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**Possible Use of Vaccination against Bluetongue
in Europe**

Scientific Committee on Animal Health and Animal Welfare

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1. Request for opinion

Bluetongue has occurred in Greece since October 1998 and in 1999 appeared also in Bulgaria. Several serotypes of the virus have been detected.

Classical methods of control such as treating the breeding locations of the vector with insecticide have been undertaken. An attenuated polyvalent vaccine is available from Onderstepoort Biological Products, Onderstepoort, South Africa.

The Committee is asked to consider the issue of vaccination against this disease and in particular the use of the Onderstepoort attenuated vaccine in the present European context.

2. The disease (including virus and serotypes)

Bluetongue virus (BTV) is a dsRNA virus that causes a non-contagious, infectious, arthropod-borne disease of ruminants. To date 24 distinct internationally recognised serotypes (based on the lack of cross neutralisation) of the virus have been identified. Vaccination against one serotype does not usually confer protection against any of the other serotypes. Although BTV is an arbovirus and is transmitted by biting arthropods it has also been shown to be transmitted directly from vertebrate to vertebrate through semen and transplacentally. However these forms of transmission are only occasional and of no consequence to virus ecology. By far the most important mode of transmission is via the bites of infected vector insects.

Cattle and goats are major vertebrate hosts of the virus, but sheep and deer are usually the only species to exhibit disease. The virus can circulate in the absence of disease in susceptible species.

Bluetongue virus naturally infects a small number of *Culicoides* species. Of more than 1,400 species of midges described world-wide, less than 20 are known to be involved in the ecology of bluetongue virus.

Because bluetongue virus infection is not contagious, meat and dairy products pose no hazard for the spread of the pathogen in ruminants. However, blood and other biologicals for cell culture or in vivo use are a potential risk for spread.

2.1 The virus

Bluetongue virus is the type species of the genus *Orbivirus* within the family *Reoviridae*. The virions have a diameter of 80 nm. There is an outer capsid comprising two viral proteins (VP2 and VP5) which encapsidates an inner capsid, or core (60 nm in diameter) that is made up of 5 proteins and a genome of 10 segments of double-stranded RNA. While the two proteins of the outer capsid are responsible for virus entry and establishment of virus infection within the host cells, the core components are responsible for replication of the viral genome. Of the two outer capsid proteins, the VP2 contains the major virus neutralisation antigen and has a variable sequence resulting in the 24 serotypes of virus that are recognised internationally. In addition, there are a number of strains within the same serotypes. The second outer capsid protein VP5, also varies considerably, although much less than the VP2. In contrast, the five core proteins as well as the three viral encoded non-structural proteins (NS1, NS2, NS3) are much less variable. This genetic diversity of BTV is a consequence of both drift (i.e., point mutations) and shift (i.e., reassortment of individual BTV gene segments).

2.2 The disease

Bluetongue is thought to infect all known ruminant species. However, severe disease usually occurs only in certain breeds of sheep and in some species of deer (Taylor 1986, MacLachlan 1994, Osburn 1994). In many parts of the world, bluetongue virus infection of sheep is usually subclinical.

All of the pathology of bluetongue can be assigned to vascular endothelial damage resulting in changes to capillary permeability and fragility, with subsequent disseminated intravascular coagulation and necrosis of tissues supplied by damaged capillaries. These changes result in oedema, congestion, haemorrhage, inflammation and necrosis.

Fever is the most usual but not invariable clinical sign. Other common clinical signs include oedema (of lips, nose, face, submandibulum, eyelids and sometimes ears), congestion (of mouth, nose, nasal cavity, conjunctiva, skin and coronary bands), lameness and depression (Mellor and Boorman 1995). There is frequently a serous nasal discharge, later becoming mucopurulent. The mouth is sore and the sheep may champ to produce a frothy oral discharge. Sheep are not strictly anorexic but eat less because of oral soreness and will hold food in their mouths to soften before chewing. Affected sheep occasionally have swollen, congested, cyanotic tongues. Lameness, due to coronary band congestion, may occur early in the disease and lameness or torticollis, as a result of skeletal muscle damage, may occur later.

If fever occurs, sheep first become pyrexia 4 to 10 days after infection. The other clinical signs soon follow with acute deaths occurring during the second week following infection. Sheep may die from more chronic disease 3 to 5 weeks after infection and usually have bacterial complications. The mortality rate and the severity of the clinical signs vary with the breed and age of animal infected, the type and strain of the virus and certain interactions with the environment (Spreull 1905, Thomas and Neitz 1947, Luedke *et al.* 1964).

2.3 Infection of the vertebrate host

Gibbs and Greiner (1988) have summarised the pathogenesis of bluetongue virus infection in ruminants. After introduction by the bite of an infected midge, bluetongue virus first replicates in the local lymph nodes and subsequently induces a primary viraemia which seeds other lymph nodes, spleen and lung, where further virus replication occurs. The virus also replicates in vascular endothelium. In most animals a viraemia, predominantly associated with the buffy coat cells and platelets, can be detected between 5 and 12 days after infection. Subsequently, it becomes more difficult to recover virus from whole blood, but isolation is enhanced if the cell fraction is washed to remove plasma antibody and is ultrasonicated.

