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Analysis of some factors influencing the epidemiology of Bluetongue Virus in ruminants. A hypothesis of control strategy through decrease of Culicoides and their associated damage in farm.

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ABSTRACT

The main object of this research has been to apply the scientific assumption that the alkalization is able to make inhospitable the larval reproductive sites in the identified area (Sant'Antioco). Moreover several studies about the reproductive behaviour of BT vectors has been conducted. First every data about BT history in Italy and especially in Sardinia had been gathered, then a epidemiologic retrospective view of the events in the whole island has been issued to study the disease prevalence and how environmental and geo-pedological factors have been able to change this prevalence. The research has been conducted in the pilot farm into the selected area of Sant'Antioco where the first hypothesis has been tested and every information about larval development has been collected. During the whole experimental period have been collected all the data about captures in the light traps and captures in the traps where midges were emerging from the larval case in both areas, alkalized and not. Furthermore have been performed the epidemiologic model taking into account the local epidemiological data and the several variables that were involved in the damage registered in every farm of the island of Sant'Antioco. Experimental data first of all have been emphasize the possibility of cut down the larval reproduction extremely in the alkalized sites then have allowed to study the BT vectors density closely and its relation with the disease prevalence in the heads presents in the near farms. The results revealed the different distribution of the disease prevalence in the several farms, referable to minute variation of the farm location. In the end it seems essential to improve the farm management through the widespread action of awakening to farmers.

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INTRODUCTION

BLUE TONGUE : A BRIEF DESCRIPTION

Virus and viraemia

Bluetongue virus (BTV) is a RNA-virus belonging to *Orbivirus* genus (*Reoviridae* family), transmitted by bite of Ceratopogonidae midges of the genus *Culicoides*. BTV infects all ruminant species, however, clinical disease can be mainly observed in sheep and goats. An exception to this rule has been represented by the clinical figures seen in cattle of Central Europe (particularly in Belgium, Germany and the Netherlands) infected by serotype 8 in 2006 (EUBT-NET). Bluetongue (BT) was first described as ‘‘Fever’’, ‘‘Malarial Catarrhal Fever’’ or ‘‘Epizootic Catarrh of Sheep’’ in the original descriptions of investigators in South Africa. The name ‘‘Bluetongue’’ is the anglicized form of the Afrikaans ‘‘bloutong’’, which was coined by Boer farmers to describe the distinctive cyanotic tongue of some severely affected sheep (Spreull, 1905,Henning, 1956). The orbivirus genus within the *Reoviridae* family, has as its type member BT virus. Members of the genus share a number of properties. Amongst these are that the viral particles have a diameter of 65-80 nm and that the particle has a M_r of approximately 60×10^6 and $S_{20w} = 550$. Physiochemical properties include a sensitivity to lipid solvents which reduce infectivity by approximately 10-fold while low pH (pH 3.0) abolishes infectivity. Orbiviruses have a bi-shelled structure, the inner of which has 32 capsomers. The genome comprises 10 segments of double-stranded (dsRNA). Each RNA segment has a single open reading frame which generally gives rise to a single translation product. However several RNA species can give rise to several translation products by initiation at an internal methionine codon. The Mr of the RNA segments range from $0.5\text{-}2.8 \times 10^6$ to give a combined genomic $M_r \sim 12\text{-}15 \times 10^6$. The genome

consists 20% by weight of the virus and the G + C content varies between 42-44%. The mature virus particle contains seven structural proteins. Minor proteins, VPI, VP4 and VP6, are contained within the inner core which is composed of two major proteins, VP3 and VP7 (M_r ~ 103 and 38 x 10^3, respectively). VP7 is the major component of the capsomers of the core surface and is the major serogroup specific protein of the virus. The outer capsid layer comprises two proteins, VP2 (M_r ~ 111 x 10^3) and VP5 (M_r ~ 59 x 10^3) (Gould and Hyatt,1994). Moreover VP2 encodes the major antigenic determinants of the virus that are important in virus neutralization. The variability of this antigenic determinants originates 24 serotypes identified (E.C. ,2000). The former polypeptide is the main antigenic determinant for neutralisation of the virus particle, although VP5 is also involved with type specificity. Three non-structural proteins NS1-3 have M_r ~ 64, 41 and 26 x 10^3, respectively. NS2 has been reported (Huismans et al., 1987) to be a phosphoprotein. The biological host range of this genus includes both insects and other arthropods. Vertebrate hosts include man, horses, monkeys, rabbits, cattle, deer and suckling mice. Transmission is through vectors such as *Culicoides*, mosquitoes, phlebotomines and ticks (Gould and Hyatt,1994). After cutaneous instillation of BTV (by inoculation or through the bite of a BTV-infected *Culicoides* vector) the virus travels to the regional lymph node, where initial replication occurs (Baratt-Boyes et al., 1995). The virus then is disseminated to a variety of tissues throughout the body, where replication occurs principally in mononuclear phagocytic and endothelial cells, lymphocytes and perhaps other cell types (Mahrt and Osburn,1986; Maclachlan et al., 1990; Ellis at al., 1993; Barratt-Boyes and Maclachlan,1994; Darpel at al., 2009). BTV promiscuously associates with all blood cells during viraemia, thus titres of virus in each cell fraction are proportionate to the

numbers of each cell type; specifically, BTV is quantitatively associated most with platelets and erythrocytes and, because of the short lifespan of platelets, virus is largely or exclusively associated with erythrocytes late in the course of BTV infection of ruminants. BTV infection of erythrocytes facilitates both prolonged infection of ruminants and infection of haematophagous insect vectors that feed on viraemic ruminants, and infectious virus can co-circulate for several weeks with high titres of neutralizing antibody (Maclachlan et al., 2009). The duration of this extended presence of BTV nucleic acid in the blood of infected ruminants is similar to the circulating half-life of ruminant erythrocytes, which is somewhat longer in cattle than sheep (Barratt-Boyes and Maclachlan,1995). It remains unclear why most virulent strains of BTV produce disease in sheep but not in cattle (Barratt-Boyes and Maclachlan,1995). The similar or identical pathogenesis of BTV infection of cattle and sheep further emphasizes this obvious paradox. Fundamental differences in the susceptibility of endothelial cells from cattle and sheep to BTV infection are perhaps responsible (DeMaula et al., 2001). Whereas BTV infection of bovine endothelial cells resulted in their activation, with the increased transcription of genes encoding a variety of vasoactive and inflammatory mediators and increased expression of cell surface adhesion molecules, similar infection of sheep endothelial cells resulted in rapid cytolysis with minimal activation. Furthermore, the ratio of thromboxane to prostacyclin, which is indicative of enhanced coagulation, was significantly greater in BTV-infected sheep than in cattle (DeMaula et al., 2002a). The significant elevation in plasma levels of thromboxane in BTV-infected ruminants suggests that platelet-derived vasoactive mediators may exert a central role in mediating increased vascular permeability in the course of BT disease. In summary, dysfunction of the vascular

endothelium is central to expression of disease in BTV-infected ruminants. BTV infection of endothelial cells lining small vessels in the oral cavity and upper gastrointestinal tract, subcutis, heart and skeletal muscle and other susceptible tissues during acute infection results in vascular thrombosis and infarction of the affected tissues. Vasoactive mediators produced by platelets and virus-infected dendritic cells, macrophages and endothelial cells likely contribute to the regionally extensive endothelial dysfunction and vascular leakage that characterizes fulminant BT, with resultant ventral and pulmonary oedema and abdominal, pleural and pericardial effusions. Similarly, African horse sickness, which is caused by a virus closely related to BTV, is characterized by profound regionally extensive vascular leakage leading to oedema of the head, neck, lungs and thoracic cavity, but without the tissue infarction and mucosal ulceration that is characteristic of BT (Coetzer and Guthrie, 2004). Mortality rates can vary substantially between epizootics, typically anywhere up to 30% (or even higher on rare occasion) in susceptible ruminants (Verwoerd and Erasmus, 2004). The signs of BT in sheep reflect congestion, oedema and haemorrhage as a consequence of virus-mediated vascular injury (Mahrt and Osburn, 1986; Verwoerd and Erasmus, 2004). Thus, sheep with BT have any combination of fever, serous to bloody nasal discharge with crusting around the nostrils, laboured breathing and profound respiratory difficulty in animals with severe pulmonary oedema, oral erosions and ulcers, lameness with hyperaemia of the coronary band, and weakness secondary to muscle necrosis. Lesions present on post-mortem examination of affected sheep can include hyperaemia, haemorrhages, erosion and ulceration of the mucosa of the upper gastrointestinal tract (oral cavity, oesophagus, fourth stomach); oedema and haemorrhage of lymph nodes; haemorrhages within the subcutis ; subintimal

haemorrhages in the pulmonary artery; pulmonary oedema; pleural and/or pericardial effusion; facial and submandibular oedema; oedema within the fascial planes of the muscles of the abdominal wall and neck, particularly around the neck ligament; necrosis of skeletal and cardiac muscle, with the papillary muscle of the left ventricle being an especially characteristic site (Erasmus, 1975; Verwoerd and Erasmus, 2004; Maclachlan et al., 2008). Histological lesions reflect the gross changes, with some variation dependent on the duration of the injury. Specifically, lesions in skeletal and cardiac muscle range from acute myonecrosis with haemorrhage, to more chronic lesions with fibrosis and infiltration of mononuclear inflammatory cells. Pulmonary oedema is very characteristic of many fatal BTV infections, but certainly is not pathognomonic of BT. Changes within small vessels in the skin and adjacent to lesions such as oral ulcers are inconsistent and often subtle; acutely affected vessels may exhibit only endothelial hypertrophy with perivascular oedema and/or haemorrhage, with subsequent variable perivascular accumulation of lymphocytes and macrophages. Animals with severe clinical disease may die, develop chronic disease with wasting, or recover fully. Some individuals that ‘‘appear to be recovering satisfactorily may suddenly collapse and die’’ and presumably these animals die from the severe and rapidly progressive pulmonary oedema that occurs late in the course of some fatal infections (Maclachlan et al., 2008). Animals that develop chronic disease usually have severe muscle loss that is manifest as extreme weakness, prostration and torticollis. They also exhibit a characteristic break in the fleece (wool break), and have a protracted recovery period (Erasmus, 1975; Verwoerd and Erasmus, 2004). The lesions of BT in cattle have been particularly well described amongst animals affected during the current BTV serotype 8 epizootic in Europe, although it should be emphasized that

these lesions are frequently subtle (Maclachlan et al., 2009). The lesions in cattle with BT can include severe and extensive ulceration of the muzzle, oral mucosa and teats; rhinitis and mucohaemorrhagic nasal discharge; epiphora and periocular inflammation; and limb oedema (Thiry et al., 2006; Darpel et al., 2007). Pleural, pericardial and abdominal effusions are apparently not as characteristic of severe BT in cattle as they are in sheep; however, severely affected cattle occasionally develop severe pulmonary oedema. Reported mortality of affected animals has been highly variable but often substantial (Meclachlan et al, 2008). A variety of free-ranging and captive (zoo) ruminants have developed disease during the current epizootic of BT in Europe, including musk ox, bison (both American and European), muflon, and yaks, whereas ruminants of African extraction are apparently resistant (Fernandez-Pacheco et al., 2008; Mauroy et al., 2008). Severe, transient corneal oedema occurs in some calves congenitally infected with BTV after suckling colostrum, suggesting that the syndrome might be related to immune complex deposition in vessels within the anterior chamber (Holzhauer and Vos, 2009). Interestingly, a potentially analogous ‘‘white eye calf’’ syndrome was previously described in Oregon in the United States and attributed to either congenital BTV or related epizootic haemorrhagic disease virus infection; affected calves failed to thrive or were born dead, and often had hydranencephaly (Reynolds et al., 1985). Transient but severe corneal oedema is also a feature of BT in adult European bison, a species that is highly susceptible to infection with BTV serotype 8 (Meclachlan et al., 2008). In the sheep congenital lesions are different in order to the disease occurs in the early or in the sustained pregnancy (Lelli et al., 2000). Lesions of acute necrotizing meningoencephalitis progressing to hydraenencephaly and subcortical cysts were described in approximately 20% of the foetuses born to ewes that

were vaccinee on the 40th day of gestation (Young and Cordy, 1964). The pathogenesis of BTV infection is similar in sheep and cattle and, most probably, all species of ruminants (Maclachlan et al., 2009). However, there are marked differences in the severity of desease that occurs in different ruminant species or breeds after BTV infection, and infection of the same species or sheep breed with different virus strains (Gard,1984; Verwoerd and Erasmus, 2004). BTV infection of cattle, goats and most wild ruminant species is typically asymptomatic or subclinical, although specific strains of BTV like the strain of serotype 8 currently circulating in Europe can induce severe disease in other species including cattle and camelids (Darpel et al., 2007; Henrich et al., 2007). Other hosts have been implicated in the lifecycle of BTV infection. Serological evidence indicates that large African carnivores are infected with BTV, whereas smaller predators that cohabit with them are not, suggesting that large carnivores are infected through feeding on BTV-infected ruminants (Alexander et al., 1994). Inadvertent contamination of a canine vaccine with BTV confirmed that dogs are susceptible to BTV infection; indeed, pregnant bitches that received this contaminated vaccine typically aborted and died. There is no evidence, however, that dogs or other carnivores are important to the natural cycle of BTV infection (Akita et al., 1994).

Vector competence

In 1944 Du Toit, in South Africa, proved that the virus is transmitted by *C. imicola*, midge of the genus *Culicoides*. Among these, some are identified like disease vectors (ex.: *C. imicola*), others are only suspected or potential vectors. In North America the main vector is *C. sonorensis*, while in Central and South America *C. insignis* and *C. pusillus* are considered the principal vectors of the disease. In Australia, *C. brevitarsis* and *C. fulvus* are the species involved in BTV transmission (EUBT-NET,2000). In Asia several species of *Culicoides* are considered infection vectors, while in Africa and in the Mediterranean basin *C. imicola* is know as the major vector, although it's possible that some species of *Culicoides Obsoletus* complex play the role as vector in some areas of Bulgaria (Mellor at all.,2000). *C. imicola* is the principal vector of BTV to ruminant livestock in southern Europe also. The other potential vectors are *Culicoides obsoletus* and *Culicoides scoticus* of the Obsoletus Complex, *C. pulicaris* of the Pulicaris Complex and *C. dewulfi*. Within the Mediterranean *C. imicola* is the principal vector accounting for as much as 90% of disease transmission. However, in the Balkans BT advanced northwards and eastwards into areas where *C. imicola* is absent and also appeared unexpectedly in northern Europe in August 2006 and again in 2007 in the

absence of *C. imicola*. The appearance of the disease in areas where *C. imicola* is not present, leaves no doubt that species of *Culicoides* endemic to Europe are transmitting BTV (Conte at al., 2007). The incriminated and potential palaearctic vectors are *C. obsoletus* (Mellor and Pitzolis, 1979; Savini et al., 2003, 2005; De Liberato et al., 2005), *C. pulicaris* (Caracappa et al., 2003), *C. dewulfi* (Meiswinkel et al., 2007) and *C. chiopterus* (EFSA,2008). In one of these studies (Savini et al., 2003) BTV was isolated from mixed *Culicoides* species pools which contained *C. scoticus* thereby implicating it also in the transmission of BT disease. BT , being transmitted by vectors, is conditioned by the environment and climate: it can be detected in the late summer. The reasons why some species of *Culicoides* contain some individuals that are competent to support the replication and transmission of BTV subsequent to oral ingestion, and why others are not, are complex. Basically, when an arbovirus like BTV is ingested by a haematophagous insect during blood feeding, the virus passes into the lumen of the hind part of the mid-gut. It then has to gain access to the body of the insect proper before the potentially hostile environment in the gut lumen inactivates it or before it is excreted. If the virus is to be orally transmitted by the vector, as is BTV, it must reach the salivary glands with or without amplification in other susceptible tissues, multiply in them and finally be released with the saliva into the salivary ducts where it is available to infect a second vertebrate host during a subsequent bite. The details of this cycle (its duration, the tissues infected, the titre of virus produced, the proportion of insects infected, the transmission rate) are controlled by a range of inter-dependent variables (the virus, the insect host and environmental factors – particularly temperature). In the case of BTV, a series of barriers or constraints are known to exist within the bodies of non-vector species of *Culicoides* and even within a variable

proportion of individuals within vector species, that act to prevent infection or else restrict infection in such a way as to prevent transmission. Briefly, the major barriers that an arbovirus may have to surmount upon being deposited in the midgut of an haematophagous insect in order to develop a fully patent infection and so be available for oral transmission are as follows:

• infection of the mid-gut cells: mid-gut infection barrier (MIB)

• escape of progeny virus from the mid-gut cells into the haemocoel: mid-gut escape barrier (MEB)

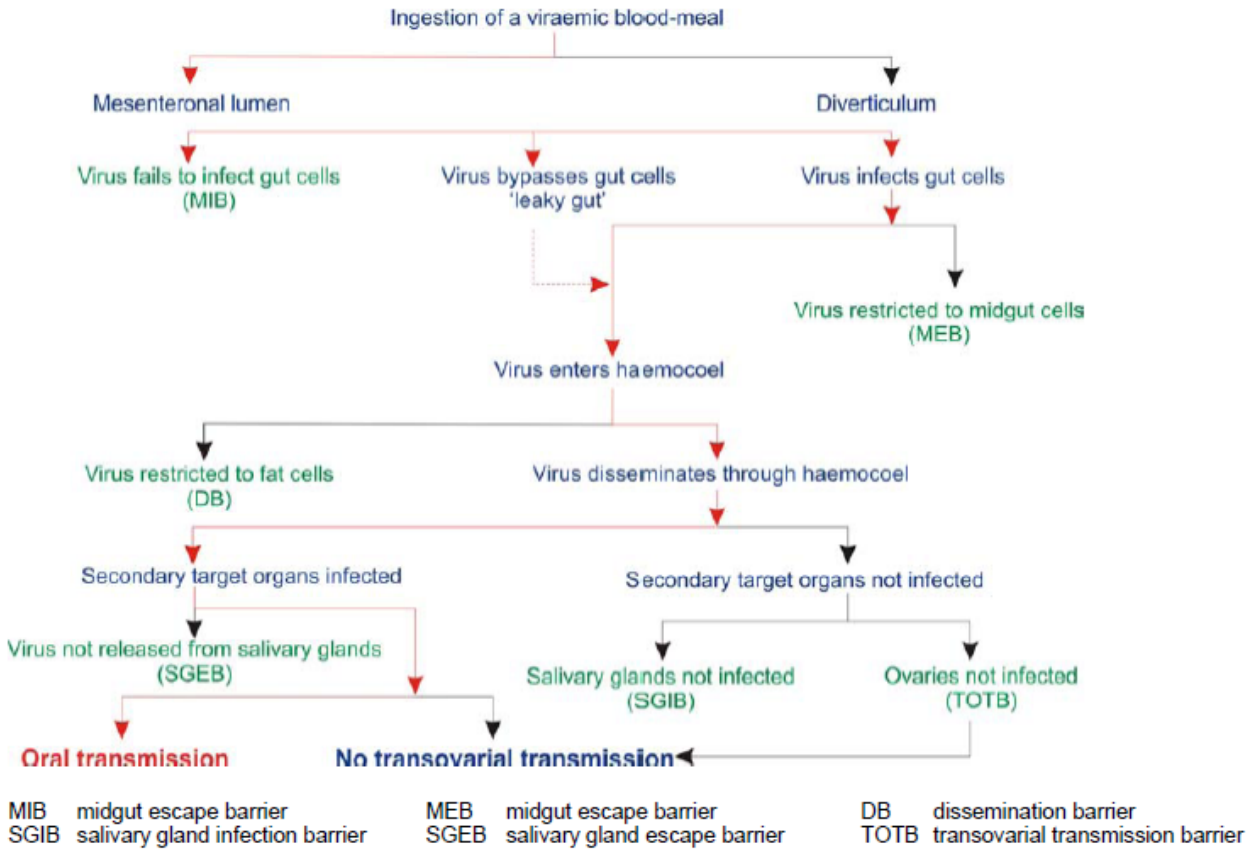
• dissemination of virus through the haemocoel to the salivary glands (and ovaries if transovarial transmission is to occur): dissemination barrier (DB)

• infection of the salivary glands: salivary gland infection barrier (SGIB)

• release from the salivary glands into the salivary ducts: salivary gland escape barrier (SGEB).

In addition, there is a further barrier to surmount if the virus is to be transmitted transovarially, the transovarial transmission barrier (TOTB). The Figure 1 descript a summary of these barriers to infection and transmission, and highlights those that have so far been identified in the BTV-*Culicoides* system.

Fig 1. Barriers to the infection and transmission of Arboviruses by insect vectors. Route of virus dissemination in the *Culicoides*-BTV system highlighted in red.



Mellor,2004.

Within a vector species of *Culicoides* susceptibility to infection is a genetically heritable trait. This means that different populations of the same species may have widely varying oral susceptibilities to infection and transmission of a particular serotype or strain of BTV dependent upon the genotypes prevalent in the parental populations from which they were derived. Consequently, results obtained by testing one or several populations of a suspect vector species of *Culicoides* cannot necessarily be extrapolated across all or most populations of that species. This situation can make it difficult to estimate the importance of a suspect vector species unless exhaustive testing has been undertaken across many populations of that species using a range of BTV serotypes and strains. As may be seen when considering the current outbreaks of BT in Europe, in practice, this can result in incorrect assessments being made of the

significance of such novel vectors (Mellor,2004). *Culicoides* biting midges are tiny flies, 1–3 mm in length (Meiswinkel et al., 1994). The life cycle consists of egg, four larval instars, pupa and adult stages. The immatures require moisture and organic matter for development and breeding sites include damp or saturated soils, bogs, marshes, swamps, tree holes, animal dung and rotting fruits or other vegetation (Meiswinkel et al., 1994;Mellor, 1996). The duration of the life cycle depends on the species and climatic conditions, varying from 7 days in the tropics to 7 months in temperate regions, where most species diapause as fourth instar larvae during winter (Braverman, 1994). The life-span of the adults is usually short and is dependent on ambient conditions, which will alter with climate change. Most adults survive less than 20 days, although occasionally they live for up to 90 days (Mellor et al., 2000). Females feed on blood, which provides protein for the development of eggs and one bloodmeal is usually required for each batch of eggs to mature. The frequency of feeding is, therefore, linked to the rate of egg development, which is itself dependent on species and the ambient temperature. Increases in ambient temperature may lead to an increased feeding frequency. This is relevant as virus transmission to susceptible hosts may occur at each feed. In the majority of species adult activity is crepuscular or nocturnal (Kettle, 1995) and activity is greatest when evening and night-time conditions are warm, humid and calm (Boorman, 1993). Climatic changes that alter these conditions may affect the activity rates of *Culicoides*. For example, windier summer nights may suppress activity and lessen the risk of virus transmission. As *Culicoides* can be carried on the wind for considerable distances, however, these same conditions may lead to more effective dispersal of infected midges to new areas (Sellers, 1992).

EPIDEMIOLOGY

The disease has a world-wide distribution in an area between 40°N and 35°S where climate and environment conditions are optimal for *Culicoides* survival. Actually drawing this line is a theoretical structure because particular environmental condition can go over this limits (Pini and Prosperi, 1999). In fact, climate change is one of the most serious environmental issues of our day. In the last century the global mean temperature has risen by 0.5°C (Jones and Wigley, 1990) and if no steps are taken to limit greenhouse gas emissions, temperatures could rise by a further 2°C by 2100. This predicted rate of change is greater than global temperatures have changed at any time

over the past 10 000 years. In addition to increases in temperature, changes in precipitation, wind patterns and climate variability are also predicted to occur (Wittmann and Baylis, 2000). The prevalence of BT is influenced by factors which can be possible the presence of vectors so the trend of the disease is closely related to the season. The impact of these changes is likely to be enormous. One of the most immediate and noticeable impacts of climate change may be an alteration in the distribution and abundance of insect species (Sutherst, 1990). First cases of BT start in midsummer, the major prevalence is observed when the summer is ending then the disease disappears because night temperature decreases below 12 °C. Cattle play an important role in the epidemiology of BT, because they generally keep the infection without show clinical symptoms. In fact the endemization of infection in temperate areas and its persistence from one epidemic season to the following are sustained by the prolonged viremia in cattle. The infective period (the longest period during which an affected animal can be a source of infection) for cattle, in fact, is considered usually to be equal to 60 days post infection but it's possible to have a longer viremia (Sellers and Taylor,1980). Usually, the transmission cycle between cattle and *Culicoides* becomes evident when sheep are infected. Viraemia in sheep is shorter than in cattle ,on average 14-31 days, but it can be longer up to 54 days (Koumbati at all,1999). Wild ruminants also , if they are several, can play an important role to keep the infection in their area. This role is proved in Africa and in North America, where animals as american deer (*Odocoileus virginianus*), the antelogoat (*American antelogoat*) and the sheep of the desert with big corna (*Ovis canadiensis*) can show clinical symptoms (Pini and Prosperi, 1999).The role of semen and embryo transfer in BTV transmission are controversial. Viraemic sires may rarely shed BTV through semen. The ability of

infected semen to transmit BT is still discussed. Regarding to embryo transfer, all Authors agree each other, considering negligible the risk of BTV transmission through embryo transfer, when the protocol of the International Embryo Transfer Society is followed. Adult *Culicoides* fly during the night (from sunset to dawn) and bite animals feeding on their blood. However, recent studies conducted in 2006 during the BTV-8 epidemic in Central Europe seem to indicate that a certain level of vector activity can be detected also during the daylight. The insects become infected biting viremic animals and they remain infected all along their life. Vertical transmission in insects has never been proved. Adult *Culicoides* need water for their reproduction. In fact, *C. imicola* lays eggs in humid soil or mud. Therefore, humid areas and water reservoirs, especially of small size, permit vector's reproduction. The environmental characteristics of *Culicoides* breeding sites, however, vary among the species and in many cases very few data are available. Adults of *Culicoides* remain in an area few hundreds of meters around the place where they are born. However, adults can be passively carried by the winds and spread for more than 300 Kilometers. Adults live for 10-20 days, but they can exceptionally survive for longer periods (up to 60-90 days). The length of their life and the survival rate are influenced by the temperature. *C. imicola* abundance decreases when minimum temperature is lower than +12°C. However, 15% of *C. imicola* population may survive longer than 15 days at –1,5°C. It is often assumed that *Culicoides* feed only on animals outdoors and that they rarely enter buildings. However, in South Africa *C. bolitinos* has been shown to enter stables whereas *C. imicola* showed some reluctance to do so. In northern Europe, during the outbreak of BT in 2006, *C. obsoletus* and *C. dewulfi*, were captured in light traps inside cattle sheds suggesting a certain capacity to attack the cattle housed inside. It is not

clearly understood whether the housing of all farm animals each night will 'force' even exophagic species to enter buildings to acquire a blood meal (Meinswinkel at al.,1994). Out of the Mediterranean basin, BT is spread in the whole Africa Continent, in Giordania, Iran, Arabia Saudita, Oman, India, Pakistan, Indonesia, Australia, in the Caribbean, Mid America and South America, in some areas of the USA and Canada (OIE 1999).

