EVOLVING THREAT OF EQUINE ’FLU: HOW BEST TO DEAL WITH MENACE

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ROMAIN PAILLOT, NEIL BRYANT, RICHARD NEWTON discuss the virology of a condition that remains a leading cause of infectious respiratory disease, and the vaccination options available worldwide

A DISEASE of horses that was clinically indistinguishable from influenza and referred to as a “distemper” was described as long ago as 1732.

In the 1930s, Germa n researchers comparing epidemic coughing in young racehorses with the then recently isolated swine influenza virus showed, for the first time, that the disease was due to a filterable virus and that it was experimentally reproducible. Equine influenza (EI) became recognised as a highly contagious respiratory disease, characterised by acute pyrexia and a harsh, dry cough that rapidly spread both within and between groups of young horses, particularly in racing yards. There were few deaths or complications when horses were rested and recovery usually occurred in two to three weeks.

The first demonstration of a virus as the cause of equine influenza occurred in 1955, when it was shown that horses in a respiratory disease epidemic in Sweden seroconverted to a soluble human influenza virus antigen. Shortly afterwards, an influenza virus was isolated for the first time from coughing horses in Czechoslovakia, and this prototype virus was the Prague/56 H7N7 virus.

Retrospective serological surveys found that the virus had been the cause of disease in many areas of the world. Serological and virological monitoring of epidemic respiratory disease

demonstrated that this prototype virus continued to be responsible for disease outbreaks in predominantly young horses in many countries between 1957 and 1963.

In January 1963, there was an outbreak of rapidly spreading acute respiratory disease among horses at several racetracks in Miami, USA. A characteristic of this outbreak, which differentiated it from earlier outbreaks, was that it affected all ages, rather than predominantly younger horses. A novel H3N8 virus Miami/63, antigenically distinct from Prague/56, was isolated and was subsequently responsible for an equine influenza pandemic.

A serological survey of H3N8 antibodies, following the epidemic of H7N7 influenza in the UK in 1963, demonstrated that British horses were completely susceptible to the new virus and an epidemic was predicted. Despite the start of production of a vaccine, insufficient stocks were available before the disease was first seen in February 1965, on two studs in Sussex. One stud had recently received mares from France, where the disease had been present for a month.

Since the pandemic of equine influenza H3N8 in the 1960s, much work has been done on developing and improving dedicated equine influenza virus vaccines and vaccination protocols. The remainder of this article considers aspects of equine influenza virology and vaccinology that today deliver veterinary surgeons, horse owners and equestrian legislators with some of the most technologically advanced vaccines in the world.

Despite this, however, equine influenza continues to pose a significant threat as one of the most highly infectious diseases in horses, and it has the ability to travel long distances in a short period of time via international air transport.

Virology

Influenza A viruses are members of the *Orthomyxoviridae* family. They have a segmented, singlestranded RNA genome with eight gene segments in complex with the viral nucleoprotein (NP), and each segment is associated with a polymerase complex consisting of the proteins PB1, PB2 and PA. Three proteins are embedded in the virus surface: the two surface glycoproteins – haemagglutinin (HA) and neuraminidase (NA) – and the membrane channel protein (M2; see Figure 1).

The HA is the major surface glycoprotein, making up approximately 25 per cent of the virus protein, compared with five per cent for the NA. Virus particles bind to host cells via HA and are internalised through membrane fusion. The HA is also the primary target for neutralising antibodies. Influenza virus is enveloped and, as such, is not infectious for long outside the host and is rapidly inactivated by sunlight and disinfectants.

Antigenic drift occurs when mutations in the gene sequence result in amino acid substitutions, particularly in the HA protein. Immunological pressure, such as might occur with vaccination during

inter-epidemic periods, may influence antigenic drift. Antigenic drift has been identified within H3N8, to the extent that several antigenically divergent subtypes and/or clades have been identified (Figure 2). However, the rate of drift in the H3N8 equine viruses is about one-third of that of human H3 HA (Bean et al, 1992).

This process allows new virus variants to escape immunity induced by previous infection or vaccination, as was seen with the evolution of two lineages, namely the American and the Eurasian, which occurred from about 1987. The American lineage has since diverged again to form the Florida sublineage (Lai et al, 2001), which within the past 10 years has diverged again to form two separate clades (Animal Health Trust [AHT], unpublished data). Major or subtype changes in the surface glycoproteins occur as a result of recombination with other influenza viruses and are called antigenic shifts. Antigenic shifts give rise to new viruses that may result in pandemics in susceptible populations, as was seen with the emergence of H3N8 viruses in Miami in 1963.

An expert panel advises the World Organisation for Animal Health (OIE) on a yearly basis, to determine whether antigenic drift has occurred to such an extent that the update of vaccine strains is recommended.

Knowledge of outbreak virus strains allows better predictions of which vaccine would be most effective. Many of the vaccines currently available contain strains that are antigenically distant from current circulating strains (Table 1); however, a vaccinated population is undoubtedly better protected than an unvaccinated population. Experience in Europe with endemic influenza, which is largely effectively controlled by vaccination, has shown that it is important to maintain surveillance on viruses to ensure that vaccines remain updated with respect to epidemiologically relevant strains.

Vaccination

Vaccination has been shown, experimentally, to protect against virus challenge, the level of which is related to the antigenic relatedness of the vaccine to the challenge virus strain (Yates and Mumford, 2000). In the case of a significant mismatch between strains, vaccinated horses can be affected by equine influenza virus (EIV). However, our experience suggests that vaccinated horses have less severe clinical signs and shed virus for a shorter length of time, if indeed they are affected at all. Furthermore, vaccination improves recovery from EIV infection and reduces the prevalence of secondary bacterial infection, which can lead to significant morbidity and, rarely, mortality.