Verwoerd and Erasmus (1994) consider that the virus in blood is primarily associated with erythrocytes, and only to a lesser extent with the buffy coat fraction. Sellers (1981) also considers that most of the virus in the blood is associated with the red blood cells. Hassan and Roy (1999) have shown that VP2 is the cell attachment protein and is also responsible for transmission of virus via red blood cells.

MacLachlan (1996) recently reviewed the pathogenesis of bluetongue virus infection of cattle, and concludes that though it is accepted that infection of blood cells facilitates prolonged viraemia in bluetongue virus-infected cattle, the pathogenesis of infection of blood cells is not yet fully understood. The virus replicates primarily in the lungs, lymph nodes and spleen and the association of virus with blood cells facilitates a prolonged viraemia. Using polymerase chain reaction (PCR) tests, viral nucleic acid may be detected in blood cells for up to 180 days. Viraemia may last for up to 100 days in cattle but is usually much shorter. There have been no reports that infectious virus can be recovered by insect vectors from cattle with blood that is positive for viral nucleic acid by PCR analysis but negative for live virus (for more background, see Reports of the Scientific Committee on Animal Health and Animal Welfare of 21 October 1998 and 8 December 1999).

2.4 Infection of the invertebrate host

Culicoides spp. are the only significant, natural vectors of bluetongue virus. *Culicoides* are small biting flies, 1 to 3 mm in size. More than 1,400 species are known world-wide, of which 96% are obligate bloodsuckers attacking humans, other mammals and birds. However, of these, less than 20 species are known to be involved in the ecology of bluetongue virus. In Europe, Africa and the Middle east *C. imicola* is considered as the most efficient vector, in North America the major vector is *C. variipennis* and in Central and South America it is probably *C. insignis*. In Australia the major vectors are *C. wadai*, *C. brevitarsis*, *C. fulvus* and *C. actoni*. Other species of *Culicoides* have been shown to be less efficient vectors. The efficiency of vector species of *Culicoides* in transmitting BTV varies, depending on the virus strain and the local sub-population of the insect involved.

The number of midges infected after biting a bluetongue viraemic animal depends on the animal's level of viraemia, the midges' vector competency and the attack rate. The blood of an infected animal is more infectious for competent vectors when virus titres are high soon after infection before immune mechanisms develop. As viraemia titres drop there is less chance of a biting midge imbibing an infectious dose of virus. However, a proportion of highly competent midges will be infected even with low-titred blood after a lengthy period of viraemia, and it is possible that the viraemia will always be effective to some extent if the vector's competency and attack rate permit.

Larvae of *Culicoides* go through four stages. A generation of *C. variipennis* (egg to egg) requires a minimum of 15 days at 25°C (Mellor pers. comm.). The period of development from egg to adult for *C. loxodontis*, under natural conditions, is 8-12 days

(Meiswinkel, 1992). Adult *Culicoides* usually survive for fewer than 10-20 days but exceptionally they may survive for much longer periods (63 – 90 days) (Nevill, 1971, Mellor *et al.* 2000). Arthropods may gain infection with great efficiency from ingestion of a viraemic blood meal, yet fail to transmit virus because the infection has failed to spread to and replicate in the salivary glands. Hence it is important in vector studies in the laboratory to distinguish between arthropod infection rates and transmission rates (Swanepoel, 1994).

Meiswinkel *et al.* (1994) report on investigations of the effect of desiccation, submersion in water and exposure to low temperatures on different life stages of *C. imicola*. Low temperatures adversely affect *C. imicola* egg viability. If the eggs are kept below 6.5 °C, viability starts to decrease after 7 days while none hatch after 37 days. At temperatures as low as -1.5 °C, 15% of *C. imicola* adults lived beyond 15 days.

Temperature is partly responsible for seasonal fluctuations in populations of *Culicoides*. Rainfall or other sources of water are vital for the development of the semi-aquatic immature stages of most *Culicoides* spp. excepting those which breed in dung and rotting vegetation. Different species of midges vary in their temperature and moisture requirements and this is one reason for the differences in seasonal population dynamics between species. Populations of some species peak in spring - summer, others in summer - autumn. Adult midges are susceptible to translocation by wind, although the importance of this in expanding the range of the insects, and the micro-organisms they carry, is arguable (Meiswinkel *et al.*, 1994).

Gibbs and Greiner (1988) and Meiswinkel *et al.* (1994) discuss the various mechanisms which have been suggested for virus maintenance. Cycles of infection in midges and animals will continue in tropical and subtropical climates where midges are active to a sufficient extent all year and where susceptible animals are available. Similarly, this cyclic activity will occur in more temperate latitudes when seasonal conditions allow continuance of the vector life cycle. Virus may survive in long-lived adult vectors or in animals experiencing extended viraemia over short periods of less favourable climate.

3. Epidemiology of bluetongue in general

Bluetongue, the clinical disease is mainly seen in certain fine-wool and mutton breeds of sheep which, seemingly by chance, are found in countries at the limits of the virus' distribution (Spain, Portugal, Turkey, Cyprus, USA, South Africa) (Taylor 1986). Elsewhere, the disease is likely whenever similarly susceptible animals are transported to countries within the bluetongue enzootic areas e.g. Nigeria (Bida *et al.* 1975), Cameroon (Ekue *et al.* 1985) and Indonesia (Sudana and Malole 1982). However, since indigenous, unimproved sheep, goat and cattle breeds are usually highly resistant to the clinical effects of infection, the vast majority of bluetongue episodes throughout the world are completely silent (Mellor 1994a). This covert presence of the virus, alternating with occasional outbreaks of severe disease has had a considerable adverse effect upon international trade on bovine and ovine species, and their germ plasms, as countries free from bluetongue attempt to maintain that status (Mellor 1994a).