Bluetongue in the world

BT first was reported more than 125 years ago with the introduction of European breeds of sheep into southern Africa. Sheep experienced a severe febrile disease with high morbidity and high mortality. The viral etiology of the disease was demonstrated in 1906 (Walton, 2003). BTV is traditionally understood as occurring around the world in a broad band stretching from about 35°S to 50°N although in certain areas it may

extend up to around 50°N . By and large this distribution is a reflection of the distribution of its *Culicoides* vectors and the temperature required for BTV replication in and transmission by the vectors (Dulac et al., 1989; Zhang et al., 1999, 2004; Lundervold et al., 2003). BT was first recognized in South Africa at the turn of the 20th century, and the disease was confined to Africa till 1943, when it spread to Cyprus, although the date is in question as it may have been as early as 1925. The virus was rapidly disseminated further and to other continents however limited by the distribution of its vectors. The plurality of the BT virus serotypes was only recognized later with 12 serotypes dating to pre 1960, an additional 4 serotypes being confirmed between 1960-1970 and the remaining 8 serotypes recognized post 1970. At the present day BT situation does not enjoy the same attention now as then maybe because of the endemic nature of its presence in South Africa. If we summarise BT diagnostics from 1983 – 2003 we are aware of the following statistics. The four most prevalent serotypes were 1, 3, 4 and 2 and then the 3 least often seen serotypes were 10, 13, and 15. Four were not found at all during the period under review namely 18, 19 22 and 23. This translates roughly into groupings previously made of serotypes with high or low epidemic potential and those present at low levels every season (Gerdes, 2003). BTV was isolated for the first time in new world from sheep with "sore muzzle" in California, U.S.A. in 1952. The confirmation of the disease in the U.S.A. was associated with an extensive epidemic, but might indeed have been regulated to a post-script in history were it not for a even more dramatic epidemic of BT, in 1956/57, in Portugal and Spain, which killed large numbers of sheep. This epidemic aroused great concern throughout the sheep rearing areas of Europe; equally, it caused alarm in Australia. BTV is currently recognized to infect domestic ruminants on the continents

of Africa, Asia, North America, South America (SA) , and Australia and several island in the tropics and sub-tropics. Several countries, such as the U.S.A., that are close to the subtropics also have endemically infected livestock. Within large countries spanning different latitudes, such as the U.S.A. and Australia, there may be areas where virus activity is absent (Gibbs and Greiner, 1994). BTV distribution in North America is limited by the range of the vector, *Culicoides* spp. With the exception of sporadic occurrences in the Okanagan Valley in British Columbia, Canada is considered free of BTV. Regional differences exist in the United States. The north eastern U.S. states are free of BT. BTV activity occurs most of the year in the southern region of the United States and in Mexico. In more temperate regions of the United States, evidence of BTV infection is seasonal. Peak transmission in the temperate zones occurs in late summer and early autumn. In the United States, the seroprevalence of BTV antibodies in slaughter cattle from selected northeastern and northern states is examined regularly. BT disease in sheep or cattle is infrequently reported in the United States. In addition to diagnostic testing, examination for evidence of BTV infection is often required to certify U.S. animals or animal products for international export. BTV serotypes previously identified in North America are serotypes 2, 10, 11, 13, and 17. Isolation of BTV serotype 2 in the United States is quite rare. However, a BTV serotype 2 isolate was obtained in 1999 from sheep with clinical disease residing in Florida. All other strains of BTV are exotic to the United States (Ostlund et al., 2003). The first published report of BTV infection in South America was done by Brazil in 1979. They detected serological evidence of infection in livestock in Sao Paulo and Rio de Janeiro States. Since then several serological surveys have determined that the infection is widespread in South America. Only four outbreaks of BT disease have been reported so far in SA

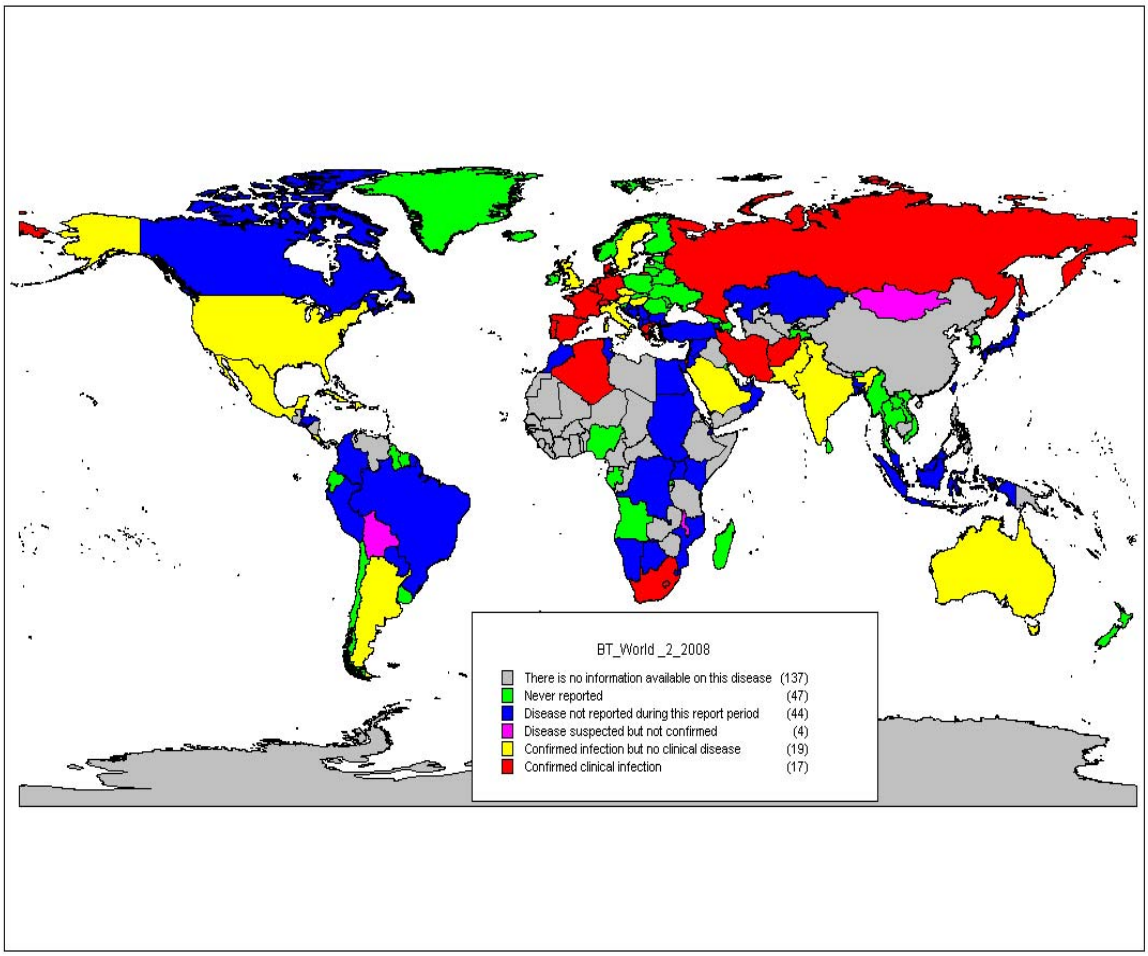
and they all occurred in the last three years, in Párana State, Brazil. The first was in June 2001, with 9 cases that affected seep and goats. The second was in February 2002 in goats and the last two in March 2002 affecting sheep and goats. The first BTV isolation from SA naturally infected animals was BTV serotype 4 from zebu cattle that were imported from Brazil to the USA. Brazil and Argentina are the only SA countries where BTV has been isolated. By serology, the serotypes detected in SA are : 4, 6, 14, 17 and 19 inBrazil; 12, 14 and 17 in Colombia; 14 and 17 in Guyana and 6, 14 and 17 in Suriname. *C. insignis* is the predominant vector detected in the area. However, as BTV was isolated from *C. pusillus* in Central America and the Caribbean and this species is also present in SA, it is possible that *C. pusillus* could be also a BTV vector in the area. The virus has not yet been isolated from the vector in the region. The pattern of BTV transmission in SA, seems to be very similar to other endemic areas. The highest incidence of infection occurs when the climatic conditions favour the breeding of competent vectors i.e. in the late summer and autumn (February to May) (Lager, 2003). In 1977 the Australian animal health authorities were advised that a virus isolated 2 years previously had just been identified as a BT virus. As a country with a population of more than 140 million sheep, Australia had exotic disease plans to respond to an incursion of BTV. Consequently, the livestock industries struggled with this news, given that, although the virus did not produce disease, it halted export of animals and animal products. During the following 15 years, 8 serotypes were identified. In the last decade, research efforts have been directed at reducing the impact of BT viruses on the export of livestock, semen and embryos. In 1993 the National Arbovirus Monitoring Program (NAMP) was formally established as a co-operative initiative between the livestock industries, national and state governments. The main

emphasis of NAMP has been on the definition of the distribution of BT viruses and their vectors, together with monitoring annual fluctuations of viruses and vectors. These objectives have been achieved by sampling of sentinel cattle at key locations around Australia and by light trap collection of insects at these sentinel sites. Despite the presence of some pathogenic viruses, Australia remains free of BT disease. However, the economic impact is considerable due to disruption to trade. Vaccination for BT has never been practiced, providing no opportunity for changed virus characteristics though genetic re-assortment between vaccine and wild types. Collectively these factors, when combined with virus and vector monitoring data that has been gathered over more than 25 years, have allowed the accurate delineation of BT free zones and zones of possible BT transmission in accord with OIE guidelines (Kirkland, 2003). More recently, it has been shown that BT virus serotypes exist with vector species of *Culicoides* in predictable, but finite, geographic and ecologic cycles or ecosystems around the world. Despite the almost certain movement of BT viruses, livestock, and *Culicoides* species between these ecosystems, there has been little evidence that BT virus serotypes have been moved between and persist in these ecosystems. Rather, periodic cyclic extensions and remissions of these virus vector ecosystems permit the viruses and the disease to move into and recede from adjacent non-endemic areas in a pattern characteristic of many otherknown arthropod-borne viruses (Walton,2003). Since 1950 four Arboviral diseases have been recorded and identified in Israeli livestock: BT in small ruminants and cattle, bovine ephemeral fever (BEF) in cattle, West Nile virus (WNV) in geese and horses, and Akabane in cattle and small ruminants. All four diseases appear in springtime, with the majority of outbreaks occurring at the end of summer. In 2002, following a wave of congenital blindness and hydrocephaly in calves, a serosurvey for

BT antibodies in cattle revealed that 79% of the herds were seropositive while in sheep only 30% were positive. What role BTV has in causing congenital malformations in dairy cattle is still not clear. Of the four arboviral diseases, BT is the only one that appears regularly in Israel; since 1976, 318 outbreaks were recorded in sheep herds, only 4 years (1980-1981, 1999-2000) were BT free. Viewing the annual number of BT infections it can be observed that every few years there is a peak in the number of outbreaks. Five serotypes, 2, 4, 6, 10, and 16 have been implicated in BT outbreaks in Israel; serotypes 4, 6, and 16 are the most common. BT vaccination is voluntary. The annual number of applied vaccine doses decreased from 77.000 in 1989 to 9.000 last years (Yadin and Van Ham, 2003). Although BTV likely has long been present throughout much of the world, the global distribution of BTV infection very recently has drastically altered (Wilson and Mellor, 2008; Purse et al., 2008). It has been proposed that climate change is in part responsible for this profound change in the global distribution of BTV, presumably by its impact on the vectorial capacity of resident *Culicoides* insect populations in previously virus-free regions such as much of the Mediterranean Basin (Gerry et al., 2001; Purse et al., 2005, 2008; Wilson and Mellor, 2008; Gould and Higgs, 2009). Similarly, multiple novel serotypes of BTV have recently spread northward from the Caribbean Basin to invade the southeastern United States, where they previously did not occur, and additional serotypes of BTV have recently incurred into northern Australia (Johnson et al., 2006, 2007). BT in sheep has its most significant economic impact in the Republic of South Africa and California. In both of these temperate areas, outbreaks of disease occur on a regular basis, often annually. The disease is usually seen in late summer and early autumn and outbreaks cease after the first frost of winter. Elsewhere, such as in Cyprus and

Turkey, many years may elapse between epidemics. Where disease occurs infrequently, epidemics are usually associated with only one serotype of virus (exs. Serotype 10 in Portugal in 1956; serotype 4 in Cyprus in 1977) but where disease is common, a random distribution of serotype may be isolated during the course of an epidemic (Gibbs and Greiner, 1994). Different strains of BT, topotypes which are carryed by several species of *Culicoides* are present in specific areas in the world. BT topotypes and the species that are BT vectors, that are present in every episystem, are constant (Caporale V., 2008). Worldwide BTV has been estimated to cause direct (disease) and in direct (trade, vaccines, etc) losses of over $ 3 billion per year (Tabachnick et al., 1996). Currently situation is reported in Figure 2.

Fig. 2 Bluetongue in the world. Currently situation.



Bluetongue in the Mediterranean countries.

Over last 60 years BT virus caused several outbreaks in the Mediterranean basin. Disease was reported in Morocco in 1958, in Israel in 1943 and in Turkey in 1944. Since 1968 disease have been occurred in Israel. Serotypes reported in these country are several : 10 in Morocco, 4 e 9 in Turkey, 2, 4, 6, 10 e 16 in Israel (Shimshony, 1987). In 1956 disease arrived at Spain and the south Portugal (serotype 10) where disappeared after only one epidemy. Then disease occurred in Cipro in 1977 and in the grecian islands, Rodi e Lesbo in 1979. Greece was pronounced immune from BT in 1991, but in october of 1998 a new epidemy hit Rodi, Kos e Leros islands (sierotipo 9). In autumn 1999 BT spread in several islands of Dodecanneso and hit the east part of the continental Greece (4, 9 e 16 serotypes)(commissione europea, 2000). Between june and novenber 1999 BT was reported in the south east Bulgary also(9 serotype) nearly the border between Turkey and Greece . In Bulgary, however, the presence of *C. imicola*, the major vector of BT, has never been proved, but midges that belong to *C. obsoletus* group are suspected that were played the role of BT vectors (European commission,2000). In January 2000 BT was reported for the first time ever from Tunisia, in areas in the NE of the country. The time of the incursion was estimated as early December 1999 and the virus was typed as BTV-2 (Mellor et al., 2008). The origin of the incursion is uncertain, however, as foot and mouth disease virus had also entered Tunisia (and Algeria) during 1999, probably via cattle from Coˆte d'Ivoire and Guinea (Knowles and Davies, 2000), it is possible that BTV could have used the same or a similar route. Cattle in Africa often experience sub-clinical infections with BTV, and BTV-2 is common in sub-Saharan West Africa (Herniman et al., 1980, 1983). No new cases of BT were detected during February–May 2000 but from June further

outbreaks of BTV-2 were reported from 10 Districts in the eastern and central parts of the country. These persisted in some areas until October 2000 (Mellor et al., 2008). Control measures implemented in 2000 included isolation of infected flocks, spraying animal holdings with insecticide and mass vaccination (in 2000, 2001 and 2002) with a monovalent, live BTV-2 vaccine (Hammami, 2004). In December 2002, a limited number of new outbreaks caused by BTV-2 occurred in central Tunisia in unvaccinated flocks but subsequently and up to early 2006 no further evidence of virus circulation was reported. In July 2000 BTV-2 was reported from eastern Algeria close to the Tunisian border and outbreaks continued into September, including areas up to 250 km to the west of Tunisia (Mellor et al., 2008). In addition, samples taken from animals near Algiers, over 400 km to the west of Tunisia also recorded positive for BTV antibodies (Mellor and Wittmann, 2002). Control was based upon the use of insecticides and clinical surveillance (Hammami, 2004). Further BTV activity in the country has not been reported up to early 2006. In late 2000, BTV antibodies were recorded in animals from a number of provinces across northern Morocco but there was no evidence of clinical disease (Mellor et al., 2008). Despite serological monitoring no further evidence of BTV activity in Morocco was detected in the succeeding 3 years (Hammami, 2004). However, in 2004 the situation changed and starting in August and continuing to the end of the year a further incursion of BTV was recorded, this time clinically affecting sheep in 14 provinces in the north-west (Anon, 2004a–g). Surprisingly, this incursion was caused by BTV-4 (Anon, 2004b), and is therefore separate from the 2000–2002 incursions of BTV-2 into other areas of North Africa. So far the origin of this incursion of BTV 4 is unknown. BTV was recorded for the first time on the French island of Corsica, in October 2000 and the virus was identified as

BTV-2 (Anon, 2000j). A vaccination campaign was quickly organized using a monovalent live vaccine and commenced during the winter of 2000–2001. However, full cover was not achieved as a second epidemic of BTV-2 occurred in July 2001 affecting animals, mainly in unvaccinated flocks, in the north and south of the island. Three hundred and thirty five flocks were affected, a sevenfold increase on 2000 (Breard et al., 2004). Accordingly, a second vaccination campaign was mounted during the winter and spring of 2001–2002 which seems to have been successful as no cases of BT were reported during 2002. In October 2003 a further BTV incursion was recorded into Corsica affecting vaccinated sheep in the south part of the island. The virus was identified as BTV-4 and a third vaccination campaign mounted from early November using a bivalent live, vaccine. In August 2004 and for the fourth year out of five BT was reported in Corsica, BTV-4 occurring in the north and west of the island, and BTV-16 in the south west (Anon, 2004h). A further vaccination campaign was mounted using live vaccines against BTV-2, -4 and -16, and further BTV transmissions were not detected after September 2004. The first outbreaks of BT in Spain commenced on two Balearic Islands (Menorca and Majorca) in September–October 2000 (Mellor et al., 2008). Three hundred and five outbreaks occurred and the virus was identified as BTV-2. A vaccination campaign in sheep using a monovalent live BTV-2 vaccine was mounted on both islands from October 2000. BTV transmission was not recorded beyond November 2000 but a further vaccination campaign in sheep was mounted in the spring of 2001 and was extended to Ibiza even though no cases of BT were detected there. However, in October 2003 a second incursion of BTV was reported, into the eastern part of Menorca. The virus was identified as BTV-4 and a vaccination campaign based upon a live monovalent vaccine against that serotype mounted. In

October 2004 a further incursion of BTV-4, this time into mainland Spain, was identified. The outbreak commenced in the southern Province of Cadiz in Andalucia and spread rapidly through Andalucia and part of Extramadura; the Spanish enclave of Ceuta in north Morocco was also affected (Gomez-Tejedor, 2004). Once again a vaccination campaign was launched in sheep using a live, monovalent BTV-4 vaccine. After a pause during the winter period the outbreak of BTV-4 in mainland Spain continued to spread during the summer of 2005, reaching further north than ever before, until by November 2005 it involved provinces in Andalucia, Castilla la Mancha, Castilla y Leon, Extramadura and Madrid (see: http://rasve.mapya.es/). Also in 2005, a single isolate of BTV-2 was made from a herd of sentinel bovines in mainland Spain. The origin of the 2004–2005 outbreaks of BTV-4 in mainland Spain was undoubtedly northern Morocco where the same serotype of virus was detected just prior to and during the Spanish outbreaks. Molecular studies also confirm the close similarity between the Spanish and Moroccan isolates of BTV-4 (Mellor et al., 2008). The origin of the single isolate of BTV-2 is difficult to determine. However, molecular analysis has confirmed that it is virtually indistinguishable from the BTV-2 vaccine virus previously used in several countries and zones in southern Europe including the Spanish Balearic islands. BTV was first detected in sheep in Portugal in November 2004 in the Evora District of Alentejo, close to the border with Spain, and by December had spread to the Castelo Branco District of Beira Interior (Anon, 2004a,b). The virus was identified as BTV-4 and a vaccination campaign in sheep mounted using a live BTV-4 vaccine. At the same time a countrywide serological surveillance system was initiated. In 2005, seroconversion to BTV but no disease occurred in sentinel bovines until at least October. Further information from Portugal is not available. The

Portuguese outbreaks are clearly an extension of the Spanish mainland incursions which originated in Morocco (Mellor et al., 2008). In June 2006, BT virus, an arboviral pathogen of ruminants, appeared in northern Europe for the first time, successfully overwintered and subsequently caused substantial losses to the farming sector in 2007 and 2008 (Carpenter at al,2009). This outbreak, caused by a BTV-8 strain thought to be of sub-Saharan origin (Maan et al., 2008), occurred 900 km further north than the northern latitudinal limit of previous European incursions. During this initial year, the financial costs of the direct effects of the disease were outweighed by the impact of animal movement restrictions employed to control its spread; there were 2000 infected holdings across Germany, Belgium, the Netherlands, mainland France and Luxembourg and few animal losses across the outbreak area (Elbers et al., 2008). After a brief winter interruption to transmission, the virus re-emerged in 2007, subsequently infecting tens of thousand of holdings and causing devastating, but as yet poorly defined, losses of livestock across the affected areas and an expansion in range to include Denmark, the UK, Switzerland and the Czech Republic. This pattern of expansion was repeated in 2008, when BTV-8 again successfully overwintered in several countries and spread to Sweden, Hungary, Austria and Italy. The outbreak of BTV-8 was eventually to cover an estimated 170,000 km2 of territory reaching almost to latitude 538N, which is the furthest north BTV has penetrated anywhere in the world. It is assumed that the virus arrived in one or other infected animal but all avenues of enquiry have failed to identify either the port of entry or the exact location of the index case. Speculation around its mode of entry includes the postulate that infected specimens of the principal Afro-Asiatic vector of BTV i.e. *C. imicola* entered the region on an aeroplane but has not been substantiated (Meiswinkel et al., 2008). In addition, a second BTV strain was

discovered in the Netherlands, this time of serotype 6. The origin, spread, likely clinical impact and overwintering potential of this second strain are currently unknown (Carpenter et al., 2009). When, on the 29th August 2006, BTV-8 was reported as the causal serotype, it became clear that the distance to a recent BTV-8 outbreak elsewhere was even larger, not only in distance but also in time, because this serotype had not been ever identified in Europe (Darpel et al., 2007; EFSA, 2007). Earlier circulation of BTV-8 has been established in Nigeria, Kenya, Sudan, Malawi, India, South Africa, Dominican Republic, Trinidad, Barbados and Puerto Rico (Vallema, 2008). After sequencing genome 2 of the isolated BTV-8 strain from northern Europe, IAH, the Community Reference Laboratory for BT, reported that the results pointed to an origin in sub-Saharan Africa and not to the BTV-8 vaccine strain used in Bulgaria in 2000 (EFSA, 2007; Elliott, 2007). Actually, after the 1999 BT outbreak, a vaccination campaign had been conducted in Bulgaria; it started after the lambing season in early 2000 and involved 100,000 lambs being vaccinated with a commercially available pentavalent live-attenuated vaccine containing BTV serotypes 3, 8–11 (Panagiotatos, 2004). Although Bulgaria stopped vaccinating afterwards,BTV-8 specific neutralizing antibodies were detected in sentinel calves in November 2006 (EFSA, 2007). The route of introduction of BTV-8 in northern Europe still remains unclear (EFSA, 2007), despite extensive consideration of the main four possible pathways:

- import by means of infected ruminants
- introduction of infected vectors along with horses
- introduction of infected vectors along with exotic plants
- use of contaminated or unstable vaccines.

After the outbreak of BT in northern Europe (Figure 3), a monitoring and surveillance programme started and is based on Council Directive 2000/75/EC (EC, 2000) and Commission Decision 2005/393/EC (EC, 2005). Its main objectives are as follows:

- monitoring of the dynamics of the disease in restricted zones
- surveillance to confirm the absence of the disease or to early detect the entry of virus into free zones
- gathering data for the assessment of the risk of entry or spread of virus into free or infected areas. This work is still going on.

Recently, Meiswinkel et al. (2007), presented the preliminary results of their entomological investigation on one of the BT-affected dairy cattle farms in the Netherlands. In 35 collections, 15 species of *Culicoides* were found, of which *C. obsoletus*/*scoticus* and *C.* (subgenus *Avarita*) *dewulfi* were the most abundant species; the only pool PCR positive for BTV was a pool of *C. dewulfi*, which makes this species, which breeds in cattle dung, a potential BTV vector. No specimens of *C. imicola* were found. Clinical signs of BTV-8 infection have been extensively described in sheep and cattle under field situations, as well as in sheep and cattle and in sheep and goats after experimental infection (Vallema, 2008).

