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## Review

# Immunoprophylaxis against important virus diseases of horses, farm animals and birds

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## ABSTRACT

Since the refinement of tissue culture techniques for virus isolation and propagation from the mid 1960s onwards, veterinary virology has received much academic and industrial interest, and has now become a major global industry largely centred on vaccine development against economically important virus diseases of food animals. Bio-tech approaches have been widely used for improved vaccines development. While many viral diseases are controlled through vaccination, many still lack safe and efficacious vaccines. Additional challenges faced by academia, industry and governments are likely to come from viruses jumping species and also from the emergence of virulent variants of established viruses due to natural mutations. Also viral ecology is changing as the respective vectors adapt to new habitats as has been shown in the recent incursion by bluetongue virus into Europe. In this paper the current vaccines for livestock, horses and birds are described in a species by species order. The new promising bio-tech approaches using reverse genetics, non-replicating viral vectors, alpha virus vectors and genetic vaccines in conjunction with better adjuvants and better ways of vaccine delivery are discussed as well.

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## 1. Introduction

The first pioneering demonstration of the principle of vaccination to control a highly pathogenic infection was for smallpox in humans at the turn of the 19th century by the English doctor Edward Jenner (1749–1823) who used the ruminant counterpart of small pox, cowpox virus [1]. Since this early vaccine, the principle has been applied to control diseases caused by members of many virus families affecting humans and animals with much benefit. Although many viral diseases of production animals are truly global, there are viruses that are at present only found in some parts of the world. These viruses may however spread to other geographical regions and/or broaden their host range as has been the case for Bluetongue Virus and West Nile virus (WNV). The global viral diseases have vaccines against them while many of the regional viral diseases have no immunoprophylaxis for their control. In this respect, noteworthy example is that for the newly emergent paramyxoviruses in the genus nipah virus.

The goal of vaccination is to induce immunity in a particular species to prevent clinical disease, excretion or infection by a pathogenic micro-organism. Since the first smallpox vaccine by Jenner, numerous different approaches for vaccination have been developed. Traditionally vaccine formulations are divided in two major classes, live and inactivated (Table 1). Live vaccines contain

attenuated or non-pathogenic strain of a pathogen or an expression vector. Such a vaccine is unable to induce disease but is able to induce suitable immunity; over-attenuation is a potential risk a vaccine scientist has to be aware of. It is the challenge for the vaccine developer to find the right balance between the virulence of the vaccine virus and its ability to replicate sufficiently in order to induce immunity. Attenuation can be achieved by many different ways (some examples are given in Table 1). For this, approaches used have been passaging of a pathogen in cell cultures, derivation and/or isolation of temperature sensitive mutants, which are restricted for significant replication in internal body organs. The use of related apathogenic strain by Jenner is yet another example. More modern methodology would involve the use of molecular biological techniques to remove virulence genes from a pathogen or to engineer non-pathogenic, replicating agents to express immunity inducing antigenic epitopes of a pathogen. Replication-defective vectors have also been developed as safe effective vaccines for some viral diseases. Live vaccines generally induce both Th1 and Th2 immune response and have been used in situations where antibody responses are not correlated with protection or where existing circulating antibody interferes with the induction of immunological response; interference due to maternally derived antibody (MDA) in un-weaned host is a common problem, particularly for killed vaccines administered parenterally.

For many viral diseases, killed or inactivated vaccines have been widely used as a safe option. Killed vaccines generally induce humoral immune responses and often require the use of adjuvants to boost the immune response. Adjuvants can play a major role in the direction of the immune response. Aluminium hydroxide for instance is a strong inducer of humoral immune responses, whereas others such as saponins and derivatives may induce cellular responses as well [2, and references therein]. As is the case with live vaccines, different inactivated vaccine formulations are known, such as inactivated whole virus vaccines, subunit vaccines, peptide vaccines, and split virion vaccines.

In this overview, we look both at existing vaccines and at current approaches towards developing new, prospective vaccines for livestock, horses and birds. At this stage it is relevant to refer to the regulatory legislation controlling viral diseases of livestock in the European Union [3–5] and the vaccine development process from an industrial perspective as such [2]. It is important to point out in this respect that essential performance data are frequently limited to company registration dossiers and little information on safety and efficacy is therefore in the public domain. For many diseases

**Table 1**  
Examples of classes of vaccine formulations.

Class	Subclass	Example/reference <sup>a</sup>
Live vaccine	Passaging	PRRSV, human polio
	Cold-adapted/temperature sensitive	Equine influenza [18] Equine herpesvirus [6]
	Vector	Bovine adeno-FMDV [126]
	Deletion mutant	Bovine herpesvirus-1 [31]
	Chimera	Aujeski-CSFV [69]; WNV-yellow fever virus [21]
Inactivated	Whole virus	FMDV, AI
	Subunit	African horse sickness VP2 [24]
	Split virion Expression	Human influenza CSFV [66]

This table serves as an example of currently used vaccine formulations and is not meant to be a complete list.

<sup>a</sup> See text and reference for abbreviation and detail.

validated challenge models to assess efficacy are non-existent and moreover, for some vaccines, efficacy data often come from field trials. Notwithstanding these drawbacks, in this article views on the shortcomings of some vaccines are offered and some ideas for improvements are proposed.

## 2. Horse viruses

### 2.1. Herpes viruses

Of the five herpes viruses naturally infecting horses, two alpha viruses, equine herpesvirus-1 (EHV-1) and herpesvirus-4 (EHV-4), are important. Both EHV-1 and EHV-4 occur worldwide and cause febrile respiratory disease in all types of horses. Additionally, EHV-1 is associated with abortions and/or paresis and late-term transplacental infection and neonatal foal disease. The biology and control of EHV-1 and EHV-4 infections has been recently reviewed [6].

Vaccines against both viruses are in common use. There are at least 12 multivalent, mostly killed, vaccines using tissue culture grown EHV-1 and EHV-4, and many also contain egg-grown equine influenza H3N8 and H7N7 viruses. Most products, however, only claim protection against respiratory diseases due to EHV-1 and EHV-4 and none is protective in foals with MDA, which are considered important in the epidemiology and transmission of these viruses [see [6]].

Two vaccines, one in the EU [7] and one in the USA [see [6]], claim protection against EHV-1 abortion after three parenteral vaccinations at the 5th, 7th and 9th months of gestation, but these vaccines have been found to offer limited protection under field conditions. It is generally accepted that vaccines with improved efficacy, particularly those with an ability to protect against EHV-1 abortion and paresis, and EHV-1 and EHV-4 infection of passively immune un-weaned foals (and to prevent spread of infection) are required. However, much effort towards the goal to derive deletion mutant live EHV-1 vaccines on similar lines to other alpha herpes viruses, notably pseudorabies virus (PRV) and bovine herpesvirus-1 (BHV-1) have been disappointing [6].

An experimental live temperature sensitive (ts) EHV-1 vaccine has shown remarkable efficacy against abortions and paresis after EHV-1 challenge of pregnant mares 6 months after a single intranasal (IN) vaccination [8]. This experimental vaccine also cross-protected yearlings against EHV-4 febrile respiratory disease and virus shedding [9] and was also significantly protective to foals with MDA to EHV-1 and EHV-4 upon EHV-1 challenge [10].

### 2.2. Equine influenza

Three important considerations in the epidemiology and control of equine influenza viruses (EIV) infections are (1) that 70% of a given population of horses needs to be fully vaccinated to prevent epidemics of influenza [11], (2) that there is no cross-protection between antibodies to H7N7 and H3N8 subtypes of EIV [12], and (3) that the incidence of EIV infections is 50–60% in countries with horse breeding and racing industries. A majority of commercially available EIV vaccines have egg-grown EIV of both subtypes and are either killed whole virus or EIV surface glycoprotein subunit vaccines containing different adjuvants [13]. These vaccines, however, induce poor or no cytotoxic T lymphocyte (CTL) response, and protection is usually short-lived and associated with the titre of antibody to virus haemagglutinin (HA) [13].