This was well demonstrated during an outbreak of EI in 2003, where vaccinated racehorses did show clinical signs of EIV infection, but horses with more complete vaccination histories showed less clinical signs of disease and shed virus for a shorter period of time. Thus, despite having vaccine strains that do not completely match circulating virus strains, and despite many pleasure horses in the UK not being vaccinated against EIV, we believe our racehorse population is relatively well protected against EIV, and is undoubtedly better protected than if they were not

vaccinated. This is illustrated by the limited spread of EIV in the UK after 2003, which coincides with some improvements in the vaccine technologies available but no significant update of influenza viral strains in these vaccines (AHT, unpublished data).

Vaccine technologies and immune responses

Natural infection with EIV confers a long-term immunity to re-infection with a closely related virus. Clinical signs of disease and virus shedding are reduced in ponies that had been previously exposed to, and infected with, equine influenza virus.

This immunity, involving both humoural and cellular immune responses, has been shown to persist for at least 32 weeks after infection, and partial protection was still achieved one year afterwards (Hannant et al, 1987; Slater and Hannant, 2000). In terms of vaccine technology, commercialised vaccines against EI can be divided into four categories (Paillot et al, 2006). Since the introduction of EIV vaccines in the 1960s, the majority of commercially available vaccines have been inactivated whole virus (Figure 3).

The main advantages of these vaccines are the absence of pathogenicity, virus replication and subsequent spread between hosts. The influenza virus has been traditionally grown in embryonated hens' eggs for the preparation of these vaccines. Viruses were inactivated with either formaldehyde or â-propriolactone. This inactivated virus is often mixed with an adjuvant that improves the strength and duration of the immune response. We believe killed vaccines, with only an aluminium hydroxide adjuvant, may be less effective than vaccines with current adjuvant, such as carbomer. Protection induced by conventional inactivated EIV vaccines is based on the stimulation of a strong antibody immune response, but remains constrained by the requirement of a correct match between vaccine and field strains of EIV.

In the past decade, a whole generation of vaccines has been designed to stimulate a virusspecific immune response aimed at mimicking immunity induced by natural infection with EIV, to provide a long-lasting protection involving both humoural and cellular immune responses and, therefore, to minimise the effect of a strain mismatch.

Current subunit vaccines against EIV contain purified HA and NA proteins. The level of circulating antibodies against HA has been shown to play an important role in protection against EI (Mumford and Wood, 1992). Haemagglutinin is a major surface glycoprotein that is essential for cell entry. The presence of HA-specific antibody can neutralise viral particles and prevent infection of cells from the respiratory tract. Neuraminidase is the other major surface protein. Antibodies against NA do not block infection, but they can inhibit the enzymatic activity of NA, which can decrease viral replication in the lungs and disease severity. Little is known about the importance of the NAspecific immune response for protection against equine influenza. The main types of subunit vaccines are either based on immune-stimulating complexes (ISCOM) vaccines or ISCO MATRIX vaccines. ISCOM particles are spontaneously formed cage-like structures resulting from the combination of

antigens with cholesterol, phospholipids and *Quillaja* saponins (Figure 4). ISCOMATRIX vaccines are identical to the ISCOM vaccine, except that the antigen is not bound within the cage structure of the ISCOM.

Live-attenuated virus vaccine contains a temperature-sensitive influenza virus that multiplies efficiently in the cooler environment of the upper respiratory tract, where immune responses are induced, but it does not replicate in the warmer environment of the lower respiratory tract in horses – therefore avoiding clinical signs of disease. This vaccine is commercialised in the USA.

Live recombinant vector vaccines are constructed by inserting HA genes from EIV into live and infectious, but non-disease-causing, canarypox viruses (Figure 5). A modified live canarypox vector vaccine is currently commercialised in Europe and the USA. The duration of immunity post-vaccination may be variable, depending on the vaccine used and response of individual horses. Despite the correct vaccination protocol being followed, some horses do not develop a normal antibody response to vaccination. This represents a very small percentage of horses, but such a phenomenon is not yet fully understood.

What vaccine to use

The vaccines that are currently available have had full safety studies performed. It has been suggested that vaccination against EI can make horses sick – and days in training can be lost as a result. Our experience suggests that, while first-generation EI vaccines (inactivated virus vaccine) did have some documented problems, the vaccines currently available have minimal associated side effects and are commonly used in actively training horses. It should be noted that full protection of any vaccine against any virus strain can only be determined using a challenge study utilising the specific vaccine and targeted field strain.

Despite this, many outbreaks around the world have been effectively controlled with vaccines that are currently available. If the population is not vaccinated, EI is likely to continue to spread through the domestic and feral horse population. The feral horse population is quite large in some countries – disease in these animals could provide a reservoir of infection that could come back and infect the domestic population later.

The strains of virus present in the vaccine and its technology are important in determining the efficacy of the vaccine. The AHT does not endorse any specific vaccine, although we recommend a frequent review of those that are commercially available and comparison of their strain composition with the last recommendations of the expert surveillance panel (ESP) on equine influenza. At the time of writing, no commercially available vaccines meet current ESP recommendations. Several influenza vaccines currently commercialised in the UK have been found to protect horses against the outbreak isolates A/eq/Newmarket/5/03 or A/eq/South Africa/4/03 (Edlund Toulemonde et al, 2005; Daly et al, 2007; Paillot et al, 2008). We have demonstrated that at least two of these vaccines were able to provide a reasonable level of protection against an H3N8 virus isolate

representative of the Australian outbreak (Sydney/07; Bryant and others, 2008).