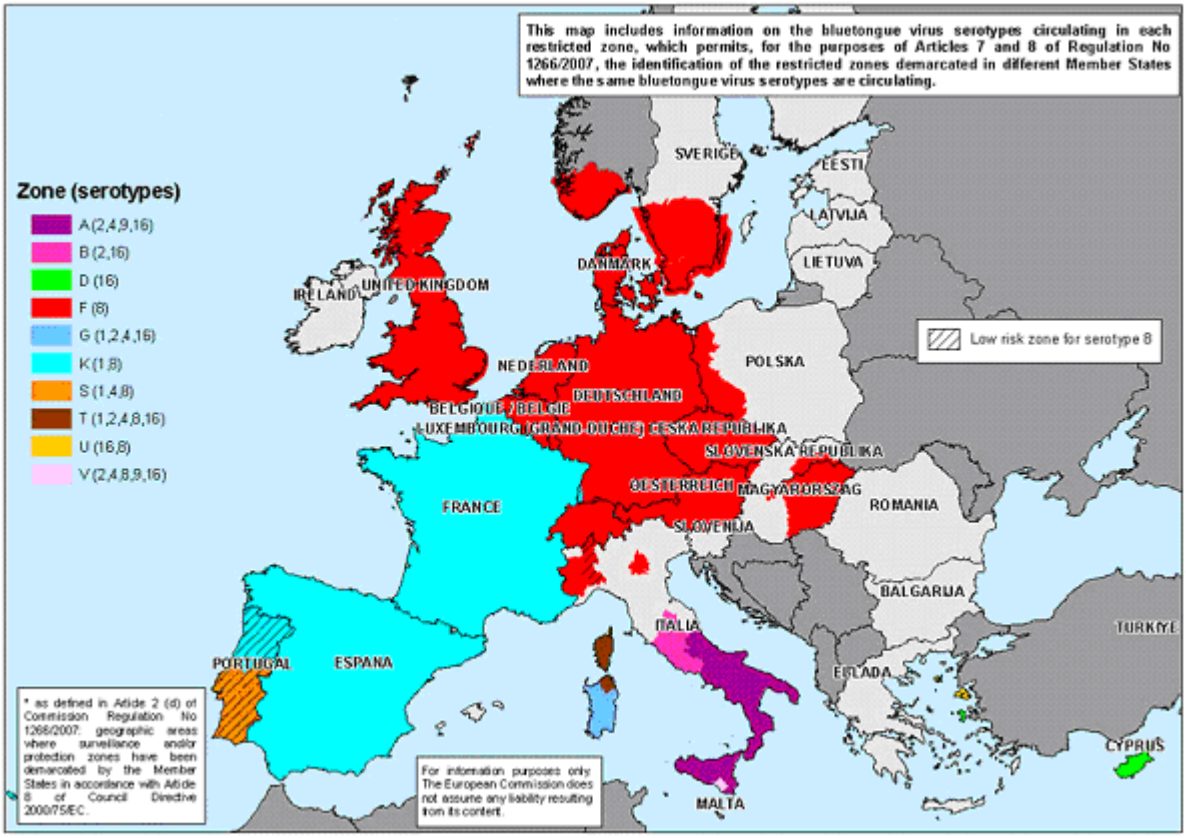
There are several reasons to explain this dramatic change of BT epidemiology, but the most are mainly three (Mellor at all,2006):

- the spread of the major BT vectors *C. Imicola* in everywhere
- the presence of new kind of vectors in *Culicoides*;
- the climate change;

It seems definitely that BT epidemies in the south of Europe are determinated by climate change. *Culicoides spp*. are carried by wind certainly , the most reliable

hypothesis to explain the appearance of BT in Sardinia is exactly that the vectors are carried by the winds which come from south and hit the region in 2000 between June and July ; the next wind wave are recorded 25 days before the disease occurred (Caporale V., 2008). This hypothesis is confirmed because also in Britannia few BT vectors are detected in samples of the entomological surveillance as far as 200 meters above sea level so it's really possible that the vectors travelled by wind from south to north (Champman et al., 2004). *C. Pulicaris* was even captured as far as the coasts of North sea and this was pointed out as a risk factor to BT can be able to arrive in the whole north Europe (Hardy and Cheng, 1986).

Fig 3. Bluetongue situation in Europe.



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Bluetongue in Italy

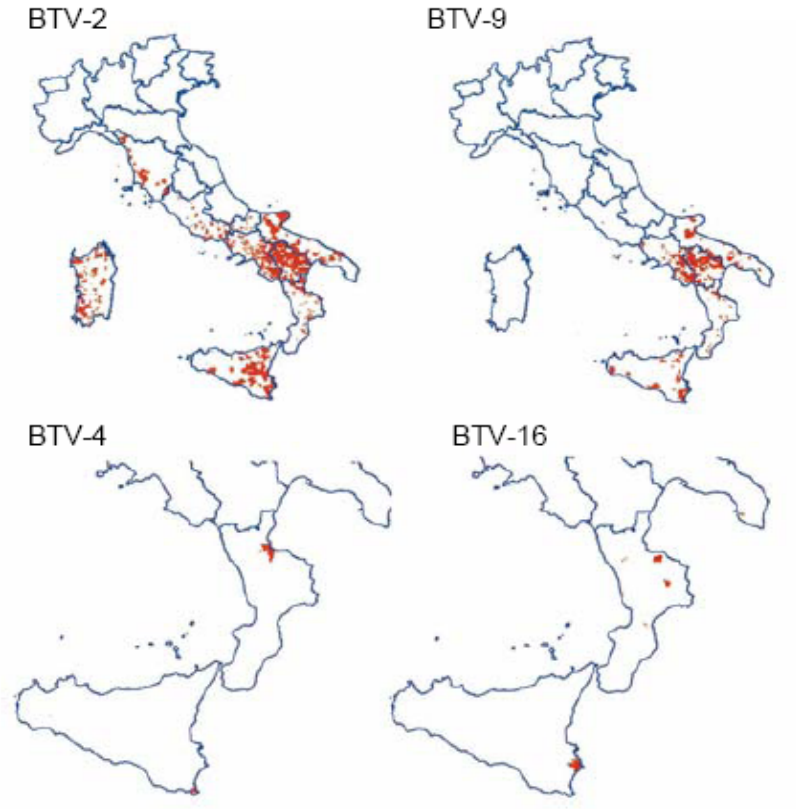
In August 2000, BTV-2 was reported for the first time in Italy. First Sardinia was hit but the BT virus spread immediately in Sicily and in the south of Italy (Anon,2000c,2000k). The occurrence of BT in Italy can be sum up through the first four epidemic:

- First epidemic (August 2000 to May 2001)
- Second epidemic (May 2001 to April 2002)
- Third epidemic (April 2002 to April 2003)
- Fourth epidemic (April 2004 to April 2005)

In 2002 Sardinia, Sicily and Calabria were hit again with Basilicata, Campania, Lazio and Tuscany there were over 6800 outbreaks and 250.000 sheep and goat died. Vaccination haven't used until January 2002 when it started in the involved regions where was vaccinated all BT susceptible species with serotype 2 (Calistri et al., 2004). The third BT epidemic started in Italy on 15 April 2002 and ended on 14 April 2003, and during this outbreak, BTV-2 and BTV-9 infection spread to the province of Benedenti, Caserta, Foggia ,Bari, l'Aquila and The total number of outbreaks detected

in the third epidemic was 432 in eight regions (Figure 4). Moreover, during the third epidemic, two new serotypes were detected in southern Italy, namely: BTV-4 and BTV-16 (Giovannini et al., 2004). Serotypes 4,9,16 have been already reported in the east mediterranean area (Turkey, Greece and area of Balkanys) but how the BT virus could be get in Italy is uncertain yet, although there are some suspect about illegal transports of susceptible animals (Giovannini et al., 2004 a, b). During the summer of 2003, the fourth epidemic commenced and clinical disease was observed in Sardinia alone in August involving BTV-4, a serotype that had not been included in the vaccination programme (Giovannini et al., 2004). Outbreaks continued into 2004 and by the end of the year circulation of one or more of BTV-2, -4, -9 and -16 had been recorded in 12 regions extending from Sicily in the south to Tuscany in the north, and from Puglia in the east to Sardinia in the west. Vaccination was carried out in all affected regions using the appropriate combinations of BTV-2, -4, -9 and -16 live virus vaccines. During 2005 for the sixth consecutive year BTV circulation was recorded, in at least 10 provinces: Lazio (BTV-2 and -16), Liguria (BTV-16), Marche (BTV-16), Molise (BTV-2 and -9), Campania (BTV-2 and -9), Puglia (BTV-2, -9 and -16), Basilicata (BTV-2, -4 and -16), Calabria (BTV-2 and -16), Sicily (BTV-2, -4 and -16) and Sardinia (BTV-2, -4 and -16), and activity continued until at least until November. Again vaccination was carried in each of the affected areas (Mellor et al., 2008).

Fig 4. Distribution of BTV infection in Italy during the third epidemic according to serotype.



(Giovannini et al., 2004).

Bluetongue in Sardinia

In Sardinia there are the most number of sheep in Italy (about 3 millions) with a density of 29 heads for Km2. Moreover, in Sardinia there are 283.000 goats and 230.000 cattle. There are also approximately 4.000 deer (Cervus elaphus), 5.000 muflon (Ovis musimon) and 1.000 fallow deer (Dama dama). The first suspect of BT was reported in 4 sheep in Pula, near Cagliari in the 18th of August 2000. First diagnosis was photosensitization the another 5 sheep were suspected in Sarroch, near Pula in the 21st of August 2000. The simplex of serum collected from the sick sheep was sent to the National Reference Centre for exotic Diseases of Animals (CESME) in the 23th of August 2000. In 24 th of August 2000, 7 out of 9 samples were reported ELISA positive and 5 out of 9 were positive in AGID. Blood and spleen from a sick animals (1

from the herd of Pula, 1 from the herd of Sarroch and 2 from a herd of Sant'Anna Arresi (Cagliari) were collected and sent to CESME, that confirmed isolation of BTV in 01st of September 2000 (serotype 2).

In 26th of August 2000 were set up BT emergency team that had to do specific functions :

- to make and to organize the action plane to better define the extent of the infected zone
- to define the temporal and geographical distribution and progress of BTV
- to give instruction to veterinary public department

Emergency team was composed of veterinaries that belong to regional, local public department of health, representatives of Regional association breeders, expert of CESME and of Istituto Zooprofilattico Sperimentale of Sardinia. In 28 th of August 2000, the National Veterinary Authority (DANSPV) issues an sanitary precautions where that were forbidden every transport of animals, semen , embryo, belonging to susceptible species from Sardinia and from the whole province of Cagliari toward another Sardinian provinces. Furthermore in another Italian regions every herd of sheep and goats which go into animals from Sardinia in the last 60 days had to check every heads and had to collect samples from cattle introduced from the 1st of June 2000.

During the epidemic have been undertaken several measures to detect the occurrence of BT at the earliest stage:

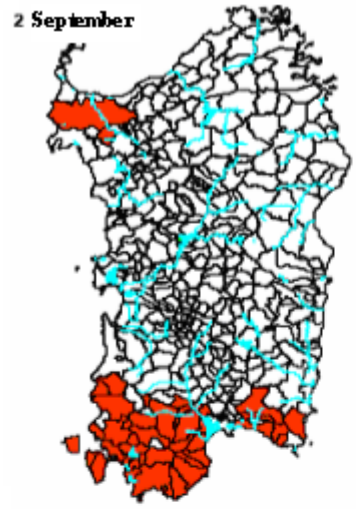
- Clinical check in all the sheep and goat herds every week;
- Exit ban to movements of susceptible animals from the restricted zone;
- Movements of animals for immediate slaughter have been allowed;
- Killing of every animal that presented BT clinical syntoms;

- Entomologic surveillance;
- Serological surveillance (between November 2000 and February 2001);
- Treatment with authorised insecticides of animals, premises and their surroundings;
- Check in the wild animals.

Since from 30th of August 2000 all outbreaks have been occurred in the province of Cagliari. In the 1st of September were reported two suspected outbreaks in the municipality district of Sassari in the north of the Island. In the 5th of September 2000 was reported the third suspect in the district of Pozzomaggiore (Sassari). The disease was spreading anywhere so in the 5th of September were banned the movements of susceptible species in the whole region. In the 2nd of September 2000 there were 263 outbreaks in 36 different municipality district. There were 62813 heads in the outbreaks, 1698 were sick and 608 were dead. In the municipality district involved there were 420000 sheep and goat. Since first days of September the disease have been spread fast (30 km at week) toward the coast and along Flumendosa, Tirso and Coghinas rivers (figure 5). Last outbreak reported in Sardinia , in the province of Nuoro, was in the 9th of February 2001. Morbidity was on average 18.1%, the death rate was 3.4%. In the figure 6 there are shown the municipality district involved. It's interesting to observe that there were 78 outbreaks in the goat where the morbidity was on average 20.7% and mortality on average was 10.2%. Epidemic peak was recorded in October where over 600 new outbreaks were reported every week (Figure 7). About the possible source of infection, epidemic enquiry conducted have not revealed any import of live animals, semen or embryo of BT susceptible species from infected countries. From November 2000 and February 2001 have been conducted an serologic

enquiry to estimate the BT antibody rate in the Sardinian sheep and goat. Blood samples collected from sheep and goat present in the outbreaks and survived the disease. Samples were collected taking into account the time the disease started in every flock. 5990 sheep and goat were tested and 22.6% were found positive (Figure 8). In April 2001, when the last case of BT during the first epidemic was reported, 80% of the island had been infected. The only area not infected was the central mountainous region. During the first epidemic, the disease was observed in 6264 flocks (25% of the total flocks in Sardinia) causing 293 901 cases (Calistri et al., 2004). After a five-month seasonal interruption, BT reappeared in August 2001 both suddenly and intensively, whit a similar trend as 2000, but in different geographical areas of the Region (Figure 9).

Fig 5. Epidemic 1: Bt spread in the first period.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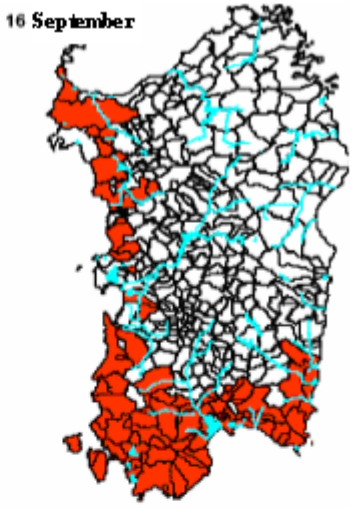
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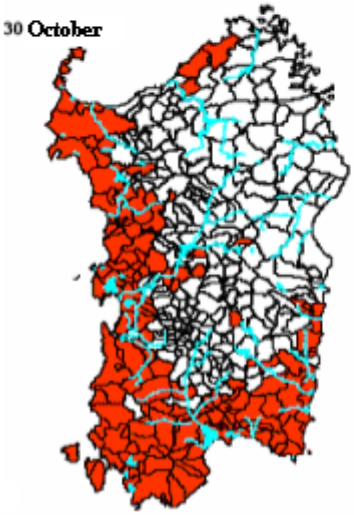
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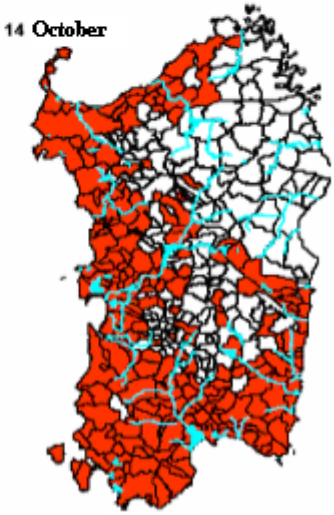
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Fig 6. Epidemic 1: BT geographical spread of outbreaks in Sardinia

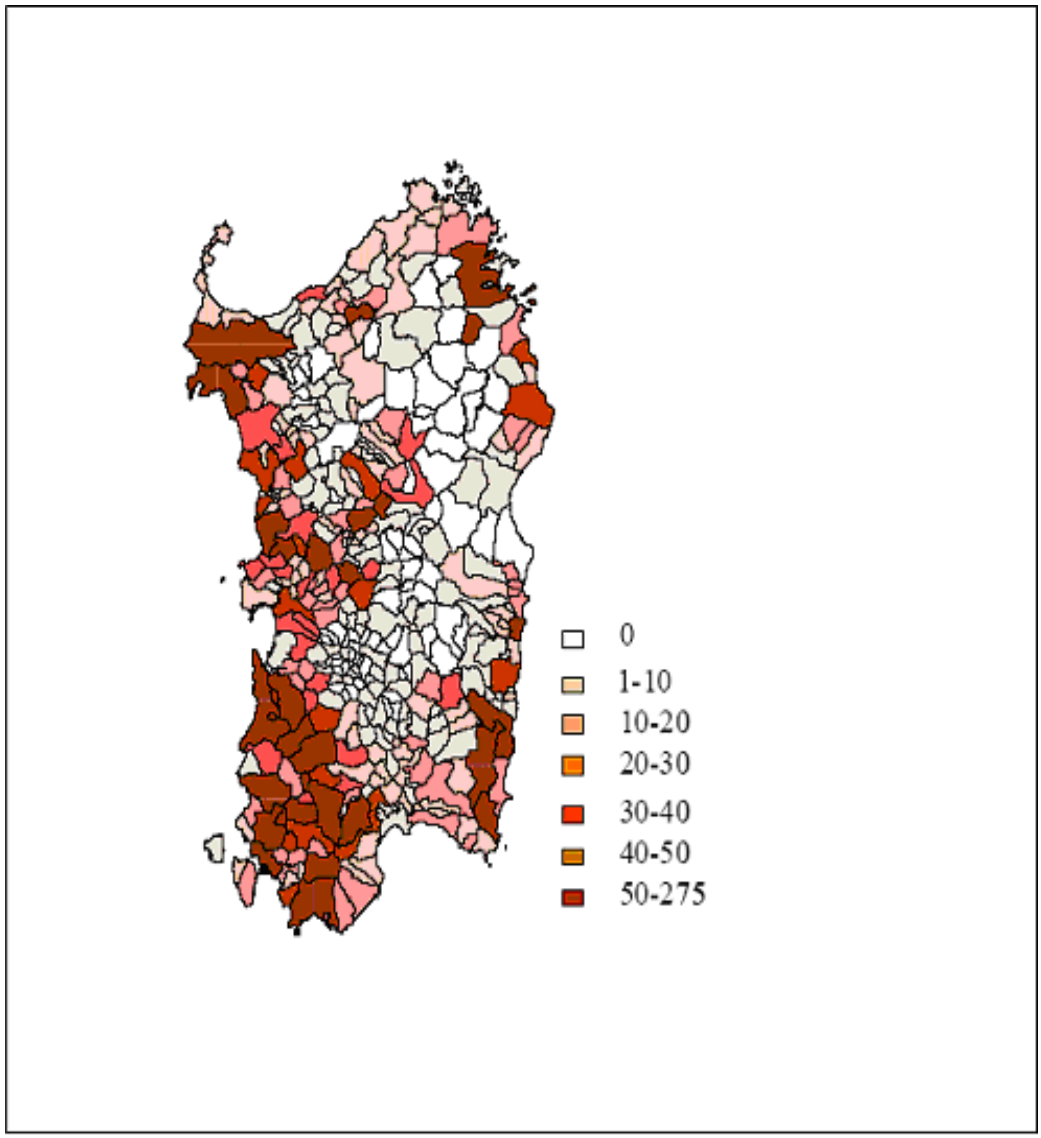


Fig 7. Epidemic 1: temporal distribution of BT outbreaks in Sardinia

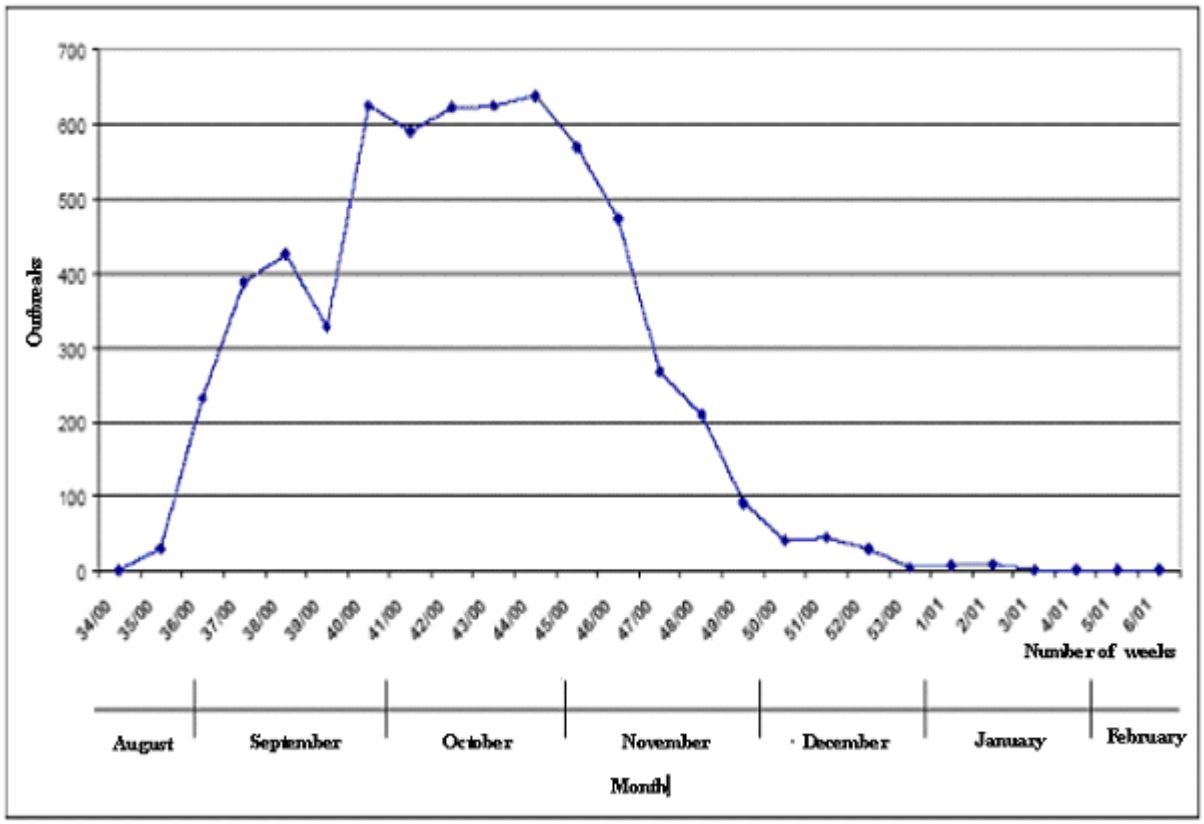


Fig 8. Geographical distribution of BT seroprevalence in Sardinian sheep.

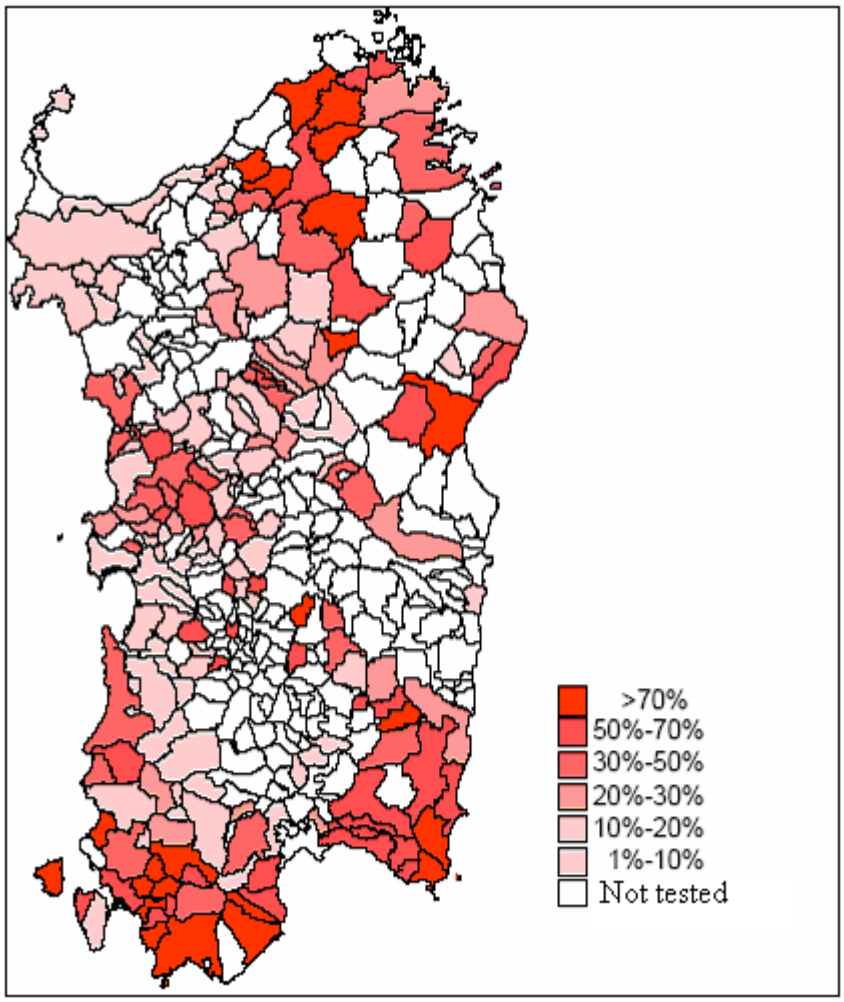
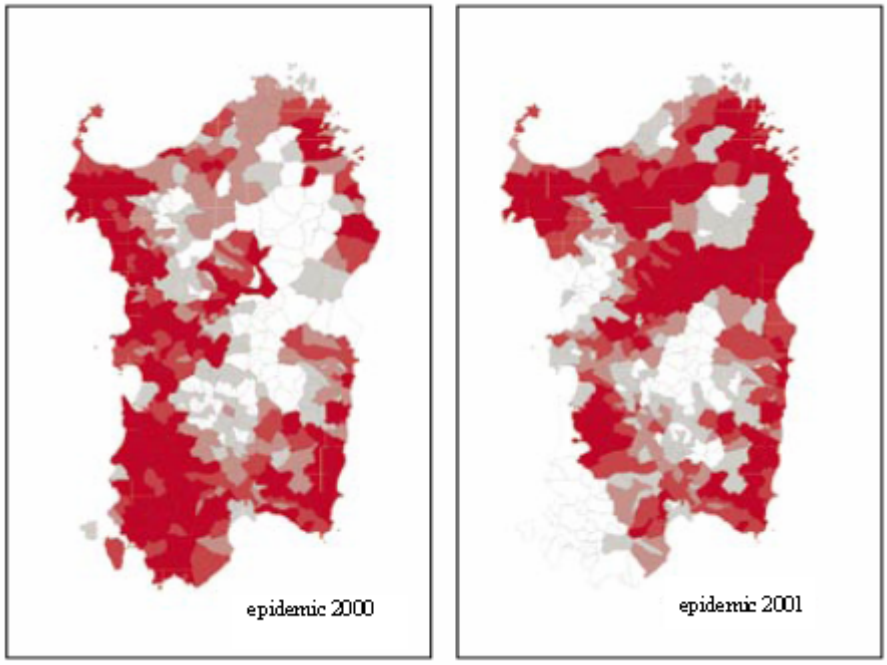


Fig 9. Territorial distribution of first and second BT epidemic in Sardinia



This second epidemic of BTV-2 recrudesced on the north-eastern coast of the island and spread eventually across the entire region, with 6.090 outbreaks and 312.130 animals dead. In October 2001, serological surveillance of sentinel animals commenced and the information integrated with that derived from clinical and serological surveillance. The data showed BTV circulation to be more extensive than revealed through clinical surveillance. Furthermore, the sentinel system demonstrated that virus circulation during winter and spring was not limited to the Cagliari Province but occurred also in the other Provinces of Sardinia (Calistri et al., 2004). Losses are similar as 2000, on average 21.73% [(dead heads + killed heads)/ total heads] while in the 2000 losses were on average 22.93 % . The lack of measures against BT will be possible the same losses or higher than these occurred in 2000 (Rolesu, 2004). For this reason ,after the epidemic that occurred in 2000 the Italian Ministry of health ordered

the vaccination of susceptible animals of all domestic ruminants species in infected and surrounding areas because the vaccination strategy stemmed from a risk assessment demonstrating the possibility to prevent both most direct economic losses and to significantly reduce also virus circulation through. The risk assessment defined two scenarios : in the first one there is a level of herd immunity up to 70% where the susceptible population are safe , while in the second one the level there is a level under 70% where the susceptible population have to be protected. In order to know what was the immunity level in the Sardinian herds were tested serum samples of Sardinian cattle, taking into account that the percentage of BT outbreaks in sheep and goats herds was lower in areas where the disease was occurred recently and cattle density for km 2 was higher. Moreover a sample survey will not be able to define the percentage of level immunity in the whole Sardinian region, so it considering the date of the outbreaks which the disease first occurred and cattle density for km2, the Sardinian municipal districts were classified in municipal districts where the cattle density are lower and where the disease occurred early, municipal districts where the cattle density are higher and where the disease occurred early, municipal districts where the cattle density are lower and where the disease occurred later municipal districts where the cattle density are higher and where the disease occurred later, and municipal districts where the disease have never occurred. Outbreaks are classified earlier or later if they have occurred before or after the 29th of September 2000 because this day was estimated to be the median of the outbreak dates. The cattle density was higher if the density for km2 was higher than 8 because this number was estimated to be the median of cattle distribution in every district . In these municipalities all the cattle were sampled and because the percentage of the level of BT immunity was lower than the

minimum preview the vaccination against BT was implemented (CESME, 2001). Thanks to vaccination (Table 1) in the epidemic 2002 there were only 10 outbreaks, although virus circulation occurred in the most part of the Sardinian region (Figure 10).