The quality of EIV vaccines varies. Some killed whole virus vaccines were found to protect naïve horses for at least 1 year after three vaccinations at 5, 6 and 12 months of age [14–16]. An impor-

tant observation was the induction of tolerance by killed vaccines in horses with MDA [17]. It is known for a long time that much more durable responses (humoral and cellular immunity, conferring protection) occur following EIV infection. Thus, live attenuated EIV vaccines mimicking natural infection should afford far superior protection than killed vaccines. However, in reality only one live attenuated ts (cold-adapted) H3N8 strain vaccine has come on the market in the USA but nowhere else yet [18]. In the EU, EIV is offered as two separate canary poxvirus recombinants expressing the HA of H3N8 EIV of European and American lineages. This vaccine, formulated in carbomer adjuvant was shown to induce both humoral and cellular immune responses [13]. Iscomatrix adjuvanted vaccines induce cellular immune responses in various animal models as well [19], although not evidenced in the horse yet. The optimal vaccination schedule for equine influenza is currently under evaluation [20]. Not all vaccines offer the best protection upon a traditional vaccination schedule [17], whereas others do [2].

### 2.3. Equine encephalomyelitis

American horses are end-hosts to three distinct serotypes of an alpha virus known as equine encephalomyelitis virus (EEV), geographically defined as Eastern USA (EEEV), Western USA (WEEV) and Venezuelan (VEEV) serotypes. All three serotypes are carried by healthy small rodents and birds and are transmitted by mosquito bites with the virus carried in the saliva. The insect bite results in viraemia and visceral viral replication accompanied by fever, depression, anorexia and death. VEEV replication is usually confined to the viscera with 10–20% mortality while WEEV and EEEV, after visceral virus replication, also localise in the central nervous system (CNS), resulting in 20–30% and 80–90% mortality, respectively. Immunoprophylaxis against these viruses is with killed, adjuvanted vaccines, some also containing WNV. For instance, Innovator EWT (Fort Dodge Animal Health) contains WNV, EEEV, WEEV and tetanus toxoid.

The horse is an end-host to WNV, which is prevalent in countries where mosquitoes thrive, notably in parts of Africa, the Middle East, Central and North America and Canada, where it became endemic over the last decade. WNV epidemiology and pathogenesis is similar to EEV. Three WNV vaccines are currently marketed for use in horses. WNV, like EEV, causes significant disease in other animal species including man. Current WNV vaccines are (1) a trivalent WNV, EEEV and tetanus product, (2) a canary poxvirus vectored live vaccine and (3) a chimerical WNV glycoprotein-yellow fever virus backbone vaccine, which is also to be licensed for human immunisation [21].

### 2.4. African horse sickness

African horse sickness (AHS) is a highly fatal, insect (*Culicoides* spp.) transmitted disease of Equidae (horses, mules, donkeys and zebras) caused by an AHS virus (AHSV), which is in the genus Orbivirus of the family Reoviridae. AHSV is pantropic, with pneumonic and visceral replication and is endemic in parts of Africa [22] and has sporadically crossed into Southern Europe and parts of the Middle East [23]. Annual vaccination is with polyvalent multi-serotype mouse-brain or tissue culture grown live virus vaccine. Future vaccines may use AHSV surface VP2 protein as a subunit bio-tech vaccine [24] or VP2 plasmid as a DNA vaccine. The pathogenesis and epidemiology of AHS have similarities to infection of ruminants by another important veterinary Orbivirus namely blue-tongue virus (see Sheep viruses below). For both viruses, biting midges of species *Culicoides* imicola and others are important in transmission. Although AHS is an important disease of equidae, the economy has not warranted vaccine development for US or EU markets.

## 2.5. Equine arteritis

Equine arteritis virus (EAV) occurs mainly in the USA and Northern Europe and causes haemorrhagic necrotic lesions in medium size blood vessels, oedema, diarrhoea, colic and abortions. A tissue culture attenuated live virus vaccine is protective, but it is contra-indicated for use in late pregnant mares. A safer option for a live intranasal vaccine might be to derive a temperature sensitive mutant on a similar line to that applied to EHV-1 abortion isolate [8].

## 2.6. Equine infectious anaemia

Equine infectious anaemia virus (EIAV) is widespread in damp areas of USA, Japan and parts of Europe. In horses, EIAV can cause haemolytic anaemia and jaundice, haemorrhages and inflammation of the spleen, liver and kidneys and fever. In general, lentivirus vaccines are difficult to develop, as evidenced by the absence of good vaccines against HIV, FIV and FeLV. However a live attenuated EIAV vaccine, developed in China, has been in widespread use there since 1983. Another live EIAV vaccine is in development in the USA [25]. In the EU, no EIAV vaccines are available.

## 3. Cattle viruses

### 3.1. Bovine respiratory disease complex

In cattle, the most widely marketed vaccines comprise those against viruses and bacteria implicated in bovine respiratory disease complex (BRDC). This is a major cause of economic loss to the cattle industry globally. Apart from *Mannheimia* spp., viruses are the main agents involved, in particular BHV-1, parainfluenza type 3 virus (PI3V), bovine respiratory syncytial virus (BRSV) and bovine viral diarrhoea virus (BVDV).

For these viruses combination vaccines are available [26–28]. BRDC vaccine presentations offered in the EU and the Americas vary with respect to valency and vaccine constitution [29,30]. Thus in the Americas, BRDC vaccines tend to be polyvalent cocktails of viral and bacterial pathogens, both as live and killed preparations, whereas in the EU, BHV-1 and BVDV abortion vaccines from various manufacturers are largely offered as monovalent products while PI3V is marketed in combination with BHV-1 and/or BRSV and *Mannheimia* [30]. Intervet's Bovipast for instance is a vaccine with such a composition (BRSV, PIV-3 and Pasteurella), which is successfully used for many years. There is a growing interest to market live and killed glycoprotein E deficient (gE<sup>-</sup>) BHV-1 vaccines [30,31] with a view for use as marker and DIVA (see below) vaccines in eradication campaigns. Recent studies have shown that an inactivated BVDV vaccine does have some properties of a marker vaccine as antibodies against viral non-structural protein NS3 are not produced or occur at low concentration in vaccinated animals [32]. The latter would however negate the marker vaccine claim since a definitive distinction between vaccinated and field infected cattle would not be possible. Also the efficacy of most registered BHV-1 vaccines is against respiratory disease, namely infectious bovine rhinotracheitis (IBR) and not genital lesions [31]. It is important to be aware that vaccination does not completely prevent virus shedding from the respiratory tract [31,27,28].

BVDV is the major cause of reproduction loss in the cattle industry through transplacental foetal infection [33]. In the Americas, BVDV vaccines with an abortion claim are rare while in the EU, BVDV abortion vaccines are considered important and currently available as Torvac-BVD (Novartis Animal Health) [34], Bovilis BVDV (Intervet International) [35] and Preg Sure BVD (Pfizer Animal Health) [36]. An interesting observation comparing these three BVDV abor-

tion claim studies is the method of assessment of the incidence of transplacental infection due to BVDV challenge. In one study animals were killed at fixed times (69–72) following the challenge and pregnancy rates assessed. The other two abortion efficacy studies allowed the ongoing pregnancy to go to term naturally when BVDV positive fetuses and newly born calves were assessed for BVDV infection. BVDV challenge methods also differed; a natural challenge by 3 persistently BVDV infected calves was performed in one study [35] whereas BVDV challenge was administered by inoculation in other two studies [34,36]. The natural challenge infected all animals quickly; interestingly the measured challenge administered by inoculation was not uniformly infectious for the foetus [34] indicating that the challenge was under potent.