Conclusion

For decades, EIV has been a leading cause of infectious respiratory disease in horses. EIV is antigenically variable and frequently evades the immune system, particularly where vaccines have not been updated recently. At the present time, equine practitioners have a diverse range of equine influenza virus vaccines to choose from. Whole inactivated virus vaccines and sub-unit vaccines remain the predominant types of vaccines in use. Protection afforded by these vaccines is based on inducing high levels of protective circulating antibodies.

However, this response is relatively shortlived and highly specific, particularly in horses that have only received a few doses. Several other vaccine technologies are now available (such as ISCOM-based and poxvirus-based vaccines). These stimulate both humoural and cellular immune responses and so aim to mimic more closely the protective immunity induced by natural infection with EIV.

Despite the availability of vaccines against EIV since the 1960s and widespread compliance with mandatory vaccination requirements for thoroughbred and sport horses since the early 1980s, outbreaks of equine influenza continue to occur worldwide, as illustrated in 2007 with the occurrence of large outbreaks in Japan and Australia.

The continued improvement of current vaccines, the further development of new vaccination strategies and ever more comprehensive surveillance are essential actions to counteract the inevitable evolution of influenza viruses.

Clinical signs of equine influenza

• After an incubation period of one to three days, the f irst clinical sign of equine influenza is an elevation of body temperature (up to 41°C), which can last for four to five days.

• A harsh, dry cough releases large quantities of virus and helps spread infection.

• Coughing is commonly accompanied by a serous and/or mucopurulent nasal discharge.

• Other clinical signs of disease may include myalgia, inappetance and enlarged submandibular lymph nodes.

• The severity of the disease depends principally on the type (such as the subtype or strain) of EIV and the immune status (from previous infection or vaccination) of the horse.