The global distribution of bluetongue virus (BTV) lies approximately between latitudes 35°S and 40°N although in parts of western North America and in China it may extend up to almost 50°N (Dulac *et al.* 1989, Guo *et al.* 1996, Qin *et al.* 1996). Within these areas the virus has a virtually world-wide distribution, being found in North, Central and South America, Africa, the Middle East, the Indian sub-continent, China, Southeast Asia and Australia (Mellor 1990, St. George and Standfast 1996). BTV has also at times made incursions into Europe, although it has apparently not so far been able to establish itself permanently in that continent (Mellor and Boorman 1995). Nevertheless the 1956-60 epizootic in Spain and Portugal resulted in the deaths of almost 180,000 sheep and is the most severe outbreak of bluetongue on record (Gorman 1990). As a general rule, therefore, BTV can be considered as infecting livestock populations in all countries lying in the tropics and subtropics (Gibbs and Greiner 1994). Additionally, several countries that are close to the subtropics, such as the USA, have endemically infected livestock. It is also the case that those countries whose territory spans a wide range of latitudes (e.g. USA, Australia) frequently include large geographical areas where BTV activity is apparently absent (Gibbs and Greiner 1994).

Genetic studies indicate that BTV tends to exist in discrete, stable ecosystems. For example, the BTV serotypes that circulate in the Caribbean are largely different from those found in North America, probably due to co-evolution of different strains of virus within each of the two ecosystems. This assertion is based on the fact that, nucleotide sequence data has revealed close genetic relationships between orbiviruses from the same geographic region. For example, VP3 sequences show that BTV-1 in Australia is related to the Australian toptype consisting of serotypes 3, 9, 16, 20, 21 and 23, whereas BTV-1 in South Africa is closer to the South African serotypes of BTV-3 and BTV-9. When "new" serotypes are introduced into different ecosystems they may 'die out' perhaps for lack of an efficient vector. Therefore, topotyping may be used to monitor the introduction, and long-term survival of BTV serotypes into different ecosystems. Also topotyping could be helpful in disease risk assessment. For example, it can help establish whether an actively circulating serotype belongs to an indigenous or an exotic toptype. BTV is transmitted between its ruminant hosts almost entirely by the bites of certain species of *Culicoides* biting midges. In consequence, its world distribution is restricted to

areas where these vector species occur and transmission is limited to those times of the year when adult insects are active. In epizootic zones this usually means during the late summer and autumn, and this is, therefore, the time when bluetongue is most commonly seen (Mellor and Boorman 1995). Throughout almost the whole of North America the major vector of BTV is *C. variipennis*, in Central and South America it is *C. insignis* and *C. pusillus*. In Africa the major vector is *C. imicola*, though in some areas *C. bolitinos* (Venter et al. 1998) may be of equal or even greater importance. In Asia several vector species have been cited, these include *C. imicola* (in the Near and Middle East, India, Sri Lanka, China, Thailand, Laos and Vietnam), *C. schultzei* grp., *C. fulvus* and *C. actoni* (in China), and *C. actoni*, *C. brevitarsis*, *C. fulvus*, *C. orientalis* (in Indonesia) (Mellor et al. 2000). Other species have also been suggested but without supporting evidence. In Australasia, *C. fulvus*, *C. wadai*, *C. actoni* and *C. brevitarsis* are considered to be most important. The major vector in Europe is considered to be *C. imicola* although recent evidence suggests that in certain areas other vector(s), possibly including *C. obsoletus*, may also occur (Mellor et al. 2000).

In the northern hemisphere BTV tends to cause annual bouts of disease in late summer and autumn (July to October), separated by periods of apparent quiescence (Yonguc et al. 1982, Osburn et al. 1983, Mellor 1994b). This pattern is related to the population densities of the vector species of *Culicoides*, which usually peak in late summer and is controlled by certain climatic variables (Mellor and Boorman 1995, Mellor et al. 2000). In such situations the annual bouts of disease may represent entirely new introductions (from adjacent infected areas) or may be the visible evidence of low-level persistence from year to year. Annual introduction is possible if enzootic foci of the virus are geographically close by, since infected *Culicoides* may be transported on the wind as aerial plankton over several 100 kilometres (Sellers and Pedgley 1985, Sellers et al. 1977, Sellers et al. 1979) or, alternatively, infected animals may be moved. Since BTV is not transmitted transovarially through the vectors (and is only transmitted very rarely in this way through its vertebrate hosts) persistence is only possible in those areas where adult active vectors are present throughout the year. In this situation, if vector-free periods do occur then they must be of shorter duration than the maximum period of viraemia in the local susceptible vertebrate population (54 days in sheep, approx. 100 days in cattle), otherwise the last infected vertebrate host will have died or recovered before new vectors arrive on the scene (Mellor 1994b). However, persistence within a country or within a particular geographical area should not be interpreted as meaning "static". Once a vertebrate host is infected with BTV it either dies or else mounts an enduring antibody response and so becomes resistant to further infection. This means that within any small geographical area (a farm or village) most or all of the initially susceptible hosts are likely to become "unavailable" to the virus within a fairly short space of time. BTV can only survive under such constraints by continually moving to new locations occupied by naïve vertebrate hosts. These movements are via the agency of viraemic hosts or of infected vectors. BTV is therefore a peripatetic virus and even within its enzootic areas its activity may be envisaged as a pattern of endlessly shifting viral "hot spots" (Mellor and Boorman 1995).

