Chapter 11
Prevention and Control of Influenza Viruses

Abstract The 2003–2004 outbreaks of highly pathogenic avian influenza (HPAI) have proven to be disastrous to the regional poultry industry in Asia, and have raised serious worldwide public health apprehension regarding the steps that should be taken to urgently control HPAI. Control measures must be taken based on the principles of biosecurity and disease management and at the same time making public aware of the precautionary measures at the verge of outbreak. Creation of protection and surveillance zones, various vaccination strategies viz. routine, preventive, emergency, mass and targeted vaccination programmes using live, inactivated and recombinant vaccines are the common strategies adopted in different parts of the globe. The new generation vaccines include recombinant vaccines and recombinant fusion vaccine. The pro-poor disease control programmes, giving compensation and subsidies to the farmers along with effective and efficient Veterinary Services forms integral part of control of HPAI. Following biosecurity principles and vaccination forms integral part of control programme against swine and equine influenza as well. Use of neuraminidase (NA) inhibitors (Zanamivir and Oseltamivir) for the treatment of human influenza has been widely accepted worldwide. The threat of increasing resistance of the flu viruses to these antivirals has evoked interest in the development of novel antiviral drugs for influenza virus such as inhibitors of cellular factors and host signalling cascades, cellular miRNAs, siRNA and innate immune peptides (defensins and cathelicidins). Commercial licensed inactivated vaccines for humans against influenza A and B viruses are available consisting of three influenza viruses: influenza type A subtype H3N2, influenza type A subtype H1N1 (seasonal) virus strain and influenza type B virus strain. As per WHO, use of tetravaccine consisting of antigens of influenza virus serotypes H3N2, H1N1, B and H5 is the most promising method to control influenza pandemic. All healthy children in many countries are required to be vaccinated between 6 and 59 months of age. The seasonal vaccines currently used in humans induce strain-specific humoral immunity as the antibodies. Universal influenza virus vaccines containing the relatively conserved ectodomain of M2 (M2e), M1, HA fusion peptide and stalk domains, NA, NP alone or in combination have been developed which have been shown to induce cross-protection. The T cell-based vaccines are another recent experimental approach that has been

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shown to elicit broad-spectrum heterosubtypic immunity in the host. As far as HPAI is concerned, various pandemic preparedness strategies have been documented.

11.1 Control of Avian Influenza Viruses

A four-component strategy is required for control of low-pathogenicity notifiable avian influenza (AI) control and is considered as a primary strategy for highly pathogenic avian influenza (HPAI) and H5/H7. They include: (1) education (2) biosecurity (3) rapid diagnostics and surveillance and (4) infected poultry elimination. Vaccination can be added as an additional tool within a wider strategy of control whenever immediate control is not feasible that leads to maintenance of livelihoods and food security. This also helps to control clinical disease till the development of a primary strategy and its proper implementation to eradicate the disease (Swayne 2012a).

During 2003–2004 in several Southeast Asian countries, devastating outbreaks of HPAI had been recorded raising serious public health concern at the global level. Estimated death and destruction of domestic poultry was recorded to be over 150 million with more than 100 people contracting the disease (including 60 deaths) since May, 2005. Economic losses of $10 billion had been incurred by the poultry sector in Asia with further agony to the poor poultry owners despite adoption of control strategies. Initiation of a stepwise and consultative process of global strategy development has been undertaken jointly by FAO/OIE Global Framework for the Control of Transboundary Animal Diseases (GF-TADs) and WHO; the first step being the strategy development in the major HPAI crisis zone of Asia. In mid-May, 2005, a formal consultative meeting has been held in order to complete this step via key Asian stakeholders. There has also been expansion of the global strategy along with adoption of similar plans for Central Asia, Africa; Americas and Europe because of the rapid spread in other regions (http://www.fao.org/ag/aga/agah/empres; http://www.fao.org/ag/againfo/subjects/en/health/default.html.).

In order to decrease the load of virus in susceptible avian species and environment, recommendations for eradicating HPAI at its source has been given by OIE consequently leading to reduced chance of human infection. The adoption of such a strategy will protect production sector as well as trade resulting in safeguarding of food security and rural livelihoods particularly in the in developing world. There is an urge to develop a control strategy that complements the policy of stamping out because of serious impact of unparalleled and nearly global spread of HPAI infections in animal as well as human health.

The first line of defence includes rapid response following detection of the disease at an early stage depending on awareness among veterinarians, animal owners and high quality of veterinary services. A liberal compensation mechanism

positively affects AI reporting as well as notification by farmers in infected and at-risk countries. Control strategies based on a combination of rapid diagnostics, appropriate and effective surveillance, improved hygiene and enhanced biosecurity, movement restrictions, stamping out and emergency vaccination, education/training of poultry workers could maximise eradication and control of AI.

For effective control programmes aimed at eradication of AI virus infection, the prerequisites are:

- Disease awareness
- Early detection
- Culling and Stamping out
- Disposing affected birds properly
- Notification timely
- Stringent biosecurity measures
- Isolating, zoning and quarantine
- Live bird market control
- Vaccination strategy judiciously.

A key factor is continuous global surveillance of influenza.

Prevention of exposure of flocks and elimination/culling of the infected birds rapidly are the best possible ways to check the spread of HPAI.

The important aspect in the overall strategy for prevention and control of AI is prevention of initial introduction of virus and restricting its spread if it is introduced (To et al. 2012). Most human H5N1 infections occurred due to direct transmission from infected poultry (Yupiana et al. 2010); therefore, controlling the infection in poultry is important to prevent human infections. Various studies reported that H5N1 virus infection first occurs in ducks and geese in which most of these infections remain asymptomatic (Henning et al. 2010); therefore, the separation of chickens from these poultry birds is very important. Apart from this, live ducks, geese and quails are now banned in the retail markets of Hong Kong (Guan et al. 2007). Legislative measures such as strict quarantine and trade limitations for birds and their products have been used, from time to time, by many European countries, Canada, US and many other countries of the world to prevent their introduction into their territory (Pittman and Laddomada 2008; Henning et al. 2009). Under the Health of Animals Act of Canada, it is mandatory to report all suspected cases of AI to the Canadian Food Inspection Agency (CFIA). Many of the older legislations to control AI such as European Union (EU)'s Council Directive 92/40/EEC, EU Directive 92/65, Diseases of Poultry Order 1994 and The Diseases of Poultry (England) Order 2003 of UK have now been amended or replaced by new legislations. Many EU Member States have witnessed their wild birds and poultry getting affected by the HPAI H5N1 strains that originated from Asia. However, additional control measures taken by EU legislation and its implementation in Member States were effective in limiting the impact of these viruses on animal and public health (Pittman and Laddomada 2008). The European Communities (AI) (Precautionary Measures) Regulations 2008 (S.I. No. 7 of 2008) are amended to a

recent legislation, i.e. European Communities (AI) (Precautionary Measures) (Amendment) Regulations 2011. Statutory Instruments. S.I. No. 11 of 2011. The older legislation has been amended by the UK government agency DEFRA in the form of 'The AI and Influenza of Avian Origin in Mammals (England) (No. 2) Order 2006'. Recently, a document 'Notifiable Avian Disease Control Strategy for Great Britain' against AI and new castle diseases virus, Version 1.0 was released in January 2012 that was revised to Version 1.1 and released in July 2012. The legislations against AI are governed by the 'Health of Animal Regulations' and 'Health of Animal Acts' CFIA, in Canada.

Stringent biosecurity measures such as removal and slaughter of infected birds, prompt incineration of carcases, disinfection of the premises after removal of infected litter, prevention of the movements of the birds and people from infected to clean areas and giving an interval between slaughter and repopulation help in containment of an outbreak. The virus can be inactivated by formalin and iodine compounds. However, such measures do not affect wild birds, especially migratory birds. Therefore, steps should be taken in high-risk areas to prevent access of wild birds to poultry farms. The success of any control or eradication strategy is dependent upon surveillance and diagnostic procedures that ascertain the AI status of a flock, farm and region. Less than optimal virus isolation or serologic procedure or incomplete surveillance strategies may provide negative data that can give false sense of security.

11.1.1 Biosecurity Principles

- Adapt isolation, traffic control and sanitation which are the basic tenets of biosecurity.
- Follow stringent cleanliness, quality sanitation as well as hygienic practices together with decontamination and disinfection procedures suitable on the farm premises.
- Spreading of virus via mobility of birds, crates or vehicles /trucks to other farms and/or market. Vehicles coming from other poultry farms or poultry market should be sanitised before and after arrival.
- Prevention of flocks being exposed in order to eliminate the birds infected with virus.
- Have a check on human traffic and avoid visitors.
- Employees including crews should wear clothing that are freshly laundered supplied each day at the farm. Place disinfectant boot dips to reduce the probability of introducing and spreading the infection.
- Avoid contact of poultry with migratory/wild/free-flying birds and waterfowls. Prevent stagnant water accumulation which is a centre of attraction to migratory avian species.
- Educate employees about the dangers of live birds markets.

- Sick or dying and dead birds should be appropriately and immediately submitted to recognised laboratories for a timely diagnosis.
- Culling and slaughtering (stamping out) of all the infected or exposed poultry flocks. Dead birds should be disposed off properly by burial or incineration.
- Washing of hands and feet frequently with soap and water and suitable disinfectants after handling affected birds or contaminated materials.
- Regular surveillance and monitoring of AI in order to acquire knowledge regarding the status of the disease.

11.1.2 Disease Management for Preventing the 'Bird Flu' Outbreaks

- Informing authorities immediately about suspicion regarding disease outbreak and leaving poultry handling to experienced personnel (veterinarians, cullers, sweepers etc.).
- Skilled veterinarians should handle either dead birds or poultry suspected to suffer from bird flu without conducting necropsy in field.
- In case disease is detected in the country, all movements of birds from area where disease has appeared should be strictly restricted.
- Culling and slaughtering (stamping out) of infected or exposed flocks of poultry. Extra care should be taken regarding wearing of protective clothing and gloves, face masks, goggles, gown, rubber boots etc.
- Field veterinarians should be trained for collection and dispatch of appropriate samples and the suspected samples should be immediately diagnosed.
- While handling dead or sick poultry follow appropriate safety measures such as wearing protective clothing, gloves, nano face masks and goggles, gown, rubber boots etc.
- Submission of live birds in confinement in boxes without returning to the farm. Plastic bags that are leak proof and double packed, sealed and can be transported under chilled condition must be used for transport of dead birds for immediate laboratory investigation.
- For safe burial of dead birds, assistance from local animal husbandry authorities must be provided.
- Local health authorities must closely monitor all persons exposed to bird flu-infected chicken or suspected farms.
- Liaison with neighbouring countries for international trade should be monitored to check the influx of AI. There must be strict regulation or complete banning of cross-border trades with affected countries. International flights, train, and surface transport should also come under strict surveillance.
- Bird flu being a 'Notifiable' disease should be immediately reported to the regulatory authorities and officials.

- Wide public awareness on the prevention and control strategies as well as the zoonotic impact of bird flu should be created using mass media. Along with it education and training programmes should be organised for veterinary para-professionals and farmers, marketers, poultry transport contractors, egg collectors and concerned professionals.
- Handling of any contaminated items must be followed by washing of hands each time.
- The importance of virus sequence analysis in the management of flu outbreaks have been described (Jonges et al. 2013).

The countries that have got the status of 'freedom from infection' are at lesser risk than those having weaker capacity of prevention and control of the disease. It is thereby necessary to shift majority of infected countries towards the category of 'freedom from infection in defined compartments' and 'freedom from infection after stamping out'. This in turn ensures freedom of such countries from HPAI reincursion assuring disease control swiftly at the time of infection without change in the demography of new areas of endemicity.

A stepwise disease control programme is proposed to meet this objective. Time frames of such approach ranges from immediate to short (1–3 years), short to medium (4–6 years) and medium to long term (7–10 years) that strengthens the prevention and awareness capacity in countries at risk in the short term. As per region and country, the effort for prevention and control will fluctuate per region unavoidably taking into consideration the wider range of veterinary infrastructures round the globe. All infected as well as non-infected countries in Asia, Middle East, Europe and Africa are included in the geographical focus of the strategy requiring strengthening of capacity in surveillance of disease progressively including wild birds and emergency preparedness (FAO 2004; Scott and Rose 1996; Miyabayashi and Mundkur 1999).

11.1.3 Salient Precautionary Measures for General Public

- Follow appropriate sanitation, hygiene and safety measures during bird flu outbreaks to avoid infection.
- Avoiding poultry farms and markets of bird selling during an epidemic is must. Children must be kept away from dead or sick poultry/birds.
- Birds and poultry products that include eggs and egg products, chicken and duck meat may carry the disease due to faecal contamination of the egg shell and objects.
- The virus in poultry meat and eggs gets destroyed if cooked properly. Follow good kitchen hygiene practices, and eat properly cooked eggs and poultry meat/products.