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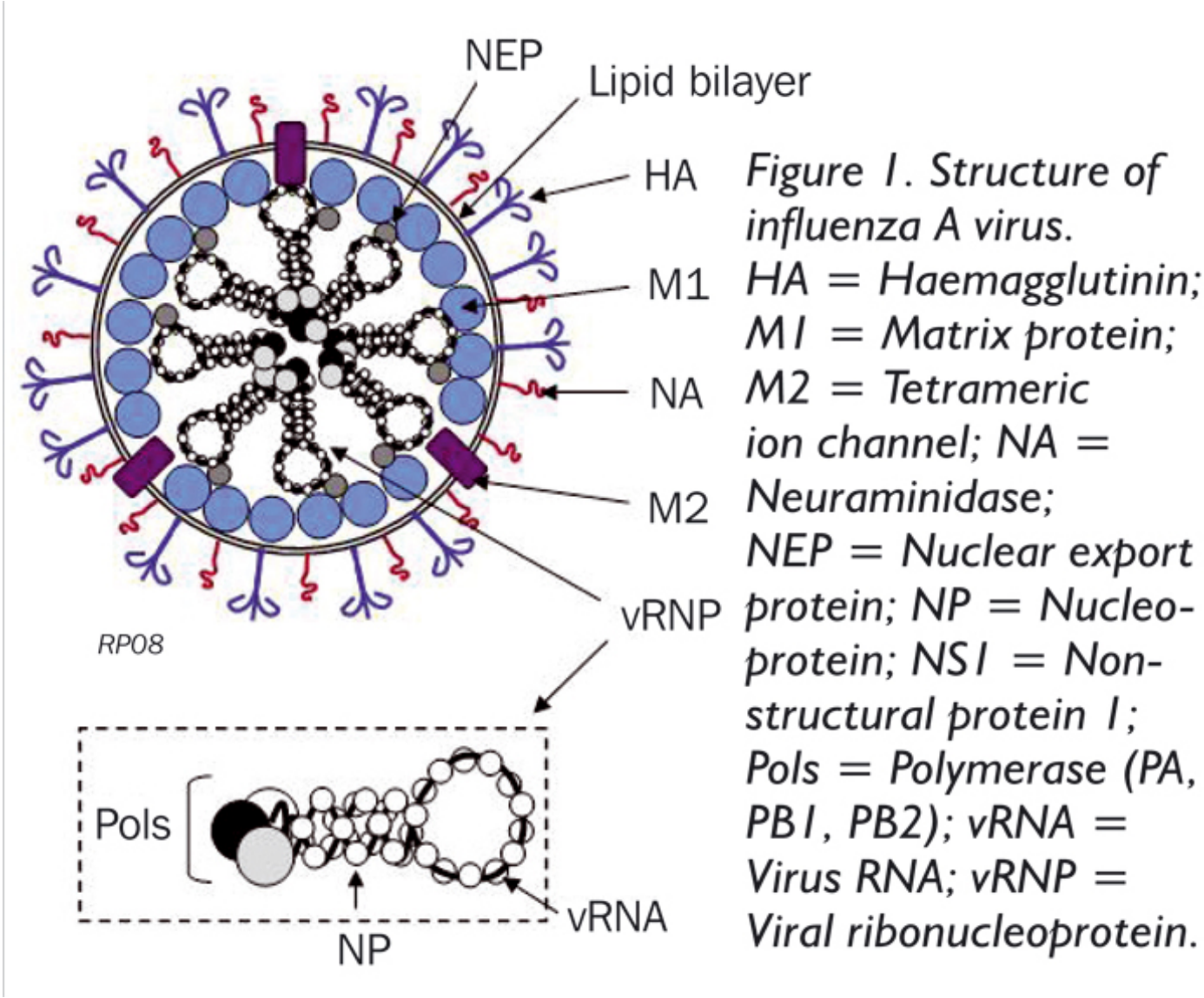
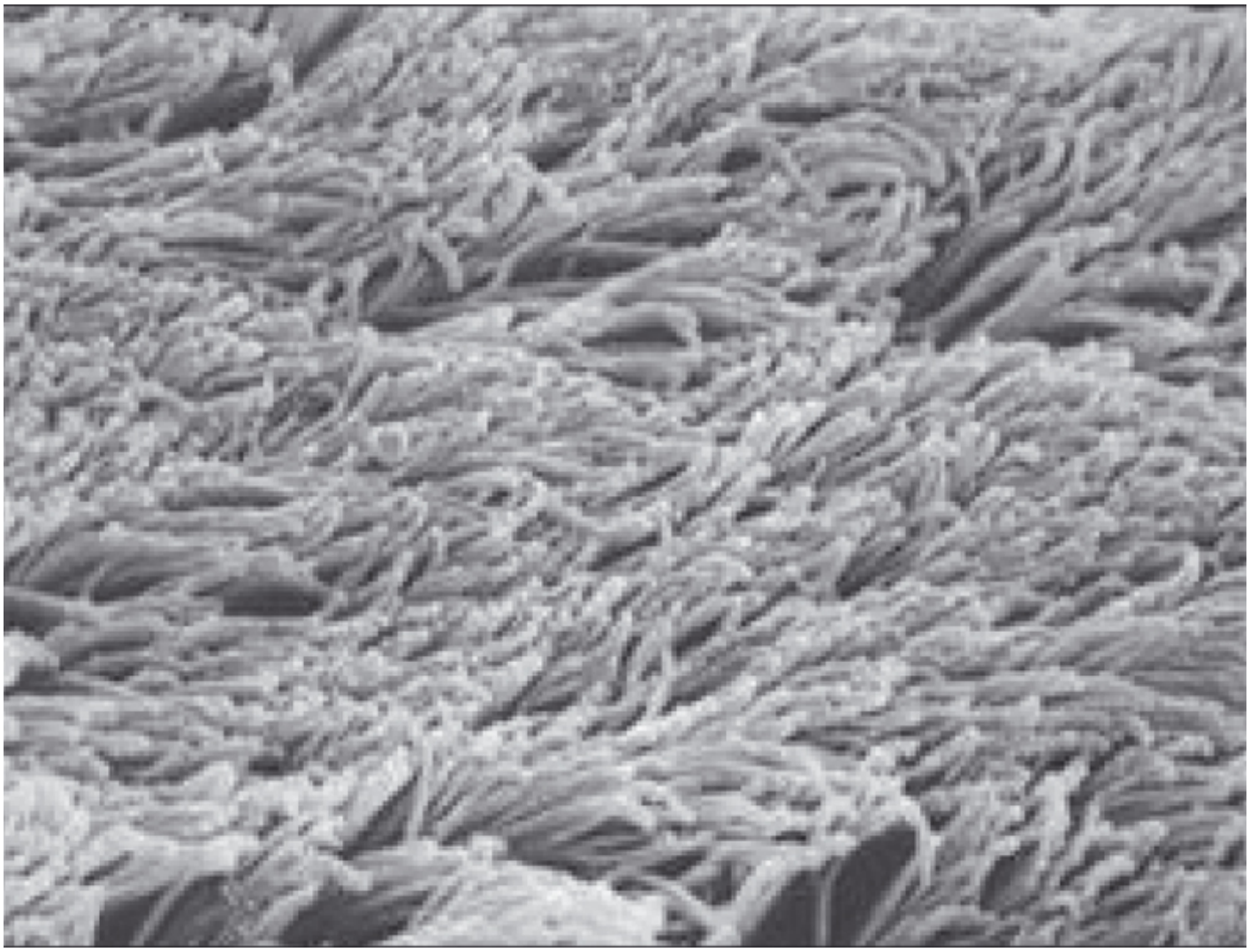
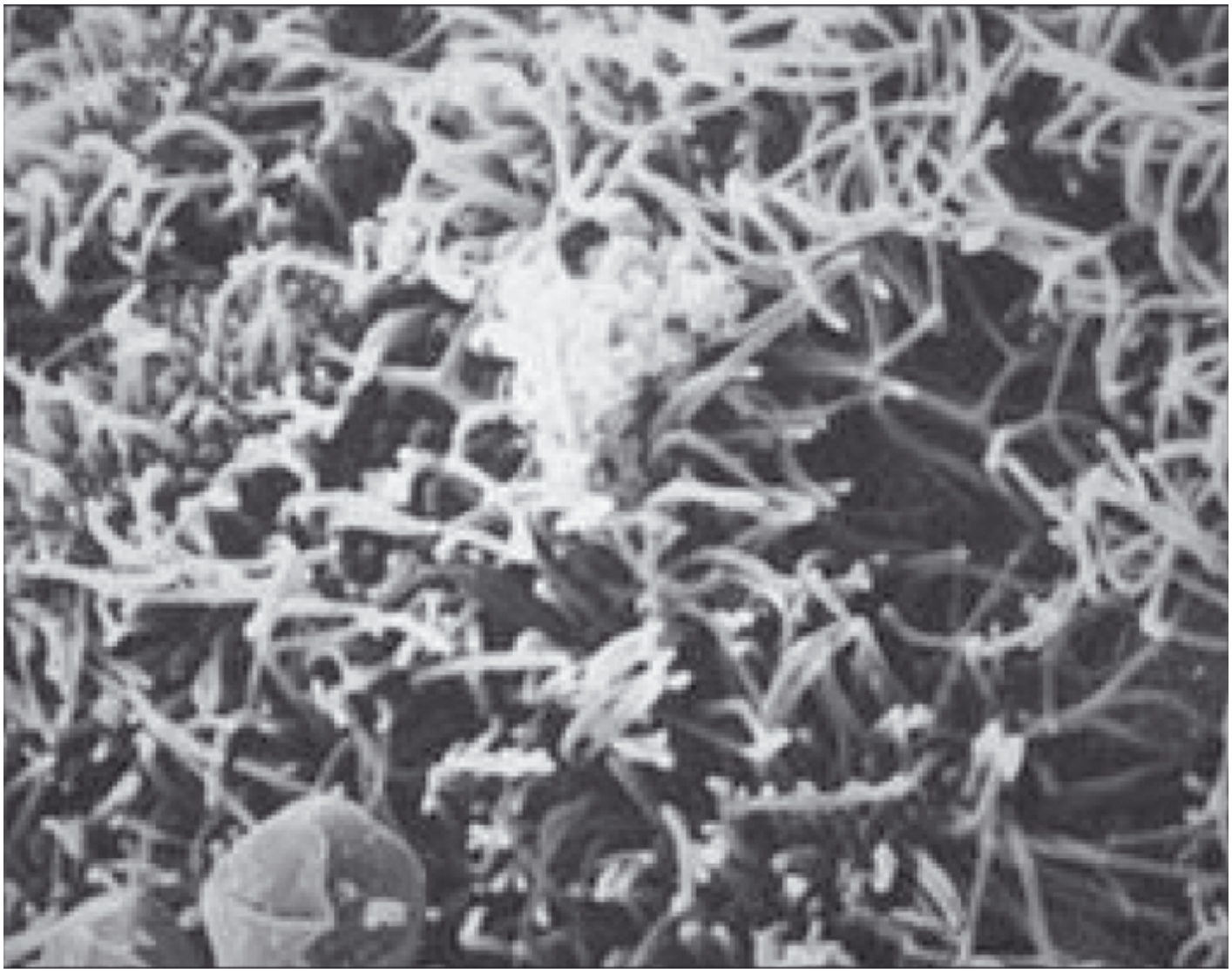