4. The 1998-2000 bluetongue virus incursions into the Mediterranean basin.

In Greece, the first report of bluetongue goes back in the autumn 1979, with an epidemic of clinical disease in sheep on the East-Aegean island of Lesbos, which was a part of an extensive BTV 4 epidemic through Asia Minor. On the following years new BTV subclinical infections were detected and stamping out of all seropositive cattle took place. In 1986 the island was declared BTV free and consequent serological surveillance confirmed that freedom. In the meantime, surveillance in mainland Greece and routine diagnostic tests did not demonstrate presence of BTV (Rebountzakou 1985, Ministry of Agriculture reports).

In October 1998 bluetongue was reported in sheep on the Greek Islands of Rhodes, Kos, Leros and Samos. The virus was identified as being BTV 9 and this was the first occasion that serotype 9 had been recorded in Europe, though serological evidence of its presence in Anatolian Turkey had been reported previously (Taylor and Mellor 1994a). The virus was reported as being widespread on Rhodes and Kos but was much less prevalent on Leros and Samos. The Greek veterinary service rapidly implemented measures to contain and eliminate the virus from their territory, and transmission seems to have ended by December 1998 - January 1999.

However, in June 1999 bluetongue was reported for the first time ever from Bulgaria. The virus was rapidly identified as being BTV 9. The incursion appears to have begun in SE Bulgaria in the Province of Burgas, near the Black Sea but rapidly spread through several adjoining provinces along the European Turkish and the Greek borders, moving in a westerly direction. Movement of animals restrictions, vector abatement measures and other zoosanitary measures were introduced by the Bulgarian veterinary authorities and BTV transmission appears to have ended at the beginning of winter in late November 1999.

In July 1999 the Turkish authorities reported the presence of bluetongue in European Turkey, in sheep flocks near to the Bulgarian border. In response to the incursion some 60,000 Turkish sheep were vaccinated with a live attenuated, BTV serotype 4 vaccine, produced in Turkey. The Turkish field virus was later identified as being the same serotype as the Bulgarian and Greek viruses *i.e.* BTV serotype 9.

In August 1999 the Greek veterinary authorities reported the presence of BTV on mainland Greece initially in the north-eastern province of Evora which adjoins the Turkish and Bulgarian borders. During September and October the presence of the virus expanded across northern Greece travelling in a westerly direction and involving provinces as far west as Thessaloniki and as far south as Larisa, Magnesia and Evia, thus making approximately one third of Greek territory "infected". Transmission continued to be reported from some areas into December 1999. Also during September 1999 and after an absence of the virus for 20 years an incursion of BTV was reported into the Greek Island of Lesbos and fresh incursions were reported into the Dodecanese. The incursion into Lesbos was particularly severe causing thousands of deaths in the local sheep. By the end of 1999 serological or clinical signs of bluetongue in Greece had been reported

from the islands of Rhodes, Leros, Kos, Samos, Limnos, Ikaria, Chios, Lesbos, Thasos, Samothraki, Skiathos, Skopelos and Evia, and also from the mainland provinces of Evros, Rodopi, Kavala, Thessaloniki, Chalkidiki, Larisa and Magnesia.

A worrying factor that emerged during the Greek BTV incursions in 1999 was that in addition to BTV serotype 9, two additional serotypes were identified (serotypes 4 and 16). Initially evidence of BTV serotype 4 came from the province of Evros near the European Turkish border but more recently this serotype has also been identified, by virus isolation from Rhodes, Lesbos and Pieria. Evidence of BTV serotype 16 comes from Rhodes, Kos and Samos. BTV serotype 4 has previously been reported in Anatolian Turkey (Taylor and Mellor 1994a) and it has also been present in Lesbos in 1979 (Vassalos 1980). BTV serotype 16 occurs regularly in Israel (Taylor and Mellor 1994a). The source of the 1999 BTV serotype 4 in Greece is difficult to determine. Because the first isolations of this serotype came from near the Turkish border, subsequent to the use of a live attenuated serotype 4 vaccine in Turkey, initially, suspicions were raised that insufficiently attenuated vaccine virus might be the origin of the incursion. However, the occurrence of a third BTV serotype (type 16) in Greece suggests that several independent waves of BTV incursion, including BTV serotype 4, may have occurred. In this context it should be borne in mind that to the east, in Anatolian Turkey, Syria, Jordan, and Israel BTV serotypes 2, 4, 6, 9, 10, 13, and 16 have been reported over a number of years, and that the westward movement of some of these and other viruses (Akabane) is well documented (Taylor and Mellor 1994a, b, Vassalos 1980, Yonguc *et al.* 1982, Burgu *et al.* 1992, Urman *et al.* 1980).

