LPF-HA) and filamentous hemagglutinin (FHA), and various toxins (pertussis toxin [PT] and dermonecrotic toxin). FHA is involved in bacterial adherence to the respiratory mucosa, whereas PT is believed to be the major protective (Sato and Sato, 1984) and pathogenic antigen (Steinman *et al.,* 1985).

Immunization. Although efficacious, the safety of the human vaccines currently in use, suspensions of killed whole cells containing protective antigens, is open to question (Robinson *et al.,* 1985). Undesirable side-effects such as screaming, collapse, encephalopathy, and other serious neurological complications have been reported in association with *B. pertussis* vaccinations (Dick, 1978). The potencies of whole cell vaccines correlate with the antigenic content of PT (Reiser and Germanier, 1986).

2. Bordetella bronchiseptica

Bordetella bronchiseptica is an obligate parasite of the upper respiratory tract of both dogs and pigs. In dogs the bacterium frequently invades the lungs as a sequela to canine distemper (caused by a morbillivirus), causing an often fatal bronchopneumonia. The bacterium is also associated with mild to severe tracheobronchitis, "kennel cough," in dogs (Bemis *et al.,* 1977). In pigs, a deformation of the bony structures of the nasal area (atrophic rhinitis) and reduction of the total volume of nasal turbinates commonly follow the bacterial infection (Ross *et al.,* 1963). Degenerative changes in the osteoblasts and osteocytes may be caused by elaboration of a dermonecrotic toxin (DNT), which is released from *B. bronchiseptica* after colonization or multiplication of the organisms on the nasal mucosa (Nakai *et al.,* 1985). The release of DNT from *P. multocida* type D is thought to exacerbate the disease.

Immunization. Vaccines to control canine *B. bronchiseptica* infections are commonly incorporated into combination packages containing attenuated live-virus canine distemper, and canine adeno and parainfluenza viruses. To control atrophic rhinitis, avirulent live, or inactivated organisms alone or in combination with *P. multocida, Erysipelothrix rhusiopathiae* and *E. coli* have been utilized in vaccines.

E. Brucella

The organisms in this group, *B. abortus, B. suis, B. melitensis, B. canis,* and *B. ovis,* cause the disease brucellosis in domestic animals

and man. The bacterium may localize in the reproductive tract which, in the female, can lead to fetal death with subsequent abortion. Brucellosis, due to *B. abortus* or *B. melitensis,* is a zoonotic disease, readily transmitted from animal to man.

1. Brucella abortus

The potentially severe consequences of brucellosis, fetal death and abortion, in the pregnant cow and epididymitis and sterility in the bull result in significant economic loss to the cattle industry. The primary source of infection is infected animals, whose mammary and/or genital secretions may contain the bacterium. Calves can become infected *in utero;* however, the main portals of infection are oral mucosa, nasopharynx, and conjuctiva of exposed animals. Immunity to *B. abortus* is dependent upon cell-mediated immunity, as the presence of serum antibodies, although a significant indicator of infection, does not correlate with the immune status of the host (Fitzgeorge *et al.,* 1967; Kaneene, *et al.,* 1978; Swiderska *et al.,* 1971; Montaraz and Winter, 1986).

2. Brucella melitensis

Most humans who contract brucellosis have been exposed either to *B. melitensis,* the etiologic agent of Malta or Mediterranean fever, or *B. abortus. Brucella melitensis,* found in the milk of infected sheep and goats, may produce fatal disease when ingested by humans. Brucellosis of sheep and goats mimics the disease as it is seen in cattle, with fetal death and abortion occurring in ewes and does and epididymitis in rams and billies.

3. Brucella ovis

Brucella ovis infects sheep, causing late fetal death and abortion in pregnant ewes and epididymitis in rams, such as *B. abortus* does in cattle.

Immunization. Brucella abortus (Strain 19) is currently used as the vaccine of choice for control of brucellosis of cattle in the United States. This is a viable, smooth strain that, while posing virtually no threat for cattle, may cause disease in man. The major objections to the vaccine are this pathogenicity for humans and the difficulty of differentiating vaccinated from naturally infected animals since persistent serum antibodies are induced by the vaccine. Killed *B. melitensis* in adjuvant or live avirulent strains have been used for vaccines to induce a high degree of immunity in sheep and goats. *Brucella ovis* bacterins in adjuvant, as well as *B. melitensis* vaccines, have been used to protect

animals from the disease *B. ovis* causes, since the antigens of these two pathogens are cross-protective (Diaz *et al.,* 1967).

F. *Campylobacter*

Pathogenic members of this genus are associated with venereal disease, fetal death, and abortion in cattle.

1. *Campylobacter fetus venerealis*

This bacterium is transmitted to uninfected cattle by coitus or artificial insemination and is an obligate parasite of the genital tract.

Immunization. Stimulation of opsonizing antibodies of the IgG class by systemic vaccination with adjuvanted vaccines is effective in preventing natural infection in bulls (Bouters *et al.,* 1973) and infertility in cows (Corbeil *et al.,* 1974; Hoerlin and Kramer, 1964), and prevents the carrier state (Wilkie and Winter, 1971).

2. *Campylobacter fetus intestinalis*

Unlike *C. fetus venerealis,* this bacterium is contracted by ingestion and is not transmitted venereally. Both sheep and cattle can be infected; however, the disease is most severe in pregnant ewes, which undergo a high percentage of abortions or premature births within a flock.