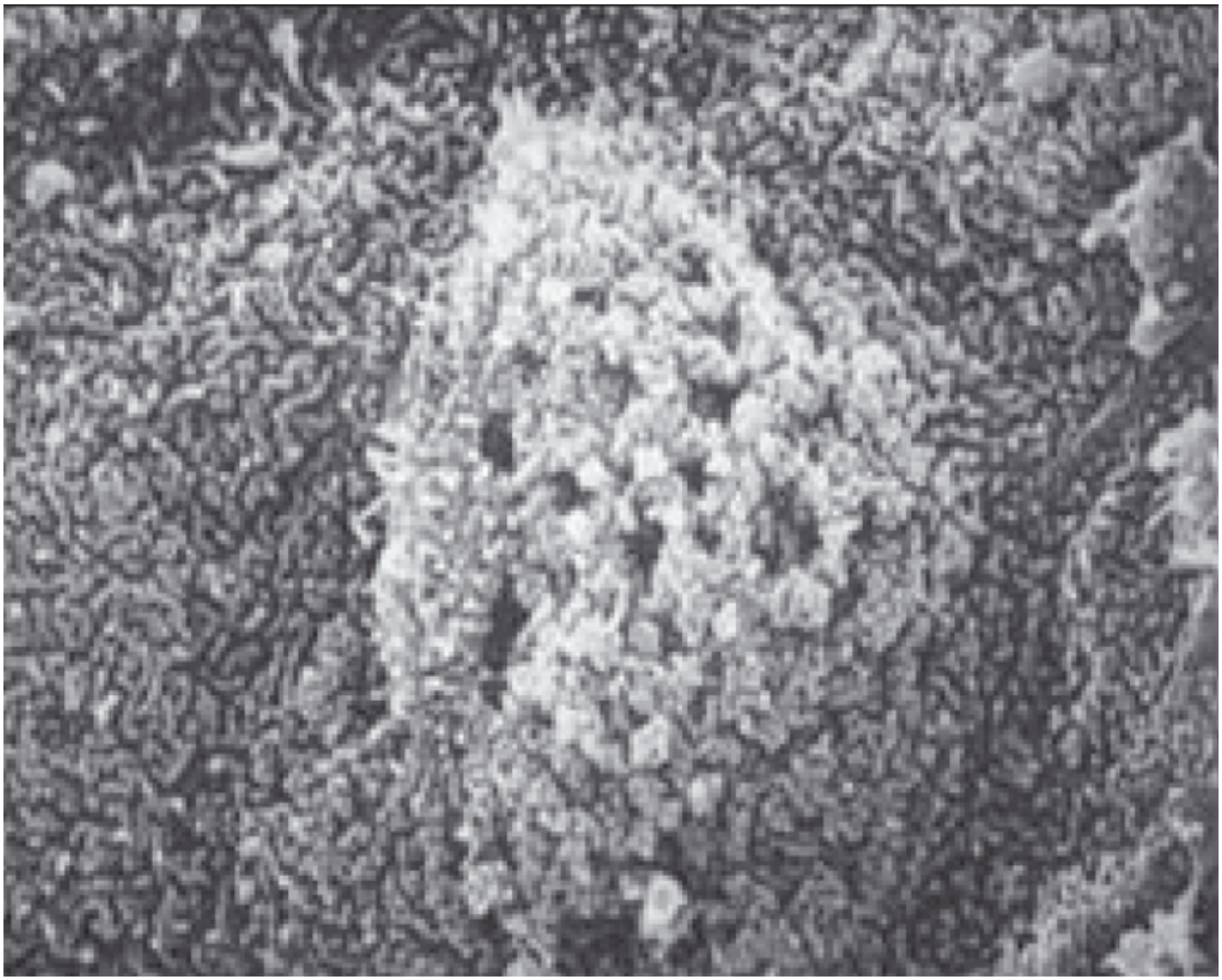
Figure 1. Structure of influenza A virus. HA = Haemagglutinin; M1 = Matrix protein; M2 = Tetrameric ion channel; NA = Neuraminidase; NEP = Nuclear export protein; NP = Nucleoprotein; NS1 = Nonstructural protein 1; Pols = Polymerase (PA, PB1, PB2); vRNA = Virus RNA; vRNP = Viral ribonucleoprotein.