During the course of the 1998-2000 virus incursions into Turkey, Bulgaria and Greece BTV vector studies were carried out in Bulgaria and Greece. In Greece the known major vector in the region, *Culicoides imicola*, which had previously been thought to be present only in Chios, Rhodes and Lesbos (as well as in much of Anatolian Turkey) was shown to also occur on the islands of Samos, Kos and Lesbos and at several locations on the mainland (Chalkidiki, Larisa, Magnesia). However, *C. imicola* has not been recorded in northern Greece (Thrace) and it seems to be absent from Bulgaria (Glukhova, *et al.* 1991) both being locations where bluetongue has also occurred. This suggests two things, firstly, that *C. imicola* is expanding its range both northwards and westwards and secondly, that in northern Greece and Bulgaria, an as yet unidentified vector may be present. In respect of the unknown vector, in Bulgaria during the course of the BTV epizootic and at locations where virus transmission was occurring, the midge *C. obsoletus* was estimated to comprise 90% of the *Culicoides* population (Mellor personal observations). *Culicoides obsoletus* has long been considered a suspect BTV vector (Mellor and Pitzolis 1979, Jennings and Mellor 1988).

In January 2000 Tunisia reported for the first time ever, an incursion of BTV into their territory. The time of the incursion was estimated as early December 1999. The virus was typed as BTV serotype 2 in late January 2000. The origin of this incursion is uncertain but is likely to be separate from that involving Turkey, Greece and Bulgaria. Since foot and mouth disease virus had also entered Tunisia during 1999, via cattle from sub Saharan West Africa it is possible that BTV could have followed the same route. BTV serotype 2 seems to be common in at least some areas of West Africa (Herniman *et al.* 1983). The vector of BTV in Tunisia is unknown, although as *C. imicola* has been

recorded elsewhere in western North Africa (Morocco, Algeria) it is probable that this is the most likely vector species (Bouayoune *et al.* 1998).

Table 1: Some facts relating to the current outbreak in Greece

- The total ruminant population is roughly 7 million breeding sheep, 4 million breeding goats and 0.6 million cattle.
- The breeding season starts in early summer with lambing from November to March. Outside that season approx. 5% of ewes and goats may be pregnant.
- In the 12 bluetongue affected prefectures of mainland Greece in 1999, the average animal population was 135,000 sheep and 90,000 goats (a total of 1.65 million sheep and 1.1 million goats).
- The average number of animals within a radius of 30 km around any outbreak is roughly 160,000 sheep, 90,000 goats and 12,000 cattle.
- The incidence of clinical disease (cases per total sheep population in each prefecture) for 1999 was as follows: Evros 0.014%, Rodopi 0.04%, Kavala 0.1%, Thessaloniki 0.08%, Chalkidiki 1.9%, Larisa 0.08%, Magnesia 0.17% and Evia 0.5%. On the island of Lesbos it was 2.33% and for the Dodekanese 6.13%.
- Contrary to the low clinical incidence, the prevalence of subclinical infections appears to be higher, especially among the bovine populations in the affected areas.

5. Bluetongue vaccines

5.1 Introduction

The most widely discussed vaccine options for the control of bluetongue are live attenuated, inactivated and recombinant vaccines:

1. Attenuated virus vaccines are cheap, easy to produce and are administered in a single dose. They are very effective in controlling clinical outbreaks of bluetongue in areas of endemic disease and in the face of outbreaks. They replicate in sheep without causing significant clinical effects and provide protection against challenge with virulent virus of the same serotype. The possibility that insects could acquire vaccine virus by feeding on vaccinated animals and then transmit it to sheep or other ruminants cannot be discounted.

2. There have been a number of attempts to develop inactivated whole virus vaccines for bluetongue during the past 25 years, but none have been commercialised. They are probably safe, but incomplete virus inactivation in some vaccine preparations has occurred in the past. Not only are they more expensive to produce than attenuated vaccines but also require at least two doses with an adjuvant to elicit a protective immune response.

Although inactivated vaccines have not been widely used in the past, they offer significant advantages over attenuated vaccines because absence of replicating virus eliminates concerns about viraemia, vector transmission and reversion to virulence. It also eliminates the danger of foetal infection, often reported for attenuated bluetongue vaccines, and eliminates the possibility of viral reassortment. Procedures for inactivating bluetongue virus using formalin, beta propiolactone, binary ethylenimine and gamma radiation and their administration with different adjuvants has been extensively reported. (Campbell 1985, Parker *et al.* 1975, Stevens *et al.* 1985, Stott *et al.* 1979, Stott *et al.* 1985).

The use of inactivated vaccines also allows a rapid response to newly emerging serotypes. Once a new serotype has been isolated from the field, it can be rapidly propagated to produce a homologous vaccine. This allows a rapid response to changes in the field, unlike the more molecular approaches which would take much longer to sequence, clone and produce a suitable recombinant expression system.

Diagnostic assays have already been developed which allow discrimination of bluetongue infected animals from those which have received inactivated bluetongue virus vaccine (Anderson *et al.* 1993). This would be invaluable in interpreting field data following a vaccination campaign. Animals vaccinated with the attenuated vaccine produce a long lived humoral antibody response, possibly lasting for the life of the animal. In comparison antibodies to inactivated vaccines are short lived, giving short term protection when needed but eventually waning to leave a seronegative population.

The procedure for bluetongue virus vaccine production is similar to that for the production of foot and mouth disease vaccine. European vaccine producers could easily

adapt to the production of inactivated bluetongue virus vaccine. The resulting product would be both produced and stored under internationally accredited GLP conditions.