The currently available BHV-1 vaccines do not completely prevent virus shedding and febrile respiratory disease, and they differ in potency [31]. In the future more BRDC vaccines will include type 2 BVDV strains both to control abortions besides the main claims on the prevention of respiratory disease.

For the control of BRDC, better protection against BRSV is desirable and approaches being investigated are live deletion mutants lacking NS1, NS2, G, SH and/or M2 genes derived using reverse genetics techniques as well as isolating cold-adapted or producing ts strains. A problem with live BRSV vaccines is that wild type virus strains grow poorly in tissue culture but good and consistent growth is a critical requirement in vaccine manufacture. Good growth is even less likely with deletion mutant BRSV strains. An interesting approach to overcome poor and inconsistent yield of BRSV antigens has been to derive persistently infected BRSV bovine cell lines [37]. All cells in such cell lines are viable and express BRSV antigens upon routine subculture [37]. A further handicap in BRSV efficacy studies is the lack of a reliable and pathogenic challenge model for BRSV in cattle, and particularly in un-weaned calves with maternal antibodies [37]. This is also a problem with the PI3V component of BRDC.

There are a number of new vaccine ideas for BVDV. These include selecting live attenuated strains using bio-tech techniques to identify virulence-associated genes and remove them, expression of virus E2 glycoprotein in vectors, both non-replicating (NRVV—see below) and replicating, subunit vaccines produced in a variety of expression vectors, and the use of new mucosal adjuvants such as multi-component liposomes. One attractive proposal is to develop an edible vaccine, in transgenic clover for example [38]. It is however questionable whether this approach is feasible from a regulatory and economic perspective [2].

A classical vector virus-based but somewhat unorthodox approach was taken recently [39] for a BVDV vaccine where the workers assessed the efficacy of EHV-1 recombinant virus expressing BVDV type 1 structural proteins C, E<sup>RNS</sup>, E1 and E2. Simmental calves were vaccinated intramuscularly (IM) twice about 3 weeks apart and challenged IN with BVDV type 1b strain. This unusual approach gave disappointing results and protection against viraemia, leucopenia and nasal shedding was partial along with a poor virus neutralising antibody response. A more direct vector approach [40] was that of expressing BVDV type 1 E2 glycoprotein in BHV-1 but to the best of our knowledge the recombinant has not been tested for efficacy.

Currently, the control of bovine diarrhoea is to immunise pregnant cattle to elevate their milk antibody titres against enteric viruses and bacteria [41]. Three killed polyvalent, rota virus, corona virus and *Escherichia coli* F5 antigen vaccines and one with live viruses and killed *E. coli* are presently marketed.

A general criticism of current BRDC vaccines is that the currently licensed vaccines were assessed using different challenge models (challenge virus strains, animals of differing ages, backgrounds and doses and strains of challenge virus inocula). It is paramount that a unified and validated challenge method is established and used

by vaccine companies in addition to the same validated laboratory tests and reagents.

### 3.2. Foot-and-mouth disease

The European Union stopped routine vaccination against foot-and-mouth disease virus (FMDV) at the end of 1991 and the control of sporadic outbreaks of FMD within the EU is now by slaughter, movement control, stamping out and disinfection of infected areas [4]. In the EU, legislation foresees the need for emergency vaccination. For this eventuality EU has FMDV antigen banks for all seven serotypes [4]. The policy of ring vaccination and culling contributed in FMD control in the Netherlands's 2001 outbreak. However, the development of serological tests to discriminate vaccinated from infected animals is considered desirable in emergency vaccination, the so-called DIVA principle (see below). In endemic areas, killed whole virus vaccines with different adjuvants in single or multi-serotype formulations are used in large quantities. Here it is important to highlight the difficulties in the immunoprophylaxis against FMD. Lack of cross-reactivity between the seven serotypes and the virus strain variation in each serotype pose major difficulty in diagnosis and control. This is further compounded by continued circulation (in endemic regions) of the virus, and high mutation rates ( $10^{-3}$  to  $10^{-5}$  per nucleotide site) per genome replication add to the difficulty in vaccine-based control of disease in face of increasing globalisation [42,43,5].

Currently, all FMD vaccines are produced by growing live virus in BHK-21 cells in roller bottles or in suspension under bio-secure conditions in large volumes. The antigen is then inactivated and blended with an adjuvant. The current killed FMDV vaccines are effective in terms of clinical protection, but do not give sterile immunity. Also they do not elicit a quick onset and long lasting immunity. In general the onset of immunity is 3–4 weeks after primary vaccination. These difficulties aside, there is much effort, some based on viral vectors (Sendai virus, replication-defective adenovirus, Capri pox virus and vaccinia virus) to express VN epitopes of FMDV VP1 protein and/or peptides thereof, T cell epitopes on viral non-structural proteins and selected cytokine genes. Some adenovirus-FMDV and/or cytokine recombinants are promising vaccine candidates with respect of quicker onset and longer duration of immunity as well as fulfilling marker and DIVA vaccine criteria (see below). This work is ongoing [5,44,45]. Some of these vectors may be developed to deliver protective epitopes from antigens of other important disease causing viruses of ruminants in order to have multi-disease vaccine [5].

It is also pertinent to note that a limiting factor in FMD virus R&D is the few laboratories in the world able to work with live virus. The core laboratories in the Global FMD Alliance (GFRA) with their aim to research, design, construct and develop a new generation of vaccines are: (1) the Pirbright Laboratory at the Institute of Animal Health in UK; (2) the joint US Departments of Agriculture and Homeland Security Laboratory at Plum Island, New York, USA; (3) the Australian Animal Health Laboratory at Geelong; (4) the National Centre for Foreign Animal Diseases, Winnipeg, Canada; (5) the International Livestock Research Institute (ILRI), Nairobi, Kenya.

### 3.3. Rinderpest

Rinderpest, or cattle plague, is a highly contagious infection of cattle spread by virus aerosol, typically causing fever, erosion of the mucous membranes of the mouth and the upper respiratory tract and severe diarrhoea, leading to dehydration and death with a case mortality of 50–90%. The causative virus (RPV) belongs to the genus Morbillivirus in the family Paramyxoviridae and the disease is now confined to parts of Africa. In enzootic areas, mass vaccination is practised with calf kidney cell attenuated live virus vaccine and

the disease has nearly been eradicated. This vaccine is also used to protect sheep and goats against related Morbillivirus peste des petits ruminants (PPRV). A conventional vaccine is effective [46].

There is ongoing work in UK and French laboratories to develop and test new capri pox virus vector vaccines aimed at controlling major virus diseases in Africa and RPV is one candidate on their list [47–49,5].

### 3.4. Lumpy skin disease

Lumpy skin disease (LSD) of cattle is caused by strains of Capri poxvirus, the LSD virus (LSDV). The disease is characterised by fever, nodules on the skin, mucous membranes and internal organs, subcutaneous oedema, enlarged superficial lymph nodes and sometimes death and abortions. In enzootic areas (parts of Africa) live vaccines of cattle, sheep and goat origins are used. These poxviruses affect 250 million cattle as well as 650 million sheep and goats in developing countries, particularly Africa. As mentioned above, there is much expert effort to develop new capri pox virus vectors as multi-disease vaccines. Should LSDV cross into the EU, control measures would be as those applied to other exotic notifiable infections such as FMDV.