- Food handlers must practice hand washing thoroughly and frequently using suitable disinfectants at home as well as in restaurants thereby helping to avoid infection on a routine basis.
- Local health authorities should closely monitor all persons having exposure to infected chickens or to farms.

11.1.4 Protection Zone

A 3 km radius area surrounding the infected farm is declared as 'protection zone' for a period of no less than 21 days from the date of identification of the influenza virus. During this period different samples taken from birds will be tested for the presence of influenza virus. Various biosecurity measures on all poultry farms within the zone will be implemented with austerity. Controlled entry and exit into the protection zone will be put in place strictly. The vehicles and other materials that leave the premises have to be cleaned and disinfected before their exit. The litter or manure is not transported out of the protection zone. Utmost care should be taken to prevent wild birds from coming in contact directly or indirectly with the domestic poultry within the zone by construction of temporary enclosures on the premises. Public awareness about the disease has to be increased. Live poultry shows, displays or markets are discouraged. A complete ban has to be imposed on bird hunting within the zone.

11.1.5 Surveillance Zone

'A surveillance zone' of a 10 km radius area surrounding the infected farm is to be formed for a period of no less than 21 days from the date of detection of the influenza virus. Complete list of all the domestic or captive bird flocks within the zone should be recognised. The flock owners in the zone must implement appropriate farm biosecurity measures. Only restricted movement of poultry and hatching eggs is allowed during this period within the zone. Once a surveillance zone is established, poultry and captive birds are not allowed to be transported out of the surveillance zone for some duration of time. Steps should be taken to restrict or completely prohibit the bird hunting. Permission will not be given to host bird fairs, bird shows or bird markets inside the zone that lead to assembly of large number of birds within a limited area. The already planned or announced poultry and other bird's fairs, shows or markets will have to be cancelled.

Informing the regulatory authorities and officials is mandatory. Training of the field veterinarians for collecting as well as dispatching of appropriate samples for diagnosing the disease in time is of utmost importance (Dhama et al. 2005; Kalthoff et al. 2010). Monitoring the influx of AI via forming a liason with neighbouring countries is an important aspect (Koh et al. 2008). Stringent

surveillance and vigilance for the virus of bird flu at international airports, as well as in railways and surface transport are required (Gowthaman et al. 2010; Dhama et al. 2013a). For para-veterinary professionals and farmers, marketers and poultry transport contractors, egg collectors and concerned professionals, organisation of education and training programmes are mandatory. Heightening of sanitation and hygienic measures, which include thorough washing of hands with soaps/detergents after handling of contaminated material, should be given priority (Kataria et al. 2005; Bunn et al. 2011; Tiwari and Dhama 2012).

11.1.6 Vaccination as Part of a Control Strategy of Avian Influenza Viruses

A powerful tool to support eradication programmes is vaccination when used in conjunction with other control methods and it increases resistance to field challenge and help to decrease levels of virus shedding in vaccinated flock thereby reducing transmission (Capua et al. 2004; Van der Goot et al. 2005). In order to control AI, all these effects of vaccination are contributors. However, it has been experienced that in order to eradicate the infection, a wider control strategy with vaccination as its integral part must be taken including biosecurity, monitoring infection and its evolution (Capua and Marangon 2006).

For eradication of AI in a vaccinated flock it is necessary to detect field exposure which is allowed by the vaccination system. This can be achieved by use of inactivated vaccines (conventional) and vector vaccines (recombinant). The viral subtype similar to the field virus is contained in the conventional vaccine enabling field exposure detection when regular testing of the unvaccinated sentinels is done. Even though the system is applicable in the field but rather impracticable especially to identify sentinel birds in premises (containing birds raised in floors). On the basis of the detection of anti-NS1 antibodies a more encouraging system has been developed and has found its use in all vaccines provided they possess haemagglutinin (HA) subtype similar to field virus (Tumpey et al. 2005). The NS1 protein synthesis occurs only during active process of replication of the virus and this forms the basis of such system. It is therefore rarely present in inactivated vaccines. Only after field exposure, birds vaccinated with such vaccines will develop anti-NS1 antibodies (Tumpey et al. 2005; Dundon et al. 2006).

For the use of a vaccination strategy the scientific basis is the induction of a protective immunity in the target population. The level of protective immunity can be raised along with increase in the resistance to infection due to good vaccination programme. The clinical presentation of AI infection must be less severe along with reduction in viral shedding in terms of amount and duration if the birds are vaccinated properly. Vaccination of poultry also reduces the risk of exposure of human to AI viruses with zoonotic potential and subsequent decrease in human cases.

From the deadly pandemic of 2009 influenza A H1N1 virus to the looming threat of bird flu H5N1, and recently the swine flu H3N2 virus outbreak, and the emergence of antiviral drug-resistant H1N1, vaccination remains the main strategy for prevention (Jin and Mossad 2012), but it cannot replace other control measures. It should only be used as an additional tool in a control strategy. The tactical use of AIV vaccination will supplement any stamping out control policy by slowing/stopping the virus distribution and spread within the population (To et al. 2012; Swayne 2012b). The goals of vaccination are (i) to create of a buffer zone between infected and non-infected areas (ii) protection of AI free areas considered at high risk of infection and (iii) vaccination of poultry flocks meant for first replenishment of the areas that were earlier found to be infected with avian flu virus (Lee and Suarez 2005).

In order to reduce both morbidity and mortality, routine vaccination is mandatory which can decrease the prevalence of infection in the longer term where stamping out and surveillance can be applied. Routine vaccination can then be continuously used with proper employment of suitable contingency plan in place dealing with possible re-emergence of the disease. In recent years, in several occasions, vaccination against AI infections due to H5 and H7 subtypes of AI viruses has been used with the objective to control and to eradicate the disease up to certain extent. It is important to note that in order to contain AI infections effectively vaccination should be used as part of a comprehensive strategy of control including biosecurity and quarantine, surveillance and eradication along with elimination of infected poultry and poultry at risk. Potent vaccination can lead to increase in the resistance to infection, reduction in virus replication as well as shedding and reduction in transmission of virus. Under experimental conditions, even though a wide variety of vaccines against AI have been developed and tested, only whole AI virus vaccines (inactivated) and recombinant H5-AI have been licensed and used widely in various countries. In local conditions, adaptation of AI vaccination programmes is required in order to guarantee efficacy and sustainability. In diverse situations, modulation of the vaccination programmes is required according to the strain of the virus involved, poultry producing sector and its characteristics and capacity of the infrastructure of the veterinary services along with available adequate resources (Ellis et al. 2005; Marangon et al. 2008).

Different vaccination strategies and schedules are required for different situations (Swayne et al. 2011, 2013).

Different Vaccination Strategies for AI. Vaccination against AI can be used as a preventive, emergency or routine practice in control programmes for HPAI and Low-pathogenicity notifiable avian influenza (LPNAI).

Routine vaccination. If the HPAI or LPNAI becomes widespread and enzootic, routine vaccination may assist in reducing disease incidence and allow the continuation of poultry production in rural settings, to maintain the livelihoods and food security of the rural poor. A routine vaccination programme requires a steady, direct supply of commercial vaccine and cannot rely upon an emergency AI vaccine bank. It is done in the following situations:

- Disease is endemic
- Enforcement of containment and eradication of infection is not possible
- Movement control cannot be instituted
- Occurrence of disease is extensive and widespread
- Effective application of DIVA strategy in endemic areas cannot be performed.

Preventive vaccination. Preventive vaccination programmes may take a slightly longer time to implement than emergency vaccination programmes but are most effective when kept small in size and targeted to high-value or high-risk populations, such as genetic stocks of commercial poultry, zoo birds, rare birds or endangered species. Preventive vaccination programmes require less planning in advance than emergency vaccination programmes, but a vaccine bank and some logistical infrastructure may be necessary for rapid implementation, should an outbreak occur in the border area of a neighbouring country. It is accomplished in the following situations where the probability of virus spread is quite high:

- Identification of AI infection in areas with a highly dense poultry population
- Data facts and proof indicate that the culling of infected, suspected or dangerous in-contact poultry holdings by themselves will be insufficient to control the outbreak
- Used as complementary eradication method to other strategies such as movement restrictions, slaughtering, controlled marketing and division of the flocks into small zones and compartments.

The OIE recommends preventive vaccination against H5 and H7 subtypes of AI viruses should be carried out using DIVA strategy under two defined situations. A bivalent vaccine, containing H5 and H7 antigens, should be administered if infection with either H5 or H7 subtype occurs from exposure to potentially infected wild/migratory birds. On the other hand, a monovalent vaccine containing either H5 or H7 immunogens would be a preferred vaccine choice when the source of infection from these two subtypes is known/or can be determined, such as from live bird markets or from outbreaks in neighbouring countries or trading partners can be a better choice (OIE 2006).

Emergency vaccination. It is done in the face of an epidemic or in a situation where colossal and fast spread of the infection is suspected. Vaccine banks are a necessary part of any emergency vaccination plan when other disease control measures alone are insufficient to contain the outbreak. In addition, implementation of an effective emergency vaccination programme also requires fully developed application plans and an understanding of the logistics of a vaccination campaign in the field.

Mass vaccination. As the name indicates, all the susceptible birds in a particular geographical region/country are given the mass vaccination. It can be used in the form of an emergency vaccination, preventive vaccination or routine vaccination. This option is usually chosen in those circumstances when various other control measures are predicted to be ineffective in controlling the current outbreak, or an outbreak that is suspected to be forthcoming.

Ring vaccination. It is only relevant to an emergency vaccination carried out in an earmarked area around an outbreak with the objective of prompt control of the outbreak. This type of vaccination is to be used in the context of a DIVA strategy, and will be a supplementary to other control measures.

Targeted vaccination. This type of vaccination is administered to specified categories of birds and will be influenced by risk analysis on biosecurity levels of the farm, economic value of the flocks and the threat perception and magnitude of the infection. The targeted vaccination will also be influenced by the willingness to report and notify disease outbreaks which in turn will depend on whether adequate compensation will be given or not. If the farm holds different host species, a decision may be taken to vaccinate only those species that are more at risk to infection. The FAO has classified the poultry production system into four sectors (1–4). Sector 1 represents 'industrial and integrated sector', sector 2 and 3 include 'commercial poultry production', and 'village and backyard production' comes under sector 4. A choice may be made to vaccinate birds of only one or more of the FOA poultry production sectors. In a situation where mass vaccination is preferred but due to insufficient vaccine availability, only valuable parent flock may be vaccinated.

Commercial oil-emulsified, inactivated AI vaccines have been used as a tool to control and eradicate multiple subtypes of LPAI in poultry since the late 1970s (Swayne and Kapczynski 2008). Many countries such as the USA, Italy, Mexico, Guatemala and El Salvador. have used vaccines against LPNAI H5 and H7 subtypes with and without controlled culling (Capua et al. 2000; Swayne and Kapczynski 2008; Villarreal 2007). Vaccination against HPAI in poultry farms were used for the first time in 1995 in Mexico against H5N2 HPAI (Villarreal 2007) and in Pakistan against H7N3 (Naeem and Siddique 2006), against H5N1 HPAI during 2002 in Hong Kong and soon thereafter in 2004 in Indonesia and China (Swayne and Kapczynski 2008). Subsequently, Russia, Egypt, the Netherlands, France, Vietnam and Pakistan also implemented vaccination programmes against H5N1 HPAI in poultry (Swayne and Kapczynski 2008).

The control components used in HPAI and LPNAI outbreaks from 2002 to 2010 primarily focused on vaccines and vaccination as one of the measures in a comprehensive AI control strategy. During the 2002–2010 period, at-risk national poultry populations of over 131 billion birds were given more than 113 billion doses of AI vaccine. It was estimated that the average national vaccination coverage rate was 41.9 % and the global AI vaccine coverage rate was 10.9 % for all poultry with the assumption that vaccines were used at two to three doses per bird in these 15 vaccinating countries. Hong Kong had the highest ($\sim$100 %) national coverage rate for poultry, while Israel and the Netherlands had lowest national coverage (<0.01 %) for poultry. During this period, the proportion of the inactivated AI vaccines and live recombinant virus vaccines against AI was found to be 95.5 and 4.5 %, respectively. Four countries (People's Republic of China, Egypt, Indonesia and Vietnam) employed 99 % of these vaccines; majority of these vaccines were used in the H5N1 HPAI panzootic. The World Organisation for Animal Health in 2006 made it mandatory to report LPNAI due to the potential of

some H5 and H7 low-pathogenicity avian influenza (LPAI) viruses to mutate to HPAI viruses. The number of outbreaks reported for LPNAI has been fewer than of HPAI, and only six countries used vaccine in control programmes, accounting for 8.1 % of the total H5/H7 AI vaccine usage, as compared to 91.9 % of the vaccine used against HPAI. Mexico, Guatemala, El Salvador and Italy have been the biggest users of vaccine to control LPNAI (Capua et al. 2009; Cecchinato et al. 2011).