3. Recombinant vaccines (e.g. recombinant virus-like particles (VLP) or single BTV antigens) are safe and have been shown to be efficacious (see chapter 6). VLP vaccines are the only vaccines that have been subjected to a number of clinical trials in different countries. They require two inoculations of low doses for long lasting protection. Due to recent advances in the manufacturing of insect cell cultures, VLP vaccines could be very cost effective (see Chapter 6). The only reason why these vaccines are not available commercially is due to the lack of interest of BTV vaccines in developed countries.

5.2 The Onderstepoort Bluetongue Vaccine

5.2.1 Current use

In the Republic of South Africa live attenuated vaccines have been used for more than 50 years and they are known to induce effective and long lasting immunity. They are currently produced in cell culture, freeze-dried and subjected to tests to conform to stringent Onderstepoort and international standards. The present Onderstepoort Bluetongue Vaccine (Reg. No. G 358 Act No. 36/1947) comprises 3 bottles (Vaccines A, B, and C) and includes the following serotypes of BTV:

Bottle A: BTV serotypes 1, 4, 6, 12 and 14

Bottle B: BTV serotypes 3, 8, 9, 10 and 11

Bottle C: BTV serotypes 2, 5, 7, 13 and 19

The three bluetongue vaccines are administered separately at 3-week intervals. Each vaccine contains 100 doses, and all are in stock. A monovalent bluetongue vaccine, or combination of vaccines, is only produced on special request but requires two months for its production and quality control.

Onderstepoort BTV attenuated virus strains currently used in vaccine production: identification of strains, their origin and passage history

<u>Virus type</u>	<u>Strain Identification</u>	<u>Origin</u>	<u>Passage History</u>
BTV-1	Biggarsberg/8012	RSA, 1958	50E 3P 4BHK
BTV-2	Vryheid/5036	RSA, 1958	50E 3P 4BHK
BTV-3	Cyprus/8231	Cyprus, 1944	45E 2BHK 3P 5BHK
BTV-4	Theiler/79043	RSA, ~1900	60E 3Pa 9BHK
BTV-5	Mossop/4868	RSA, 1953	50E 2BHK 3Pa 6BHK
BTV-6	Strathene/5011	RSA, 1958	60E 3Pa 7BHK
BTV-7	Utrecht/1504	RSA, 1955	60E 3Pa 7BHK
BTV-8	Camp/8438	RSA, 1937	50E 3BHK 10 Pa 7BHK
BTV-9	University Farm/2766	RSA, 1942	70E 2BHK pp 3BHK 7P 6BHK
BTV-10	Portugal/2627	Portugal, 1956	E81
BTV-11	Nelspoort/4575	RSA, 1944	35E 3P 5BHK
BTV-12	Estantia/75005	RSA, 1941	55E 3P 4BK
BTV-13	Westlands/7238	RSA, 1959	45E 2BHK 3Pa 4BHK
BTV-14	Kolwani/89/59	RSA, 1959	60E 3Pa 4BHK
BTV-19	143/76	RSA, 1976	29E 3Pa 3BHK

Attenuated BTV-16 is also available:

BTV-16	Pakistan/7766	Pakistan	37E 3P 2BHK 1 Vero
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No. E	Number of passages in eggs
No. BHK	Number of passages in baby hamster kidney cells
No. Vero	Number of passages in green monkey kidney cells
No. P	Number of large plaque selections
No. p	Number of small plaque selections
A	small plaque variant

These attenuated vaccine strains were validated for use in sheep only; their efficacy in other ruminants has not been determined.

5.2.2 Safety, transmissibility, and reversion to virulence

There are no data available that would prove or disprove the possibility of *Culicoides* midges transmitting the Onderstepoort attenuated vaccine strains from vaccinated to unvaccinated animals. Also not determined is the possibility of reversion to virulence, following multiple sheep-insect passages. Finally, no virus transmission trials between vaccinated sheep-vector-unvaccinated ruminants have been conducted. Since the generation of such data is either extremely difficult or not feasible, this part of vaccine validation is rarely sought. Only attenuated viruses that generate titres of less than 1000 plaque forming units (PFU) per ml of blood at the height of viraemia in test animals and elicit neutralising antibody are selected for vaccine production. It is considered that

viraemias lower than 1000 PFU/ml will ensure that the virus is not transmitted by blood-sucking insects (OIE Manual, 1996).

Potential safety problems associated with live attenuated vaccines although probably very low, are the following:

1. Some attenuated bluetongue vaccine strains were shown to be teratogenic (in sheep) when administered during the first half of pregnancy. However, no quantitative studies have been done to assess this problem.
2. Although, only very limited data are available, it has been suggested that attenuated bluetongue viruses could be transmitted by vector midges.
3. In the field attenuated virus may recombine with a wild type virus and so create viruses that have new biological properties. However, there is no evidence that recombination has led to the emergence of new, or more, virulent strains.
4. The release of attenuated virus into the environment may result in a reversion to virulence. However, if attenuated viruses cannot be transmitted by insects from vaccinated to unvaccinated animals, reversion to virulence becomes a theoretical possibility only.