Tab 1. BT vaccination : the positive effect.

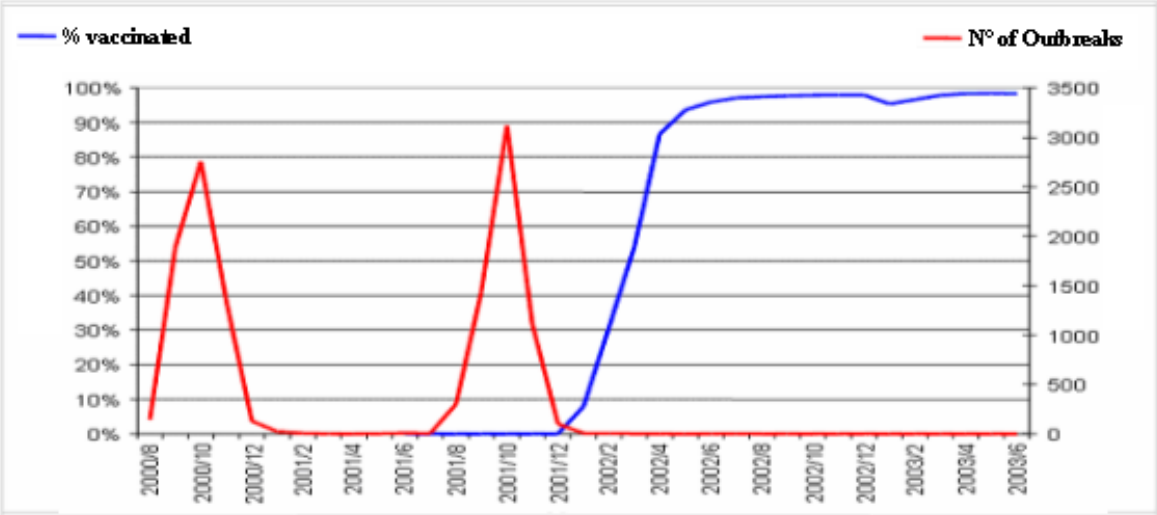
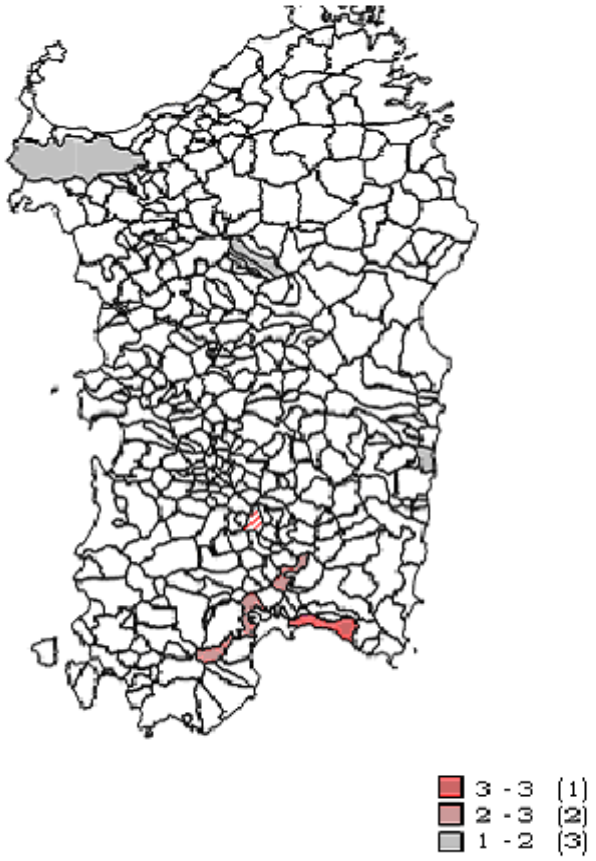


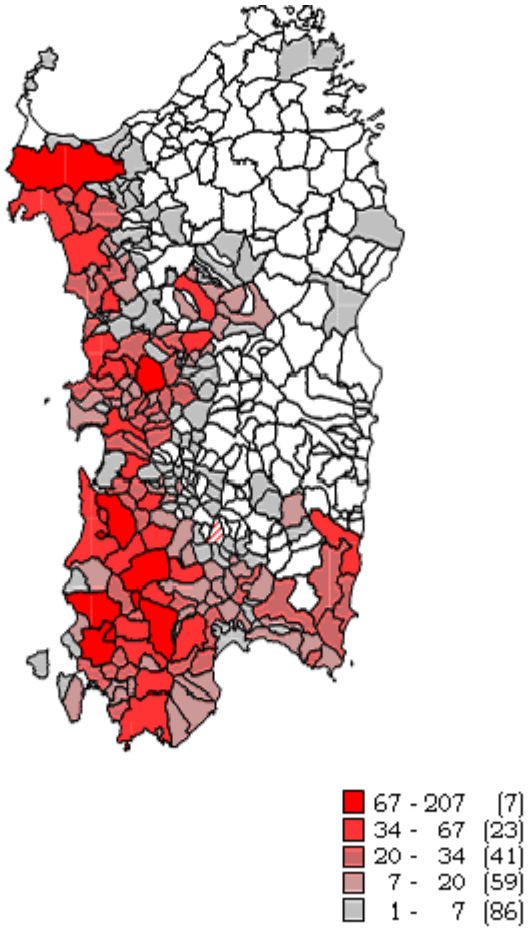
Fig 10. Number of outbreaks in the BT epidemic 2002.



In the August of 2003 the disease occurred again (fourth epidemic) as similar as first epidemic, it started from south-west of the region (Figure 11). The arrival of a new serotype (SBT-4) got back to the alarming situation of 2000. Were recorded 3716 outbreaks and 99000 dead heads more or less. Consequential damage adjusted a 2.97% (Rolesu, 2004). The fifth epidemic started from the 15 th of April 2004 and the 14 of April 2005. In this period were confirmed 129 outbreaks In despite of the worries because has been registered a far lower number of herds under the vaccination programme than before, the epidemiologic trend has been absolutely favourable. In fact in the 2004 had been occurred only 92 outbreaks, while in the 2003 in the same period the outbreaks had been over 2000. The most present serotype has been the 2, but in the South of Sardinia has been the serotype 4 and in Olbia the serotype 16 caused a weak

symptomatology. This epidemic caused a few dead animals, but it caused a consequential damage because the commercialization from territory with infection was forbidden. It was important to notice that the territory with infection was that territory included also only in part in the area within a 20km radius marked from the outbreak or from the herd where there was a seroconversion.

Fig 11. Number of BT outbreaks in every municipality district in the epidemic 2003.



The comprehensive evaluation of BT epidemic believes that BT serotypes present in Sardinia in the considered period were 2, 4, 16 , even if in different degree. In 2004 was used a attenuated live vaccine created by the combination of three different serotypes 2,4,16. After vaccination there were a lot of notice about adverse effects; in fact 2114 out of 7617 (27.75%) herds where sheep and goat were vaccinated reported a lot of adverse effect. The most indicated were lameness, respiratory difficulty, inappetence, loss of weight and decrease of milk production (Table 2).

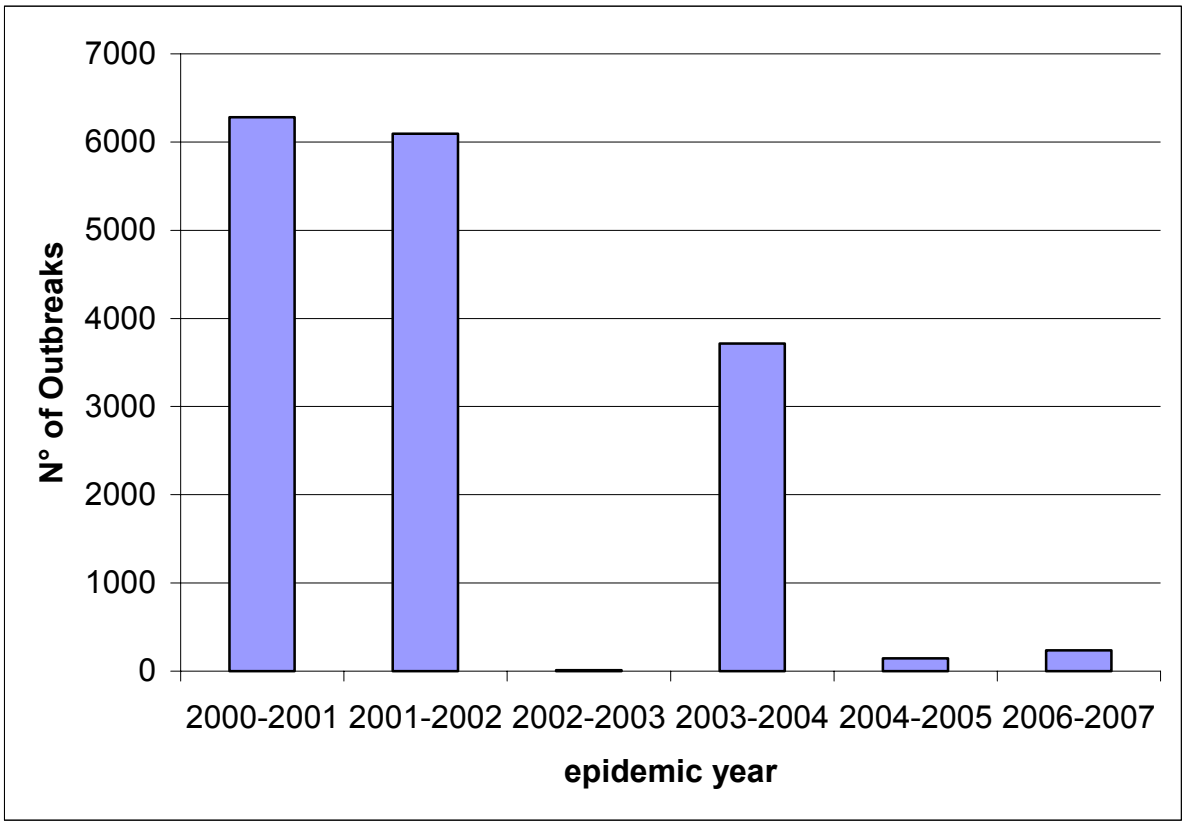
Tab 2. Adverse effects of BT vaccination 2004 (vaccine serotypes 2-4-16).

Vaccineted farms	**7368**
Farms with adverse effect	**1903**
%	**25.83**
Vaccinated heads	**996855**
Deads heads	**2802**
deads heads	**0.28%**
Lameness	**11.35%**
Respiratory patology	**5.10%**
Decrease milk production	**14.38%**
Inappetence, loss of weight	**13.12%**
Wool loss	**0.33%**
Stillbirthrate	**0.14%**
Sudden deadh	**0.35%**
Other	**5.55%**

In the year 2005 was used for the first time the inactivated against BT serotype 2 and serotypes 2 and 4. Were vaccinated very few heads (2% of sheep and goat were vaccinated with a the inactivated and 1% with live attenuated) and in not homogeneous way in the whole regional territory. In the year 2005 there was a situation of BT epidemiologic silence, but in the end of 2006 a new BT serotype came and this was a cause of alarm in the European Community. In fact in epidemic occurred between the end of 2006 and the beginning of 2007 the new serotype , in despite of unsuitable

temperature because there was the autumn, serotype1 (BTV-1) showed it to be able to cause major damage , overall in the municipality district where outbreaks occurred first, as similar as the worst epidemic in the past. Outbreaks reported were 232 and there were around 3000 dead heads. Ministry of Public Health decided that CESME will be able to produce the live attenuated vaccine against the serotype 1, because there wasn't available any inactivated vaccine (D.MIN.SAL, 2007). The epidemic observed in 2006-07 have been gone the same way of preview epidemic , because there was a new outbreak that started from a preview outbreak reported in the 20 km of radius. This happens, of course, in absence of animal movements that can be able to transfer the virus over this distance. The most representative measure was the mortality that is included in a range from 5% where the epidemic arrived later to 17.48% in the municipality district where the epidemic started. For this epidemic serological surveillance was up 50%, especially in the zones where the infection hasn't arrived yet (Sassari). Entomological surveillance confirmed that activity of BT vectors in Sardinia is continuous during the year, altough a lower activity are reported in the winter. (Rolesu, personal comunication). In the years 2007 and 2008 serological surveillance carried on and susceptible species which are destined to commercial exchange were vaccinated against serotypes 1, 2, 4 (Figure 12). In the January of 2009, in the north of Sardinia, province Olbia Tempio several seroconversion have been detected in the sentinel animals and a new serotype (BTV-8) which has never been to Sardinia arrived.

Fig 12. Bluetongue outbreaks trend from 2000 to 2007.



CONTROL MEASURES

The prophylaxis towards BT, not being a realistic struggle for winged carriers over large areas is essentially based on controlling the infection and/or the use of vaccines. No country in the world that has been reached by the disease, except Spain and Portugal, where the disease retreated spontaneously, has been able to permanently eradicate it from it's territory. The control measures are essentially based on:

- creating protection and surveillance ranges, respectively of 100 and 150 km around the infected territories;
- blocking the receptive animal's movement from the protection and surveillance ranges;
- entomological surveillance through traps for *Culicoides;*
- arranging groups of sentinel animals on the territory that will be serologically checked every 7-15 days can rapidly evidence viral circulation (CESME,2001).

Greece has implemented the measures reported above and in addition have treated, in the protection and surveillance ranges, all receptive animals with insecticide sprayed directly on the animals. Nevertheless, what regards the struggle with these insects through the use of insecticide, it is necessary to explain that products with proven and certified activity against *Culicoides* do not exist. Not because *Culicoides* resist insecticides, because, in adults and in larva, it is hardly attackable in the environment in a significant way. The use of insecticides in the environment that are allowed by law (usually natural pyrethroids or the synthetic), if on one side it can determine a temporary reduction of insects, therefore also the *Culicoides*, it is not able to prevent

infection and transmission of the virus in a significant way, moreover, for the negative effects on the environment and on animals (for example, possible residues in milk) it must be attentively estimated with the veterinary service. Also, the use of repulsive insecticides to spread directly on animals must be carefully valued and is not considered a method of prevention that lasts and that is of proven efficacy. Even biological struggles to these insects through the use of pathogen bacteria for the insects and innocuous for vertebrates, such as Bacillus Thuringiensis, have demonstrated until this day completely useless (IZS TERAMO). Breeding hygiene is also one of the first measures of prophylaxis that can be effective in reducing the density of the carrier. In fact, *C. imicola*, the main carrier of the infection in our Country, to reproduce on land, needs humid and rich organic material, even in small dimensions. Water heaps (ponds, lakes, swamps, etc.), unlike mosquitos, do not represent a very good place for the reproduction of these insects, as the larva is not able to float and it drowns. These insects prefer a humid and muddy soil situated at the border of the water heaps. The little pools of water, in fact, with manure residues and other material are an optimum place for the reproduction of these insects. So it is obvious that where, for the structural conditions of the breeding, it is possible to keep the land and the surface as dry as possible and where there is organic material, such as manure, not accessible for the insects, their density reduces more or less sensitively (IZS TERAMO). As for the vaccination prophylaxis, many studies have been done for the production of inactive vaccines, live-attenuated and recombined.

Inactive vaccines

Inactive vaccines offer the advantage of absence of repetition of the utilized virus, zeroing substantially the risks attached:

- eventual pathogenical residual of the vaccine virus;
- viremia of vaccine virus with a consequent possible diffusion of the vaccine virus through arthropod carriers;
- eventual recombination and reversion phenomenons.

Live-attenuated vaccines

When the sickness is introduced in Italy the vaccines attenuated are used in the USA and in South Africa. The only commercial vaccine that has been authorized by the UE, exclusively reserved the authority to order the purchase to be used in European Union, is a live-attenuated vaccine for the immunization of sheep, produced by the Veterinary Institute of Ondersterpoort, South Africa. As the immunity is serotype-specific, immunization of animals must take place with a vaccine that containes the same serotypes that circulate on the interested territory. The vaccine produced by the Veterinary Institute of Ondersterpoort has been used in different African Countries (South Africa, Tunisia), Australia, Bulgaria, Balearic Islands. In these regions it has been used only on ovine. The vaccine is obtained starting from the virus and through serial passages on embryonic eggs and on BHK cellular cultures. The vaccine must be stored at 4-8 °C. The dose is 1 ml to be administered under the skin. According to the instructions for use of the Veterinary Institute of Ondersterpoort:

- the sheep must be vaccinated before the seasonal peak of BT (late summer and fall). The vaccination of sheep should begin at latest 9 weeks before the reproductive season;

- it is not advisable to vaccinate animals during the first half of pregnancy as there can be problems such as abortion and foetal malformation (vaccination in the Balearic Islands, even among sheep in advanced pregnancy, did not have inconveniences of any sort);
- rams should be vaccinated after the mating season in order to avoid unwanted effects on the production of semen due to eventual fevers due to the vaccination;
- lamb born from vaccinated mothers must be vaccinated around the 6th month forward because the absence of maternal antibodies can interfere with the vaccine immunization (in Sardinia, where about 18% of the ovine population presents antibodies, probably all lamb will have to be vaccinated after the 6th month).
- sheep must be vaccinated annually;
- weak or sick animals should not be vaccinated;
- some animals can develop a slight fever. These animals should be protected from sunlight. Rams that are vaccinated for the first time can develop a temporary infertility;
- immunity develops 3-4 weeks after the administration of the vaccine. (CESME,1991)

Recombined vaccines

The recent technologies on recombined DNA have permitted during the last years to develop recombined vaccines that could, in a theoretical line, offer advantages, respect

live-attenuated vaccines, as they can permit to distinguish the vaccinated animals from the infected. Many experiments have been conducted to attempt the production of recombined vaccines towards BT. In particular the surface proteins VP2 and VP5 have been used, able to induce immunity towards homologous serotypes and the protein of *core* viral particle (VP3 and VP7) able to induce immunity towards homologous as towards heterologous (Roy at all,1994). Recombined vaccines towards BT, though not being until today available in commerce and for the use on vast populations, could represent in the near future a valid way to fight the sickness, also because they could be "marker" vaccines (European commission, 2000). Towards the use of vaccines for controlling BT, the Scientific committee of the UE during the 2000 stated that:

"There are basically two options to react on BT outbreaks:

· 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;

· 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 BT 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."(CESME, 2001). The vaccination in the sensitive population began in Italy in January 2002 and when the new epidemic arrived the vaccination coverage varied enormously on the entire territory. This stroked with a manifestation of different levels of data loss of different quantities of circulating viruses. This was performed using two different vaccines, depending on the serotypes observed in the different zone: the monovalent BTV-2 used in Sardinia, Tuscany and Lazio, and the bivalent (BTV-2 and BTV-9) was used in Southern Italy. When the new epidemic began, in July 2002, 57% of the eligible animals in Italy had already been vaccinated but vaccination coverage in the various regions varied greatly. Sardinia and Tuscany were able to vaccinate approximately 90% (97% in Sardinia and 87% in Tuscany) before the commencement of the new epidemic. In Basilicata, on the other hand only 2% of the population was vaccinated before the new epidemic started in July 2002 and only by the end of December 2002 had 84% of the eligible population been vaccinated. In the other regions (Sicily, Latium, Calabria and Campania) than twothirds of the populations were vaccinated. The different levels of vaccination had clear consequences on disease occurrence. In the two regions in which approximately 90% of the ruminant population was vaccinated, clinical disease either disappeared. In Tuscany it went from 158 outbreaks to 693 deaths during the epidemic in 2001/2002, to 0 outbreaks during the next, in Sardinia it reached a drastic reduction from 6090 outbreaks and about 293 thousand deaths, to only 10 outbreaks and 28 deaths in 2002. A clear demonstration of the effectiveness of vaccination was observed in Sardinia where, in August 2003, a new epidemic due to BTV-4 started, causing 850 outbreaks in six weeks. The vaccination of ruminant populations was

conducive to a progressive reduction of virus circulation and consequently of the zones in which movement restrictions were applied. Sardinia has been taken as an example to evaluate the effect of vaccination on animal trade for the following reasons:

- before the BT epidemic, cattle were traded extensively between Sardinia and continental Italy, especially northern Italy;
- after the appearance of the disease in Sardinia, the export of cattle from the island to disease free areas in continental Italy came to a complete standstill;
- the progressive relaxation of movement restrictions paved the way for the resumption of exports to disease-free areas in northern Italy (Giovannini et al., 2004).

In 2001, 1019 cattle were transferred to the peninsula, and the 92% of these, during the last two months of the year, when the effects of the vaccination were visible and vast areas of north Italy were free from the carrier and could accept animals from areas of surveillance without the risk of compromising their unaffected state (CE 2001, OIE 2003). At the end of 2002, a new risk analysis conducted the authorization for the transferring of vaccinated animals from infected areas but with 80% of vaccine coverage of the population susceptible of infection. The risk analysis also brought to a new approach for the definition of the areas under transferring restriction. From January to June 2002, a total of 3097 cattle were exported from Sardinia to the peninsula, against the 8 animals from the year before (E.U., 2003, Giovannini et al., 2004). the presence of many serotypes of viruses in Italy complicates the choice of the vaccine to apply in every area: monovalent, bivalent, trivalent or tetravalent. The role played by

the illegal traffic of animals greatly obstructs the choice of a national vaccination strategy based on a steady geographic reality. One of the objectives of these strategies is the reduction of the intensity and the duration of the viremia avoiding contact between vaccinated animals and savage strains of the virus (Monaco et al., 2004; Savini et al., 2004). Having a susceptible population at hand, BT infection extends very fast. During the 2000, at the beginning of the sickness the virus extended in Sardinia at a velocity of about 30 km per week (illustration) (Calistri et al., 2004). Also in August 2003, when BTV-4 invaded Sardinia, it's velocity of extension was similar to three years before. The BT infection clinical period of incubation is between 5 and 20 days. Afterwards the infected animals are rapidly recognized clinically through a clinic examination and through serology. However, during the summer and the beginning of autumn of 2000, during the epidemic peak, BT surveillance in Italy only clinically examined the animal. The serological and virological diagnosis were used only as a confirmation method or in occasional sampling of animals in new infected areas (Calistri et al., 2004). Afterwards the importance of clinical surveillance decreased compared to the importance of a serological surveillance. This is based on the surveillance of guard animals in a net (Giovannini et al., 2004). In this matter a serological surveillance plan was instituted regarding all over Italy.

The plan has the following target:

1. obtain/exclude the viral circulation in the various Italian areas;
2. Obtain antibody coverage of the vaccinated population;
3. Together with the entomological surveillance, throughout the national territory:
   a. monitor of the sanitary conditions in the areas free of infection;
   b. locating seasonal areas free of infection.

4. Feeding the National Serum Bank constituted at the CESME.

The plan establishes the general measures for the realization of a specific surveillance system for BT on the entire national territory and is based on the regulation:

- serological checks of a network of guard cattle (Area B and C);
- guard cattle serological check or check on blood samples gathered for other purposes or gathered at the slaughterhouse (Area A);
- checks on the antibody coverage of the vaccinated population.

The surveillance system generates data and necessary information to the government and acts management and to the verification of the plan's goal. The Department of Public Veterinary Health, Nutrition and Food Safety (General Direction of Animal Health and Veterinary Pharmaceutical), in agreement with the National Reference Centre for Exotic Sicknesses (CESME) and with the Veterinary Operating Centre for Epidemiology, Programming and Information (COVEPI), on the basis of data, entomological and epidemiological information can dispose further investigations that were not foreseen in the plan.