Efficacy of vaccination can be monitored by conducting HI test on 20 serum samples per flock 1 month after the second vaccination. According to the guidelines set by Agriculture, Fisheries and Conservation Department, Hong Kong, AI vaccination is deemed to be effective, if in more than 70 % of samples tested show a HI titre >1:16. The most practical and widely used method for monitoring a vaccinated flock is the use of sentinel birds. Thirty to sixty unvaccinated sentinel birds are housed in the same shed where vaccinated birds are kept. The sentinel birds are observed for development of clinical symptoms and/or seroconversion. The ELISA or HI test is used to screen 10–20 serum samples collected every 30–45 days from sentinel birds. The flock is considered positive for AI, if the sentinel birds show clinical signs or seroconvert (Dhama et al. 2013b).

In February 2005, the United Nations Food and Agriculture Organization (FAO), in consultation with many international bodies, recommended to the governments in affected areas of South East Asia to carry out vaccination of targeted poultry flocks as the mass culling of birds is proving to be inefficient in arresting the disease. Until this event, culling was considered to be a preferred control measure by the policy makers, than vaccination. The main reason against vaccination was that vaccination does not prevent infection, or viral shedding, but will often mitigate clinical signs. Vaccinal protection is subtype specific and therefore, the vaccine will protect against that particular HA type(s) and not against the other HA types. Therefore, vaccination could allow a highly pathogenic form of the virus to infect and to replicate within a flock without being detected, and allow it to potentially spread to other susceptible birds. The differentiation between infected and uninfected vaccinated animals was not possible until couple of years back. Therefore, trade barriers were imposed even on the vaccinated animals since these were showing the presence of vaccine-induced antibodies. In spite of the threat of trade bans, poultry was vaccinated in certain countries, because it helped in bringing the outbreaks under control as the vaccination led to reduced severity of clinical signs, less shedding of the virus resulting in reduced viral load in the environment and more amount of virus needed to infect a bird.

Traditional vaccines against the prevalent strain of AIV have been used in many countries for the control of H5 and H7 viruses (Swayne 2003; OIE 2005). AI vaccines are still not permitted, specifically banned or discouraged in many countries. However, the use of emergency vaccines AI control under certain circumstances has been reserved by several countries. There are currently two major forms of AI vaccine available. There is a recombinant form using Infectious Laryngotracheitis or Fowl Pox vaccines as a carrier. The other form is a whole killed virus vaccine.

Inactivated homologous (same field strain), heterologous (same H but different N) and oil emulsion vaccines are available for poultry to protect them against AI (Stone et al. 1997; Alexander 2001; Swayne et al. 2001; Swayne and Halvorson 2003; Capua and Marangon 2003; Swayne 2003; Ellis et al. 2004). Inactivated homologous vaccines have been extensively used during AI epizootics but the drawback is that there is no cross-protection and moreover it is difficult to differentiate between vaccinated and exposed birds. No H5N1 virus vaccine is available commercially to combat bird flu. Live attenuated vaccines against any subtype of influenza A virus are not recommended in birds (OIE 2005).

Effective inactivated influenza vaccines for the control of AI in poultry are available. However, interference of vaccine-induced antibody with any serosurveillance and epidemiological programme is a major limitation of the use of these vaccines. DIVA strategy can be employed that easily differentiates the birds that were naturally infected with influenza from those that were vaccinated. The whole killed vaccine has been used in a DIVA (differentiating infected from vaccinated) strategy to vaccinate birds in and around the movement control zone of an infected area. Italian officials implemented this strategy following a series of consecutive AI outbreaks in their poultry population. They attribute the eradication of AI from their domestic poultry, in large, to the use of vaccine in addition to stamping out, biosecurity and surveillance.

The safety reasons and technical issues are a major hurdle of development and production of vaccines from pathogenic AI viruses particularly H5N1 by traditional methods. Approaches other than the use of inactivated virus vaccines are currently being evaluated. The new generation vaccines include: recombinant vaccines, recombinant fusion vaccine (Liu et al. 2012), DNA vaccines (Ledgerwood et al. 2012), and reassortant prototype strains generated by reverse genetic, vector-expressed HA or subunit vaccines.

Vectored AI vaccines using fowl pox virus (FPV) and infectious laryngotracheitis virus (ILTV), baculovirus, vaccinia virus and new castle disease virus (NDV), expressing H5, H7 AIV HA gene insert (Kuroda et al. 1986; De et al. 1988; Crawford et al. 1999; Li et al. 1999; Swayne et al. 2000a; Veits et al. 2003; Cornelissen et al. 2012) have been developed. The serological surveillance will not be impeded by such recombinant or purified HA vaccines as the recipient sera will not react in the double immunodiffusion test, because antibodies against the NP or M antigens that are common to all AI viruses and react in this test, will not be produced. However, these vaccines replicate poorly in birds that were naturally exposed to, or were vaccinated with the vector virus (Swayne et al. 2000b). It has been found that chickens receiving even a single dose of plasmids expressing H5 and H7 HAs were protected from infection by either subtype (Kodihalli et al. 1997, 2000). The promoters and cytokines can influence the development of immunity following influenza DNA vaccination (Swayne 2003; Chen 2004). The reverse genetics techniques have been exploited to develop candidate vaccine viruses against the HPAI viruses including H5N1 subtype virus (Liu et al. 2003; Neumann et al. 2003; Webby et al. 2004; Nicolson et al. 2005; Tian et al. 2005). The reassortant viruses generated using this technology, containing the same H5 and

H7 HA gene as the challenge virus, but a heterologous NA gene, can help in differentiating the infected and vaccinated birds (DIVA strategy) (Lee et al. 2004). The gene-deleted mutants may be the probable candidates of future live AIV vaccines (Swayne 2004) however; the inherent risk of generation of pathogenic disease-causing strains by reassortment of such vaccine virus with field viruses in nature should always be kept in mind. In order to differentiate infected from vaccinated flocks, a different NA is used in the vaccine to allow differentiation with the field virus infection by detection of specific antibodies against the NA of prevailing field virus in the vaccinated birds. DIVA strategy using these kinds of 'marker vaccines', with a heterologous strain differing in NA from the circulating field virus has been successfully used in Italy during the outbreaks of H7N1 (Marangon et al. 2003). The combination of a 'DIVA' strategy and efficient disease monitoring system should prove to be quite effective measure for the control of AIV infections in poultry (Capua and Marangon 2003; Bano et al. 2003; Capua et al. 2004). This strategy can help countries to escape from trade restrictions. In one vaccine used in one type of DIVA strategy, the haemmagglutinin subtype of both the vaccine strain and the field strain circulating in the population was same, but their NA subtype was different. Such a DIVA strategy was used to control the H7N1 bird flu outbreak that occurred in Italy in the year 2000–2001 with a vaccine containing H7N3 strain. Both vaccinated birds as well as infected birds developed antibodies to H7 proteins. However, anti-N1 antibodies could be detected only in the birds infected with the circulating H7N1 strain and not in the vaccinated birds which developed antibodies to N3 instead of N1. The validation and approval by the World Organization for Animal Health (WOAH), and the EU of this DIVA strategy was instrumental in putting up a new recommendation. Consequently, for the first time trade bans were lifted and the products from vaccinated, uninfected animals could be traded in the EU.

The DIVA strategy was also used in the United States. Low pathogenic H5N2 and H7N2 subtype AI viruses have routinely been detected from AI outbreaks in the poultry. The H5N1 or H7N8 subtype AI viruses have not been identified in poultry in the US. The absence of these heterologous NAs (N1 or N8) was used in DIVA. The technique of reverse genetics was employed to create influenza virus reassortants (rH5N1 and rH7N8), that were used as vaccine strains. The protective immunity induced in specific pathogen-free chickens by reassortant influenza vaccines (rH5N1 and rH7N8) was comparable to homologous H5N2 and H7N2 vaccines. The NA inhibition or indirect immunofluorescent antibody tests were able to differentiate infected and vaccinated birds due to the induction and presence of different anti-neuraminidase antibodies in the sera of the birds. The differentiation of infected from vaccinated poultry is not always based on structural proteins. The assays based on the detection of antibodies to nonstructural (NS1) protein of influenza A virus have been reported (Tumpey et al. 2005). The experimental studies on the 'DIVA' vaccines generated using reverse genetics system has also proved to be efficient in giving protection to the flocks against challenge viruses (Lee et al. 2004). The subunit and killed whole-virus vaccines have also been included in DIVA strategy. This type of DIVA strategy may fail in

two situations: (i) emergence of a field virus with a different N antigen than the existing field virus (ii) or simultaneous circulation of subtypes with different N antigens was already occurring in the field conditions.

In 1995 in Pakistan the first outbreak of HPAI caused by H7N3 subtype had been reported. In order to control the disease, a homologous aqueous-based vaccine has been prepared from the field isolate and has been employed for ring vaccination. The same vaccination approach was used later in the year 1998 during an outbreak caused by H9N2 subtype (Naeem and Siddique 2006).

In order to identify source of the virus, precedent hot spot selection, obligation of ban on transport and post-vaccination monitoring HPAI control by vaccination must go on in coordination with planned field surveillance as well as epidemiological investigations. It is easier to carry out vaccination of commercial poultry farms, whereas significant logistical and technical problems may be faced while vaccinating backyard and non-confined poultry. Reaction of domestic ducks is different from that of terrestrial poultry to HPAI vaccination on the basis of the fact that virus may be shed on challenge and therefore remain infective potentially. For monitoring vaccinated domestic flocks of duck it is essential to undertake serological monitoring by DIVA strategy along with the use of sentinel domestic ducks. To control domestic duck-borne HPAI infective reservoirs, a major step would be the successful use of HPAI vaccination that may be a major source of re-infection of terrestrial poultry. However, a different course in domestic ducks is seen during HPAI disease syndrome unique in comparison to other poultry. Virus shedding in ducks is constant but morbidity and mortality are low. Currently, the efficacy of OIE approved vaccines has not been clearly recognised in domestic ducks, thereby requiring further epidemiological as well as field studies. In domestic ducks some vaccines may be effective as per pilot studies in China but requires further investigation. The effectiveness of poultry vaccine (H5 based) in domestic ducks has to be evaluated by coordinated efforts of FAO, OIE and their partners. Its outcome in domestic ducks will have a great bearing to formulate future strategies to control shedding of virus in reservoir host (Guan 2005).

11.1.7 Role of Economic Indicators, Poultry Density and Veterinary Services on Control of HPAI in Poultry

Being notifiable diseases, the reporting of High-pathogenicity avian influenza (HPAI) and LPNAI in poultry to the World Organisation for Animal Health (OIE) is essential. The responses of various countries to AI outbreaks, the subsequent situations and circumstances, are quite variable. The economic status, capacity of diagnostic services of the countries and other factors influence these responses. Work has been carried out to determine how the HPAI control programme is

affected by a country's poultry density, the performance of its Veterinary Services and its economic indicators (gross domestic product (GDP), agricultural gross domestic product (AGDP), gross national income (GNI), human development index (HDI) and Organisation for Economic Co-operation and Development (OECD) status). It has been found that as poultry density increases for least developed countries, there is an increase in the number and duration of HPAI outbreaks and in the time taken for eradication of the disease. Member OECD Countries, i.e. those with high-income economies, transparency and good governance, had shorter and significantly fewer HPAI outbreaks, quicker eradication times, lower mortality rates and higher culling rates than non-OECD countries. Direct economic indicators such as GDP, AGDP, % AGDP, GDP per capita, GNI and HDI do not show a significant association with HPAI control data. The assessment of various countries with 'OIE Tool for the Evaluation of Performance of Veterinary Services' revealed that HPAI control measures were much better in countries that had effective and efficient Veterinary Services (Pavade et al. 2011). In this context, especially the pro-poor disease control programmes are important. Many poor people of the world depend on small-scale or backyard poultry for the sake of their occupation without having access to veterinary services. At village level, these low-income groups can be maintained by this strategy via recovery of animal health services by means of organising early warning networks that are community based. Utilisation of the existing pool of para-veterinary workers at village, increment in the general awareness of the farmers through simple guidelines of biosecurity on AI control by use of local language publications, provision of access to credit or microfinance as a tool for remedy as an alternative to reward (direct) that is not affordable by some countries, are some of the other approaches to maintain the strategy. Developing groups and/or associations of farmers help in progressing alertness as well as for broadcasting of information. However, it is important not to forget indirect costs accounted usually for in the assessment of benefits and results from abridged levels or values of production due to temporary or permanent changes to management systems/markets. Certain expenditures such as transfer costs are excluded from the total cost of an HPAI control strategy as they do not represent additional resource utilisation but only symbolises transfer of loss between the stakeholders. For estimation of the impact of an HPAI control strategy on stakeholders individually, some of them such as reimbursement and subsidies are important. Particularly important in such situation of HPAI is compensation/reimbursement. Urging for providing compensation is either: to persuade acquiescence with culling regulations and to avoid a crisis in livelihood. Heavy financial losses may be incurred upon by farmers whose birds have been culled thereby making them unable to finance the restocking cost. A decision is required while estimating the compensation cost only for the birds' market value or to include the lost production value. Receipt of full compensation for losses including 'down time' in the system of production is rare for any country or for any disease. The market value is more commonly received for the lost animal or bird or a certain portion of the value. Provision of minimum credit on no interest ground is an alternative to compensation (Vannasouk 2004).