5.3 New Vaccines

Vaccination has been a most successful methodology to combat diseases in man and livestock. Most of the current viral vaccines are prepared using attenuated or inactivated virus. This approach, although useful in many cases, has certain drawbacks, as indicated in the previous chapter. Control of Bluetongue (the disease) is particularly difficult due to the multiple serotypes of the virus. In addition, the viral genome is made up of 10 segments allowing exchanging the genes randomly between different viruses. This may cause generation of infectious virus with mixed genes.

Recent recombinant DNA technology has provided novel approaches to developing intrinsically safe vaccines, these vaccines are not yet commercially available. This technology offers substantial advantages both in terms of safety and the potential of developing a marker vaccine. The latter could be used as a prophylaxis in areas at risk, without endangering the "free" status of the region. An accompanying serological test would allow the distinction between vaccinated and infected animals. DNA recombinant technology involves the synthesis of immunogenic proteins and particles that elicit highly protective immune responses. Successful vaccine development requires systems where the engineered products mimic the authentic proteins, not just in terms of their primary amino acid sequences but specifically in terms of their three dimensional structures, i.e., the products must be as authentic as possible.

In recent years insect (caterpillar) specific baculoviruses have received considerable attention as vectors for the high-level synthesis of foreign proteins. Protein engineering systems were utilised to synthesise individual bluetongue virus proteins and core- (single coat) and viral-like (double coat) multiprotein structures (CLPs, VLPs). These

engineered particles essentially mimic the virus particles, but do not contain any genetic materials (French and Roy, 1990; French *et al.*, 1990).

The immune responses against these synthetic proteins (subunits) and empty particles (non-replicating) have been tested both *in vitro* (virus neutralising tests) and in vaccination challenge experiments (BTV susceptible sheep; Roy, 1991, 1992, 1993, 1995; Roy and Erasmus, 1992; Roy *et al.*, 1990; Roy *et al.*, 1992; Roy *et al.*, 1994a; Roy *et al.*, 1994b; Johnson and Roy, 1996; Urakawa *et al.*, 1994). Based on these initial data, a series of clinical trials have been undertaken in animals (using 50 to 200 sheep in each trial).

The initial trials were undertaken using unpurified recombinant proteins as subunit vaccines. When one unpurified protein (VP2, the virus neutralisation protein) was used for vaccination of sheep, 100 µg/dose could afford protection in sheep against virulent virus when challenged after 2½ months. However, less of this same protein (~ 50 µg) was needed for complete protection when mixed with 20 µg of the second virus outer coat protein VP5. In brief, vaccination trials in sheep with recombinant BTV-10, two outer capsid antigens, a mixture of VP2 and VP5 gave complete protection against virulent virus challenge.

Some vaccination trials with core-like particles or CLPs that contain only the two proteins VP3 and VP7 (conserved across all serotypes) had also been undertaken against homologous and heterologous BTV challenges. It was clear that CLPs could provide either partial (with only slight fever) or complete protection [depending on dose (~ 50-200µg/sheep)] against homologous and heterologous virus challenges. Sheep vaccinated with CLPs (derived from serotype 10) showed no febrile response when challenged with Australian virus BTV-1 or -23, and the level of viraemia was significantly lower ($P < 0.01$) than that in the non-vaccinated control sheep. Animals showed strong group specific antibody response, but no neutralising antibodies. Immunisation of sheep with CLPs without the outer capsid (variable), therefore, mitigate the viral infection or completely protect against heterologous virulent virus challenge. Since CLPs are conserved across the various serotypes, CLPs could have potential for candidate vaccine which may at least mitigate the bluetongue disease and inhibit the virus spread.

A number of trials have been undertaken with virus-like particles or VLPs containing VP3, VP7 as well as the two outer capsid proteins, VP2 and VP5 (cell attachment and neutralisation proteins). Various doses (10 - 200 µg/sheep) of VLPs were tested in sheep (100 - 200 animals per trial). In one of these trials, the data obtained clearly showed only 10 µg of VLPs gave long lasting protection (at least 15 months, maybe longer) against homologous BTV challenge. Therefore, it was concluded that vaccination trials of sheep with BTV-10 VLPs containing the BTV-10 VP2 (variable) were highly immunogenic even when administered at low doses (10 µg) and protected sheep when animals were challenged with homologous virus 4 months later. It is noteworthy that the sheep inoculated with 10 µg of VLPs actually received only 1 µg of VP2 (10-20% of the VLP mass).

Vaccination trials in sheep with a cocktail of VLPs representing two or five different serotypes were also assessed. A cocktail of US BTV-10 and -17, 10 µg of each VLP had

afforded complete protection when challenged not only with virulent BTV-10 and -17, but also a heterologous virus, S. African BTV-4. Further, with higher doses, i.e., 50 µg of each, protection was achieved with other heterologous serotypes (e.g., BTV-11). Similarly, with a cocktail of BTV-2, -10, -11, -13 and -17 VLPs (10 µg each) complete protection was achieved with heterologous virus (e.g., BTV-16) challenge. In summary, some evidence was obtained for cross-protection, depending on the challenge virus and amounts of antigen (10 - 50 µg) used for vaccination due to shared epitopes between the different serotypes. It is therefore likely that a mixture of VLPs representing seven serotypes (BTV-1, -2, -10, -11, -13, -17 and -23) that has been already prepared may very well afford protection against most, if not, all BTV serotypes.