### 3.5. Vesicular stomatitis

Vesicular stomatitis virus (VSV) causes an acute viral disease of cattle, horses, deer and pigs and occasionally humans. The disease in cattle resembles FMD while in pigs the lesions are similar to other viral vesicular diseases. VSV occurs in the Americas. VSV has not been seen in Europe since 1919 and then for the first and only time. The mode of virus transmission is not fully known, but both mechanical spread by mosquitoes and sand flies and contact spread are considered likely. Common signs of infection are excessive salivation and vesicles in buccal cavity, hard palate, lips, gums, tongue and teats (which may lead to mastitis). Fever, and weight and milk yield losses are additional effects of VSV infection.

### 3.6. Rabies

Cattle are occasionally hosts to rabies virus. In the EU, five killed rabies virus vaccines are marketed [30] and are recommended for use along with wildlife control, mostly of rabies in the red fox (*Vulpes vulpes*) using oral vaccine delivered with baits of fishmeal, fat and paraffin containing attenuated rabies virus or replicating recombinant vaccinia virus expressing rabies virus glycoprotein. The latter vaccine has been highly effective in reducing the incidence of wildlife rabies in mainland Europe [50,51,4]. Rabies is a major disease in cattle in areas of Latin America with vampire bats. The disease is commonly known as 'derriengue or paralytic rabies'. Control of bovine paralytic rabies in Latin American countries since late 1960s has focused on vaccination of cattle and measures to reduce vampire bat populations with variable success.

## 4. Pig viruses

Viruses from many different families infect domestic pigs and can cause significant economic losses. However, the level of sophistication of approaches used towards vaccine development is quite variable depending on the virus. Molecular biology has been usefully applied to derive candidate live attenuated or subunit marker vaccines for some virus diseases, notably those caused by PRV and classical swine fever virus (CSFV). In the case of PRV, the identification of virulence-associated, non-essential and essential genes and their functions [52–54] has led to development of effective and

safe conventional as well as bio-tech deletion mutant marker vaccines [55–59]. An interesting finding for live PRV vaccines was the superior protection that was given if the vaccines were adjuvanted [60,61].

#### 4.1. Classical swine fever

Routine prophylactic vaccination against CSFV in the EU ceased in 1990, but there is still a provision for emergency vaccination under certain circumstances [4]. This aside, however, oral vaccination of wild boars (*Sus scrofa scrofa*) with a live Chinese (C-) strain CSFV vaccine has been conducted in France [62] and Germany [63] in order to improve herd immunity. This is probably due to reduced virus shedding by vaccinated boars. The ability to discriminate vaccinated from infected animals serologically using bio-tech vaccines should be carefully assessed.

Outside the EU, CSFV vaccination of domestic pigs is widely practised using a live C-strain vaccine as well as a bio-tech CSFV E2 glycoprotein subunit marker vaccine [64,59,65]. The application of bio-tech techniques for the development of experimental or commercial vaccines includes the use of E2 subunit marker vaccines expressed in baculo virus [66], E2 subunit vaccine [67], a chimeric BVDV–CSFV marker vaccine [68], and recombinant PRV expressing CSFV glycoprotein that has been shown to be protective for diseases due to PRV and CSFV [69].

#### 4.2. Porcine reproductive and respiratory syndrome

Porcine reproductive and respiratory syndrome (PRRSV) or porcine epidemic abortion syndrome (PEARS) is due to an arterivirus (related to EAV) and is an economically important, relatively recent disease of domestic pigs, first identified in the USA and Canada in 1987 [70]. The disease struck Germany in the winter of 1990, spread to the Netherlands in 1991 [71] and then to other parts of the EU. PRRS is characterised by high pre-weaning mortality and high reproductive losses.

The type of vaccine to be used was a controversial issue, but PRRSV vaccine manufacturers finally opted for live attenuated vaccines derived by passage in monkey kidney cells (MA104). Killed vaccines do not command a significant share of the market and are poorly protective [72]. There remains a clear need for more effective vaccines but this challenge is further compounded by the fact that PRRSV readily mutates in the pig in a single episode, resulting in selection and perpetuation of more pathogenic strains in naïve pigs which have lower protective cover from existing vaccines.

#### 4.3. Foot-and-mouth disease

As in cattle and other ruminants, FMDV is an important cause of disease in domestic pigs and its control in the EU is similar to that in cattle. The domestic pig is also a host to important diseases caused by other picornaviruses, notably swine vesicular disease virus (SVDV), Teschen disease virus (TDV) and encephalomyocarditis virus (ECMV). These three picornaviruses are transmitted by the oropharyngeal route. SVDV is a notifiable virus and vaccination is not allowed in the EU although outbreaks of SVDV have occurred in EU countries notably the Netherlands, Italy and Portugal in the 1990s and also in early 2000s. The SVDV disease is very similar to FMD and the virus is antigenically related to human Coxsackie B5 virus. TDV vaccines are not available and infections are rarely diagnosed. The main clinical signs of TDV infection are in coordination and paralysis. The source of some primary outbreaks of SVDV disease in Europe was unknown, the route of SVDV transmission was frequently obscure [4]. The Americas are currently free of SVDV.

#### 4.4. African swine fever

African swine fever (ASF) is a highly contagious, fatal haemorrhagic disease of domestic pigs caused by a large double stranded DNA virus (ASFV) in the family Asfarviridae. In the EU it is a notifiable disease [4]. ASFV is enzootic in most Sub-Saharan countries and has crossed the Atlantic Ocean and outbreaks were recorded in some South American countries and some Caribbean islands. In 1980s outbreaks ASF occurred in Europe (Iberian Peninsula, Sardinia and sporadically in France and Belgium). The European foci of ASFV infections were eradicated through slaughter policy. Infected pigs shed virus in all excretions and live virus spreads both horizontally and vertically and by bites of infected soft ticks (*Ornithodoros moubata porcinos*) [73] in which the virus replicates. Killed vaccines have failed to protect against the disease while live vaccines revert to virulence or fail to infect pigs. ASFV in Sub-Saharan Africa exists in the wild through a cycle of infection between soft ticks of genus *Ornithodoros* and wild swine (warthogs and bush pigs) which remain clinically normal. This virus epidemiology makes eradication difficult. The incursion by ASFV into Europe continues. Georgia recently (7th June 2007) officially reported ASF and the virus has rapidly spread throughout Georgia with high mortality in pig populations. Outbreaks of ASF have also occurred in nearby Armenia and recently also Russia has reported ASF.

An effective safe ASFV vaccine is clearly required and there are indications that this is possible [74]. The protective effect of ASFV vaccine is attributed to structural virus proteins, which are produced as long as the vaccine virus was propagated in animals and/or pig macrophage cultures; genes of these protective antigens were lost while the virus was adapted to grow in conventional tissue culture. It was concluded that the protective proteins were not essential for virus replication [74,75]. Tissue culture is essential for vaccine production for both conventional and bio-tech virus-based vaccines [2]. It may be possible to construct a recombinant virus (ASFV or other) in which these genes are in a stable configuration but first the protective genes need to be identified and characterised.

#### 4.5. Swine influenza

Historically, two pathogenic subtypes (H1N1 and H3N2) of swine influenza virus (SIV) co-circulate in domestic pigs in the EU and the Americas. A new SIV subtype, H1N2 has recently emerged in parts of the EU [76]. Killed SIV vaccines containing H1N1 and H3N2 SIVs are available. Naïve pigs vaccinated with H1N1 SIV vaccine were not fully protected against H1N2 challenge [76]. Despite the observation that SIV can be the main cause of porcine respiratory disease complex [77], little attention has been paid to routine vaccination of pigs against influenza and the development of improved efficacious vaccines. In this regard an experimental vaccine against a recently emergent reassortant H3N2 subtype SIV in the USA was developed. The HA and the nucleoprotein (NP) genes of the virus were expressed in replication-defective human adenovirus 5. This recombinant SIV vaccine was fully protective for piglets in a controlled challenge study [78].