Electron microscopy view of healthy tracheal ciliated epithelium.



Electron microscopy view three days after infection with EIV.



Electron microscopy view six days after infection.

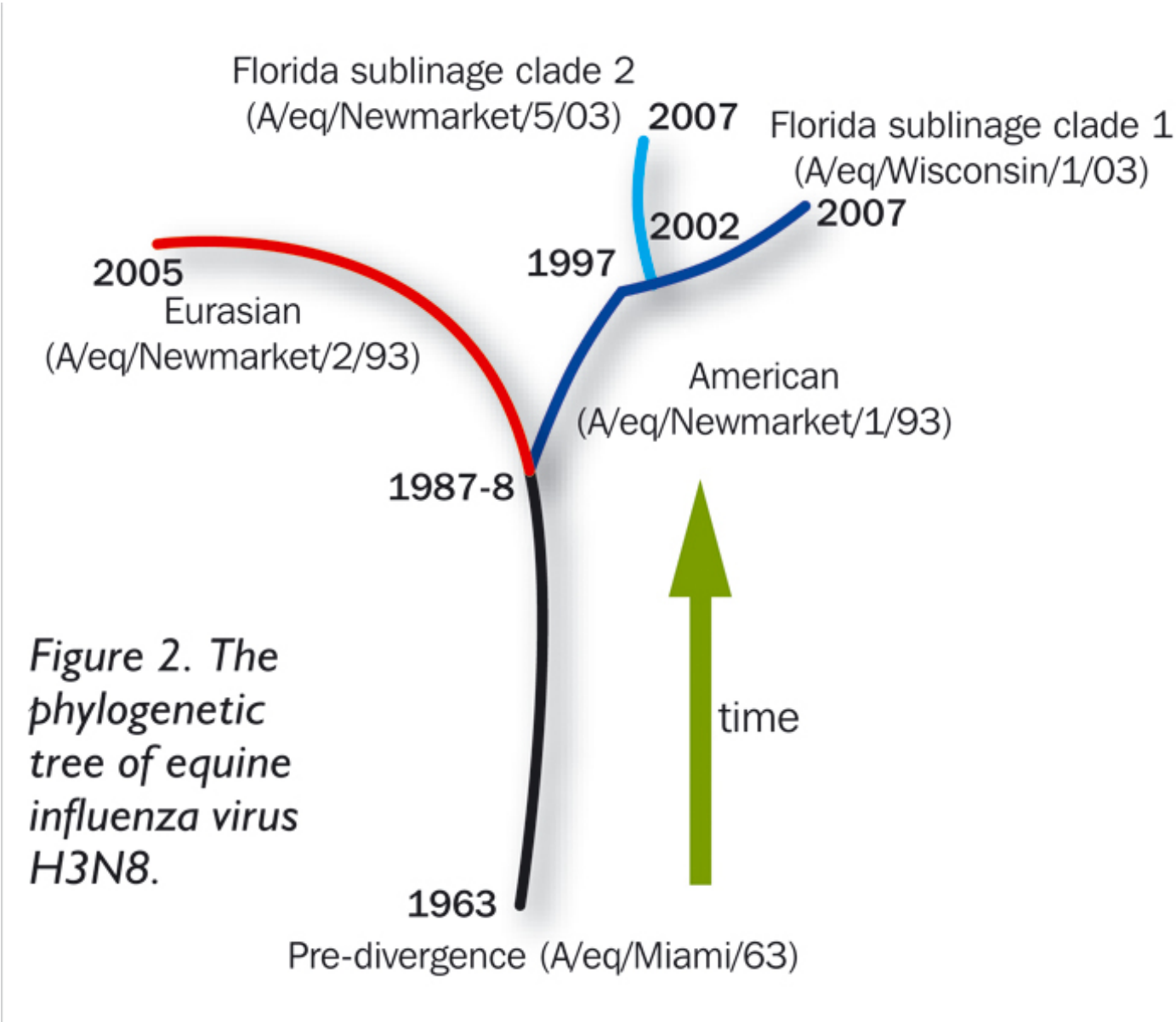


Figure 2. The phylogenetic tree of equine influenza virus H3N8.