The national territory was divided in three geographical areas with different risk levels:

Area A: area with a minor risk for the diffusion of the infection

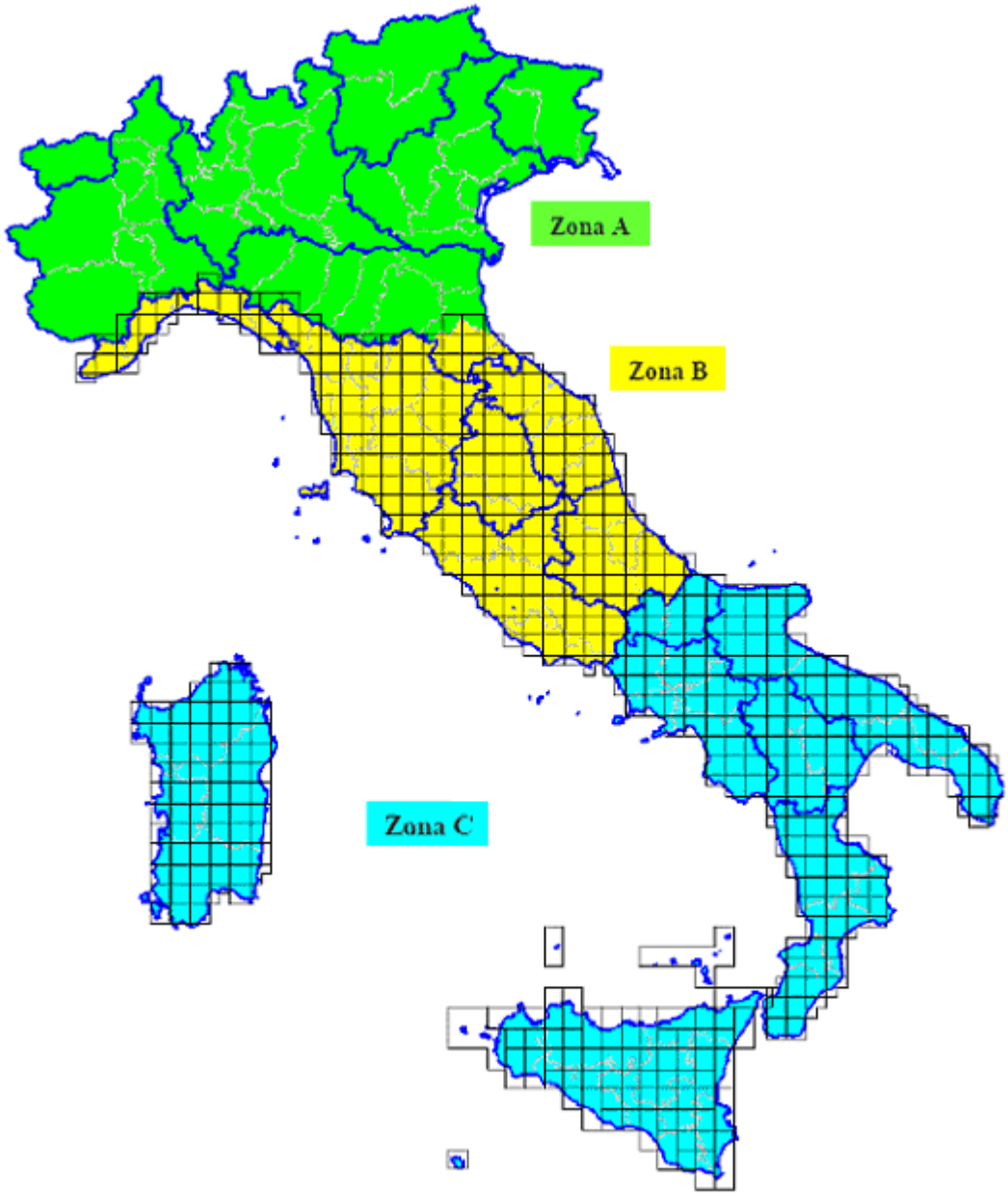
Area B: area with a major risk for the diffusion of the infection

Area C: endemic area

Area A has a geographical unit minimum regarding the provincial territory, while area B and C are divided in cells of 20 km sideways (Figure 13). In these zones the cells represent geographical unity of reference for the surveillance activities allowing capillary interventions for geographic and environmental specificity purposes that can influence the infection process and also in function of the epidemiological evolution

situation. Contextually at the vaccination activities scheduled by the effective legislature at the end of every campaign, vaccinations for the serological exams will begin, finalized to verify the vaccine coverage level in the territories obligate to vaccination.

Fig 13. Division of Italian territory in Area A, Area B and C (cells of 20 km sideways).



CESME, 2007

Area A – area with minor risk of infection diffusion (Unaffected area)

It is the area currently unaffected by the infection , in which the surveillance system has the principal aim to prematurely reveal any virus entrance, in order to interrelate all possible measures to limit the diffusion. Geographic units of reference to which the measures are applied (surveillance activity or eventual restrictions, etc.): the provincial territory. The small dimension provinces can, if requested by competent authorities, establish a single geographic unity of reference. In that case the measures will be applied on the province's territory that form the unity.

Area A includes all the provinces of these Regions:

- Piedmont;
- Aosta Valley;
- Lombardy;
- Friuli Venezia Giulia;
- Trentino Alto Adige (autonomous province of Trento and autonomous province of Bolzano);
- Veneto;

  plus some provinces of the Emila Romagna region (Bologna, Ferrara, Modena, Parma, Piacenza, Ravenna and Reggio Emilia).

Area B – Area with a major risk of infection diffusion

It is a "tampon" area and it represents the most important strategical zone for the preservation of the northern areas presently unaffected by the infection. It is fundamental that in this area the control and surveillance levels are as high as possible in order to foresee in a brief time any virus transmission on the territory. Geographic

units of reference: cells of 400Km2. In this area the territorial surface is divided in cells of 20 km sideways and includes the Italian regions that confine between unaffected areas and endemic areas.

Area B includes all the provinces of these regions:

- Liguria;
- Tuscany;
- Marche;
- Umbria;
- Lazio;
- Abruzzo;

  and the provinces of Rimini and Forlì-Cesena in Emilia Romagna;

Area C – Endemic area

It is the area of infection where the virus circulation has been demonstrated in many territorial zones and where, therefore further a further virus circulation is expected, although geographically and/or temporarily limited. In this area the serological surveillance has the following aims:

- to define from time to time the areas with a viral circulation in act, in order to provide useful indications compared to the possibilities to move the animals that come from such zones;
- monitor the geographic diffusion of the different viral serotypes, providing useful information in order to apply different vaccination schemes;

- evaluate the effectiveness of vaccination where performed. Geographic units of reference: cells of 400Km2.

Area C includes all the provinces of these regions:

- Molise;
- Campania;
- Puglia;
- Basilicata;
- Calabria;
- Sicily;
- Sardinia.

The Department of Health, in agreement with the CESME, the COVEPI and the Regional Veterinary Services, Based on the evaluation of the risk of introduction of the infection or to exclude the circulation of the virus, may decide to change the geographical demarcation of the areas or activities under surveillance in the three areas (CESME 2007).

Sentinel network

Networks of sentinel animals have been implemented in several countries to monitor the presence and spread of vector-borne diseases. A sentinel system has been used in Australia since late 1975, but the number of sentinel sites was very limited (Kirkland et al., 1991). In Canada, a sentinel programme has been in place in the Okanagan Valley since 1988 (European Council., 1992). In the United States of America (USA), periodical surveys are performed, usually serological surveys of slaughter cattle for antibody against BTV (Pearson et al., 1991). The serological surveillance networks in place in other countries were not consistent with the requirements for serological surveillance of BTV infection in Italy. The Italian system was based on the following:

- the OIE *Terrestrial animal health code*, which states in Chapter 2.1.9. that random and targeted serological surveillance should provide at least a 95% level of confidence of detecting an annual seroconversion incidence of 2% in cattle (or other ruminant species if sufficient cattle are not avaible) (Giovannini et al., 2004).
- a serological survey undertaken in the state of Queensland (Australia) in 1989 showed that the prevalence of serological positivity in cattle from locations with low prevalence of infection was on average 6.45% (Ward et al., 1994).

The sentinel animals are chosen in cattle species field. In case there is no cattle or the number is not enough to complete the sample established to represent the territory in the single cells for each geographic unit of reference (cell) or if their distribution does not allow to have a sample that represents the territory, it is possible to choose or integrate samples of animals of other receptive species, through prior agreement with the National Centre of Reference.

The sentinel animals:

- can be identified individually through electronic identification systems, through authorization from the Department of Health;
- must not have been vaccinated;
- are periodically checked to notice the presence of antibodies towards the BT virus.

On the territories subjected to vaccination or where the vaccination is expected, in order to limit the loss of sentinel animals connected to commerce, the sentinel must be choosen, if possible, inside cattle breeding, choosing those less subjected to commercial flow and possibly those that practice internal remount. In this last case, in order to compensate the loss of sentinel due to death and commerce of animals, the calf used for internal remount will replace the dead or sold sentinel. On the territories that are not subjected to vaccination or where vaccination is not expected and the area B territories to substitute the loss of sentinel connected with death and commerce of animals, it will be enough to substitute the lost sentinel with other cattle of the same breeding, through checks of their serological negativity. In case the sentinel animals are positive, the activities are diversified depending if the remark takes place at the first at the first blood sample or at the following blood sample. The activities expected if positive are aimed

to confirm/exclude viral circulation on the territory and establish the eventual extension in order to determine the sanitary measures to adopt on the territory area. When one or more sentinel animals result positive to the ELISA test at the first blood sample, on the territories included in area A, in which previously the presence of outbreaks viral circulation haven't been found and in the territories of area B.

The Istituto Zooprofilattico Sperimentale competent for territory must:

- communicate positivity to the veterinary Local Health Centre service;
- send the serum or the positive results of the serum to the CESME for the confirmation of the verified positivity.

The veterinary service of the Local Health Centre competent for the territory must:

- take a blood sample with EDTA from each animal that results serologically positive and a sample of serum of all the animals of the breeding referred above;
- Fill out the form that comes with the samples;
- Send the samples together with their card to the CESME as soon as possible.

The CESME provides the confirmation of the serum positivity giving communication as soon as possible to the veterinary Local Health Centre service competent for the territory, to the Veterinary service that belongs to that region of the Local Health Centre, to the IV office of General public veterinary health direction, alimentary and nutrition of the Health Government, to IZS competent for the territory.

The veterinary service of the Local Health Centre competent for the territory, in case the CESME confirms the positivity, in order to verify/exclude the viral circulation on the territory must:

- perform a census of all the animal farms that are sensitive to BT in the range of 4 km from the farm in which the serological positivity was confirmed and detect the geographic coordinates related to the location of each breeding;
- Placing a trap for *Culicoides* in each farm where the positivity was found;
- Effecting the first capture within 24 hours from when the positivity took place and effect one capture per week until the next sample of the guard animal takes place;
- Send the captures with the duly filled out card to the CESME within 24 hours of the capture;
- Start with a wide epidemiologic research in collaboration with the IZS competent for the territory, in order to establish the provenance of the animals present in the farm or in the farms where the positivity was confirmed, within 48 hours of the confirmation and immediately sending the copy of the research to the CESME;
- Effecting clinical examinations periodically for at least 15 days in all ovine and goat breeding in the range of at least 4 km of the farm or the farms where the positivity was confirmed. In every ovine and goat breeding, the examinations must be at least 2 at not less than 7 days from each other. In case the clinical examinations do not evidence the presence of clinical symptoms, a blood withdrawal must be done on every ovine and goat sample from every farm.
- Effecting within 30 days a blood withdrawal from a sample of animals of all the bovine breeding within the range of at least 4 km from the farm where positivity was found.

The veterinary service competent for the territory and the CESME, each for their own responsibilities, give immediate communication to the Department of Health of the established presence of the viral circulation. The breedings in which the positivity was found must be considered breedings with infection in action and the territory in which they reside in, territories with infection in action. Consequently, they must take the sanitary measures according to the national and community laws in case of breeding grounds. When one or more guard animals result positive to the ELISA test to one of the blood samples after the first, the IZS and the veterinary service of the Local Health Centre competent for the territory must, each for their own responsibilities, other than carrying out the activities mentioned above, give an immediate communication to the CESME. In case positivity is confirmed by the CESME, the breedings in which positivity is confirmed must be considered breedings with infection in action and the territory in which they reside in, territory with infection in action. Consequently, they must take the sanitary measures according to the national and community laws in case of breeding grounds (CESME,2007). The value of about 5% prevalence in areas of low prevalence of BTV infection was confirmed by the ad hoc monitoring conducted in Sardinia to evaluate the involvement of the cattle population in the BT epidemic. Therefore, it was decided to divide Italy into two main zones, based on the risk of infection . The lower risk zone was subdivided into a grid of square cells of 400 km per side (1600 km2 per cell) and 148 sentinel animals were monitored in each cell (in compliance with the OIE requirements for free countries or zones to provide a confidence level of at least 95% to detect annual seroconversion incidence of 2% in cattle). In the higher risk area, a finer grid was designed, with squares of 20 km per side (400 km2), to have a more precise definition of the distribution of infection. A total of

58 sentinel animals were included in each cell to confirm that the prevalence of infection was less than that observed in low prevalence areas (i.e. to provide at least a 95% level of confidence of detecting an annual seroconversion incidence of 5% in cattle). Since the minimal movement restricted zone is a circle of 20 km radius around the infected holding, the geographic density of sentinels is able, for any area of 1 256.6 km2 (equivalent to a circle with a radius of 20 km), to provide at least a 95% level of confidence of detecting a seroconversion incidence of 1.6% in cattle (Giovannini et al., 2004). In consideration of the particularity of the epidemiological situation, in the region of Sardinia the following modifications were brought to the plan mentioned above:

- serological surveillance on cattle guard animals will be effected only in cells that do not include territories in which cases of sickness have been observed for less than 60 days. (figure 14);
- the frequency of samples on cattle guard animals is set at 15 days with exclusion of the first sample within October 10, 2001.

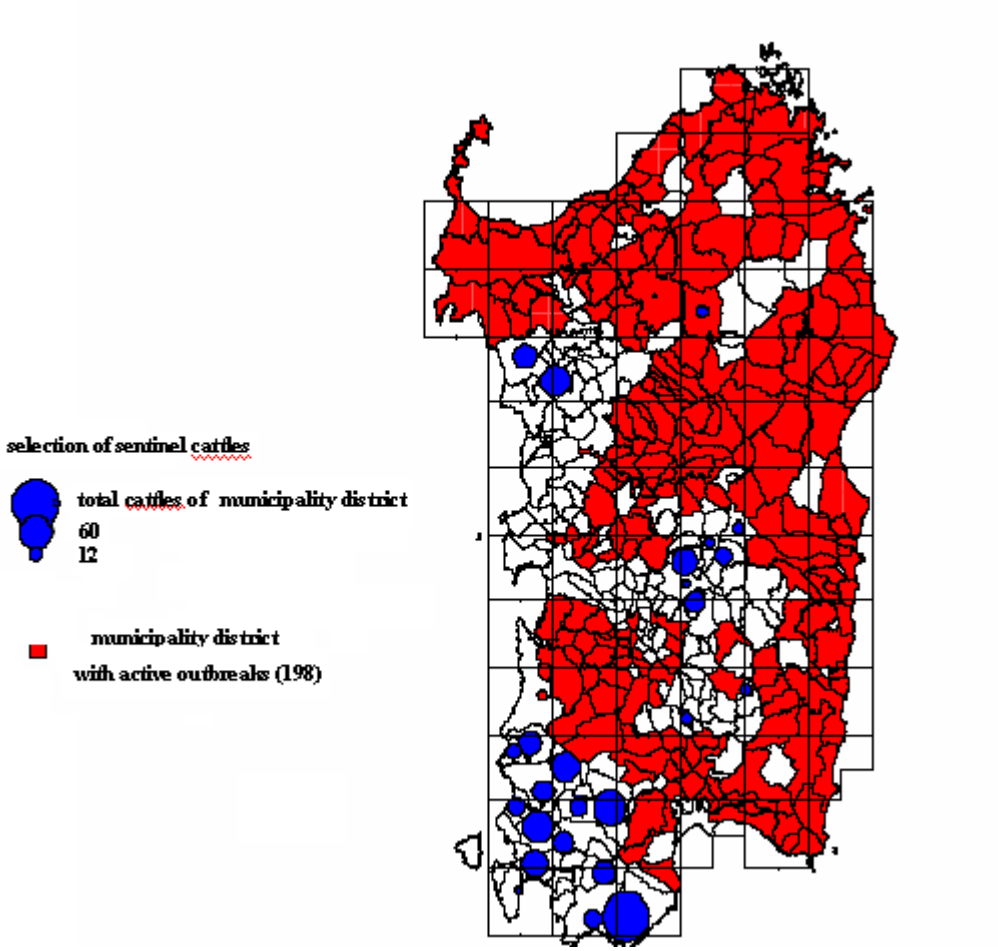
For the choice of negative cattle guard animals, serological surveillance data conducted during the first months of 2001 and registered at the the Zoological Experimental Institute of Sardinia will also be used. In case, through the selection of animals of the cattle species, it is not possible to guarantee the presence of at least 58 guard animals per cell, the cattle breedings can be substituted, at the same conditions, by ovine breedings.

In case of seroconversion of a guard animal, the action to perform will be following:

- clinical surveillance in ovine and goat breedings in the range of 5 km from the farm in which the positive animal is located;

- positioning a trap in the farm where the positive animal is located with at least 2 captures of insects with the aim to verify the density of the carrier (Sulis et al., 2000).

Fig 14. Cattle guard animals, selection 2001.



Serological surveillance based on monthly check ups on guard animals provides at present the controlling of 48 guards of 20x20 km per side, for an expectation of total

check ups of 2900 heads distributed in the 8 provinces (figure 15). The criticality noticed, in the recent years in particular, whether for the availability on behalf of the breeders or for the ojective difficulties to trace the guard animals in some grills, in an adequate manner according to the contents of the surveillance plan, orders a verification system that considers the process in means of breeding grounds and the seroconversion of the territory.

Fig 15. Order of guard animals on regional territory.

Hypothesis of new order of the serological surveillance system.

Sardinia's territory is divided in 377 municipalities. Inside these territories, during 2000-2008, BT concerned about 10000 ovine goat farms, with 345 municipalities involved by breeding grounds (table 3, figure 16). This prevalence is distributed diffrently depending on the average altimetry of the municipality territory. Subdividing the municipalities in function of the average altimetry, we obtain four areas (area 1 from 0 to 280 meters amsl; area 2 from 280 to 430; area 3 from 480 to 610; area 4 > 610). the average prevalence observed is subdivided on average following a altimetric gradient (figure 17). By combining the two factors, altimetry and prevalence, brings to the individualization of seven "risk areas". A redistribution of the guard animals in terms of risk is proposed, through a mixed provincial model that keeps regard of the typology of the territory. In particular of the altimetry and prevalence of the area observed during 2000-2008. The number og guard animals to be controlled will be 100 per risk area, for a total of 1050 animals (Rolesu 2009).

Tab 3. Prevalence and number of outbreaks in the different provinces.

PROVINCIE	Medium Prevalence	N. of Municip. With outbreaks	Standard deviation
CAGLIARI	,36	62	,20
CARBONIA-IGLESIAS	,55	23	,14
MEDIO CAMPIDANO	,27	25	,15
NUORO	,34	40	,24
OGLIASTRA	,29	22	,23
OLBIA-TEMPIO	,39	25	,23
ORISTANO	,53	83	,26
SASSARI	,39	65	,23
Total	**,41**	**345**	**,24**

Fig 16. Outbreaks prevalence distribution in municipality territories. 2000-2008 period.

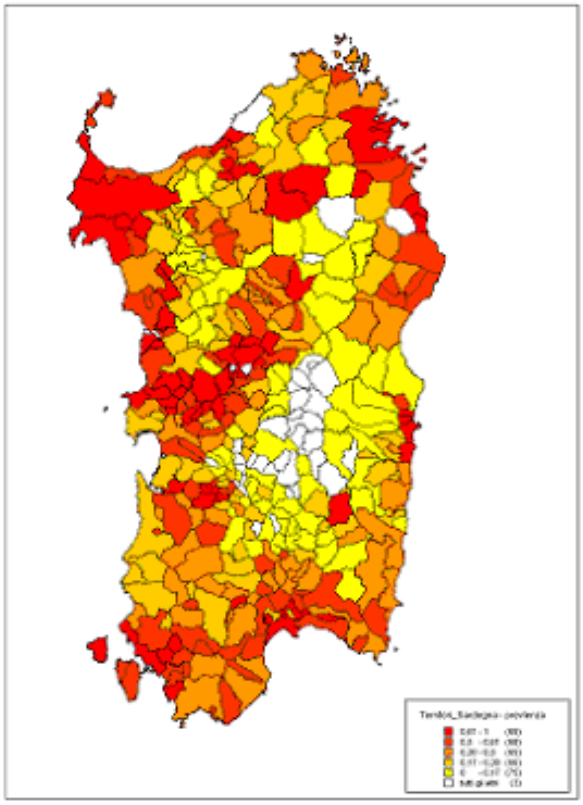
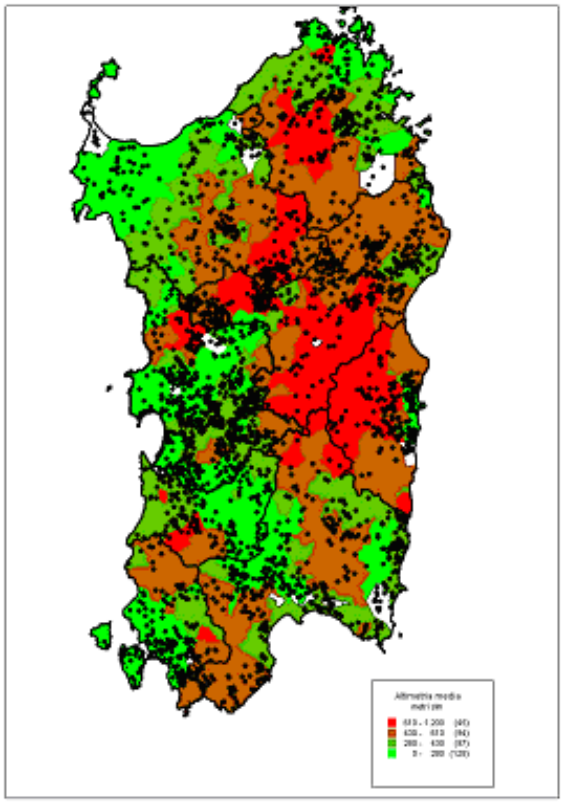


Fig 17. Altimetric areas and present distribution of guard animals.



Entomology surveillance

The methods commonly used to control *Culicoides* worldwide were reviewed as part of the previous EFSA report on BT. These methods can be broadly divided into those that involve a chemical application and those that utilise physical methods. Chemical methods include:

• treating livestock with insecticides, repellents or systemic antiparasitic drug (e.g. avermectins)

• treating larval breeding sites or adult resting areas with insecticides

• treating animal housing and/or transport with insecticides Physical methods include:

removal or reduction of larval breeding sites on farm holdings

housing livestock in screened buildings at times of high levels of *Culicoides* activity.

There is a need to differentiate between repellent effects and irritation. Repellent effects will prevent an insect to land (and feed) on a treated host. It has no killing effect and the insect will be able to land and feed on an untreated host. Irritation does not prevent the

insect from landing and, sometimes, feeding. However, due to irritation ("hot feet effect") the insect may eventually be so irritated that it will take off prior to feeding. But it has picked up insecticide and may consequently become paralyzed before it will die. Most of the pyrethroids do not possess repellent effects (EFSA, 2007a). There are currently no veterinary medicinal products or biocidal products authorised in the EU specifically for use in any control role against *Culicoides*. Hence, interest has centred upon the large number of products that are available, licensed and in widespread use against other Dipteran pest species. It is important to note that the use of certain type of product formulation has been restricted recently, particularly in northern Europe, due to both environmental and operator safety concerns. A wide range of compounds are used to control both ecto- and endo-parasites on ruminants and some of these have the potential to impact upon *Culicoides* that attempt to feed upon them. These can be divided into products for topical use, such as pour-on formulations (usually applied along the back-line of the animal), ear tags attached to animals, dipping formulations (where the animal is immersed in the product) or injectable systemic formulations. Pour-on, ear tags and dipping products are usually based around synthetic pyrethroids (e.g. cypermethrin, deltamethrin, permethrin, flumethrin, cyfluthrin, cyhalothrin) or organophosphates, although other active ingredients are also sometimes used (e.g. macrocyclic lactones). These compounds exert their effect in two ways: primarily they are highly toxic to insects landing on the treated animal, often killing within minutes of their landing; secondarily they exert a contact irritation that may reduce the probability of the arthropod successfully initiating or completing a blood meal from the host. While toxicity of different active ingredients is usually relatively easy to define, this latter effect is not usually so well defined. However, it is usually far less effective than that

exerted by dedicated repellent compounds (e.g. DEET). Injectable systemic compounds are most commonly based around macrocyclic lactones (e.g. avermectins) and toxicity occurs following ingestion of a blood meal containing the active ingredient. As outlined by a previous EFSA opinion on BT (EFSA, 2007b), in the absence of a clear identification of breeding sites of species (as for example from the *Obsoletus complex*) in Europe, larval control remains not feasible. Although a recent publication gave some information about the breeding sites of *Culicoides* on European farms (Zimmer et al., 2008), these observations require both extension and repetition before they can be generalised across the region of interest. Broad-scale Ultra Low Volume (ULV) insecticidal treatment of potential larval development sites with synthetic pyrethroids or organophosphates is not acceptable under current legislation in the UK, particularly given the poor level of knowledge regarding adult resting and larval development habitats of most of the potential vector species. *C. obsoletus* presents a particularly difficult problem in that it inhabits a wide range of habitats as larvae at low densities, making wide-scale removal unrealistic. The only potential role for insecticides in the UK is in extremely contained habitats (*e.g.* dung or compost heaps) where run-off to water sources are less likely to occur. Trials of the effect of both, chemical and husbandry changes, have not been carried out to date. Further information has been obtained regarding the larval habitats of *C. imicola* in Israel. This species breeds almost entirely within the perimeter of the animal compounds (Braverman et al., 2001). Besides weekly manure removal, oral treatment of cattle with tetrachlorvinphos reduced the numbers of adult *C. imicola* (as monitored by suction light trap) by killing the larvae in the animals' faeces (Braverman, unpublished data). However, since no maximum residual limits (MRL) is established, the use of tetrachlorvinphos, as a

veterinary medicinal product, is not currently permitted in food producing animals in the European Union. Insecticides recommended for use in vehicles in the UK during animal movement all contain synthetic pyrethroids as active ingredient. None have been tested against the northern European *Culicoides*, but they outperform organophosphates in wind tunnel trials carried out in other regions with other vector species (Floore, 1985; Kline et al., 1981). Spraying is limited to animal housing/abattoirs (or similar) and the vehicle itself. In Spain, trials using insecticide impregnated nets on stable openings conferred a relatively protection to animals by reducing the abundance of *C. imicola* into stables when compared with a control stable (Calvete et al., 2007). Insecticide-treated nets have been found to reduce *Culicoides* numbers by 70 – 80% within suction traps placed inside pens in which animals were stabled in Kumasi, Ghana (Maia et al., 2005). No insecticidal products are currently authorised specifically against *Culicoides* in the EU although a wide range of untested products are available. Historical testing of toxicity worldwide has demonstrated that pyrethroids-based products are more effective in the laboratory against *Culicoides* than organophosphate-based products. Hence, the use of the former is preferred except where other issues (environmental impact and legislation) preclude it. Novel data concerning the efficacy of pyrethroid-based pour-ons and ear-tag products have been obtained but are equivocal. While all trials showed some mortality in *Culicoides* feeding on treated animals, the extent of this mortality was poorly measured and was not related to reduction in BTV transmission. Thus the development of efficacious pyrethroid-based insecticides for reducing *Culicoides* attack rates (and thus the sequential transmission of BTV) has yet to be realised. Nevertheless, a number of recent studies, despite shortcomings in experimental design and the lack of controls,

have shown promise and leads us to conclude that they may prove useful following additional future development. However, a common result after treatment with pour on formulations or ear tags was the decreasing insecticidal efficacy from the back line to the belly and legs of treated animals. This is related to the limited spread of the insecticide and is common to topically applied products of non-systemic activity. Dipping products have not been assessed to date in this role in Europe. Systemic products have also not been assessed, but based on past experience, are unlikely to be used to lower *Culicoides* population levels thereby preventing BTV transmission on a wide scale. No new data have been provided regarding the treatment of housing or transport for animals. Treatment of breeding sites remains difficult as habitats are poorly defined for most species. Further research is required to investigate products with the potential for use against *Culicoides*. Preference should be given to products that already fulfil legislative criteria for use in EU member states. For testing of insecticidal/repellent activity against *Culicoides*, it is recommended to use the standardised *in vitro* method of the WHO for lethality and repellency testing where this is compatible with field populations. Field studies should always include a negative, untreated control group and, if available, a positive control. Abbot's (Abbot, 1925) correction for mortality should be implemented and the efficacy trials should be performed in accordance with relevant VICH (International Cooperation on Harmonization of Technical Requirements for Registration of Veterinary Medicinal Products) and European CVMP (Committee for Medicinal Products for Veterinary Use) guidelines for veterinary medicinal products.Although clear, confirmatory data are not at present available, pyrethroid-based pour-ons or ear tags may limit the population of *Culicoides*, thereby reduce the risk of sequential BTV transmission. Pyrethroid-

based insecticides should not be used as a stand-alone measure to protect animals against *Culicoides* attacks. The use of sprays or dipping may be more suitable for immediate effects and short term treatment. Also, these methods should be preferred in order to ensure equal distribution over the entire body surface. The use of these products, however, should be considered with regard to adverse environmental and health effects. All use of chemical treatments should be carefully assessed with regard to environmental impact, user risk and the potential for development of resistance in *Culicoides* populations (EFSA,2008). In Mediterranean countries, *C. imicola* breeds in close proximity to animals, particularly in intensive cattle farms. Removal of the manure, which is an important component of the breeding site, should therefore reduce drastically the population of *C. imicola*. Manure removal can be done by converting the manure to compost, biogas and/or by ploughing the manure in the animal yard, as it is already practiced in Israel in some dairy farms. Wherever there are manure heaps that can't be entirely removed, the top 6 cm should be removed every 10 days (*Culicoides* being likely to breed on the surface of heaps). Keep animal constructions and premises as dry as possible by preventing leakage of water installation and overflow of water troughs. In northern Europe the situation is more complicated as potential vector species breed in a wide variety of habitats and due to higher levels of precipitation it is more difficult to identify the wetter areas that support *Culicoides* larvae. In addition at least one species, *C. obsoletus*, is known to breed in a large diversity of habitats. Therefore, measures to reduce population density will be difficult to define. Given the knowledge regarding *C. scoticus* is even poorer than that for *C. obsoletus* it will be similarly difficult to target this species. In the case of *C.dewulfi* and *C. chiopterus*, the literature, to date, indicates that these species breed in dung, as indicated in the previous