Freedom from AI is the most favourable status for a country having export of poultry products. Export markets are lost for countries that become infected which creates urge to take necessary steps to regain the former status of trading faster than before. Stamping out infection should be the primary response to an outbreak. The threat of loss of export market automatically if vaccination is practiced properly may not always happen (Botteron and Aquilino 2004). This is subjected to full compliance (as per OIE recommendations) assuring virus-free status in any country/zone/compartment especially in those wherein strict surveillance is implemented.

Better HPAI control measures are seen in countries having effective and efficient veterinary services as assessed using the OIE tool for evaluating performance of Veterinary services. The Official Veterinary Services as a part of the Animal Health Infrastructure has provided the mandates for dealing with transboundary animal diseases via central as well as field services. Apart from this the diagnostic laboratory network along with broader groups of stakeholders that include: industry, veterinarians at private sector, district/village animal health workers and smallholders also contribute to such dealing. The key players in this process are Official Veterinary Services which must work with industrial partners/the private sector/the veterinary profession and/stakeholders closely. This is particularly important at the time of implementation of measures of disease control having a major crash on producers as well as consumers of poultry products (FAO 2004; Pavade et al. 2011).

11.1.8 HPAI Control via Greater Awareness of Policy Issues

The countries which are pretentious or at risk now can get acquainted with the urge for reinforcing their frameworks of regulatory policy to put into effect animal disease control measures along with supporting formal intra-regional and global trade. These will have to take measures for realignment of their veterinary regulations as well as policies for meeting WTO/OIE standards. Quality and evaluation of veterinary services, animal quarantine reforms at institutes; introducing OIE standards, guidelines and recommendations for livestock and livestock products trade, export certification and designing out disease-free zones and compartments are the various mechanisms that are included. National or regional preventive interventions of HPAI on long-term basis should be supported wherever needed. These countries additionally support major goals for poverty diminution distinguishing HPAI control and other TADs that will significantly affect production of livestock; ease of access to regional as well as global markets along with improved rural livelihood (http://www.wetlands.org/default/htm).

11.1.9 Poultry Sector Restructuring

The destructive effects of HPAI can be prevented by reforming the poultry sector and is one of the most conducive interventions to be undertaken that requires acceptance of the total socio-economic system. At different levels of the poultry sector in several countries different approaches are required for restructuring via unique infrastructure and marketing properties; comparison of backyard and commercial production of poultry and impact on socio-economy. Restructuring must be regularised which will affect several segments of the sector in various ways and rates. These variations have resulted in undertaking general principles only (www.saarc.org; http://www.ecosecretariat.org/Directorates/dem.htm) which are listed below:

(1) Rationale for restructuring must be based on a concrete analysis of socio-economic impact considering the stakeholders' interest.
(2) Government commitment with full-fledged support from stakeholders is important and should abide by a long-term strategy.
(3) Livelihoods of small-scale poultry farmers, who stand for greater proportion of poultry in several HPAI-affected countries must be taken into consideration.
(4) Market forces should prepare the reconstructing strategy taking into consideration commercial as well as small-scale poultry producers.
(5) Public and private sectors should collaborate and show transparency to execute restructuring strategies.
(6) Restructuring should be an integral part of an overall disease control strategy including biosecurity and vaccination, zoning and/or compartmentalisation following the guidelines of OIE and FAO and to take into account human and food safety issues.
(7) Public awareness must be promoted for gaining support from producers and consumers, government agencies and private sector institutions, and other stakeholders.

11.2 Control of Swine Influenza Viruses

Stringent biosecurity measures along with disease surveillance and monitoring programmes provide adequacy to the prevention and control measures. Application of advanced diagnostics, stockpiling of drugs like Tamiflu along with novel vaccine development via utilisation of advanced tools and techniques and finally judicious vaccination strategies have got paramount importance in order to prevent the disease. The epidemic potential is limited by such measures which ultimately prevent the occurrence of a human pandemic (Pawaiya et al. 2009; Mak et al. 2012). Potential pathways for introducing and spreading disease must be identified by developing a swine influenza biosecurity plan. Prevention of the occurrence and

spread of the disease is the best way to deal with the disease as influenza viruses can be transmitted crossing species barrier between humans and pigs (VanReeth and Ma 2012). Human–pig interactions must be considered under biosecurity plan especially when pigs are exposed to persons with illness similar to influenza. Supportive therapy is the primary treatment and a dry, clean and dust-free environment are required by infected pigs. Proper rest must be provided to people suffering from swine flu without their free roaming in public places where there is gathering of numerous people (Dhama et al. 2012). Any secondary bacterial infection must be treated and controlled by antibiotics. Herd treatment involves use of expectorants administered in the water for drinking. In Europe and North America, vaccines are available commercially against H1 and H3 (Kothalawala et al. 2006). Nasal shedding and lung tissue infection along with lung pathology have been reduced markedly by vaccination of animals having exposure to the virus as evident from results of several studies. Pigs aged 5 weeks having clinical disease with infection of the lungs are protected by maternal antibodies from vaccinated sows but nasal shedding of the virus is not prevented. Even though vaccines are available to prevent swine influenza, 100 % efficacy is not proven. Either whole or split virus vaccines are commercially available and adjuvenation and inactivation along with preparation of whole-virus vaccine are done in embryonated egg of hen or in cell lines. Major drawback of such vaccines is that they do not bestow every time cross-protection against emerging subtypes. Autogenous multivalent inactivated vaccines may be prepared in individual farms specifically against the strain of the virus circulating in swine population.

In the USA, this is only legalised for use on the farms for which the vaccine has been produced. Currently, modified live influenza virus vaccines are not available for swine, although results of recent studies of gene-deleted vaccines have been reported (http://www.epa.gov/oppad001/influenza-disinfectants.html). Vaccination of pigs by using adenovirus recombinant virus, Swine origin influenza virus (S-OIV) vaccines) (Wesley et al. 2004; Wesley and Lager 2005), an NS1-truncated modified live virus vaccine (Richt et al. 2006; Vincent et al. 2007) and other vaccination strategies (Thacker and Janke 2008; VanReeth and Ma 2012) for the control on swine influenza viruses has been reported. The H1-encoded recombinant equine herpes virus-1 (EHV-1) of A (H1N1)pdm09 is found to be protective for pigs against itself or any other kind of influenza virus (Said et al. 2013).

11.3 Control of Equine Influenza Viruses

Strict quarantine and controlling interstate movements are the major strategies required for effective control of the disease. The newly infected animal can shed the virus till 21 days and OIE has suggested isolation of the infected animals for 28 days and such animals need to be placed 100 m away (minimum) from healthy animals. Personal care must be followed during handling animals. The water and feeds should not get mingled between non-infected and infected animals. Quick

diagnosis of respiratory diseases of equines by examining nasal swabs as well as serum samples is necessary for instant control of the disease. However, after quick detection and restriction of the disease in a limited area, it is required to achieve containment followed by eradication by quarantine as well as movement restriction without requirement of vaccination (Wood 2009). Most of the licensed equine influenza virus vaccines available internationally consist of inactivated whole virus of subtypes H7N7 and H3N8, or subunit vaccines (Park et al. 2003) which are given intramuscularly. The duration of protection provided by current vaccines is limited and they have less ability to control infection (Nelson et al. 1998) but if booster vaccinations are given then the disease occurrence and severity can be minimised. Vaccinated dams gives protective immunity to the ponies for the initial period but by 1–2 months it declines, so it is essential to protect the young ones using vaccines developed from strains similar to those circulating in the region (Townsend et al. 1999, 2001). The vaccination strategies will vary among horses of different classes and age groups such as foals, breeding mares and stallions, performance horses and recreational horses. Crouch et al. (2004) carried out vaccination of the young horses intramuscularly with the immuno-stimulating complex (ISCOM) vaccine involving the American lineage H3N8 and the challenge studies were done using the reference strain (A/eq/Newmarket/1/93). The vaccinated ponies were significantly protected as there were no evidence on clinical signs and virus excretion on challenge studies.

11.4 Control of Human Influenza Viruses

11.4.1 Vaccines for Human Influenza Viruses

The influenza vaccine (commonly flu shot) is used to protect against the highly variable influenza virus (Couch 2008). However, its efficacy is not always high which may not be due to the vaccine itself, rather because of the frequent antigenic changes in the virus (Horimoto et al. 2008). Commercial licensed, inactivated vaccines for humans against influenza A and B viruses are available, which consists of three influenza viruses: two influenza type A subtypes H3N2, and H1N1 (seasonal) virus strain, plus a strain of influenza type B virus. As per WHO, use of tetravaccine consisting of antigens of influenza virus serotypes H3N2, H1N1, B and H5 is the most promising method to control influenza pandemic (Onishchenko et al. 2007). In March 2012, US Food and Drug Administration (USFDA) approved quadrivalent flu vaccine administered as nasal mist (Traynor 2012), which was reported to be more beneficial than trivalent vaccines as it includes one more influenza B strain (Campos-Outcalt 2012; Barr and Jelley 2012; Block et al. 2012; Ambrose and Levin 2012; Reed et al. 2012). Seasonal influenza (SI) vaccines in immunologically naive hosts may sometimes lead to a different kind of situation in which the generation of heterosubtypic immunity against

potentially pandemic strains is actively inhibited (Bodewes et al. 2009). The operational challenges, in rapid production, procurement and deployment, and delayed and non-uniform access to vaccines and their implications during the A (H1N1) influenza pandemic in 2009, H5N1 and H7N9, have been described (Jorgensen et al. 2013; Mei et al. 2013).

The WHO and US Public Health Service recommends the viral strains to be included each year for the annual vaccination programme (Firore et al. 2008). The principal updates and changes of the 2008 recommendations are: (1) All children between 5 and 18 years of age should be given annual vaccination, starting from the 2008–2009 influenza season, (2) all children aged between 6 months through 4 years will continue to get the annual vaccination, (3) healthy persons aged 2 through 49 years can be vaccinated with either trivalent inactivated influenza vaccine or live, attenuated influenza vaccine (LAIV) (instead of the previous recommendation in which LAIV was administered to person aged 5–49 years); (4) vaccines containing the 2008–2009 trivalent vaccine virus strains A/Brisbane/59/2007 (H1N1)-like, A/Brisbane/10/2007 (H3N2)-like, and B/Florida/4/2006-like antigens should be used and (5) Update on resistance of influenza viruses to antiviral drugs in the United States should be given.

Live attenuated vaccines to influenza viruses are also commercially available in the market (Belshe et al. 1998; Nichol et al. 1999; Yin et al. 2012) and are found to be more effective due to the induction of both mucosal and systemic immune responses (Boyce et al. 1999; Piedra et al. 2005a, b). Different vaccines and different vaccination schedules may be required for populations of different age groups such as paediatric, young adult, adult, elderly adult and pregnant women (Grohskopf et al. 2013). Other approaches to human influenza virus vaccines include cold adapted (ca), temperature-sensitive (ts) mutant or reassortants derived from them or avirulent parents having HA and NA genes from other parents and recombinant vector vaccines (Belshe et al. 1998; Maassab and Bryant 1999), virus-like particles (Galarza et al. 2005; Pushko et al. 2005, 2007; Bright et al. 2007; Quan et al. 2007; Matassov et al. 2007; Bright et al. 2008; Lee et al. 2014a), viral vectors, DNA-based vaccines and universal vaccines (Lambert and Fauci 2010; Shaw 2012). In 2010, Centers for Disease Control and Prevention (CDC) approved high dose (4x) influenza vaccine for people aged 65 years and over, who have weak immune response (Centers for Disease Control and Prevention (CDC) 2010). A number of different approaches have been used for the development of influenza virus vaccines (Kemble and Greenberg 2003; Subbarao and Katz 2004; Wood and Robertson 2004; Neumann et al. 2005; Matassov et al. 2007; Cox 2008; Du et al. 2008; Murakami et al. 2008; Mayrhofer et al. 2009; Friesen et al. 2014; Huang et al. 2014; Huber 2014; Keitel and Piedra 2014; van der Velden et al. 2014). A subunit vaccines against H5 or H9 subtype AIV and infectious bursal disease viruses (IBDV) using viral protein 2 (VP2) of IBDV as cargo protein to display a 12-amino-acid (aa) immunodominant epitope derived from N-terminal M2 extracelluar domain (nM2e) of H5 or H9 subtype AIV was developed (Tang et al. 2012). An avian live attenuated master strain that may be used for the development of vaccines for epidemics and pandemics caused by influenza viruses has been

reported (Hickman et al. 2008). Researchers are putting in efforts to develop a universal flu vaccine that need not be reformulated each year (Du et al. 2010; Shaw 2012; Jang and Seong 2013). The extracellular portion of the influenza matrix 2 (M2) protein and conserved epitopes from the influenza NP, matrix 1 (M1) and HA proteins are being developed as candidate-inactivated 'universal' or 'common type' influenza A vaccine (Fan et al. 2004; Neirynck et al. 1999; Lambert and Fauci 2010). The route of inoculation and delivery system can also affect the outcome of influenza virus immunisation/vaccination (Belshe et al. 1998, 2007; Holland et al. 2008; Wee et al. 2008; van Damme et al. 2009). Safe and effective immunity was reported to be induced by the low-dose influenza vaccines delivered intradermally using microneedles strain that compared well with the full-dose intramuscular vaccination. The microneedle injection device was found to be effective, safe, and reliable (van Damme et al. 2009; Widera et al. 2006; Gorse et al. 2013; Marra et al. 2013). The M2 cytoplasmic tail mutants have been used as vaccines against H5N1 influenza A virus (Watanabe et al. 2008).