#### 4.6. Porcine circovirus-2

Porcine circovirus-2 (PCV-2), a member of the genus circovirus of the family Circoviridae has been associated with post-weaning multisystemic wasting syndrome (PMWS) and also dermatitis, nephropathy syndrome, abortion and reproductive failure [79,80]. PMWS was first recorded in the USA and Canada in the late 1980s and in the EU in the mid 1990s.

Currently 4 companies market PCV-2 killed vaccines containing an adjuvant. These vaccines are: porcine kidney (PK-15) cell line

grown PCV-2; two PCV-2 ORF-2 capsid protein expressed in baculo virus; and a chimera of PCV-2 ORF-2 gene on PCV-1 backbone. The subunit capsid protein vaccines are sold in the USA, EU, Latin America, some Asian countries while the conventionally produced PK-15 vaccine has marketing authorisation for some EU countries. The PCV-ORF2 vaccines have demonstrated good efficacy in the US and Canada. Interestingly, the chimera of PCV-2 ORF-2 on apathogenic PCV-1 backbone [81] is not offered as a live vaccine. A reason for this may be difficulties in licensing a live GMO product in EU. The DNA clone of the PCV-2 and PCV-1 chimera was also protective in challenged pigs [81]. In insect cells, baculo virus construct produced self-assembling virus-like particles which when combined with an adjuvant induced both Th1 and Th2 immune responses in piglets [82].

Other vaccine approaches investigated are PCV-2 ORF1-ORF 2 fusion genes inserted into an attenuated PRV strain. This live recombinant virus was immunogenic in pigs for both PCV-2 and PRV; inoculated pigs produced PCV-2 T lymphocytes and neutralising antibodies [83]. PCV-2 ORF 2 capsid gene has been expressed in adenovirus and the live recombinant was protective for pigs [84,85]. PCV-2 isolates vary genetically and pathologically [86]. Therefore current and new vaccines have to be broadly protective against all or most strains; however a current vaccine strain claims genetic stability for PCV-2. Recently a new serotype of the virus has emerged in the USA causing considerable losses in pigs nearing the fattening stage. Studies have however shown that PCV-2a infection immunity can protect against challenge with PCV-2b and vice versa in experimental pig models [87].

#### 4.7. Parvovirus

The main consequence of porcine parvovirus is transplacental infection, which in early pregnancy causes resorption. In subsequent stages, transplacental infection results in stillbirths, mummification and embryonic death.

The key stages of pathogenesis are virus ingestion, virus growth in the small intestine, viraemia, secondary infection of dividing cells in the endometrium followed by transplacental infection of embryo or foetus. Both live and killed vaccines are marketed by several manufacturers. Parvovirus vaccines do significantly prevent transplacental transmission of the virus and were shown to be economically profitable [88]. In Europe the products are mostly killed, but live vaccines are more generally available in the USA.

## 5. Sheep viruses

### 5.1. Bluetongue

Bluetongue virus (BTV) in the genus Orbivirus of the family Reoviridae currently has 24 serotypes and members have segmented RNA genome, which can reassort in vivo. BTV is non-contagious and virus is transmitted biologically by approximately 30 species of biting midges of the genus *Culicoides*. In endemic areas different species of midges are involved in BTV transmission. BT was first described in South Africa and has since been recognised in most countries of the tropics and sub-tropics. In Europe, eight BTV serotypes have been identified in the Mediterranean basin. In Africa, the Middle East and Asia *Culicoides imicola* are important vectors of BTV transmission (accounting for at least 90% of disease transmission) while other midges (the Palaearctic endemic species of *C. pulicaris* and *C. obsoletus*) are important vectors in Northern Europe but other species of midges may also become important in BTV transmission in the future. The first reported incursion of BTV in Europe was between 1956 and 1960 to Portugal and Spain. Between 1998 and 2005, five BTV serotypes (BTV 1, BTV 2, BTV 4, BTV 9 and BTV 16) swept the Mediterranean basin and affected 15 countries.

In 2006, BTV serotype 8 appeared in Northern Europe [89,90]. All the ruminants including sheep, goats, cattle buffaloes, antelopes, deer and camels are susceptible to BTV infection. The virus has also been the cause of severe disease and deaths in white-tailed deer in the USA. However BT in sheep has its most significant clinical pathological and economic impact in temperate areas of the world. While infection of animals in tropical and sub-tropical countries is common, clinical disease in indigenous species is unusual. After infection via the saliva of a biting midge, virus undergoes primary replication in the regional lymph nodes from where virus is carried in blood leukocytes to target tissues for secondary replication and then results in pathology. In sheep common disease signs and lesions are fever, hyperaemia, cyanosis of the mouth and tongue, diarrhoea and mortality. In pregnant ewes, virus may cross the placenta and lead to foetal infection causing abortion, mummification or birth of stillborn or weak lambs [91]. BTV can cause some disease in cattle. In the current BTV-8 outbreak in Northern Europe, sporadic episodes of disease and deaths in a small proportion of infected cattle herds have been consistently recorded [Elbers ARW and others 2007].<sup>1</sup> In cattle the clinical signs commonly observed have been crusty lesions of nasal mucous membrane, salivation, fever, hyperaemia and apathy. But significantly, cattle are important reservoir and virus amplifying hosts as midges feed more frequently on cattle. An important development in BTV epidemiology is the recent evidence for transplacental and contact transmission of BTV in cattle [92].

Lack of significant cross-protection between serotypes makes control of BT with vaccines a difficult task. Notwithstanding this difficulty, immunoprophylaxis tried have been killed, live and biotech vaccines. Important drawbacks against killed vaccines have been the requirement of high antigen mass and 2 doses for some ruminants in order to elicit active immunity. While, these drawbacks are not an issue for live BTV vaccines, they however have the potential to revert to virulence through reassortment [89], they cross the placenta, and may be shed in semen. These risks aside, live vaccines have been used extensively and successfully in sheep in South Africa, Israel, and the USA and limitedly in the Mediterranean Basin. Onderstepoort Biological Products Ltd in South Africa is a major manufacturer of live BTV vaccines and it is where EU has BTV vaccine bank. For the current BTV-8 outbreak, killed vaccines from 2 companies have been commissioned for use as a single dose for sheep and two doses in cattle. The BTV-8 vaccination campaign in Northern Europe should yield useful data in the future.

Newer, bio-tech approaches have been investigated, however, and hold much promise. BTV-like and virus core-like structures have been constructed using virus major (VP3, VP7) and minor (VP1, VP4, VP5) capsid proteins. The latter were produced in baculo virus multiple expression vectors. These virus-like, single and double-shelled particles emulsified in Freund's incomplete adjuvant, or Montanide ISA-50 adjuvant, were highly immunogenic and protective for naïve sheep [93]. The approach looks hopeful since baculo virus expression vectors for different BTV serotypes could be prepared in advance and stored in a bank on similar lines to FMDV serotypes [4] and brought out when an outbreak starts.

### 5.2. Capripox

Poxviruses of sheep and goats (Capri poxviruses) are closely related, but virus isolates tend to be host specific in disease severity. Sheep pox is a notifiable disease in the EU. It is prevalent in Africa, Asia, the Middle East, and the Mediterranean. Capri pox viruses affect 650 million sheep and goats in developing countries. The sheep pox virus (SPV) is highly contagious and commonly

<sup>1</sup> [www.efsa.europa.eu](http://www.efsa.europa.eu).



aerosol spread and infection in sheep is often fatal although recovered animals are immune and passive immunity is conferred via colostrum.