EFSA report on BT (EFSA, 2007a), and hence population numbers might be reduced by dung removal, however, to date no quantitative evidence of this has been provided. Inferences could be drawn from the fact that manure removal from animal yards reduces dramatically the numbers of houseflies and it is reasonable to assume that it will reduce also the dung breeding *Culicoides* and species that develop in dung contaminated development sites. Other potential vector species including the subgenus *C. pulicaris* have a wide range of habitats that are not presently well enough defined to allow treatment or removal of breeding areas that are difficult to target e.g marsh land. In order to minimise *Culicoides* breeding where possible, animal dung should be removed from sheepfolds, cowshed and their premises and suitably managed. Research should be done to evaluate the effectiveness of different manure management options to minimise *Culicoides* breeding under different husbandry conditions existing in the EU (EFSA,2008). Given the lack of knowledge on the distribution of vectors in Italy, an entomological surveillance programme was implemented in infected and adjoining areas at the beginning of the BT epidemic in October 2001 to map the distribution of vectors (with particular reference to *C. imicola*). Blacklight traps were moved around the study areas to define the distribution of *C. imicola,* and permanent traps were operated at different sites in Italy from June to October 2001 to evaluate the effect of soil type on the presence of *C. imicola*. Entomological surveillance has been extended nationwide since October 2001. Blacklight traps were positioned in fixed locations in each province and operated weekly to monitor the population dynamics of *Culicoides* spp. Blacklight traps were also operated on a temporary basis in suspected or confirmed cases of virus circulation and whenever a more specific understanding of vector distribution was required (Giovannini et al, 2004).

From August 2000, a series of control measures and of surveillance of the disease have been adopted:

- clinical weekly check ups of all animals in ovine goat breedings;
- blocking the movement of receptive animals inside the protection areas, except the animals destined to be butchered (in following phases limited and controlled movement were allowed);
- slaughter of sick animals;
- anti-*culicoides* environment treatment with insecticide (piretroidi);
- blocking the movement of live animals, sperm, ovules, embryos of sensitive species in the province of Sardinia;
- clinical check ups on animals in all ovine goat breedings that have brought live animals from Sardinia, from June 1, 2000, with serological controls. In case of positivity, capture of *Culicoides* and serological test of all receptive animals on the farm;
- entomologic surveillance plan to define the presence and the density of culicoidies population in Sardinia.

An entomologic surveillance plan was made starting from August 2000 with the following objectives:

- creating a diffusion and abundance map of *Culicoides spp*. and *C. imicola*, in particular;
- identifying the areas in which *C. imicola* will possibly result absent;
- evaluate the activity of *C. imicola* during the winter and it's capacity to survive the winter season.

Sardinia's territory was divided in 281 cells. In every delimited area a trap for *Culicoides* was placed, at least for one night. The traps used were made by the Ondersterpoort Veterinary Institute, Republic of South Africa. Figure 18 shows the distribution of the number of specimens of *C. imicola* captured in Sardinia (CESME,2001). For the activity of entomoligic checks, besides the localization of the traps, it is necessary to evaluate the dinamic of the captures, in terms of absolute number of insects, *Culicoides spp*., *C. imicola*, but also in terms of growth factor (figure 19, 20, and 21). Entomologic surveillance towards BT in Sardinia, more than a duty carried out by the law, represents a fundamental detail of the whole aimed to define the effective epidemic situation of the disease.

Fig 18. Geographic distribution of the number of specimen of *C.Imicola* captured in Sardinia (Ln of maximum specimen captured).

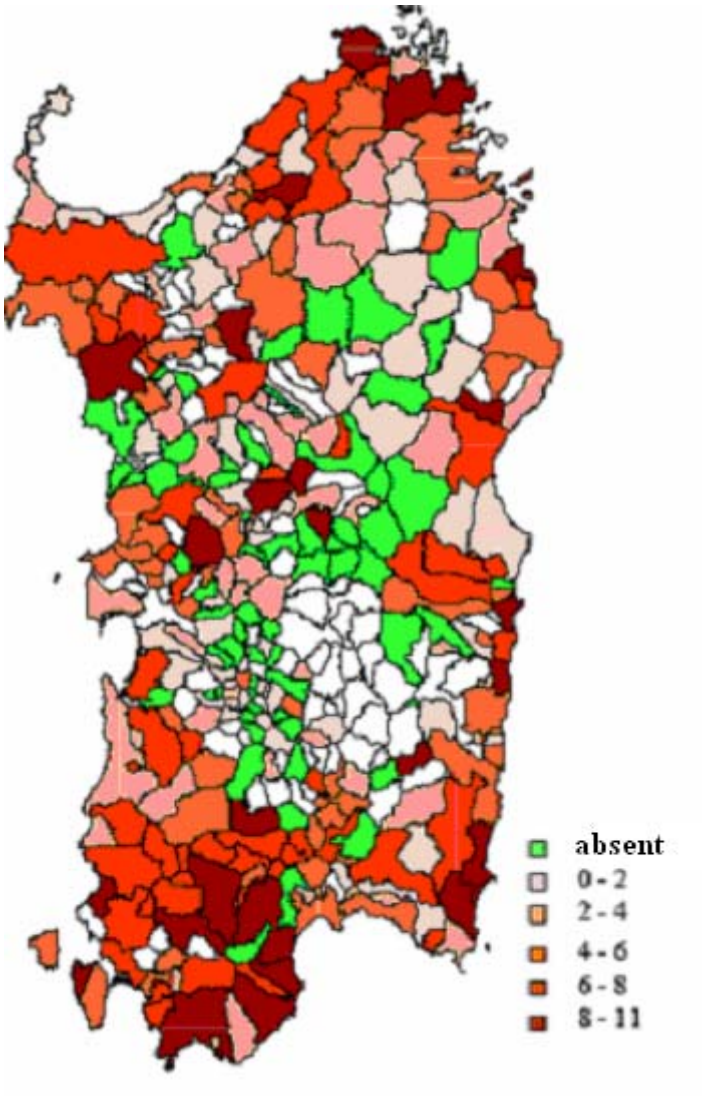


Fig 19. Average flow of the captures of *Culicoides spp*. and *C. imicola* per month considering all the traps. From the year 2000 to today , a total of 38 permanent traps have been used.

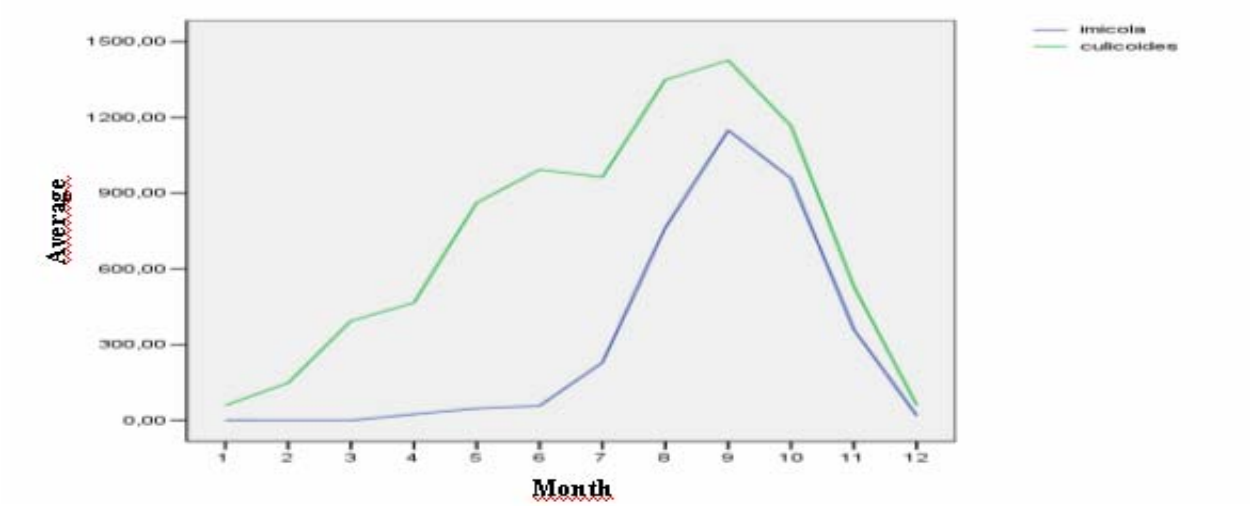


Fig 20. Outbreaks flow, during the years in which important epidemic waves took place, is similar (Aug-Nov), with peak in October. Exception is represented from 2006, with late beginning (Oct) and late autumn peak (Nov).

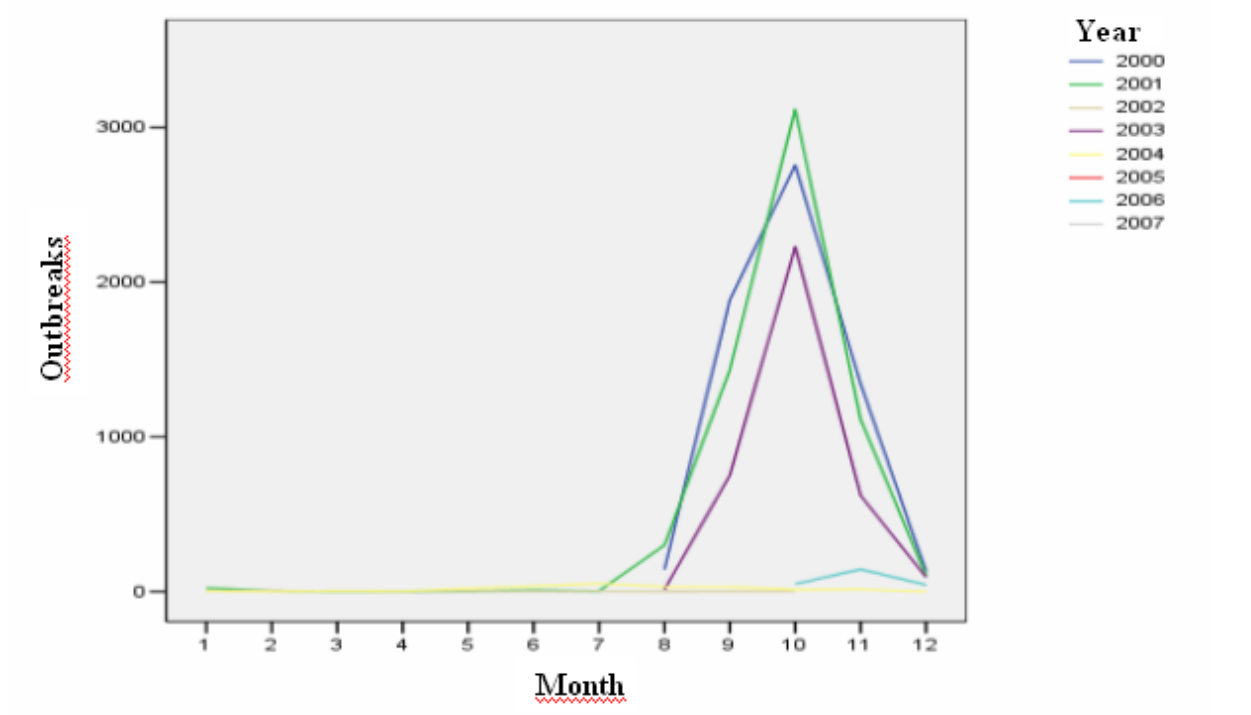
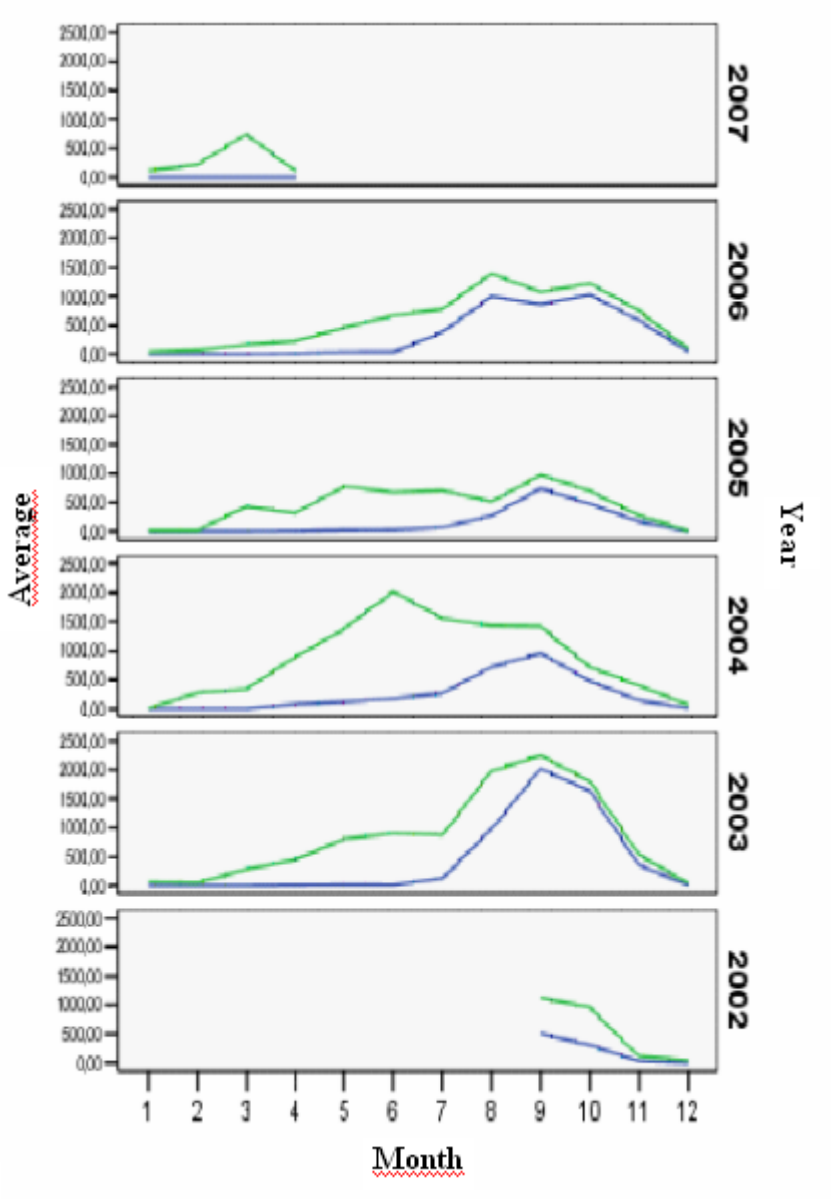


Fig 21. Average flow of captures of *Culicoides spp*. and *C. imicola* per month.

Panel per year.



For the execution of the activities connected to the entomologic surveillance, seven fixed stations (Black-Light traps Onderstepoort Institute, South Africa) were individuated and made operative, located with the collaboration of the CESME, at zootechnical farms in which, respect to analogous of the surrounding territory, increases in the population of the carrier insects of Bluetongue in ovines were found (*C. imicola*, *C. spp.*). During the years, and based on the need to identify considerable epidemiologic territories with a more accurate definition level, it was decided to increase the number of fixed stations from seven to twenty three, using the principle of the National Entomological Surveillance Plan (figure 22 e 23).

Fig 22. The positioning of permanent traps for entomologic surveillance considers all the data in possession and is configured as a body of sites more sensitive than the probabilities of capture.

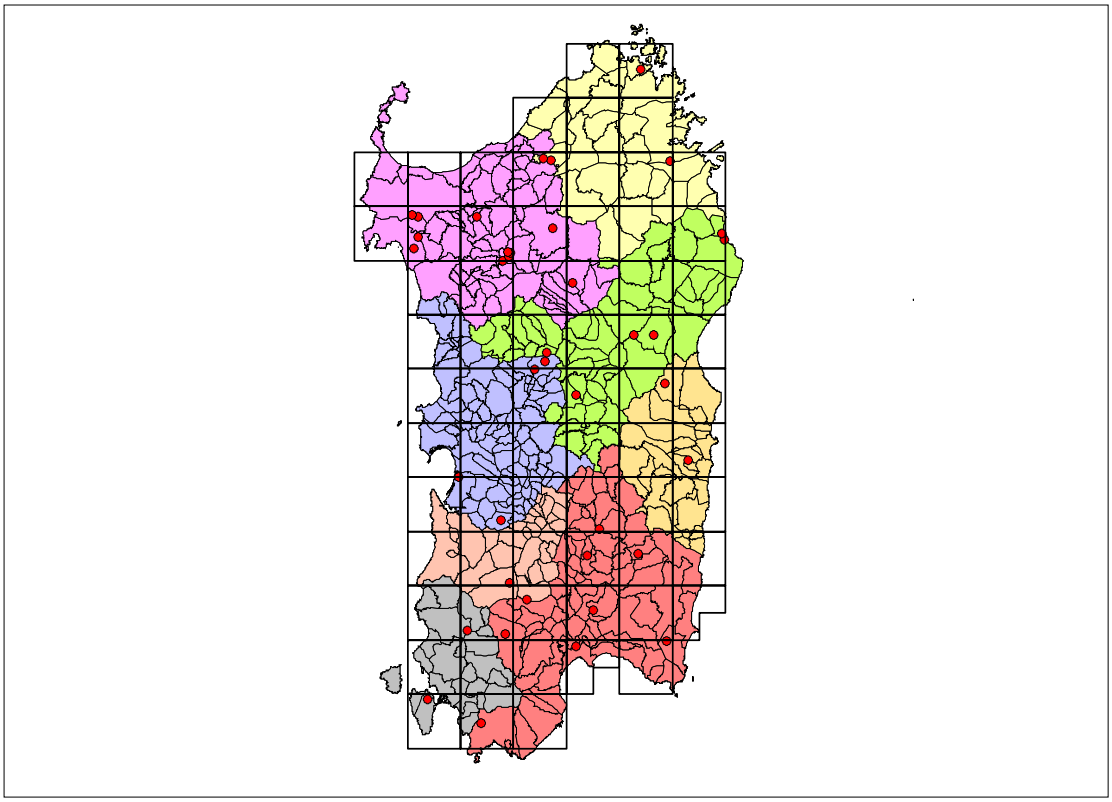
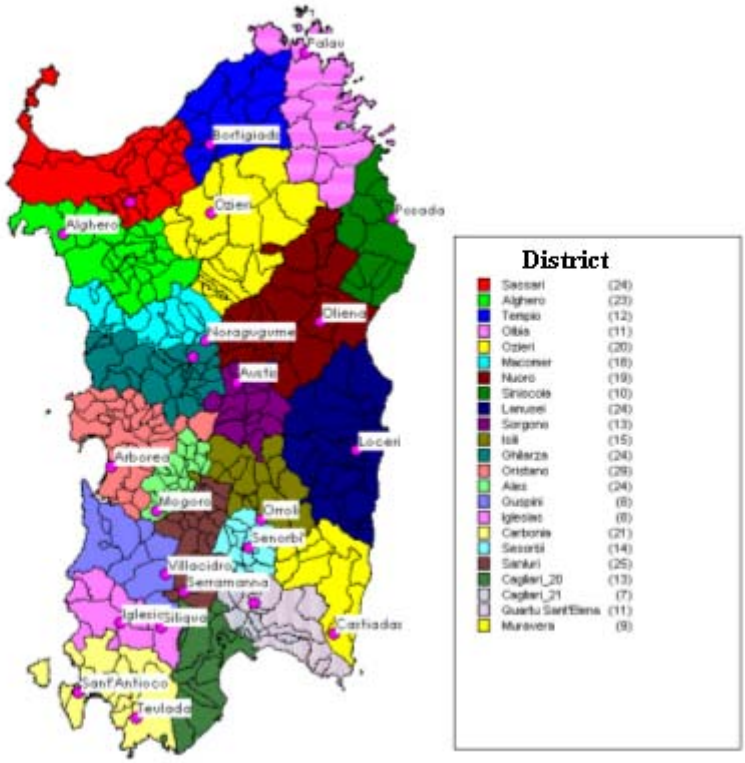


Fig 23. Positioning the traps in the various districts.



Thirteen of these in which the Surveillance Plan was performed continuously from 2002 to 2006 were chosen, depending on the weekly scheduled cadence, consenting to follow in time the evolution of the capture of the carrier insects in different areas of the island. The analysis of the capture (identification, separation, calculation of the insects captured) were made according to a standard procedure, adjusted in the entomologic sector of the Protozoology Laboratory of IZS Sardinia. Such procedure is constantly watched by the CESME, where the staff in charge was trained. On a total of 2613 captures, the average values of the *C.imicola/Culicoides.spp* (Ci/Cs), of n° of

Culicoides.spp. (Cs) and of n° of *C.imicola* (Ci) report observed during the captures of the 13 considered permanent traps, result different during the year. 2003 is the year in which a higher average value of the report (Ci/Cs) equal to 26.88, and also the number of (Cs) equal to 925.030 was observed. 2006, instead, was the year with the highest average value number of *C.imicola* (465.160). In this period it was possible to observe the constant correlation between the entomologic surveillance data and the seroconversion data and/or the clinical manifestation. The difference of the averages observed during the years in regard was statistically meaningful (value of $P<0.05$). Among the numerous factors that can influence the carrier circulation, it was considered the typology of use of the ground that characterizes the area surrounding the various stations, in order to evaluate the eventual differences in terms of environmental context, considering an area of 3 km of range around each permanent trap (figure 24 e 25) (Rolesu et al., 2007).

Fig 24. Example of use of the ground around a permanent trap. Siliqua trap.