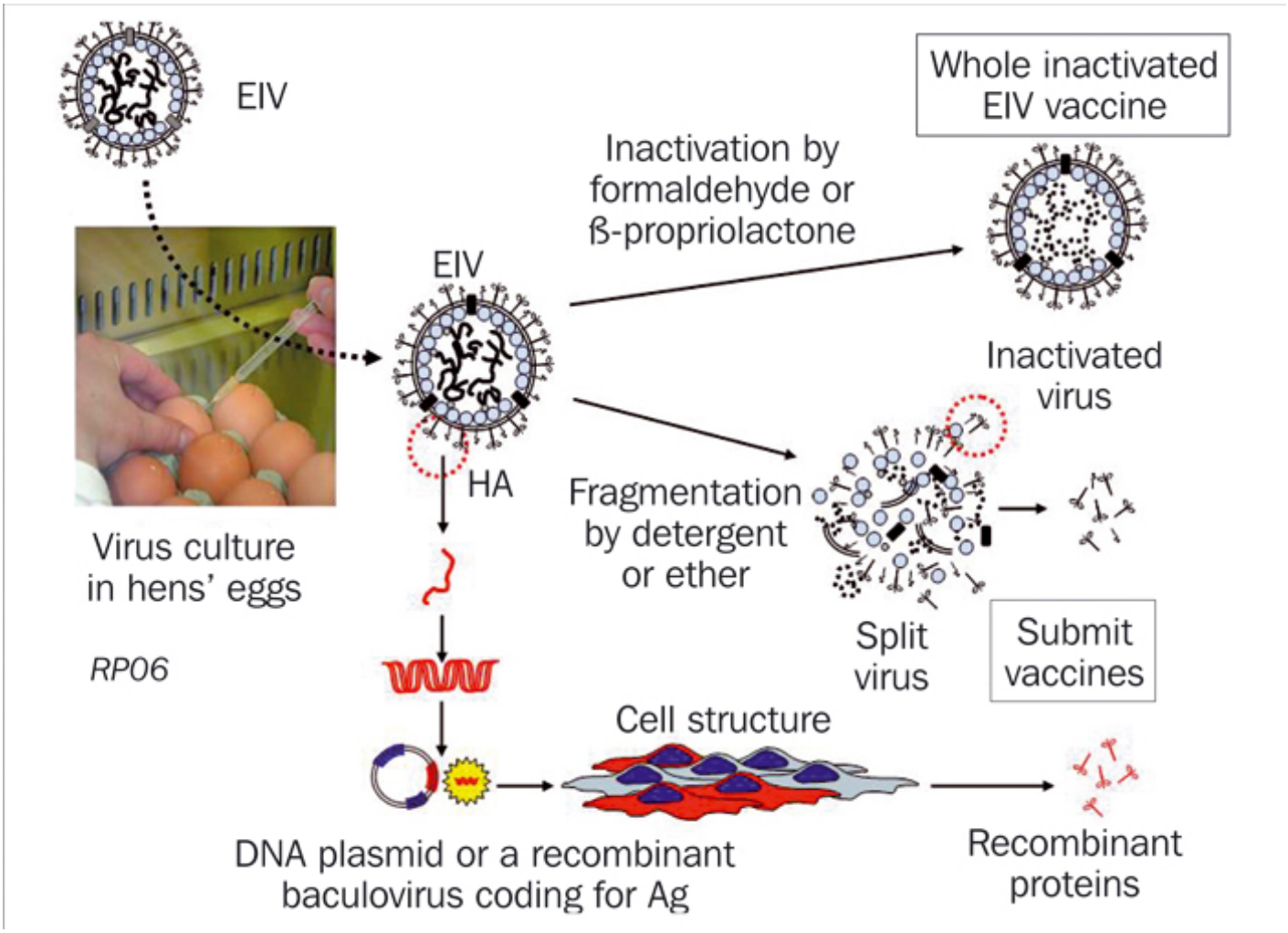


Figure 3. A whole inactivated and subunit vaccine.