11.4.2 Universal Influenza Virus Vaccine and Universal Antibodies-Based Flu Therapies

The influenza universal vaccine should be able to trigger and generate broad protective immunity against conserved antigens present in many different subtypes of influenza viruses. It usually takes about 6 months from the time composition for the next season's influenza vaccine is decided and to produce, manufacture, release and distribute a vaccine that antigenically matches with the new influenza virus strain. The prediction and forecasting of a subtype or strain that may cause the next pandemic is quite difficult or rather not possible as the influenza A viruses display huge genetic diversity of in nature. Therefore, the control of the first wave of a pandemic by existing annual vaccines will not be possible. On the contrary, the administration of the universal vaccine, being 'off-the-shelf vaccine', could provide immediate protection against a newly emerging influenza virus or pandemic strain. A universal vaccine would reduce the severity of disease, enable the host to rapidly clear itself of the virus and decrease the case fatality rate until a specific vaccine against that virus is available. For mass-scale use of these vaccines, highly efficient microbial expression system will be required for the production of universal influenza vaccines in order to reduce the cost. As there are no time constraints for universal influenza vaccines, these can be produced, distributed, dispensed and given to the targets much ahead of the emergence of new epidemic or pandemic strains. In order to generate long-lasting effective protective immunity, universal vaccines development strategy should be such that these mimic the native conformation of target antigens. Current inactivated and live attenuated influenza virus vaccines are effective and induce protective immunity only when the vaccine strains and those causing epidemics are antigenically similar.

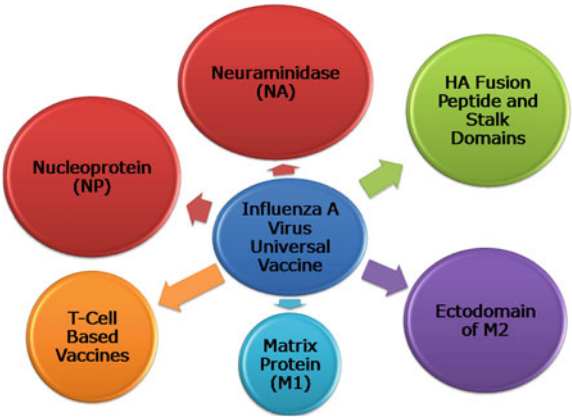

Fig. 11.1 Approaches to the development of universal vaccines against influenza A viruses. Antibody-based universal vaccines can be developed using various targets such as HA fusion peptides and stalk domains, ectodomain of M2, NP, NA and M1 proteins. Recently, efforts have also been directed to develop T cell-based universal vaccines that will protect the host by inducing cell-mediated immune response

However, these will not be effective enough and are bound to fail in preventing the emergence and spread of new pandemic or highly virulent viruses containing a substantially different HA protein. Current vaccines tend to induce strain-specific humoral immunity as the antibodies are elicited against dominant epitopes on the globular head of the HA the influenza virus which themselves are under immune selection pressure to mutate by antigenic drift. Universal influenza virus vaccines containing the relatively conserved ectodomain of M2 (M2e) (Kim et al. 2013), M1 (El Bakkouri et al. 2011; Atsmon et al. 2012; Quan et al. 2012; Zheng et al. 2013), HA fusion peptide and stalk domains, NA (Gravel et al. 2010; Johansson and Cox 2011; Quan et al. 2012; Schneemann et al. 2012, Margine et al. 2013; Eggink et al. 2014; Lu et al. 2014a), NP alone or in combination, as recombinants, VLPs or synthetic peptides have been developed which have been shown to induce cross-protection (Kang et al. 2011, 2012; Song et al. 2011a, b; Wu et al. 2012; He et al. 2013; Ma et al. 2013; Girard et al. 2013; Pica and Palese 2013; Vitelli et al. 2013). Various approaches have been used to develop effective universal vaccines against influenza viruses (Fig. 11.1).

The primary objective of the development of universal influenza vaccines is to induce broadly neutralising antibodies (Corti and Lanzavecchia 2013). However, prior influenza immunity may have its effect on the induction of such broadly protective antibodies. It was speculated that the pre-existing immunity produced in response to previous exposure to influenza virus or vaccine may impede the generation of broadly neutralising antibodies; and therefore can only be used in very young children having restricted exposure to influenza viruses or vaccines. However, mice and ferrets having pre-existing immunity to influenza virus produced broadly neutralising influenza antibodies when a prime-boost vaccine formulation, consisting of

a DNA vaccine for priming and an inactivated seasonal vaccine as booster, were used. The source of pre-existing immunity whether due to natural exposure to a flu virus or in response to vaccination was inconsequential. These influenza-immune ferrets that had received the prime-boost vaccine preparation, developed broadly neutralising antibodies against the conserved stem region of HA, and showed protection to challenge with heterotypic influenza viruses. It, therefore, opens up the prospects of developing universal influenza vaccines for humans using the conserved stem region of HA despite previous influenza exposure (Wei et al. 2012).

Induction of broad-spectrum heterosubtypic immunity by T-cell-based vaccines is another approach (Berthoud et al. 2011; Goodman et al. 2011; Hillaire et al. 2011; Lillie et al. 2012; McKinstry et al. 2012; Cargnelutti et al. 2013). However, the rapid replication of influenza viruses in the host resulting in the development of clinical signs prior to development of effective cell-mediated immune response that restricts viral replication is a challenge to the design of such vaccines. Therefore, the vaccination with these set of vaccines must induce and maintain T cells in a high effector state without causing immunopathology (Subbarao et al. 2006; Tan et al. 2013). Because $CD8^+$ T cell epitopes are often derived from highly conserved regions of influenza viruses, such vaccines need not be reformulated annually and, unlike current antibody-inducing vaccines, could provide cross-protective immunity against newly emerging pandemic viruses. A single vaccine which contains a combination of several viral immunogens that involves and stimulates different parts of the immune system would be much better than a single immunogen vaccine (Goodman et al. 2011). Identification of an immune correlate of protection and the methods to measure it is another important area that must be looked into for the T-cell-based universal vaccines (Sridhar et al. 2013; Subbarao and Matsuoka 2013).

Seasonal and pandemic influenza can be prevented primarily by vaccination (Geary and Beckett 2012). A universal vaccine that elicits broad protective immunity against multiple subtypes of influenza A viruses is highly desired. Broadly neutralising 'heterosubtypic' antibodies (BnAbs) have been recognised that bind to a extremely conserved regions on the stalk of HA present in all Group 1 influenza viruses (Sui et al. 2009; Ekiert et al. 2009) and inhibit virus replication by blocking virus–host cell membrane fusion. The identification of these broadly neutralising antibodies recognising the stalk domain of influenza virus HA has given impetus to develop a universal influenza virus vaccine in which only these epitopes are present, but the variable and immunodominant epitopes situated in the globular head of HA are absent.

There are 18 subtypes of HAs that have further been classified into two Groups. The Group 1 HA subtypes are H1, H2, H5, H6, H8, H9, H11, H12, H13, H16, H17 and H18; the Group 2 HA subtypes are H3, H4, H7, H10, H14 and H15 (Fig. 11.2). The influenza virus haemagglutinin molecule possesses two structurally distinct domains: (i) a globular head domain and (ii) a stalk domain at the membrane-proximal region. The globular head is composed of part of HA1, and the stalk structure is made up of portions of HA1 and all of HA2 (Wilson et al. 1981). The sialic acid binding pocket present in the globular head domain helps the virus to

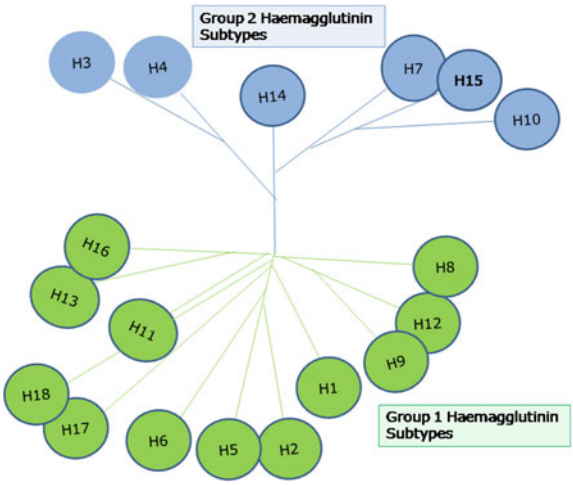

Fig. 11.2 Classification and phylogenetic relationship of various influenza A virus haemagglutinin subtypes. There are 18 subtypes of HAs that have further been classified into two groups. The group 1 HA subtypes are H1, H2, H5, H6, H8, H9, H11, H12, H13, H16, H17 and H18; the group 2 HA subtypes are H3, H4, H7, H10, H14 and H15

attach to the host cell, whereas the, HA2 fusion peptide present in the stalk domain, causes the fusion of the viral envelope with the endosomal membrane of the cell. These functions allow the virus to enter the host cell and production of progeny virions subsequent to replication, transcription and translation of the viral genome. Majority of the antibodies produced after influenza virus infection are directed against specific immunodominant regions located in the globular head domain of the HA and are strain specific. However, generation of broadly neutralising antibodies against various epitopes located on the HA stalk domain of the influenza virus have been reported (Corti et al. 2011; Ekiert et al. 2011; Sui et al. 2011; Wrammert et al. 2011).

It has been reported that the human immune system can produce BnAb, not only to the conserved areas on the HA stem of Group 1 viruses, but also to unknown common epitope(s) present in Group 1 and 2 influenza A viruses (Sui et al. 2011). Recently recombinant influenza viruses expressing chimeric haemagglutinins encompassing a variety of globular head and stalk mixtures have been generated, not only from different haemagglutinin subtypes but also from diverse haemagglutinin phylogenetic groups (Hai et al. 2012).

Full coverage and protection is not conferred by the current flu vaccines against SI virus strains. The identification of broadly neutralising antibodies such as V_H1-69, that work against majority of group 1 influenza A viruses have been identified. A human neutralising monoclonal antibody (CR8020) with broad specificity against most group 2 viruses, including H3N2 and H7N7, has been reported. The epitopes in the HA stalk recognised by the V_H1-69 group 1 antibodies and the CR8020 monoclonals are distinct. The development of a universal flu vaccine and broad-spectrum antibody therapies got a boost from this observation that most subtypes of influenza A can be neutralised by just a mixture of two antibodies (Ekiert et al. 2011).

Novel immunogens were constructed that contained epitopes from the HA stalk but lacked the variable and immunodominant epitopes located in the globular head of HA. Studies were conducted to determine whether the 20-residue A-helix of the HA2 chain that makes up the main constituent of the epitope of broadly neutralising antibodies CR6261, F10 and others is by itself adequate to induce antibodies with comparable broad antiviral activity. Antibodies produced by the mice immunised with VLPs exhibiting the A-helix identified multiple HA subtypes from group 1 but not from group 2; and the nature and properties of the induced antibodies was similar to those of CR6261 and F10, except that the anti-A-helix antibodies were not able to neutralise influenza virus. Therefore, further manipulation of the transplanted peptide, and presence of additional regions that display some more parts of the epitope, is required to achieve protection (Schneemann et al. 2012).

Studies were carried out to determine whether the various strains within the highly diverse H5N1 HPAI viruses are able to elicit broadly neutralising antibody by constructing DNA plasmids encoding codon-optimised haemagglutinin (HA) from 17 representative strains covering all reported clades and subclades. Mice immunised with the triclade DNA vaccine encoding HAs of (sub)clades 0, 2.3.2.1, and 7.2 produced broadly neutralising antibodies against all H5 clades and subclades and were protected by these induced BnAbs against challenge with a lethal heterologous H5N1 virus (Zhou et al. 2012).