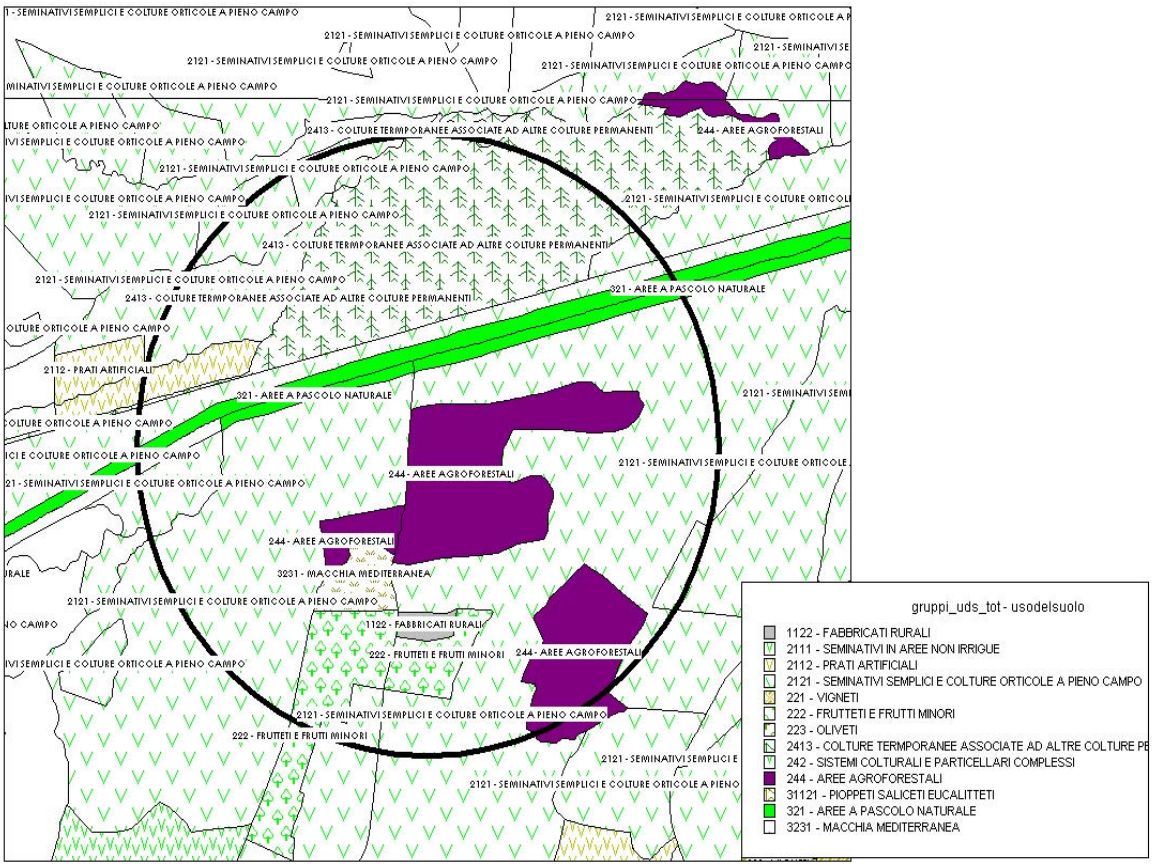
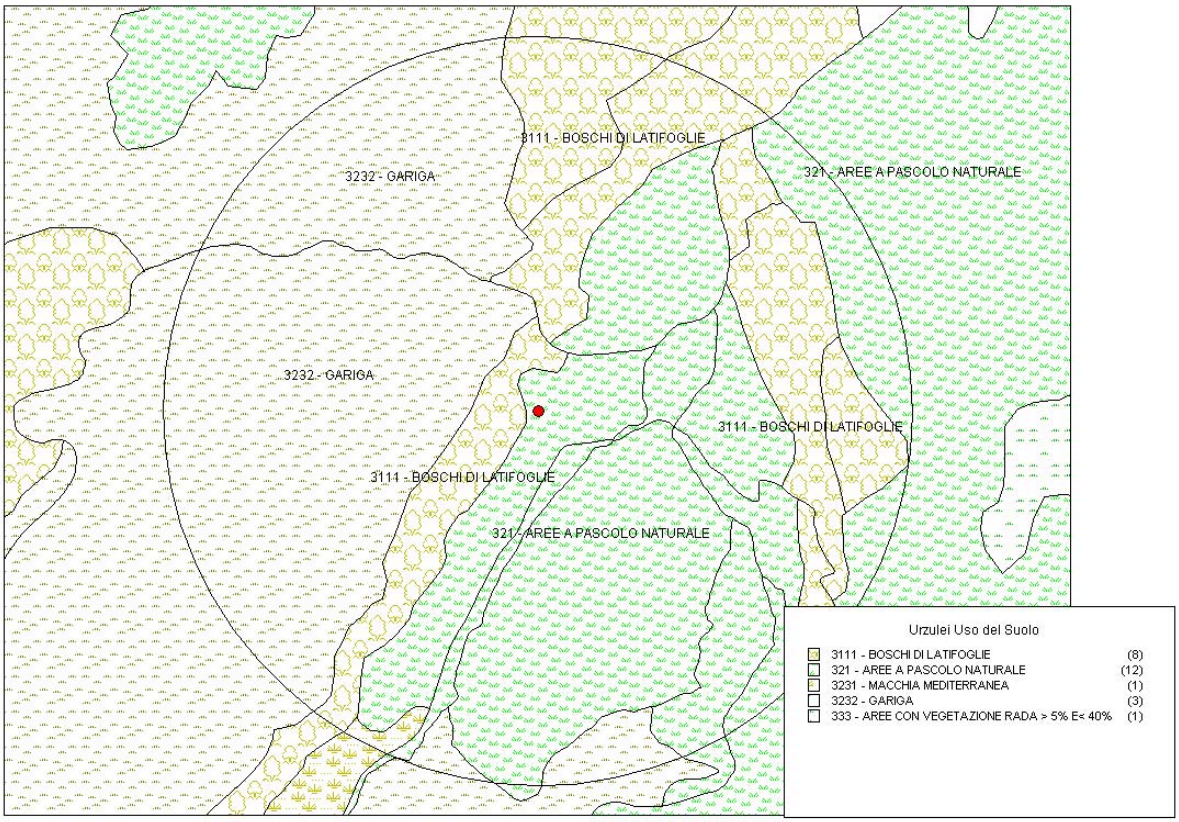


Fig 25. Example of use of the ground around a permanent trap. Urzulei trap.



Objectives

The object of this research could be sum up in the following aspects:

- Description of the study conducted in some areas of the island where the disease prevalence was lower than another areas to ex plane the reason of this.

- Analysis through the study about the use of the ground and geopedology in the farms where occurred the first epidemic of Bluetongue, taking into account the influence of this factors on Bluetongue prevalence.

- Analysis of differences into desease prevalence in the whole region through the use of the ground.

- Performance of the mathematical model using several esplicative variables to explain the disease spread in the farms.

- Application of the scientific assumption that the alkalization is able to make inhospitable the larval reproductive sites in the pilot area (Sant'Antioco) and the study about the reproductive behaviour of Bluetongue vectors. Collected data have suggested to apply a wide treatment to reduce larval sites extremely so that the number of *Culicoides* able to transfer disease should be lower.

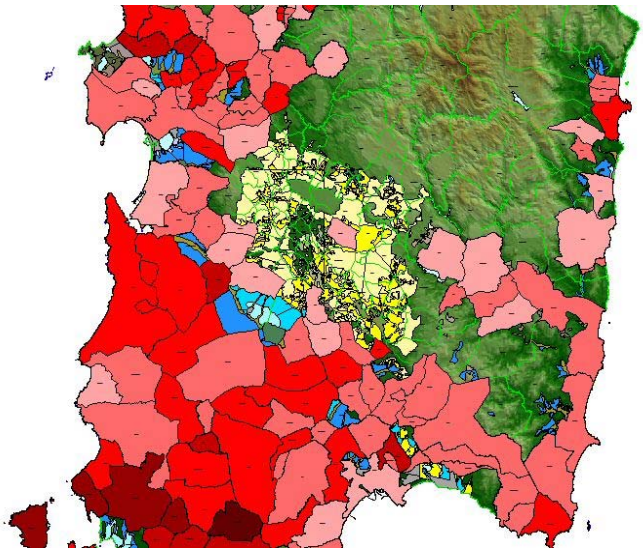
Territorial Epidemiologist survey

Materials and Methods

In South Africa areas of quarantine for horses exist in order for them to be moved to foreign countries without exporting the African Horse Sickness, a pathology that has the same carrier of Bluetongue in ovine. These areas are characterized by abundant salty lands (Meiswinkel et al., 1997). This date, combined with the observations performed in Sardinia during the different epidemic waves that followed each other from 2000, made it possible to point out how some breedings in specific territories (Peninsula of Sinis, Giara di Gesturi etc.) showed an extremely reduced morbidity-mortality respect the surrounding breedings, suggested to effect inspections finalized for the geologic evaluation (Rolesu et al, 2007). it was also evaluated the compatibility of these territories for the ideal habitat of the carrier insect and at the same time with other noticed risk factors, correlated with the *C.Imicola* development and as a consequence, the introduction of viruses and BT breeding grounds. These areas are object of inspections finalized to verify on the field the elements to support the first hypothesis, or that, in analogy to some particular areas of South Africa, there should be correlation between these territories (salty) with the ones referred to by the bibliography (sandy hills). In particular, to compare the analysis, the GIS technology based on essentially two software was used: Mapinfo and ArcGis. Both software were used to process geographic data that represent arguments of interest, for decoding and transforming them into interchangeable formats, (for example starting from files in

Autocad format). By the first analysis that was effected on the territorial municipality basis, it is possible to notice a strong correlation between the lands classified in "alkaline, sub-alkaline" with the presence, in the same territories, of a number of breeding grounds clearly inferior respect the ones that were expected on the basis of the dynamic of the BT diffusion. This analysis based on municipality was followed by an accurate analysis based on geographical coordinates of principal farm figures of the ovine goat breedings in which, during the first epidemic wave, BT cases were observed. A cohort of breedings that during this epidemic wave had the first symptom signals in a limited time period (one month) were used, in order to avoid confusion effects determined by the age of infection. At this point it is possible to correlate the georeferentiation site with the type of land corresponding. It was able to verify that the damages caused by blue tongue in the striked breedings, localized on the sub-alkaline-alkaline types of land were significantly inferior ($p<0.05$), respect the breedings situated in acid type lands. Using the GIS technology, it was able to analyse in detail the many theme aspects of every single breeding. Figure 25 shows the synthesis and the overlap of the different thematic characters used. The first theme represents the number of Blue Tongue breeding grounds observed during the first epidemic wave. The transparent territories (not coloured in increasing tones of red) are municipalities without signals. The second level is the representation of the altimetry (green), while the third represents the typology of the lands.

Fig 26. Stratification of the thematic levels.



Increasing the detail level of the analysis (Figure 27), it is possible to attempt to equalize these territories on the basis of the ground. In particular we can see how the territories apparently exempted from blue tongue (and most likely from carrier insects in a number able to guarantee, in presence of virus, the outbreak), are almost exclusively of alkaline/sub-alkaline type. This typology of ground is present at the same way in the peninsula of Sinus, where the few cases observed, although in the municipalities close, blue tongue raged, made it possible to believe that there were the characteristics to make the territory unsuitable for the carrier insects. The altimetry

representation (Figure 28) is necessary since a gradient correlated is noticed at a negative type of altitude respect the number of insect carriers per capture. In the matured experience during these years (2000-2006) from the relative data to the entomologic surveillance as a rule this altimetry gradient finds comparison also in terms of observed blue tongue breeding grounds and give a total observation, independently by the age of the first signalling.

Fig 27. Detail of the typology of land in the area of Giara of Gesturi.

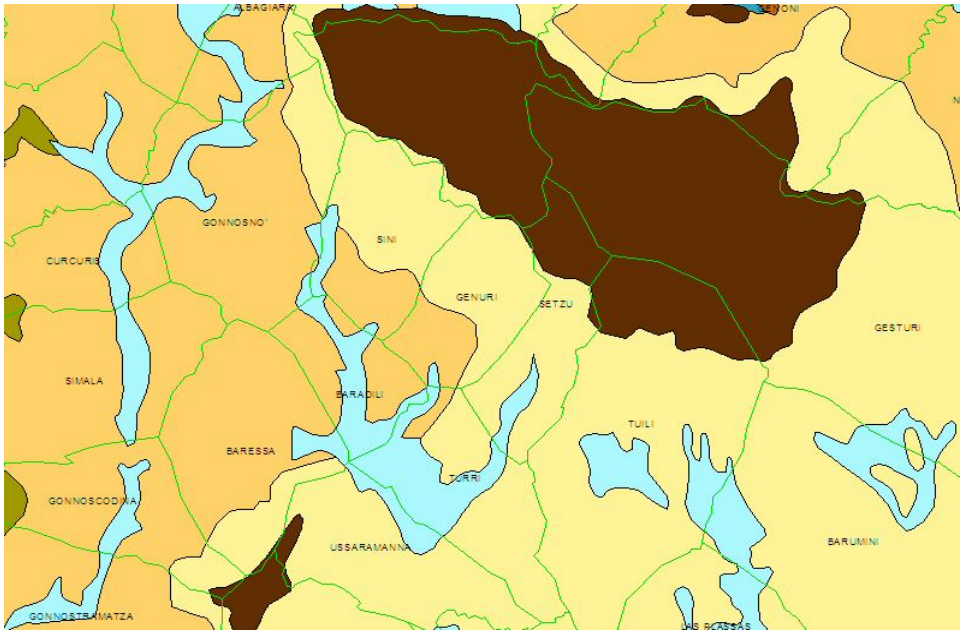
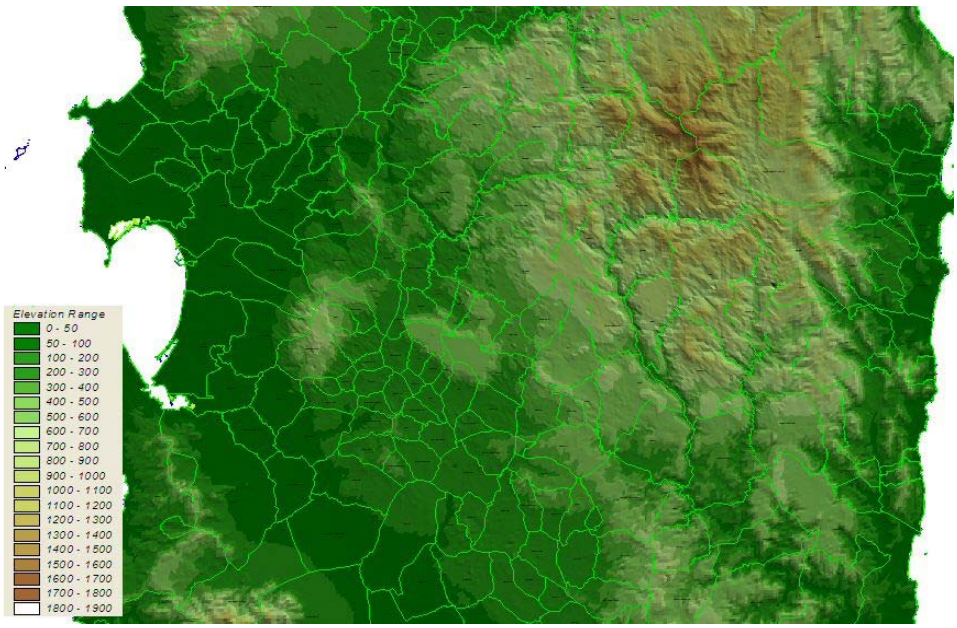


Fig 28. Detail of the altimetry.



The thematic character represented by figure 29 is exactly the number of breeding grounds of blue tongue observed during the first two epidemic waves (2000-2001 and 2001-2002). in relation with the previous image, the strong correlation between the two representations is evident. The map of figure 30, instead, represents the same data but expressed in terms of breeding grounds found/breedings present (prevalence). Some territory "enclaves" begin to be evident, where the cases observed are inferior respect the expected, in fact in some municipalities (white territories) are completely absent.

Fig 29. Number of Outbreaks during the first two epidemics.

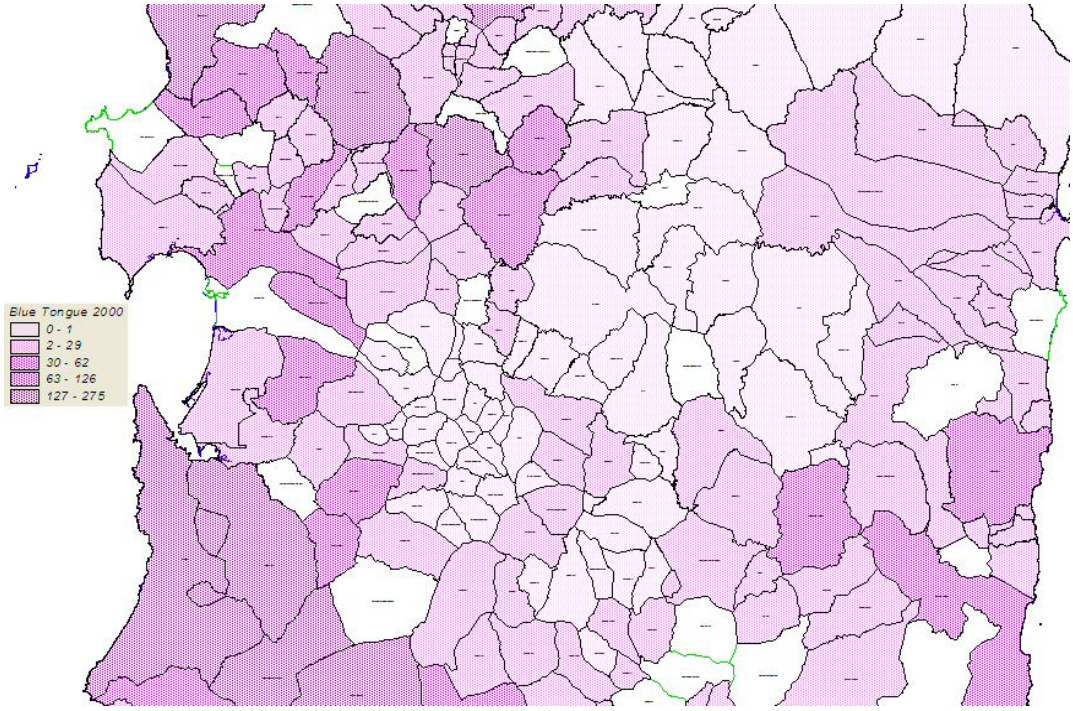
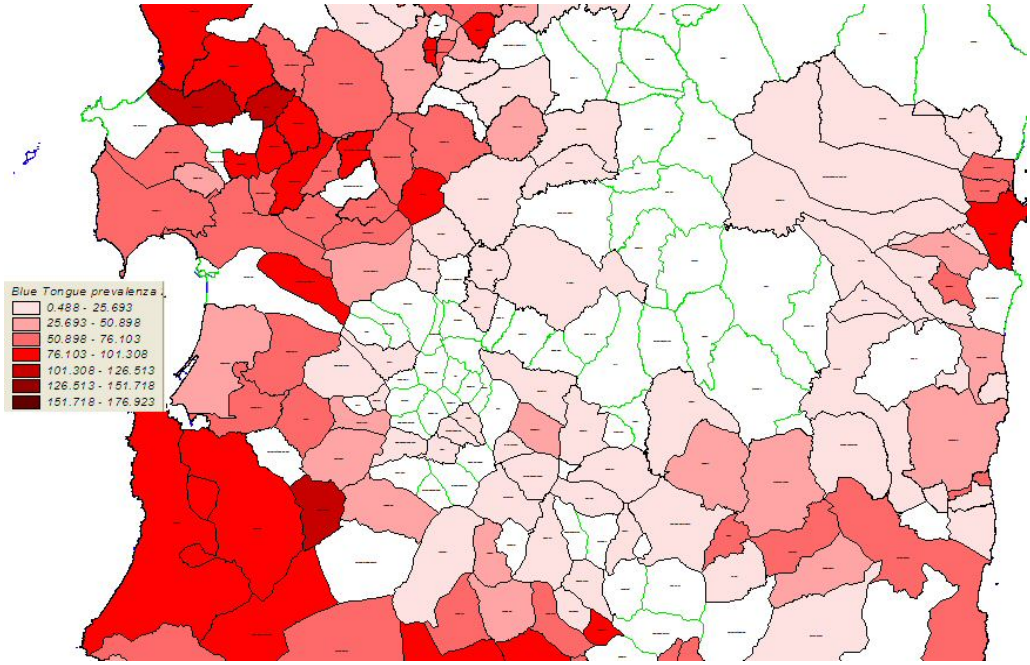
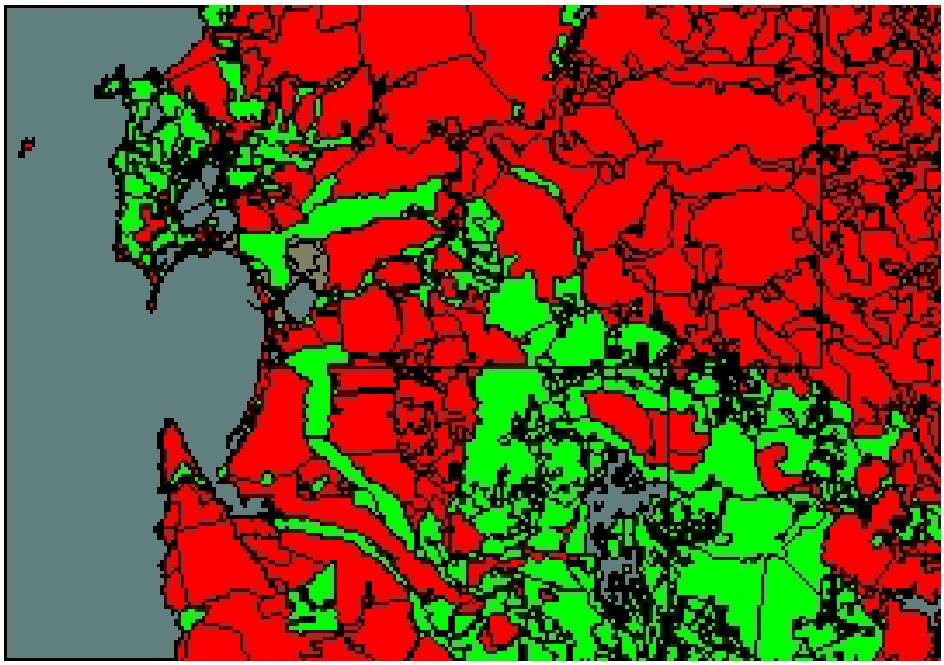


Fig 30. Prevalence of the first two epidemics.



If we represent the ground with a thematic character that takes into account the typologies without a grouping, beyond a vague specification of well defined contexts, it is not very evident the correlation between the two key territories. If we reclassify the ground on a Ph basis, in acid type lands (red), and alkaline/sub-alkaline type ground (green), it is evident and with no doubt, the strong correlation between the two territories (Figure 31). Starting by these observations, synthesized above, we moved to an overall evaluation of the Sardinian territory, using the methodology of analysis described for the pilot area of the Sinis.

Fig 31. Classification of the land in Ph base.

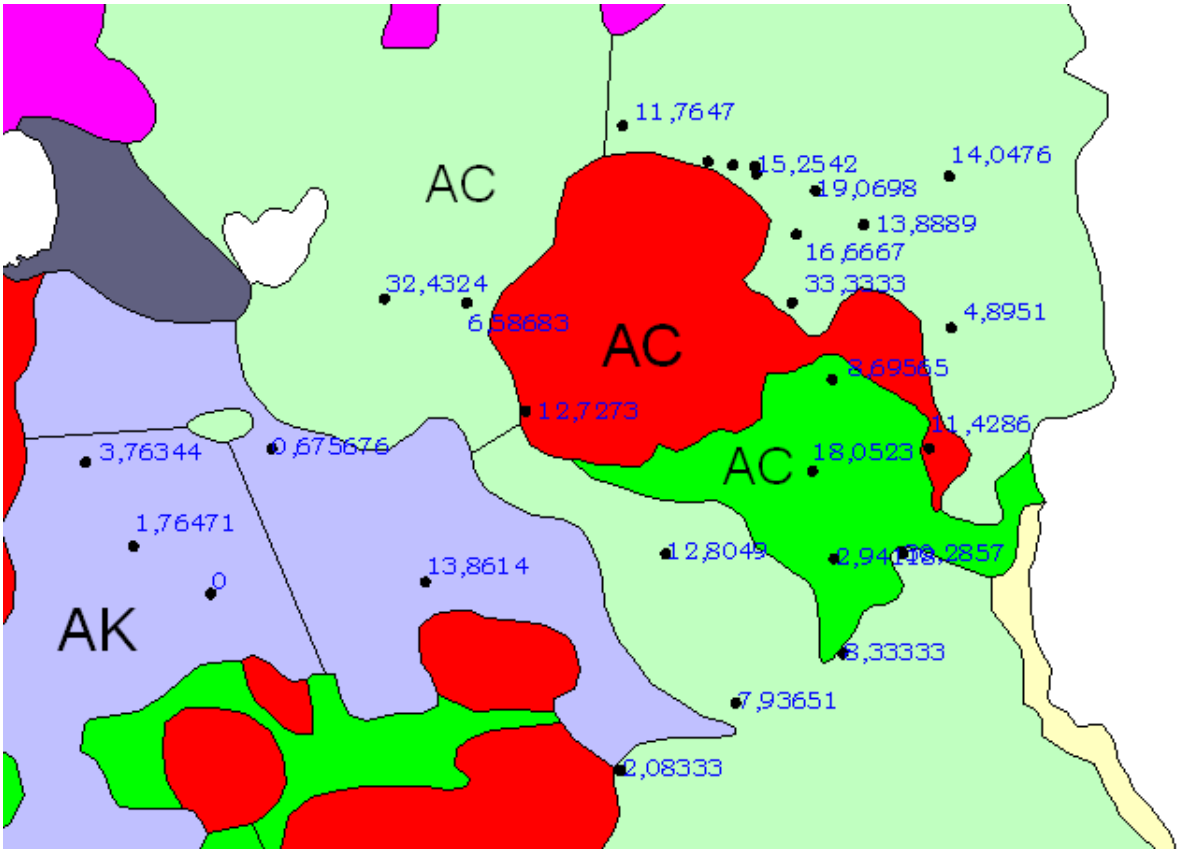


In order to cross the scientific assumptions mentioned in the introduction (salty lands) on the basis of observations effected it was verified what happened during the first outbreak in some areas of Sardinia, for which were in possession the georeferencing of the breedings site of the breeding grounds, which helped to establish the type of soil in which these breedings insist.

Results and Discussion

An analysis of the data showed that there were strong correlations between the type of ph of the soil, together with the particle size and the drainage of the same. So there were two categories of farms within the outbreak created according to the type of soil (figure 32), it was then tested the difference between the total damage observed in the breedings belonging to the two groups (AC vs AC) that was interesting (table 4 and 5).

Fig 32.Outbreaks in acid territories (AC) and Sub-alkaline/Alkaline (AK): Damage(slaughtered+deceased/present)*100.



Tab 4. Significant differences between acid and alkaline territories.

Groups	Average Dammage %	N	Standard deviation
Ac	15.637	196	15.775
Ak	11.308	65	15.168
total	14.559	261	15.710

Tab 5. Significant differences between acid and alkaline territories.

Anova	Origin of variance	Sum of square	Gdl	Average of square	F	Sig.
	Between groups	914.865	1	914.865	3.746	0.054
	Into the groups	63257.391	259	244.237		
	Total	64172.256	260			

Conclusion

This finding of great interest has confirmed the working hypothesis that some territories are less conductive for the development of *C. imicola,* and that exists a strong correlation with the pH of itself ground. Based on the first analysis conducted in the municipality district it was evident the strong correlation into the alkaline and sub alkaline ground and the number of BT outbreaks occurred in these territories. This number were far lower than the number of expected BT outbreaks in order to the well-known sequence of events. The damages registered in BT outbreaks that were in alkaline or sub alkaline territories were interesting ($p < 0.05$), than the herds collocated in the acid territories with a statistical significance. In particular it is interesting to notice that the territories without BT outbreaks where the number of BT vectors were not as many as required to transmit the infection, were nearly all alkaline or sub alkaline. This typology of ground has been found in the Sinis peninsula where have been occurred few outbreaks , although in the closer territories had been registered a lot of BT outbreaks. This observation shows that in the territory of Sinis there were particular characteristics so that BT vectors couldn't be able to develop. It seems favourable to analyse the possible improvement in the herd condition in terms of optimum habitat for the insect vector, reducing every possibility of reproduction for the BT vector. This behaviour will allow to have a BT lower risk and consequently it will be possible to reduce the correlated damages.