A variety of live vaccines exist and most are given annually. Current vaccines are effective but some cause injection site lesions but work is ongoing to develop better vaccines. The aims for new vaccines are to provide one vaccine to protect against sheep and goat pox and preferably also against other major virus diseases of ruminants along with a longer duration of immunity. Vaccines effective in the young in face of maternal antibodies would also be a step forward. This work is ongoing for multivalent capripox—RPV, PPRV, FMDV and BTV [47,5,94,49,48].

### 5.3. Other sheep viruses

Sheep are susceptible to FMD and are an important host in FMD epidemiology. Some European companies offer killed vaccines containing oil as single or double emulsion or aluminium salts plus saponin as adjuvant for sheep immunisation in FMD endemic areas on similar lines to cattle and pigs.

Rabies vaccination of sheep is indicated in areas where wildlife (foxes, skunks, raccoons and bats) rabies is endemic. There are killed adjuvanted vaccines available.

Louping-ill virus (LIV) is a typical flavivirus and it has been reported in parts of British Isles and some European countries; ticks, particularly *Ixodes ricinus* is considered the natural vector in LIV transmission and the reservoir hosts are grouse and shrew. In sheep clinical signs are fever, depression, and anorexia. Virus may invade the central nervous system resulting in muscular tremors, incoordination, ataxia and characteristic louping gait [95]. Killed vaccines are available and one has been shown to be protective [96].

Orf virus in the genus Para poxvirus is considered to cause significant economic loss to sheep farmers. The virus has a more severe effect in neonatal lambs and nursing ewes. Live and killed vaccines are available and in demand. In UK, approaching 2 million doses are used annually. Orf virus has been used to deliver PRV protective glycoproteins and the vector protected pigs from PRV disease after challenge [97].

## 6. Avian viruses

### 6.1. Avian influenza

Avian influenza (AI) is a complex infection of birds caused by viruses of the Influenza A genus [98]. At present, 16 haemagglutinin (H) and 9 neuraminidase (N) subtypes have been recognised and at least in theory, combination of different H and N subtypes is possible but in practice do not arise. The very virulent AI viruses (AIV) belong to the H5 and H7 subtypes, although not all H5 and H7 viruses are highly pathogenic (HPAI). All other AIV cause a milder, primarily respiratory disease (low pathogenic AI, LPAI), unless exacerbated by other infections or environmental conditions.

Since the 1990s, AI infections due to two subtypes, LPAI H9N2 and HPAI H5N1 and H7 AIVs (LPAI H7N2, HPAI: H7N7, H7N3 and H7N1) caused disease in poultry and some also in humans and some have spread across vast areas. There is thus considerable pressure to use vaccination as a part of a control policy. On an international scale, however, this would be a major undertaking since the ecology, particularly the spread and transmission of AIV is complex [99] although there is relatively good understanding of some aspects of AIV epidemiology. Notwithstanding this difficulty, autogenous killed vaccines against some LPAI viruses have been used mainly in turkeys in parts of USA and Italy.

Vaccination against HPAI H5 and H7 AIVs was actively discouraged as it may interfere with serological diagnosis of HPAI

infections. Now, however, the emphasis is changing due to the high rise in HPAI and LPAI infections [98]. Like human influenza virus vaccines, AIV vaccines are grown in chicken eggs. For human influenza vaccines the process is greatly aided by the use of H1N1 human influenza A virus A/PR/8/34 (PR8) to produce high growth PR8-vaccine virus H and N (6 PR8 internal proteins and vaccine virus H and N glycoproteins) reassortants by the method of Kilbourne [100] and reverse genetics [101] to match the epidemiological field situation in the vaccine. For avian influenza H5, however, it has been shown in the field that classically grown, inactivated vaccines formulated with potent adjuvants can offer cross-protection against related pathogenic strains [102]. In the EU, H5 and H7 viruses are in the OIE list A and the outbreaks in poultry are notifiable [2,3]. This is also the case in the rest of the world. Following a major outbreak of HPAI H7N1 virus in Italy in 1999–2000, a targeted preventive vaccination campaign using the differentiating between infected and vaccinated animals (DIVA) strategy was developed and applied in regions where LPAI viruses frequently occurred. This involved vaccination with an inactivated LPAI virus vaccine followed by regular serotyping of vaccinated birds in order to identify birds with antibodies to HPAI virus and thus onset of HPAI disease. Upon identification of HPAI virus infection, emergency eradication via culling is implemented. AIV vaccination may be taken up elsewhere. The USA currently considers vaccination as one option for its influenza management and India is considering its use. China is using AIV vaccines widely. Vaccination to control AIV disease is however not universally accepted.

Types of AIV vaccines also vary, most being egg grown, inactivated whole virus preparations with an adjuvant. Prospective bio-tech vaccines are being investigated which hold promise. There are several such investigations ongoing; examples are that for the Mexican HPAI H5N2 virus-fowl pox virus recombinant vaccine and Newcastle virus expressing H5 and H7 of AIVs [103–105].

### 6.2. Paramyxoviruses

For avian species, important paramyxoviruses are Newcastle disease virus (NDV), avian paramyxovirus-3 (APMV-3) and turkey rhinotracheitis virus (TRTV). NDV is a worldwide disease of gallinaceous birds—chickens, turkeys, guinea-fowl, pheasants and also pigeons. NDV strains have been classified, based on three tests of virulence, as lentogenic, mesogenic and velogenic, to signify avirulent, moderately and highly virulent isolates, respectively.

Vaccination of poultry is almost universal, using egg grown, live lentogenic NDV vaccines given as coarse spray from 1 day of age or in drinking water from 1 to 3 weeks of age. Lentogenic strains used are Hitchner B1, Ulster 2c, VG/GA; mesogenic LaSota strain has also been used as a live vaccine. The oral or aerosol live NDV immunisation serves to bypass maternal immunity, which is low at these sites. Immunisation against NDV is also offered as killed polyvalent vaccines in combination with IBV, IBDV and/or TRTV (see below for abbreviations of viruses). These polyvalent vaccines are recommended for use as booster vaccines at 14–20 weeks of age following live vaccine priming. Birds are often boosted at point of lay and every 5 months with killed oil adjuvanted vaccines, or mesogenic (La Sota strain) live vaccines. Now it is possible to modify the sequence of virus HN glycoprotein protease cleavage site by reverse genetics in conjunction with infectious C DNA cloning of anti genomic RNA. Also the lentogenic NDV strains are being investigated as a candidate vaccine vector for other poultry diseases, such as HPAI viruses, infectious laryngotracheitis virus (ILT), infectious bursal disease virus (IBDV) to produce multi-disease live vaccines for mass vaccination by spraying. Experimentally the NDV vector approach has been successful in some studies but not all. HA of HPAI H5N2 virus expressed in lentogenic NDV vaccine strain using reverse genetics fully protected spray-vaccinated chickens against

both NDV and HPAI virus diseases [104]. A similarly produced H7 (from LPAI H7N2 virus)-NDV (lentogenic Hitchner B1) vaccine was only partially protective [105]. It should be pointed out that both these experimental vaccines are marker/DIVA vaccines.

APMV-3 is prevalent in Europe, USA and other poultry producing countries and causes respiratory disease and stunting of turkeys. Laying turkeys are vaccinated with killed adjuvanted vaccines. Primary in ovo (see below) live virus vaccines may be more effective. APMV-3 is also a candidate for multivalent turkey virus vaccines with for instance TRT virus and HVT. This may be feasible using HVT (see below) as a vector for APMV-3 and TRTV immunisation of chickens but possibly not for turkeys since apparently HVT did not protect against Marek's disease in turkeys when it was tested in France.