Although the potential of influenza B viruses to undergo genetic changes and emerge into dangerous pandemic is quite low, however, they have a considerable involvement in the annual flu sickness in humans. Identification of broadly neutralising antibodies against influenza A viruses has paved the way for the prevention and control of influenza through development of monoclonal antibody-based immunotherapy and universal vaccines. However, two co-circulating, antigenically distinct lineages of influenza B viruses are largely responsible for the annual flu losses. Mice given three human monoclonal antibodies (CR8033, CR8071 and CR9114), were found to be protected against lethal challenge with both of these lineages. However, the nature of these monoclonals was different. The CR8033 and CR8071 antibodies identified distinct conserved epitopes in the head region of HA of the influenza B and prevented the exit of virus particles from infected cells. On the other hand, the CR9114 monoclonal was observed to bind with a conserved epitope in the HA stem and conferred protection against lethal challenge with influenza A and B viruses by preventing pH-triggered conformational change in the HA and subsequent fusion of the virus with the outer membrane of a host cell that is required for virus replication. The protection given by these antibodies against broad range of influenza virus strains indicated that they are directed against relatively conserved epitopes from one flu virus strain to the next. The information derived from these antibodies may help in building strategies for long-term protection against a variety of flu viruses prevailing over a long period of time, based on the development of monoclonal antibody-based immunotherapy and universal flu vaccines (Dreyfus et al. 2012).

In September 2012, it was reported that the scientists from The Scripps Research Institute and Sea Lane Biotechnologies have solved the co-crystal structure of a human antibody (code named: C05) that can neutralise influenza viruses in a unique way. They hypothesised that the bone marrow can provide the complete fossil record of all the antibodies made by a person in his lifetime. An all-inclusive library of billions of flu antibodies, from the internationally and locally collected bone marrow from patients, who during their lifetime, got infected with some important strains of flu, was generated. The unusual new antibody (C05) was isolated by screening this huge library for antibodies that could bind to proteins from a variety of influenza A viruses.

The C05 recognises and blocks the receptor binding site (RBS), located on the heads of viral haemagglutinins and is responsible for viral attachment to host cells. The RBS is a very important region on flu viruses and is comparatively much more exposed to the immune system than many other components and areas of the virus. Except for the small size of RBS relative to an antibody's usual grip area, it makes up an ideal target for antibodies. The binding of the targets by the antibodies is done with the help of two arm-like structures, each of which is made up of six protein fingers or loops. Both the RBS and some of the adjoining areas in the head, which differ among various flu strains, have to be captured by a usual antibody. The firm grip obtained by an antibody on this region for one flu strain generally will be lost due to mutation in the virus strain. This is the reason for the shift of focus more on the haemagglutinin stalk than on the head for the development of universal flu vaccine.

Instead of capturing the hypervariable regions around the flu RBS, the C05 uses a single-elongated protein loop to reach in and make a one-handed or one-fingered seize of the RBS itself. The antibody appears to produce maximum effect when two of these active loops, one on each arm, grab two viral RBSs on separate haemagglutinins, indicating a need for these antibodies to cross-link two haemagglutinins. The important function of the RBS does not allow it to change much from strain to strain; and thus variety of different harmful influenza A viruses can be neutralised by C05. Therefore, the C05 and other similar more potent antibodies may be used for protection from severe influenza infections. A universal flu vaccine designed to elicit such antibodies in people should be significantly more effective. Detailed studies on the mechanisms of action of such a broadly neutralising antibody has revealed that the replication of flu virus is inhibited by prevention of the membrane fusion during viral entry by insertion of the heavy chain of these antibodies into a conserved pocket in the stem region of virus HA (Ekiert et al. 2009; Sui et al. 2009).

The C05 also protected cells cultures infected with these flu viruses. Relatively low doses of C05 prevented infections in mice even after exposure to a lethal dose of influenza A virus. It was also found to confer complete post exposure protection of infected mice when this antibody was given up to 3 days post exposure.

It is not only important to select the correct target for the universal influenza vaccines, but the vaccine delivery method has to be unique and innovative as these conserved epitopes that might provide broad heterosubtypic immunity, are weak

immunogens. The objectives, prospects and expectations of a commercial universal vaccine, whenever these become available, need to be well defined. Who should be the target group for such vaccine, whether there is a need for booster or revaccination and when should it be undertaken. What should be the life of such a vaccination before its composition and formulation needs updating (Subbarao and Matsuoka 2013).

11.5 Treatment

- No specific drug therapy is practiced in birds and/or animals.
- To reduce the secondary bacterial infections, antibiotics and supportive therapy have been recommended.
- Anti-Flu drugs (Amantadine, Rimantadine, Zanamivir/Relenza and Oseltamivir/Tamiflu) can be given for both prevention of people from getting infected and treatment purpose also. They can turn the illness into a milder form and help in preventing serious complications. These must strictly be prescribed by a medical doctor only. Antipyretic medications and suitable anti-inflammatory drugs are also prescribed.
- Relenza and Tamiflu are NA inhibitors, which hold back the flu viruses from reproducing within the host cell, and are found to be most effective. Tamiflu is the drug of choice.

11.5.1 Treatment for Human Influenza Viruses

There are two classes of antiviral drugs: NA inhibitors (Zanamivir and Oseltamivir) and adamantanes (Amantadine and Rimantadine), which inhibit a viral protein called Matrix-2 (M2). These commercial antiviral drugs for treatment of the human flu are available only on the prescription of a medical doctor (Monto 2003; Das 2012; Nguyen et al. 2012). Adamantanes were used initially in the outbreak of H5N1 in Hong Kong in 1997 (Yuen et al. 1998). Amantadine and Rimantadine are effective against many subtypes of human influenza A viruses but not against H5N1 (Li et al. 2004), H1N1 (Parmar et al. 2011), or influenza B or C viruses (Vargese et al. 1983). These two drugs exert their antiviral effect by two mechanisms. First, the release of transcriptionally active ribonucleoprotein complex for transport to nucleus is prevented by blocking the influx of the H^+ ions into the core of the virion from the acidified endosome due to its effect on viral M2 protein. Second, the HA maturation during transport from the ER to the plasma membrane is blocked (Hay 1992). However, resistance of these two drugs to influenza viruses has increased to a very large extent and is widespread (Anonymous 2006; Weinstock and Zuccotti 2006; Ilyushina et al. 2007; Lan et al. 2010; Nguyen et al. 2012); and it is due to L26I or

S31 N mutations of the M2 protein. The binding of the drug is decreased by S31 N mutation (Pielak et al. 2009). The other two drugs Zanamivir and Oseltamivir exert their antiviral action against all influenza A virus subtypes (including H5N1) and influenza B viruses by inhibition of the viral NA (Tumpey et al. 2002; Moscona 2005a, b; Parmar et al. 2011; Hsu et al. 2012; Vijayan et al. 2012). Oseltamivir can be taken orally, but the optimal effect of zanamivir can be achieved after inhalation or intranasal administration only (Hsu et al. 2012). Various other NA inhibitors viz., peramivir and CS-8958 are also active against the H5N1 virus (Boltz et al. 2008; Kiso et al. 2010a). NA inhibitors can be used to combat pandemic influenza (Democratis et al. 2006). However, the resistance of influenza A viruses (Gupta et al. 2006; Monto et al. 2006; Alexander et al. 2007; Sheu, et al. 2008; Stittelaar et al. 2008; Fleming et al. 2009; Hauge et al. 2009; Burch et al. 2009; Naughtin et al. 2011; Nguyen et al. 2012), and influenza B viruses (Hatakeyama et al. 2007; Sheu et al. 2008) to NA inhibitors has been reported. Oseltamivir resistance can emerge and leads to treatment failure more commonly due to H274Y and N294S (N2 numbering) and less frequently due to V116A, I222L, K150 N and S246 N substitutions within the NA (de Jong et al. 2005; Renaud et al. 2011; Nguyen et al. 2012; Jarhult 2012). Oseltamivir resistance was observed in majority of the human influenza A (H1N1) viruses during the 2008–2009 influenza seasons in the United States (Anonymous 2009a) and Australia (Hurt et al. 2012). The S-OIV is susceptible to oseltamivir and zanamivir but resistant to the adamantanes (Anonymous 2009b). Pyrosequencing is used to determine the molecular markers of antiviral resistance in influenza A (H5N1) viruses (Deyde et al. 2009). Therefore, it becomes crucial to monitor the antiviral resistance among the field influenza viruses (Hurt et al. 2012; Nguyen et al. 2012; Jarhult 2012) to inform public health strategies for the control of influenza infections. Studies have been conducted to determine the effects of combination therapy for highly pathogenic H5N1 influenza virus infection in mice and cell cultures (Ilyushina et al. 2008; Smee et al. 2009). The antiviral activity of Carbocyclic Cytosine nucleosides against H5N1 has been demonstrated (Chu et al. 2008; Rao et al. 2009). Corticosteroids, when given for a long period of time or at higher doses can cause serious adverse reactions in influenza A (H5N1) virus-infected patients. Antibiotic may be given for suspected bacterial co-infection in patients with A(H5N1) virus infection. Oxygen may be given for correction of hypoxemia (WHO 2007). Apart from these, rest, good plan of nutrition, quitting smoking and adequate exercise will help the body to fight against influenza viruses.

The use of viral M2 inhibitor, adamantanes has been discontinued. The NA inhibitors are facing increased resistance, and the number of such mutants is feared to increase rapidly due to their indiscriminate and continuous use, putting the population at risk to a drug-resistant epidemic (Baranovich et al. 2011; Sheu et al. 2011). The development of increased resistance to FDA-approved antiviral drugs against influenza virus has encouraged a paradigm shift in the strategies for the development of antiviral drugs for influenza virus (Barik 2012; Eyer and Hruska 2013; Hayden 2013; Motohashi et al. 2013; Lee et al. 2014b). Various approaches

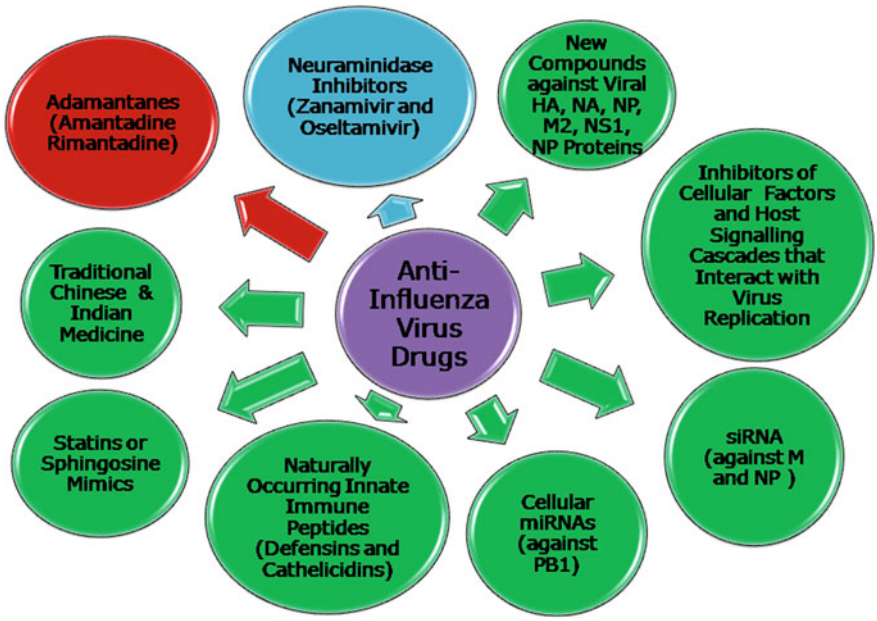

Fig. 11.3 Various approaches (past, present and future) to anti-influenza virus drugs. The admantanes have been discontinued. Currently, NA inhibitors are being used. But resistance against them has started to built in. A variety of approaches as mentioned in the illustration are being developed

have been used to develop antivirals against influenza viruses (Fig. 11.3). The inhibitors of cellular factors and host signalling cascades that directly or indirectly interact with virus replication are potential new development candidates (Karlas et al. 2010; Ludwig 2011; Shaw 2011; Muller et al. 2012; Lee and Yen 2012; Loveday et al. 2012; Beyleveld et al. 2013; Edinger et al. 2013; Terrier et al. 2013, Zhao et al. 2013). The cellular miRNAs have been shown to inhibit influenza viral replication by degradation of the viral PB1 gene (Song et al. 2010). The siRNA have been observed to inhibit influenza virus replication targeting the M and NP genes, and thus can be used as potential antiviral therapeutic agent against influenza virus (Ge et al. 2004; Barik 2010; Raza et al. 2011). A cocktail of multiple drugs against influenza virus, which may target two viral functions or one viral and one cellular function, can be developed (Nguyen et al. 2010). New compounds have been screened against various viral targets, such as NA (Kiso et al. 2010b; Ikematsu and Kawai 2011; Ivanenkov et al. 2013; Chen et al. 2014), HA (Prasad et al. 2013; Shen et al. 2013; Zhu et al. 2012; Yang et al. 2013a), the NP (Kao et al. 2010; Chenavas et al. 2013), M2 ion channel (Balannik et al. 2009), and NS1 (Jablonski et al. 2012). Nucleozin was found to trigger the aggregation of NP and inhibits its nuclear accumulation that is required for influenza virus replication (Kao et al. 2010). A mathematical model has been developed for building an antiviral strategy against influenza A infection (Hur et al. 2013).