In conclusion, results from various vaccination studies indicated that VLPs are highly immunogenic, even at low doses. There are several possible explanations. First, when assembled into VLP the conformational presentations of the relevant epitopes on VP2 mimic those on the authentic virus. Second, both VP2 and VP5 are present. Third, internal coat proteins (VP3 and VP7) may also confer a protective immunity. BTV CLPs and VLPs offer particular advantages as potential vaccines over other systems. The large quantities of CLPs and VLPs can be produced due to the high expression capabilities of baculovirus vectors (produced in serum-free medium), and can be purified using a one-step generic protocol based on the physical properties of the particle. More importantly, these particles are devoid of any detectable amount of insect, or baculovirus proteins or nucleic acids and thus pose no potential adverse effects. Together with a suitable diagnostic test detecting antibodies against viral proteins not present in the vaccine a new BTV vaccine would allow the distinction between vaccinated and infected animals (marker vaccine).

Most importantly, since this VLP vaccine only contains four structural proteins of the seven proteins, and lacks all the three non-structural proteins (NS1, NS2, NS3), therefore antibodies against these vaccines are easily distinguishable from those against infecting field virus. Extensive research has been undertaken to develop diagnostic reagents based on NS1 and NS2 to specifically identify both the viral antigens and antibody and these are available to use.

6. Conclusions

1. BTV infections occurring in Third Countries bordering the EU pose a constant threat to southern EU Member States. The 1999 outbreaks of bluetongue in Greece illustrate this imminent danger. Bluetongue infections can cause heavy losses in sheep, whereas the infection is subclinical in cattle and goats. The latter species serve as an efficient reservoir for BTV. There are basically two options to react on bluetongue outbreaks:
 - 2.1. No vaccination. This option bears the risk that BTV causes considerable economical losses in sheep and that the virus becomes endemic in the area for as long as the climate remains favourable.
 - 2.2. Vaccination. For safety reasons the use of inactivated vaccines would be preferable. However, at present only live attenuated vaccines are available. The tentative control of bluetongue in Europe by vaccination should ideally be based on the use of live attenuated vaccines that include local strains. This would avoid the possible introduction of new BTV topotypes from different (e.g. South African) ecosystems, in case vaccine strains revert to virulence. However, this is unlikely to be possible in the short term (at least one year) since such vaccines are not available and their production would first require their attenuation and secondly assessment of safety and efficacy. Due to these circumstances the only practical option would be to use currently available attenuated live vaccine strains. The type of vaccine used would depend on the BTV serotype(s) (mono-, bi- or trivalent) prevalent in EU countries and in countries bordering the Mediterranean Sea and which are liable to affect the nearby parts of the EU.
3. The available live attenuated vaccines are licensed in South Africa for use only in sheep. Accordingly vaccination is only suitable in order to reduce economic losses in that species. Further research is necessary in order to establish efficacy and safety of the available live attenuated vaccines in cattle and goats. Since cattle and goats rarely develop clinical bluetongue, "efficacy" in this context means "ability to prevent the development of a viraemia on subsequent infection with a field virus", thereby preventing these species being involved in the covert transmission of the virus.
4. A substantial amount of research has been conducted in the field of BTV vaccine research and development. Safe vaccines with distinct efficacy, either based on DNA recombinant techniques or inactivated preparations have been developed. However, neither of these products has been licensed or is ready for large scale production, respectively. The introduction of such vaccines, preferably with marker properties would be a useful tool both for the control and possible eradication of BTV infections in endemic areas and for the prophylactic protection of livestock in endangered regions of Europe. However, the market for a recombinant or an inactivated BTV vaccine is likely to be small, and may not be an economic proposition for a commercial company.

7. Recommendations

General

1. In those areas of Greece where BTV infections occurred in the last season, it is recommended that sentinel herds of cattle are established or designated in order to detect new outbreaks and to indicate whether BTV is established in the respective areas.
2. In areas where BTV infections re-emerge, it is recommended that judicious vector control is carried out.

Use of currently available live vaccines

3. As an emergency action, based on a cost/risk benefit analysis and the epidemiological situation, in order to prevent suffering and economic losses it is recommended that live vaccine which is available from Onderstepoort, South Africa is applied to sheep in those areas of Greece where BTV infections reappear in the current season. The vaccine should contain only those serotypes which have been identified in Greece and neighbouring countries.
4. Vaccination campaigns should take into account the topography of the affected area, e.g. rivers, lakes, valleys and mountain ranges.

Use of vaccines to eradicate the bluetongue virus

5. Further research is recommended in order to establish efficacy and safety of the available live attenuated vaccines in cattle and goats. With the efficacy and safety of the live attenuated vaccine in cattle and goats established, vaccination could then be extended to all three ruminant species in order to stop transmission of the virus. It is expected that this policy would eventually lead to the eradication of BTV from the affected areas.

Planning for the future

The European Union is at risk from bluetongue, both from the south east Mediterranean and from North Africa. Because of the highly susceptible sheep population, the economic losses and poor animal welfare in affected regions would be considerable.

6. Vaccines effective against all likely serotypes suitable for use in all ruminant species need to be developed, tested and placed in a European strategic bluetongue vaccine reserve (vaccine bank), ready for use when required.
7. New efficacious and safe vaccines with marker properties, either based on DNA recombinant techniques or inactivated preparations, which exist but which are not commercially available should be developed for registration and commercial

distribution. Because of the small commercial market, some public funding may be necessary to achieve this objective.

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