Turkey rhinotracheitis virus (TRTV) belongs to the genus Pneumo virus in the family Paramyxoviridae and was first isolated in the mid 1980s. Respiratory disease is the main consequence in the field. The virus is readily attenuated by passage in eggs or tissue culture. Both live and killed vaccines are marketed but with variation in vaccination regimes. One regime for live TRTV vaccine use comprises spray vaccination of 1-day-old chicks and subsequent booster vaccination. Live vaccines do however commonly result in respiratory disease since vaccine virus is known to revert to virulence upon bird to bird passage. Hence further development of safer vaccines, without loss of efficacy may be warranted. The use of reverse genetics may be helpful in this respect.

An interesting and potentially useful, conventional vaccine for TRT arose from a study reporting isolation of an avirulent TRT virus strain (15a/01) from the wild Canada goose. A live inoculum of the virus was protective in a controlled challenge study in turkeys [106]. For bio-tech vaccines, TRT virus F gene DNA vaccine induced protection in turkeys [107].

### 6.3. Marek's disease

Marek's disease virus (MDV) occurs worldwide and infects chickens of all ages causing visceral, neural and other lesions including T cell tumours and death. MDV strains vary considerably in virulence, and strains of birds show marked differences in susceptibility. Virus spread is by aerosol of cell-free virus shed from feather follicles [108,109].

Turkey herpes virus (HVT) has been widely used as a heterotypic live cell-free virus or cell-associated virus vaccine for broilers. HVT is being investigated as a vaccine vector for other avian viral diseases. All layers are routinely vaccinated with HVT, HVT+SB1 (see below) or CVI 988 (see below). Other MDV vaccines used are tissue culture attenuated serotype 1 CVI 988 Rispens strain [110] and its tissue culture passaged variants [111] but these variants are less effective than CVI 988. Serotype 2 SB1 strain and other tissue culture attenuated serotype 1 strains of MDV have also been commonly used as vaccines. Vaccination is performed in ovo (at 18 days of incubation) or at 1 day of age. Sometimes a booster vaccination is given. Development of immunity may be influenced by viral maternal antibodies, which delay vaccine virus replication [112,109]. MDV field strains are gradually evolving to more pathogenic phenotypes [113,114]. MDV vaccination is by parenteral routes. Cell-associated live MDV (live chick embryo fibroblast cells [CEFC] infected with virus) vaccine is difficult to consistently produce at the required potency (concentration of viable infected CEFC). For each batch of live cell vaccine CEFC are infected under defined conditions and incubated for a set time when viable cells are harvested and cryopreserved in liquid nitrogen.

Combinations of different vaccines against MDV are also used often to counter more pathogenic field MDV infections. SB 1 and HVT combination is used to vaccinate broilers while the preferred combination for layers and breeders is CVI 988 and HVT.

However new vaccines will be needed to control emergent MDV pathotypes.

### 6.4. Infectious laryngotracheitis

Gallid herpesvirus-1 in genus Iltovirus of Alpha herpesvirinae, commonly known as ILTV, is prevalent wherever poultry are kept and spreads by aerosol causing haemorrhagic tracheitis and bronchitis with high (90–100%) morbidity resulting in 10–60% mortality. Conventionally attenuated live virus vaccine is given by spray. Surprisingly, the choice of ILTV vaccines is limited. ILTV is a member of alpha herpesvirinae and there are approaches available for new vaccines for example as those successfully applied to BHV-1 and PRV.

### 6.5. Duck herpes virus

Duck herpesvirus-1 (DHV-1), also known as duck plague or duck viral enteritis, is transmitted by aerosol and is present in most duck producing countries. In ducklings, the virus is pantropic infecting blood vessel endothelium thus giving rise to multiple petechiae generally but particularly in the heart, liver, kidney and intestine, causing high mortality. Live attenuated chicken egg-grown vaccine is given subcutaneously (SC) to ducklings <2 weeks of age. A drawback with DHV-1 vaccine is its availability possibly due to very short shelf life and the low demand.

### 6.6. Avian infectious bronchitis

Avian infectious bronchitis virus (IBV) infects chickens of all ages and breeds and is a common cause of chronic respiratory disease, frequently in dual infection with *Mycoplasma* spp. and/or septicaemia causing *E. coli*. IBV spreads rapidly by aerosol or direct contact, primarily replicating in respiratory and urogenital tract epithelia. Respiratory signs include catarrhal tracheitis, bronchitis and sinusitis while urogenital tract infection results in nephritis.

Chick vaccination is with live egg-grown attenuated virus strains (Massachusetts M41, H120, 4/91, Ma5 and other serotype strains) by spray at 1 day old and in drinking water at 2–3 weeks old. Adult chickens are revaccinated with killed adjuvanted (usually oil) vaccines given parenterally or more virulent live vaccines by the respiratory route. Extensive use of live IBV vaccines has been suggested as the main cause for the emergence of pathogenic IBV variants [115]. These authors developed a vaccine using a recombinant fowl adenovirus expressing the IBV S spike glycoprotein as a strategy to avoid the problem. Demonstrating its further potential would involve precise epidemiological investigations possibly in different geographical locations.

### 6.7. Infectious bursal disease

Serotype 1 of IBDV belonging to the family Birnaviridae and commonly known as Gumboro disease virus is a significant cause of diarrhoea, immunosuppression and mortality in chickens. Virus transmission is via infected food and water, virus primarily replicating in the oropharynx resulting in viraemia and infection of the bursa. Non-pathogenic serotype 2 strains of IBDV from turkeys also occur and the determinant of pathogenicity appears to be the tropism for bursa of Fabricius [116]. A difficulty in controlling IBDV disease in the field is the emergence of variants against which existing vaccines are partially protective.

Control of IBDV is with egg and/or tissue culture attenuated live vaccines. They are given by spray or drinking water when residual MDA has declined sufficiently for the vaccine to take, and laying hens are boosted with killed oily vaccine by the SC or IM route. Serotype 2 IBDV strains cause sub clinical infection in chickens and

turkeys. Efficacy of primary in ovo inoculation of an experimental live IBDV vaccine was reported recently [117]. The next generation of IBDV vaccines are likely to be multivalent bio-tech vaccines namely one vaccine for two or more poultry virus diseases.

### 6.8. Chicken anaemia

Chicken anaemia virus (CAV) belongs to genus gyro virus in the family Ciroviridae and has low morbidity, typically resulting in atrophy of thymus, spleen, caecal tonsils and bursa stemming from infection of precursor bone marrow cells which also results in anaemia. Skin lesions are also commonly observed along with 5–15% mortality [118,119]. Choice of vaccines is limited. A live tissue culture grown vaccine for IM or SC application is on the market.

In the case of vertically transmitted infections due to avian adeno virus (egg drop syndrome), avian reovirus and infectious bursal disease virus (Gumboro disease), adult birds are vaccinated in order to provide passive protection to chicks via maternal antibodies.

## 7. Future developments

### 7.1. New vaccine approaches

There is a good prospect of new improved veterinary virus vaccines in the future. From 1990 onwards, non-replicating virus vectors (NRVVs) were developed with two virus families, avipox and adeno viruses. Recombinant avipox vectors shown to afford protection were those against rabies, canine distemper and poultry viruses including HPAI H5N2 virus [120–123,103]. There are however exceptions as was the case with recombinant canary pox—EHV-1 vaccine [124]. Examples of adenovirus NRVVs are those that have been developed for human adenovirus-5 expressing FMDV capsid and 3C protease tested in pigs, HA and NP genes of newly emergent reassortant SIV in the USA [125] and bovine adenovirus-3 [126].