Various novel peptides that inhibit influenza viruses have also been documented (Jones et al. 2006; Rajik et al. 2009; Matsubara et al. 2009, 2010; Triana-Baltzer et al. 2009). Defensins and cathelicidins, the naturally occurring innate immune peptides, have been tried as antivirals against influenza virus, though their mechanism of action has been observed to be different (Salvatore et al. 2007; Barlow et al. 2011; Tripathi et al. 2013). The antiviral activity of serum amyloid P against influenza A viruses has recently been demonstrated (Job et al. 2013). A different approach in the form of immunomodulatory therapy against severe influenza has been reported (Fedson 2006, 2013; Marsolais et al. 2009; Darwish et al. 2011; Viasus et al. 2011; Walsh et al. 2011) in which statins or sphingosine mimics have been used with the assumption and hypothesis that the cytokine-storm induced mortality and morbidity will be reduced. Broad subtype-specific DNA aptamers that bind with high affinity to influenza A viruses have recently been described (Shiratori et al. 2014). Traditional Chinese Medicine (Ge et al. 2010; Yang et al. 2013b; Lu et al. 2014b) and plant-based compounds (Song et al. 2005; Hsu et al. 2010; Lee et al. 2013), have also been tried for the treatment of influenza virus. Indian traditional system of medicine, Ayurveda, promotes the immunity of the host and involves the intake of herbs basil (*Ocimum basilicum*), Ginger (*Zingiber officinalis*), garlic (*Allium sativum*), gooseberry (*Embelica officinalis*), aloevera, camphor and eucalyptus oil (Parmar et al. 2011), *Ginkgo biloba* leaf extract (Haruyama and Nagata 2012), Red Sea grass (*Thallasodendron ciliatum*) (Ibrahim et al. 2012), flavanoids of various plants (Costa et al. 2012) and acidic polysaccharides from *Coccomyxa gloeobotrydiformi*, a green alga (Komatsu et al. 2013). The intake of these herbs may have beneficial effects during influenza infection.

11.6 Pandemic Preparedness

The WHO uses certain parameters and criteria to define various phases of influenza virus pandemic (Fig. 11.4). Rough guidelines for the responses that may be undertaken during a pandemic cycle have been documented (Fig. 11.5). Influenza is classified as a C category agent in potential bioterrorist agent (Rotz et al. 2002). Although the past pandemics of influenza were events naturally occurring but an influenza strain deliberately altered can lead to initiation of pandemic. With the increase in transportation facilities, urbanisation and introduction of new influenza virus subtypes (Starbuck et al. 2012), WHO and health authorities have recognised that the world is under threat of influenza pandemic worldwide, which could potentially have serious impact on health of human as well as animal population (Patriarca and Cox 1997). During pandemics of influenza in the twentieth century, death toll was millions and enormous social as well as economic losses were incurred globally. The forecasting of pandemic influenza virus, its pathogenicity and the extent of susceptible population is very difficult. So, the pandemic preparedness for influenza may help in reduction of virus transmission, and thus

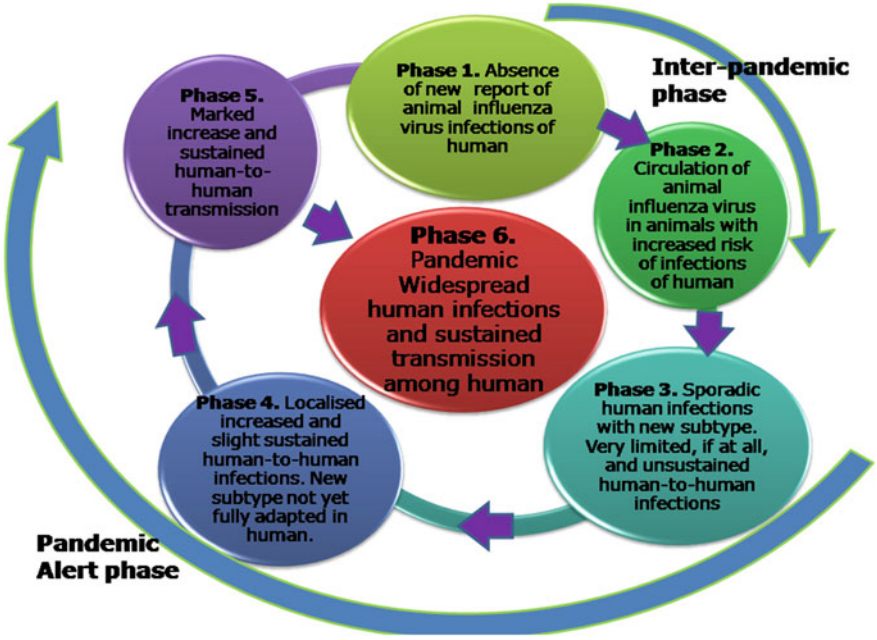

Fig. 11.4 Pandemic phases as classified by World Health Organization. The World Health Organization has classified the influenza virus pandemic into six phases, 1–6, using certain parameters and criteria as shown in the illustration. The Phases 1–2 are inter-pandemic phases. The phases 3–5 are pandemic alert phases and on crossing the 5th phase, the WHO declares a new pandemic of influenza virus

reduce the clinical cases and deaths. The preparedness varies from country to country and requires the commitment and input facilities. Planning healthcare for influenza pandemic is quiet tough and is not be possible within days or a month but require multicentric approach involving government, experts from various departments viz., policymakers, legislative persons, human and animal health professionals, pathology laboratories, extension workers and volunteers. On healthcare resource capacities, the AsiaFluCap Simulator can provide evidence-based and illustrative information during future pandemics and help to generally understand dynamics in resource capacities (Stein et al. 2012).

Sound epidemiological approach is the basis for pandemic awareness for controlling HPAI in Asia and other parts of the world but complete eradication is difficult due to the presence of the virus in wild reservoir birds. The range of epidemiological scenarios existing in different systems of poultry production in several Asian countries is considered in this approach. A high incidence of disease with greater frequency of outbreaks in poultry and humans are included in the epidemiological scenarios along with incidence of disease at low frequency; sporadic outbreak of disease or risk of contracting disease. Combined accurate disease control options (*FAO Position paper: Recommendations on the prevention,*

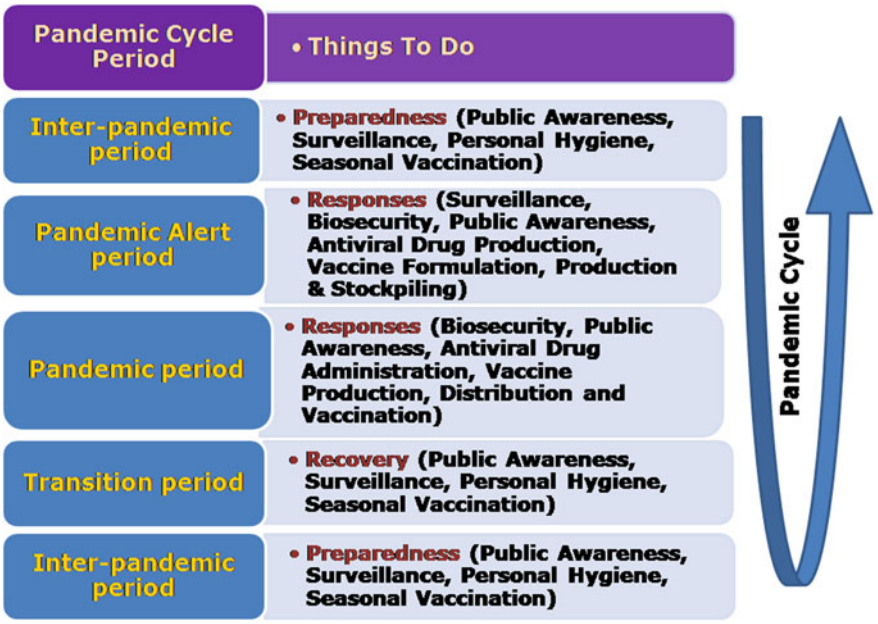

Fig. 11.5 Pandemic cycle and various responses to be undertaken. A pandemic cycle consists of inter-pandemic period in which continuous surveillance is carried out, general public is made aware of the benefits of personal hygiene and seasonal vaccination is done. During the pandemic alert in addition to the above, drug production, vaccine formulation, production, stockpiling and administration is carried out. During the pandemic period, besides the above legislation enforcement, vaccine distribution and vaccination and drug administration is done. The transition period is the period of recovery

control and eradication of HPAI in Asia, September 2004, http://www.fao.org/ag/againfo/subjects/en/health/default.html*)* is received via approval and support of OIE. Depending on the stage at which various nations and farming systems have reached along with the variable disease status, this approach received both approval and support of OIE.

Following the outbreaks of SARS, HPAI and swine flu (H1N1), many countries learned lessons from these outbreaks but there is always a space for further improvement so there should be regular revision of pandemic preparedness which may be divided into following steps:-

Emergency preparedness. For starting of pandemic preparedness for any disease, human resources (policy making) as well as financial resources (funding) are important and need to be taken care of. The persons should be identified from various organisations for contribution in the planning. For influenza pandemic preparedness planning, the persons to be included are epidemiologists, virologists, experts from human and animal health professionals, administration, military and paramilitary persons, representatives of NGOs, press and media persons and schools (Rebmann et al. 2012). The proper coordination among these persons

requires appropriate command and control structure. For carrying out essential functions, there is a need to develop standard operational procedures and role. Individual and group responsibilities during a pandemic should be known to all. In order to fill the gaps in knowledge about the influenza pandemic and especially vaccine in poorer nations, extension works should be done where considerable differences in education and access to media are evident (Kouassi et al. 2012; Cantey et al. 2013; Sims 2013).

Surveillance. Surveillance is collection of data along with their interpretation and dissemination for developing the evidence-based interventions. In influenza pandemic, surveillance for influenza-like illness (ILI) must be established in man and animals along with creation of links between animal and human health professionals. The highly dynamic nature of influenza viruses demands regular systematic surveillance of influenza in both humans as well as animals for getting all-inclusive picture of the prevalence of influenza viruses. For this, there is a need for simple and easy to perform test for the characterisation of influenza virus that has recently emerged (Mak et al. 2012).

Case investigation and their treatment. For confirmation of suspected influenza cases, diagnostic laboratories for human as well as animal should be well equipped with immunofluorescence (IF); reverse transcriptase polymerase chain reaction (RT-PCR) testing along with training facilities. Daily reporting of the influenza cases is compulsory. To ensure treatment of the affected individuals, clear-cut guidelines should be there.

Prevention of disease spread in the community. In case of influenza pandemic, medicines and vaccines are limited in developing countries, so the focus should be on non-medical interventions to check the spread of the disease. In animals, it may be possible up to some extent, but in humans, it affects the behaviour and rights of humans and needs firm educational and legal support. There is a need to strengthen the personal respiratory hygiene. Enzootic circulation of HPAI viruses at present requires increased ability to produce pandemic influenza vaccine globally which becomes one of the important components of pandemic preparedness plans, and its targeted use during pandemic alert or in early pandemic situation is likely to mitigate the consequences of an influenza outbreak (Plosker 2012). There must be some strategies to ensure rapid and quick supplies of vaccines, their distribution and importation to countries that may bear the brunt of a future epidemic (Mihigo et al. 2012; Sims 2013).

Border controls. In the event of an outbreak of HPAI, the movement of birds and products internationally is controlled as per OIE recommendations. Countries must restrict import of poultry and products from infected countries until safe trade recommences, which will depend on whether or not the infected country or countries are adhering to recommendations laid down by OIE. Border control measures decrease but cannot rule out risk of infection. Entire banning of smuggling cannot be done. In some instances, advanced measures to quarantine may give rise to a lucrative environment for smuggling especially if there is shortage of products. Geographical, economical, social and political reasons, individually or collectively, ensure reasonably well-secured borders. The chances of borders

leakage must be thought by veterinary authorities while disease control and prevention strategy are developed. International borders cannot be identified by wild birds. Veterinary authorities must take steps to prevent infected wild bird population from coming in contact with domestic poultry and transmitting the infection to them (WHO 2005).

Maintaining essential services. To keep a society going about with their daily chores, essential services are required. To reduce the morbidity and case fatality rates, it is very important that health services should be functional all the time. The requirements of human and animal healthcare professionals, their sources and their training, disposal of corpse, animal carcasses and their infected belongings etc., have to be identified in advance. Thorough understanding of the contributory factors to the willingness of healthcare authorities to report to work during a public health crisis is significant in planning for pandemic preparedness (Devnani 2012). Duty to healthcare has been identified as a pressing ethical issue in contemporary discussions of pandemic preparedness, and women still dominate the front lines of healthcare work (Godderis and Rossiter 2012). There is a need of tight coordination, communication, integration, and alignment in any management structure (Fieldston et al. 2012; Sims 2013).