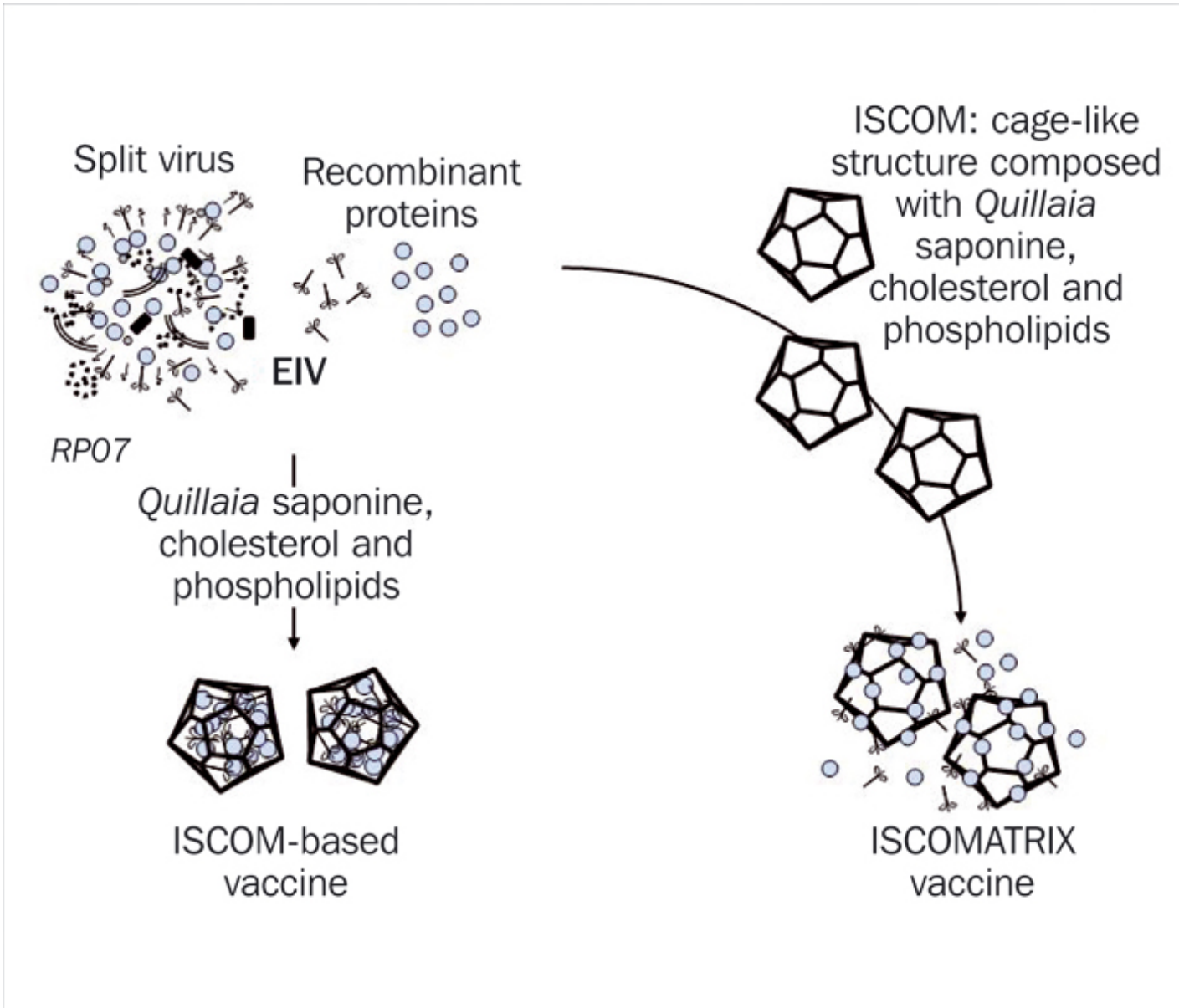


Figure 4. Immune-stimulating complex (ISCOM) vaccines.

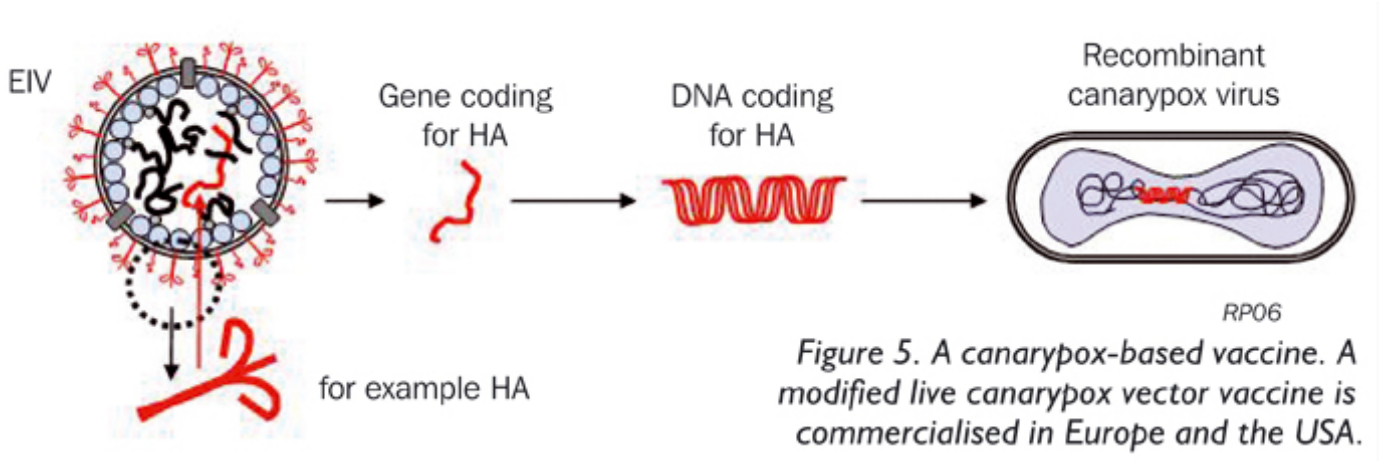


Figure 5. A canarypox-based vaccine. A modified live canarypox vector vaccine is commercialised in Europe and the USA.

Manufacturer	Vaccine name (general)	Vaccine strains			Nature	Licence
		H7N7	H3N8 American	H3N8 European		
Intervet	Prestige II/V/VVE	No	Kentucky/93 & 02	Newmarket/2/93	Inactivated	USA
Schering-Plough	Equip FT	Newmarket/77	Kentucky/98	Borlänge/91	ISCOM-based subunit	EU
Fort Dodge	Fluvac Innovator	Prague/56	Kentucky/97	No	Inactivated	USA
Boehringer	Calvenza EIV	Newmarket/77	Kentucky/95	Newmarket/2/93	Inactivated	USA
Merial	ProteqFlu	No	Kentucky/94	Newmarket/2/93	Canarypox-based	EU/USA
Fort Dodge	Duvaxyn IE plus	Prague/56	Newmarket/1/93	Suffolk/89	Inactivated	UK/
Intervet	Equilis Prequenza/Resequin	Prague/56	Newmarket/1/93	Newmarket/2/93	Iscomatrix subunit	UK
Intervet	FluAvert	No	Kentucky/91	None	Live attenuated	USA
Boehringer	Equi-Flu	Yes	Yes		Inactivated	USA