Replicating vectors have also been developed. The prototype virus vector is vaccinia and for bacteria, attenuated *Mycobacterium bovis*, bacilli Calmette-Guèrin (BCG), isolated in 1938, and Ty21, a strain of *Salmonella typhi*, are being investigated [127]. NDV has been used as live vector as well for instance for to express AI H5 [128] or as a maker vaccine [129].

Other potentially useful approaches being pursued include the generation of chimerical viruses belonging to the same genus and employing full length C-DNA clones used to transcribe infectious virus RNA and exchanging protective gene(s) from vaccine candidate virus with all other genes from donor attenuated virus. This approach has been applied to positive strand RNA viruses such as BVDV-CSFV and WNV-yellow fever virus combinations belonging to genera Pestivirus and Flavivirus, respectively, in the family Flaviviridae [130,21,131].

Reverse genetics has proved a useful technique with negative strand RNA viruses, with and without segmented genomes [132,133,129]. The technology has proved helpful in the mapping of virulence motifs in genes, producing virus chimera, and in deleting and altering undesirable motifs/base sequences in genes thus attenuating the virus and identifying gene function. Rabies virus vectors provide highly stable gene expression [134]. Furthermore, it has been proposed to use recombinant rhabdoviruses as vaccines for HIV and other diseases [135].

Genetic vaccines in the form of DNA vaccines were introduced in the early 1990s and have been investigated for a wide range of infectious and malignant diseases. In contrast to vaccines, which employ recombinant viruses or bacteria, genetic vaccines consist only of DNA (as plasmids) or RNA (as mRNA) or self-replicating genetic vaccines (see below) which are taken up by cell and trans-

lated into proteins. These vaccines are delivered by needle injection (commonly into muscle or skin) or by gene gun for which they are precipitated onto inert particles (usually gold beads). For delivery, mucosal surfaces (respiratory tract or gut) have also been investigated.

Considerable work in this field has identified several parameters for optimal vaccine performance. These include the use of: (1) strong viral promoters to enhance expression; (2) immunodominant epitopes from antigens in the form of mini genes or as buried component(s) within unrelated but highly immunogenic core sequences (particularly for full length toxic or immunosuppressive antigens); (3) defined epitopes for B or T cells; (4) targeting sequences designed to direct intracellular trafficking; (5) helper epitopes such as hepatitis B core-antigen which activate B cells and elicit T cell responses; (6) non-methylated, palindromic DNA sequences containing CpG-oligonucleotides (usually from bacterial DNA) to activate innate immune response via activation of monocytes, natural killer (NK) cells, dendritic cells and B cells.

One promising approach for genetic vaccine development has been the use of alpha viruses such as Sindbis, Semliki Forest, VEEV and others as self-replicating vaccines [136]. These positive-stranded RNA viruses encode their own RNA replicase which functions in a broad range of cells (mammalian, avian, reptilian, amphibian and insect cells) and replication takes place in cytoplasm. A limitation for such vaccines is that they are not recommended for use for diseases due to alpha viruses because of the risk of recombination between the alpha viruses. The usual vaccine construct involves foreign gene(s) in place of the alpha virus structural genes linked to alpha virus replicase and a strong heterologous promoter such as that from cytomegalovirus virus. Theoretically up to 200,000 copies can be produced in single cell within 4 h and expression of encoded antigen can be as much as 25% of the total cell protein [137].

In ovo or embryo vaccination is a growing trend for commercial poultry vaccines to overcome interference due to MDA [138]. This is an interesting development but data directly comparing the new with old methods are required to reach an objective conclusion although the method has been in use for about 20 years. In ovo inoculation of chicken embryos is performed at 18 days of incubation into the amniotic cavity. Interestingly over 80% of commercial broiler flocks in USA receive MDV vaccine by the in ovo route for the primary inoculation. Other live poultry vaccines that could be given by in ovo inoculation include those against NDV, IBV, and IBDV.

### 7.2. Adjuvants and vaccine delivery

Adjuvants and vaccine delivery are important in the design of new killed vaccines and have been discussed in more detail by us and others [2], and references therein. For completeness, however it is pertinent to briefly consider some new thinking – including vaccine delivery methods – that can induce primary immune response in the very young in the face of maternal antibodies after a single inoculation, and delivery systems that provide sustained release of antigen. In the case of the former, mucosal immunisation is favoured—whereas for the latter various approaches are possible such as oral baits, edible vaccines, liposomes, microparticles and polymeric capsules.

Single shot acid-resistant liposome vaccines with multiple components are becoming feasible candidates. The components might include several antigens and multiple adjuvants such as lipophilic muramyl dipeptide derivatives, monophosphoryl lipid A and bacterial toxins, cytokines such as interferon (IFN)- $\gamma$ , interleukin 2 (IL-2) and IL-4. The genetically modified innocuous bacterial toxins (cholera toxin B, *Pseudomonas* exotoxin A, *E. coli* heat stable toxin) are safe candidate mucosal adjuvants [139] and are needed since most infections begin at a mucosal site. Such a 'do-it-all-in-one' lipo-

some approach is attractive but costly. For veterinary vaccines cost is an important aspect for vaccine development and manufacture [2].

There have been important advances in the design of controlled release systems for antigen delivery in vivo. Polymers of polylactic–polyglycolic esters are non-toxic biodegradable vehicles for the slow release of antigens for periods of up to several months depending on the relative amounts of the polymers [140]. An acid-resistant hydrophobic matrix comprising the adjuvant saponin or its derivatives with antigen, cholesterol and phosphatidyl choline, commonly known as Iscoms (immuno-stimulating complexes), are immunogenic by the intramuscular as well as oral/nasal route and are potent T-helper cell (Th 1) adjuvants for enveloped viruses such as influenza virus [141,13,19]. In fact an Iscom equine influenza vaccine is available. An up-to-date account of the technology, ISCOM-MATRIX, in which better defined saponin fractions are used and in which the antigen is not present in the particle [19], is available in the context of equine influenza vaccine as well. Another idea is the use of polyester liquids, which form a gel in vivo after injection, as was the case with 'Atrigel', a drug delivery system used for pseudorabies virus vaccine in pigs [142].

As DNA plasmid vaccine technology is refined further, improved delivery methods are likely to be developed in conjunction with specific cytokine DNA plasmids to preferentially stimulate Th1 and/or Th2 immunity. There is much to look forward to but costs will inevitably decide what is commercially feasible and so what actually comes on to the market. The latter is largely dictated by margins of profit, which are minimal especially in the poultry and swine industry. However the early optimism regarding DNA vaccines has been tempered by subsequent observations that in many cases DNA vaccines perform poorly in target animals than the initial successes seen in mice. There is however exceptions for example the Fort Dodge's West Nile virus vaccine approved by FDA but yet to be marketed.

## 8. Conclusions

Since the early demonstration of disease control through vaccination by Jenner, followed 100 years later by Louis Pasteur, the science of immunoprophylaxis against important veterinary viruses has grown enormously. Historically, great strides were made and major stimuli in vaccine manufacture arose from the Frenkel method [143] of bulk production of FMDV vaccine in vitro, followed by the growth of poliovirus in tissue culture [144]. Tissue culture technologies have been instrumental in the science of vaccine development and manufacture.

Notwithstanding the fact that the majority of current veterinary viral vaccines are derived and still produced by conventional attenuation and/or inactivation processes in tissue culture, genetic engineering techniques have been widely used but remain to fulfil the promise of offering improved, widespread and economic alternatives. There is, however, no perfect vaccine and such a goal may never be realised since viruses are constantly mutating in order to survive and evade host's defences.

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