Research and evaluation. During a pandemic, the country is having an extra burden on resources to control the disease but this situation might help in understanding of the disease and effect of preventive measures, which will contribute to global knowledge. The research may include virus transmission (Fouchier et al. 2012; Van Gageldonk-Lafeber et al. 2012), antigenic and molecular characterisation of virus strain, antiviral drug resistance, vaccine efficacy and socio-economic impact of the pandemic etc.

11.7 Seasonal Influenza

Apart from the influenza virus pandemics that occur only very rarely, the annual/SI infections occur every year and are responsible for lots of morbidity, deaths and huge direct and indirect economic losses. In temperate regions, low-level flu infection/disease may occur throughout the year but the peak occurs in January/February with gradual increased incidence being observed between October to March. Thus, there is a seasonal aspect to influenza virus induced disease in temperate regions. The seasonality of influenza and its effects and impact in the tropical regions is less obvious (Cox and Subbarao 2000). The understanding of the seasonality of influenza may provide greater knowledge about the maintenance, spread and transmission of the disease (Naumova 2006) that in turn will help in putting in place better preventive and control measures. Although many theories based on influenza virus biology, host immunity at individual and population level that are influenced by factors such as photoperiod, nutritional status, crowding, ambient temperature, indoor heating, air travel, bioaerosols and paths of global wind streams (Dowell 2001; Hammond et al. 1989) and many mathematical models such as

seasonal forcing, dynamical resonance, bifurcation phenomenon (Dushoff et al. 2004; Smith 2003, 2006) have been described, yet none of them have been able to completely address the mystery of seasonality of influenza (Lofgren et al. 2007).

Every year simultaneous co-circulation of a number of different influenza virus strains in the human population is observed. The protective immunity to influenza viruses is of short duration, homotypic and is mainly mediated by antibodies. Therefore, cross-protection by antibodies generated against one influenza virus type, or subtypes of influenza A virus normally does not occur against another type or subtypes of influenza A virus or their antigenic variants (Couch and Kasel 1983). The accumulated point mutations are responsible for the frequent appearance of antigenic variants causing the annual epidemics of seasonal influenza. It is for this reason that the influenza vaccines are reformulated in most seasons to include those influenza virus strains that are expected to prevail and cause disease during the upcoming season.

SI is an acute viral infection mainly of the upper respiratory tract caused by subtypes of influenza A virus and influenza B virus strains. Presently, the H1N1 and H3N2 among the many subtypes of influenza A virus are responsible for seasonal influenza. Although SI can occur in age group, but children <2 years, adults >65 years, and people of any age with diabetes, or some chronic diseases of heart, lung, kidney, liver or immune-incompetent persons constitute the high-risk group. The main clinical signs and symptoms of SI are abrupt onset of pyrexia, dry cough, sore throat, rhinitis, headache, myalgia and severe malaise. Except for the high-risk groups where severe complications, pneumonia and death may occur, most other healthy infected adults do not need medical care and natural recovery occurs within 7 days. A rough estimate of annual influenza-associated deaths ranging between 3,000 and 49,000, during 30 seasons from the 1976–1977 season through the 2005–2006 season, have been reported (CDC 2010). The economic burden due to SI is due to complications and death in high-risk group as well as productivity losses on account of absence of the workforce. Antiviral drugs and vaccines are available for prevention of SI. Vaccines are quite effective in healthy adults and can prevent 70–90 % of influenza-specific illness in them. Reduction of severity and complications by up to 60 % and mortality by 80 % has been observed in elder persons through vaccination against SI.

The selection of the virus strains that will constitute the SI combined vaccine regimen by the World Health Organization (WHO) is a complex process and involves the national influenza centers, five WHO Collaborating Centers for Reference and Research on Influenza, Essential Regulatory Laboratories (Figs. 11.6 and 11.7). After detailed deliberations and consultations in the month of February of each year, the recommendations for the SI vaccine's composition are given by the WHO. The timeline for the manufacture of SI virus vaccine is shown in Fig. 11.8.

The WHO Vaccine Composition Meeting for Northern Hemisphere 2013–2014 and Southern Hemisphere for 2014 influenza season vaccines were held on February 21, 2013 and September 23–26, 2013, respectively, at WHO headquarters in

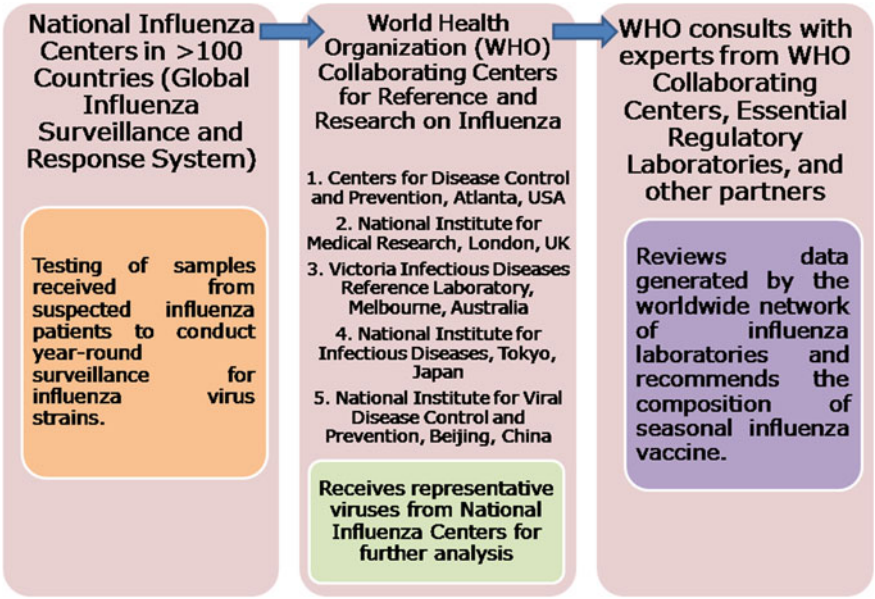

Fig. 11.6 Selection of virus strains for the seasonal influenza vaccine

Fig. 11.7 Essential regulatory laboratories that help WHO in selection of the composition of seasonal influenza vaccine

Geneva, Switzerland (WHO 2013). The recommended composition for both the hemispheres was the same. The Vaccines and Related Biological Products Advisory Committee (VRBPAC) a part of the USFDA on February 27, 2013 approved

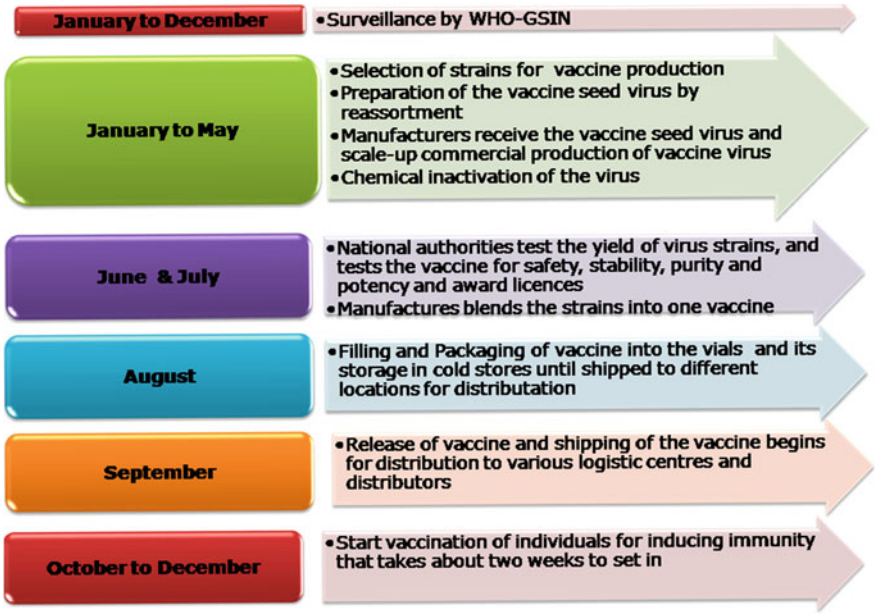

Fig. 11.8 Manufacturing timeline for the seasonal influenza vaccine

the WHO recommendations for this year's seasonal flu vaccine for the United States (FDA 2013). The equivalent agency to VRBPAC of U.S. is the European Centre for Disease Prevention and Control (ECDC) for the EU/EEA countries for this purpose.

For the 2013–2014, the SI vaccines can be Inactivated Influenza Vaccine (IIV), Live Attenuated Influenza Vaccine (LAIV) grown in embryonated eggs, or cell culture (cc) and a recombinant vaccine (Fig. 11.9). The inactivated influenza vaccines can be trivalent (IIV3) or quadrivalent (IIV4). Similarly quadrivalent live attenuated influenza vaccines (LAIV4) are available for the 2013–2014 season (Grohskopf et al. 2013). The antigenically distinct Victoria and Yamagata lineages of influenza B provide only limited cross-protection. Unpredictability of the predominance of B virus lineage during a given season and limited cross-protection led to the inclusion of one strain from each of the two influenza B virus lineage in SI vaccines (Ambrose and Levin 2012; Block et al. 2012; Reed et al. 2012). Also, for the first time, an approved and licensed trivalent recombinant HA influenza vaccine (RIV3) containing purified HA proteins is available in U.S. for use against SI in persons aged between 18 and 49 years (Protein Sciences 2013). It can safely be used in persons who are allergic to eggs.

Besides the RIV3, the other recently approved vaccines, including LAIV4, IIV4, trivalent cell culture-based inactivated influenza vaccine (ccIIV3) are expected to be in great demand in U.S. this season. Although, in those situations or target groups where more than one type of vaccine formulation is available, WHO

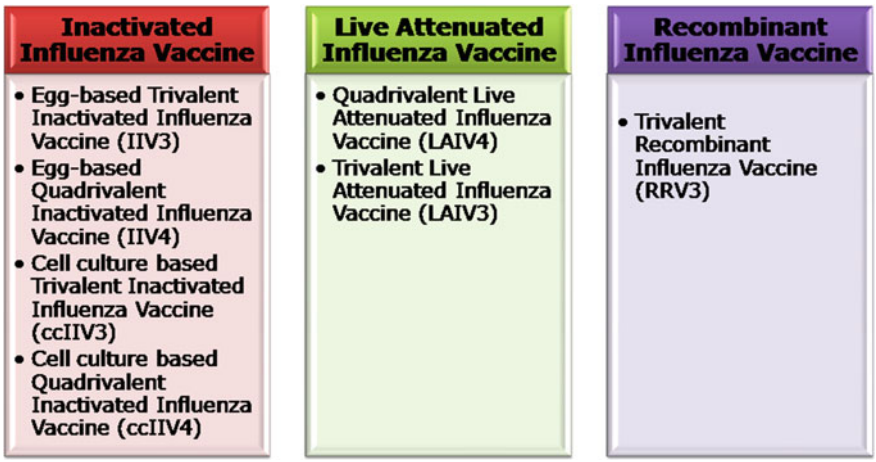

Fig. 11.9 Types of influenza virus vaccines available for 2013–2014 influenza season

is unbiased and does not recommend any favoured treatment to any category or manufacture of the influenza vaccines. It is estimated that production of about 135 million and 139 million doses of inactivated and live vaccine in the U.S. will occur for the current influenza season. The European Medicines Agency (EMA) is presently evaluating the quadrivalent LAIV. For the 2013–2014 influenza season, the ACIP has recommended routine annual influenza vaccination for all persons aged $\geq$6 months who do not have contraindications. The ACIP has, in its report, has also given specific recommendations and guidance on the use of alternative influenza vaccines for the high-risk groups etc. such as the persons aged $\geq$65 years and <2 years, pregnant women, persons allergic to eggs, and those with medical conditions that confer high risk for complications from influenza (Des Roches et al. 2012; Erlewyn-Lajeunesse et al. 2009; Jamieson et al. 2012; Grohskopf et al. 2013).

The 2013–2014 season influenza quadrivalent influenza vaccines will contain haemagglutinin obtained from the following:

- an A/California/7/2009 (H1N1)-like virus,
- an (A/Texas/50/2012 (H3N2) virus,
- a B/Massachusetts/2/2012–like (Yamagata lineage) virus,
- a B/Brisbane/60/2008–like (Victoria lineage) virus.

The trivalent influenza vaccines for 2013–2014 season influenza will be similar to the quadrivalent influenza vaccines except that this will not contain a B/Brisbane/60/2008–like (Victoria lineage) virus.

The composition of the influenza vaccines for the following influenza season is recommended two times a year, after analysing the influenza virus surveillance data generated by the WHO Global Influenza Surveillance and Response System

(GISRS) after detailed discussion with advisory group of experts. The WHO meeting to deliberate on the composition of influenza virus vaccines for the Northern Hemisphere 2014–2015 is going to be held on 17–19 February 2014, in Geneva, Switzerland.

In order to create public awareness about the advantages of continuing influenza vaccination, National Influenza Vaccination Week (NIVW) is celebrated every year. The NIVW for last year was observed from December 8–14, 2013.

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