Immunization. In sheep, vaccination with polyvalent adjuvanted vaccines is efficacious in preventing disease (Thompson and Gilmour, 1978).

G. *Vibrio*

The most important organism of this genus, *V. cholera,* causes severe, acute enteritis in humans and nonhuman primates.

Virbrio cholera

The etiologic agent of cholera in humans, *V. cholera,* causes a potentially fatal diarrheal disease. Infection results in the production of a powerful enterotoxin in the small intestine, which stimulates an increase in cyclic AMP in intestinal epithelial cells and causes a profuse outpouring of isotonic fluids. *Vibrio* possesses immunogenic heat-labile flagellar (H) protein and heat-stable (O) lipopolysaccharide somatic antigens. The cholera toxin is immunologically and functionally similar to the heat-labile enterotoxin of *E. coli* (Yamamoto *et*

al., 1984). It consists of six light subunits (L) that assist in toxin adherence to intestinal cell receptors and one heavy subunit (H), which is the toxic entity.

Immunization. Present cholera vaccines are administered by parenteral or oral routes. Parenteral vaccines consist of formalin/phenol-inactivated bacteria, whereas oral vaccines employ killed or live bacteria. Vaccines given by either route provide protection for approximately 3–9 months. The predominant immune mechanism is antibacterial rather than antitoxic (Levine *et al.,* 1979); antibacterial antibodies prevent attachment of the bacterium, whereas antitoxic antibodies inhibit toxin adherence to cell receptors. Because current vaccines often produce adverse side-effects (Feeley, 1970), synthetic and semi-synthetic vaccines are currently under investigation. For the latter, a nonpyrogenic, bivalent cell-surface protein–polysaccharide conjugate is being investigated (Kabir, 1986).

H. *Leptospira* spp.

The pathogenic genera of this family can penetrate the gastrointestinal mucosa and abraded epidermis. Leptospires are transmitted through contact with the urine of animal carriers, either directly or in contaminated water or soil. Rodents are the primary reservoir of the bacteria which, due to its ability to synthesize urease, colonizes the renal nephron and subsequently is shed into the urine. Leptospirosis causes economically serious disease in cattle and swine by causing fetal death, abortion, and infertility. Recovery from infection with one serotype lends immunity only to that serotype. This immunity is predominantly humoral, since agglutinins (IgM) are responsible for the initial clearance of the bacteria; neutralizing antibodies (IgG) are also protective (Hanson, 1974; Negi *et al.,* 1971). Canine leptospirosis infections may be severe, occurring more commonly in male dogs than in females. Man is a dead-end host for leptospires; infection in man is accidental and usually related to occupational exposure.

Immunization. Killed, multivalent, leprospira vaccines protect against clinical disease in cattle and swine; however, in pigs, immunity does not protect against renal colonization (Stalheim, 1968). Dogs can be vaccinated successfully with formalin or phenol-killed vaccines that contain antigens from the two most common infecting serotypes, *L. canicola* and *L. icterohemorrhagica* (Kerr and Marshall, 1974). Vaccines for humans, prepared from chemically inactivated cells of leptospires, have been used extensively in certain areas of the world.

I. *Neisseria*

Neisseria meningitidis

Neisseria meningitidis is a frequent cause of endemic purulent meningitis in human infants and adults, although the incidence of the disease is substantially higher in young infants (Hoffman, 1986). Bacterial invasion of the meninges is usually hematogenous from the upper respiratory tract and is a life-threatening affliction. *Neisseria meningitidis* has been classified into at least nine groups (A, B, C, W-135, X, Y, Z, L, 29-E) on the basis of its capsular polysaccharides (Morse, 1986).

Immunization. Protection against meningococcus meningitis results primarily from the presence of antibodies against the capsular polysaccharide of *N. meningitidis* (Frasch *et al.,* 1982). Group A and C polysaccharide vaccines are especially effective against disease in children over two years of age and in adults.

J. *Rickettsia*

Epidemic typhus fever has afflicted mankind since ancient times. It is an acute highly infectious disease with the potential for explosive epidemics in man. Significant outbreaks of the disease have been intimately associated with war and famine. The disease is characterized by sustained high fevers, headache, panencephalitis, a diffuse maculopapular skin rash, and toxic vascular damage. The fatality rate may be high. The etiologic agent, *R. prowazeki,* is transmitted from person to person by the human body louse *Pediculus humanus corporis.* Infection is established by inoculating infected louse feces into the skin by scratching.

Immunization. Although no etiologic relationship has been demonstrated between *R. prowazeki* and the bacterial strain *Proteus* OX19, these two species share a common polysaccharide antigen (Castaneda, 1934). The sera of infected typhus patients agglutinates *Proteus* OX19 and this test is now standard (Weil–Felix reaction) for diagnosis of the acute disease. Additionally, *R. prowazeki* has two major antigenic components—one heat labile and the other heat stable (Craigie *et al.,* 1946; Topping *et al.,* 1945). Typhus fever vaccine contains killed organisms propagated in the yolk-sac membranes of developing chick embryos (Cox, 1948). This vaccine not only diminishes the symptoms of typhus in immunized persons, but it also greatly reduces the mortality rate (Gilliam, 1946). The usefulness of the attenuated (Madrid E strain) vaccine is hampered because, under appropriate conditions, the strain may revert to virulence (Brezina, 1982).

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