Experimental working

Materials and Methods

The practical application of this observation was that it was experienced in the pilot site of Sant'Antioco, and experimental deals with the "Dipartimento di Scienze Applicate ai Biosistemi dell'Università degli Studi di Cagliari, Sezione di Parassitologia – Laboratorio Entomologia Medica". A procedure to "alkalize" the sites considered of great risk for the reproduction of the insect carrier situated near the sheep and goat herds, through the use of certain substances (eg. Lime milk), in amounts and arrangements evaluated according to the type of soil and the relative permeability of the same. The action was therefore directed only against pre-imaginal stages and was therefore important the location of outbreaks of the insect larvae. In fact it is well known that *Culicoides* are widespread and concentrated in all those muddy areas, even in modest extensions, which are created around the breedings. In particular, major outbreaks are shallow (less than 15 cm), rich in organic matter (with a concentration of 8% solids) determined from the effluent of animal excrements inside the farms. The larvae are found in shallow mud on the edge of the water line. The only sites capable of producing a sufficient quantity of insects to trigger the viral transmission of BT are, in fact, the large and small pools of water that are created by drinking troughs that leak,

from washing water, water reserves as ponds used for watering livestock (Figure 33, 34, 35). the treatment consists in preparing a solution of lime in the ratio of 200-300 g/1 per square meter of pond effluents containing organic predominantly consisting of the cleaning of stables or animal shelters, etc...treatment is hypothesized with these doses for wells with an average depth of about 10 cm, the quantities will be increased if the depths increase (eg. For an average depth of 20 cm the quantity of lime solution will be doubled).

Fig 33. Water stagnation near the breedings.

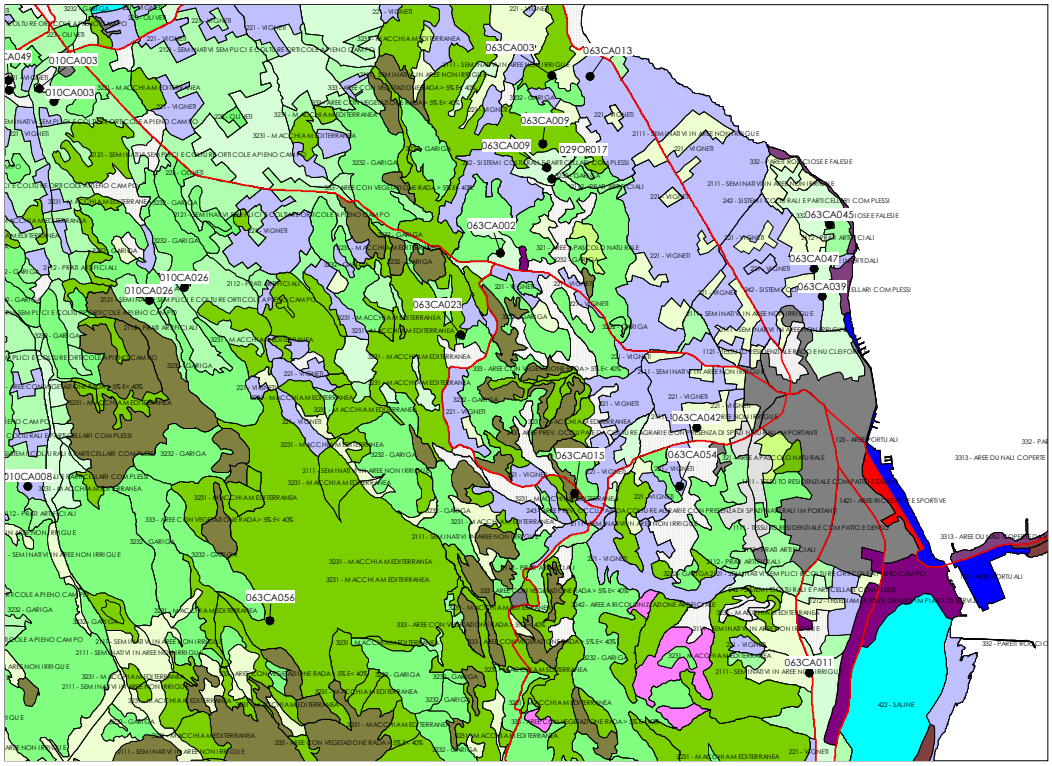
Fig 34. Water stagnation near the animal shelter.

Fig 35. Water collection with organic collection in areas of animal station

The experiments carried out at Sant' Antioco have already given more than favourable indications for the use of this effective and efficient “larvicide”. The correct georeferencing of the breedings (milking rooms, stables, etc...) and appliances in which these animals have the opportunity to stay (pasture), and the layering of information elements such as hydrography, roads, land use, soil type, vegetation, etc., with the help of estimation models effectuated with satellite observation, offers a powerful evaluation-management tool of the territory, and through this, the identification of hazards. This methodology made it possible to identify a set of correlation factors to the initial null hypothesis on the biology of the vector, in particular on the role that water has to influence the number of population of insect carriers, and in promoting the

widespread even in wide fields. The techniques of spatial statistics, at this point, can be used with greater detail and with more efficiency (Figure 36).

Fig 36. Pilot Zone of Sant' Antioco – Example of thematic stratification, breedings, use of soil, roads.



Culicoides data

The choice of the territory of S. Antioco for the experiment was dictates by the following observations:

- The presence of a limited territory well-managed, where the influence over neighboring territories in contained to a minimum by insularity.
- The presence within the territory of all susceptible species.
- Possession of the historical reference to the various epidemic waves and data on the damage they caused.

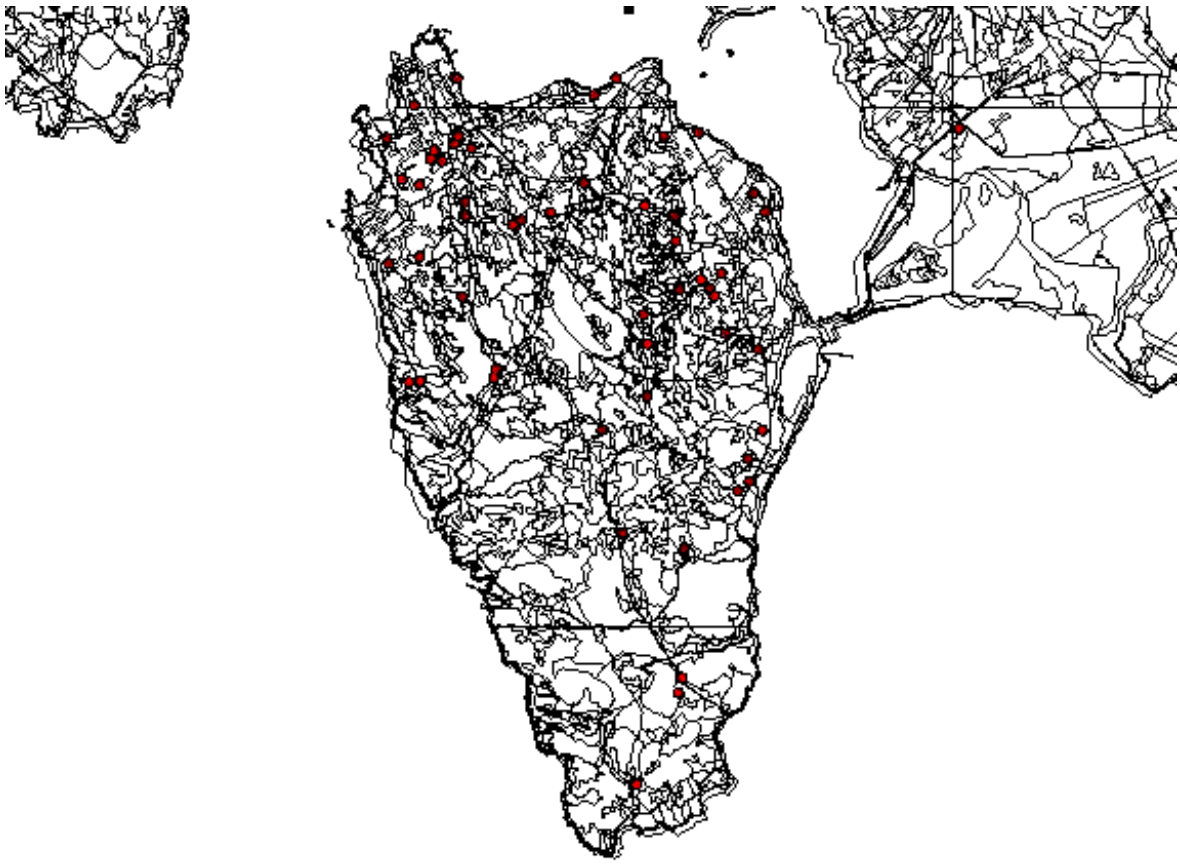
More specifically the area (108 km ²) now includes a total of 59 companies distributed in the two communities, Sant'Antioco and Calasetta (Table 6). To highlight the presence of companies dual attitude with bovine key feature of the epidemiological history of the carrier.

Tab 6. Total farms in the territory.

	Cattle	Goat	sheep	Cat/goat	Cat/sheep	Goat/sheep	Total
Calasetta	**8**	**3**	**12**	**0**	**3**	**1**	**27**
S.Antioco	**7**	**2**	**17**	**1**	**5**	**0**	**32**
total	**15**	**5**	**29**	**1**	**8**	**1**	**59**

It was also important to proceed immediately with the georeferencing of the breedings acquiring data from the national database, and positioning companies as graphically depicted in Figure 37.

Fig 37. Disposal of breedings on the island of Sant'Antioco.



In addition to the territory of the pilot site of S. Antioco, the trial took place in synchrony in other 7 pilot sites (Iglesias, Orani, Posada, Cardedu, Villagrande - in three distinct locations), which has developed research through the planned activities of the various OU. The pilot sites correspond to as many breeding farms. For Iglesias (Baschieri Company) Sant'Antioco (Ghisu Company) and Posada (Marongiu Company), the companies hosting the pitfalls of standard monitoring plan for the

National Blue tongue, allowing the comparative study traps fixed in relation to those who are flickering positioned directly on the ground within the larval outbreak (or supposed). The trap is flickering craft, and was developed using the life cycle of insects that have developed from larvae present in the first layers of soil at the time of the flickering as adults, they head toward the only opening to which this is any Erlenmeyer containing 70% ethanol. These traps allow the evaluation of the activity of single outbreak since catching the insects to the transition from larval to adult, before it can flicker in the surrounding environment (Figure 38).

Fig 38. Traps.

The farm chosen for pilot testing and breeding is Ghisu Luigino, placed 50 meters above sea level, site also already used to the placement of a trap of national entomological surveillance system. Following initial site inspections is defined as an area to be treated, near the main corporate body, where they assumed the presence of the general characteristics of the land suitable for breeding of the vector (Figure 39).

Fig 39. Satellite view of the corporate body and the area in the experiment.

Therefore the pH of a soil sample collected in the area chosen for treatment was measured.

pH of the mud = 7.45

To the mud quantities of lime milk have been added in proportion to that used in the field (1L / m²):

A = 98 c m²

Lime milk = 9.8 ml

After the addition of lime milk, the pH was immediately measured:

Surface pH = 12.25

pH at a depth of 3-4 cm = 8.52

The pH was again re-measured on 23/02/2007 (a distance of 5 months after treatment) on the treated area, with the litmus test and obtaining a value around 7.5. A sample of the same mud and in stagnant water that has been brought to the laboratory for pH measurement with finding a pH value equal to 7.96. A month after the pH was re-measured in the treated area and various points of the company, and adjacent to each trap are everywhere a value between 6.5 and 7. On 30/03/07 in the area of treatment to a depth of 2 to 4 cm are still visible traces of lime, which is laminated mud.

Probing of the soil.

On 06/04/2007 have been removed 4 cups of ground, 2 from the treated area and two in the control area at several points:

Carrot 1 zone grid: pH = 8.09

Carrot 2 grid area: pH = 8.16

Carrot 3 zone control: pH = 7.67

Carrot 4 zone control: pH = 7.47

The measurement of pH was made with the pHmeter resulting in a volume of 50 ml with distilled water, 15 ml of mud.

Event History of Pilot farm S. Antioco:

The major tasks in the company were based on periodic site inspections to review acts of the various catches and the collection of the flasks containing the insects to be classified with the department of parasitology at the IZS.

23/03/06 Placement of 8 traps (Figure 40)

Fig 40. Position of the first traps pilot farm.

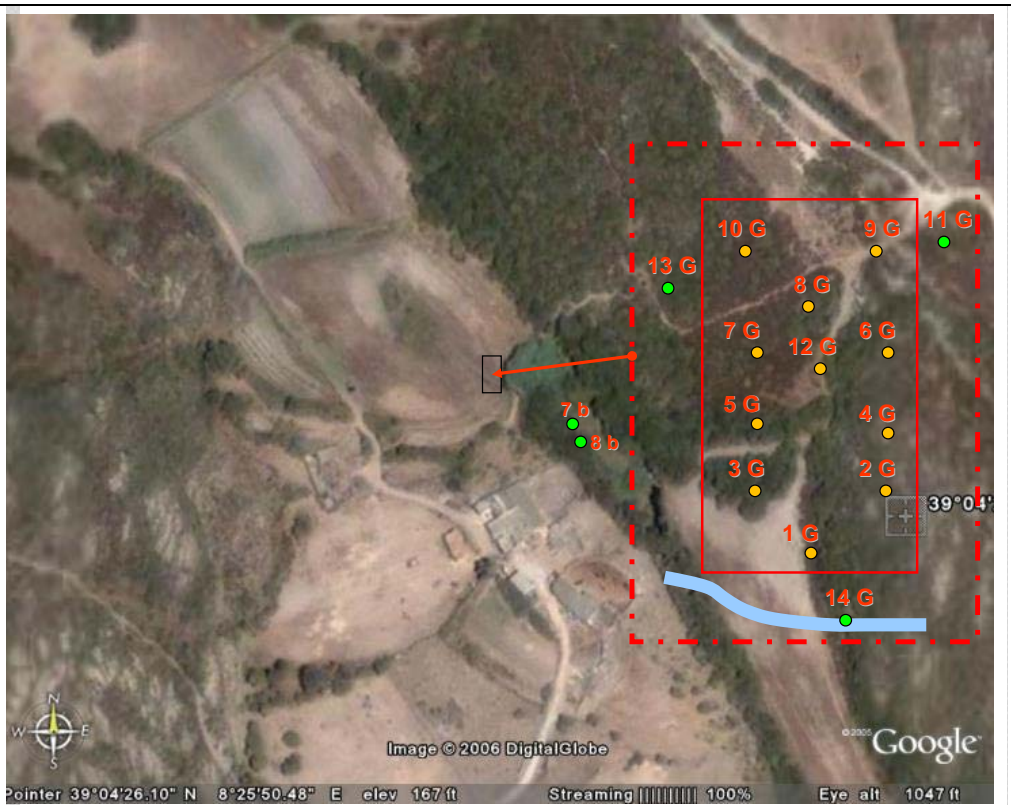
03/04/06 Placement of other 2 traps

05/06/06 Repositioning traps, protection of the trap 1a with 4 plates

28/07/06 Moving trap 3a

04/08/06 Treatment with hydrated lime to 20% in an area of 30 meters around traps 1a, 4a and 5a and in an area of 15 meters around the trap 8a (Figure 41)

fig 41. Diagram of the grid and the arrangement of traps treated.



25/08/06 Trap 1a small shift on the short side of the trap and positioning trap 1b. Removal of sheets from the trap 1a

01/09/06 Repositioning of the trap 1n a position previously occupied

15/09/06 Moving the trap 3b of about 50 cm. Removing traps 1a, 1b, 4a, 5a and 9a to start a new phase experimentation

01/10/06 Beginning of a gradual drying up of the treated area following the creation

of a drainage canal that conveys water away from the grid

27/10/06 Placement of traps 12g, 13g, 14g for the purpose of control verify a possible re-colonization of *Culicoides* after treatment

01/12/06 Displacement of the trap off the grid 1g

07/12/06 Removal of traps within the grid (2g ... .10 g, 12g) and repositioning of the 6 remaining traps (renamed 1m ... 6m). From then on the catches are taken every two weeks, are also to a shift of the traps

22/12/06 Repositioning of all the traps

05/01/07 Repositioning traps 1m, 3m, 5m and 6m

12/01/07 Repositioning of all the traps

02/02/07 Repositioning of traps 1 m, 2 m, 3 m and 4 m

16/02/07 Repositioning of all the traps

23/02/07 Repositioning of all the traps and placement of a new trap called 7m

16/03/07 Repositioning of all the traps

23/03/07 Repositioning of the single trap 5m and placement of a new trap

30/03/07 Positioning of a new trap

06/04/07 Repositioning of tr 1 ... .. 10, positioning of a new trap called tr6

13/04/07 Repositioning of tr6 alone because of the need of the farmer to plow the portion of land where it was positioned

20/04/07 Repositioning traps tr1, 2, 3, 9 and 6

04/05/07 Repositioning of tr9

11/05/07 Repositioning of tr 1, 2, 3, 4, 6, 8, 9

18/05/07 Repositioning of tr5 and 10. The area where the grid is positioned is gradually drying, the soil from last year is more dry due to the conveyance of water in channel traced by the farmer to prevent water disseminate in the treated area

08/06/07 Repositioning of tr7, 8 and 10

28/06/07 Repositioning tr8

13/07/07 Repositioning tr4 and tr9. You notice a slow progress of water of the channel of the treated area

03/08/07 Repositioning tr6 and tr8. A slight deviation the channel was produced so that the water more easily and more quickly flows to the treated area

07/09/07 Repositioning tr 1, 3, 6, 7, 8, 9

14/09/07 Repositioning tr5 and 10

21/09/07 Repositioning tr 4

28/09/07 The situation of the soil is almost identical to that of last autumn with all the treated waterlogged area (Figure 42).

Fig42. Photos of the grid treated and traps inside.

Data processing

For the acquisition of data, a database was used Access 2000 (Microsoft) and subsequent statistical evaluation was performed using software SPSS for Windows ver 15.0. The evaluation of geographic data, the realization of thematic maps and spatial overall assessment was carried out with the aid of GIS software Mapinfo Professional ver 7.8 and the ArcGIS software. The maps were acquired at altitude http://www.gdem.aster.ersdac.or.jp, while the mapping land use was acquired by the Autonomous Region of Sardinia, Department of Defense environment, map services. For the analysis of spatial variables, the entire island of S. Antioco has been divided into areas resulting from applying the theory of Voronoi. The partitioning of a plane with n points into convex polygons such that each polygon contains exactly one generating point and every point in a given polygons is closer to its generating point than to any other. The cells are called Voronoi polygons (http://mathworld.wolfram.com/VoronoiDiagram.html). The basis of the observation that the insect vector moves towards the nearest farm, we have therefore constructed the Voronoi polygons using geographical referencing for each individual farmer, thus obtaining a breeder of reference for every single point of territory of the whole island. The data on the first wave of the epidemic BT, vintage 2000-2001, were acquired and processed by the Center veterinary epidemiology Regional (OEVR), which feeds the National Information System of BT, together with entomological surveillance data on catches of insects vectors. The experimental data on the catching of insects using flicker traps were acquired by the "Dipartimento di Scienze Applicate ai Biosistemi dell'Università degli Studi di Cagliari, Sezione di Parassitologia – Laboratorio Entomologia Medica" and relate to current IZSSA 06-2004 research project

"Produzioni zootenciche biologiche: studio, sperimentazione", which also was part OEVR as Unit No. 7.

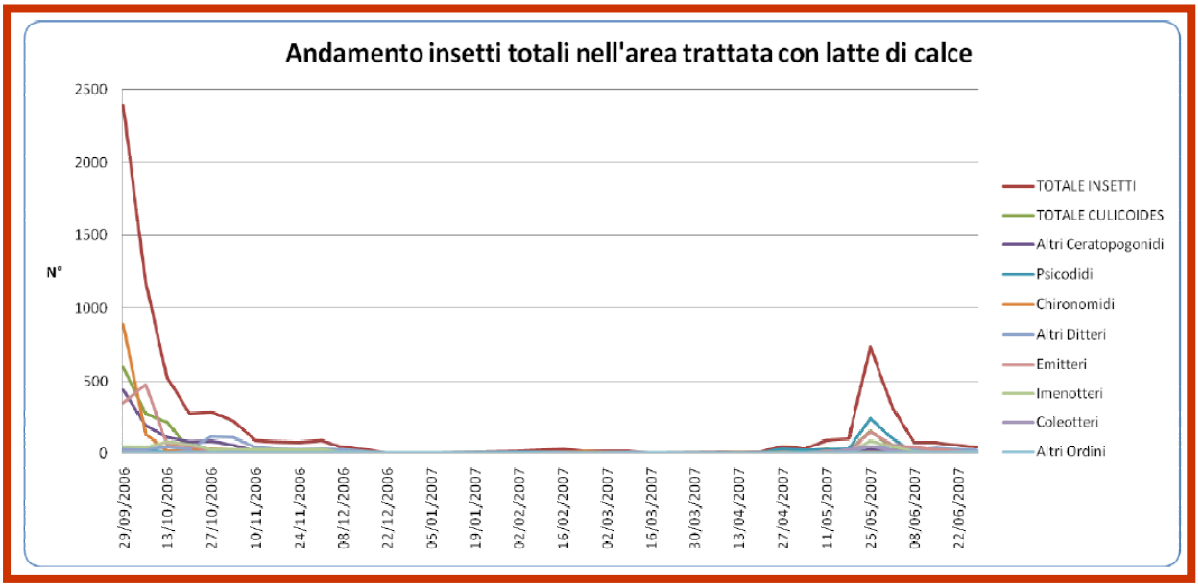
Results and Discussion

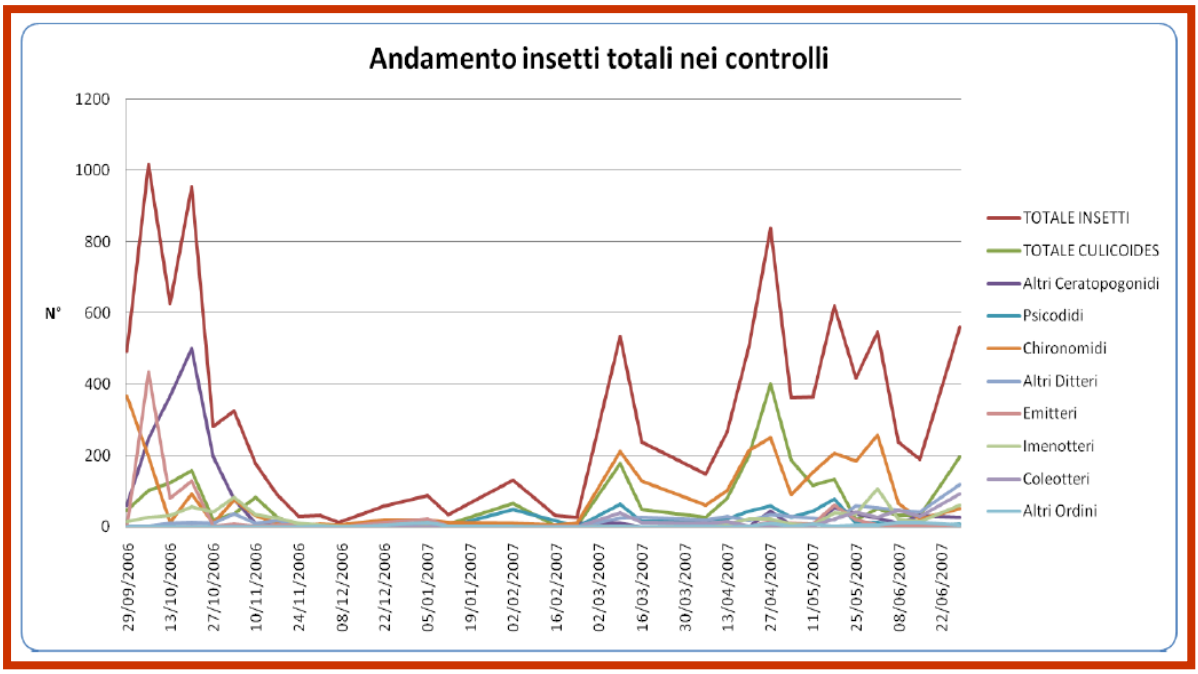
The comparative study of the fixed traps and flickering traps concerned the classification of the species *C. imicola*, *C. obsoletus* and *C. Pulicaris*, all three possible vectors of BT virus. The entomological survey provided a check that trends in populations of *Culicoides spp*. present in the different periods of the year recorded by the two traps is similar, even for the different species, particularly *C. imicola* (figure 43.44). Zeroing the larval development obtained in soil treated is considered to be the same on every part of the alkaline surface, as during the experimental period the traps within the grid were constantly moved and relocated in different areas of land to distribute the control over the entire area. The immediate slaughtering of the catch in the treated areas compared to untreated lasted for the period from 29/09/2006 to 27/10/2006, with an important permanency of the infertile soil treated (no catches) for long periods (± 5 months). E 'was also interesting to note the confirmation of the dependence in the presence of water by the larva, an aspect highlighted by the decrease of fish caught during the change of land by livestock farmers, a fact that prevented the normal influx of water to the area . The graphs of Figure 45 show the evolution of catches on traps placed in the soil treated with flicker catches on traps placed in control of ground control (untreated). It is clear the sharp decline to zero until the larval development during the period after the alkalinizzation of sites considered optimal for breeding of the vector.

Fig 43

Fig 44

Fig 45. Difference between the catches in the treated area compared to the control area.





Conclusion

The preliminary findings are encouraging, even if limited to one pilot site, and worthy of consideration. Of particular interest:

- The immediate culling of the catch in the treated compared to untreated;
- The permanence of infertility of the soil treated (no catches) for periods long (± 5 months).

This important result led us to hypothesize a treatment on a larger scale. Also, because larval breeding sites are not normally used agronomically but for the most part are places of transit and parcels of land not cultivated, it might be thought that any secondary treatment does not create any kind of damage to crops. This would ensure greater safety with a persistence of the basicity of the soil, keeping low the possible negative side effects. Crucial for the success of the intervention is the cooperation of the owner or manager of the company with the technical staff operating. This point varies from company to company, could be a source of confounding of final data. Diligent preparation and illustration of the type and arrangements need to be implemented in respect of the breeder so that it can share the purposes of research and experiment. Keep in mind that changing the area we consider to be ideal habitat for

larval development is only one piece of a broader mosaic of agronomic interventions from having to make the body corporate, such as:

- Any drainage of watercourses to drain stagnant and wells;
- Remediation and cleaning of the marginal areas of cultivated fields;
- Changing the staging areas of livestock;
- Amendment of transit times to the admissions;
- Improvement of structural conditions of the company and the milking parlor and resting;
- Possible use of insecticides.

Epidemiologic model

Materials and Methods

Besides crossing the hypothesis aroused through the testing in the pilot site of Sant' Antioco, we evaluated the BT epidemiology situation in the concerned territory having the objective the study of all the variables we considered influential about the disease. During the first epidemic wave in the communes of Sant'Antioco and Calasetta, the outbreaks of Bluetongue have been reported starting from August 21, 2000. After only one month as many as 30 (22 ovine farms, 7 mixed farms and 1 goat farm) of these present 35 farms were declared breeding grounds (85,71%), and only 5 ovine farms were free from the first epidemic wave. The damage calculated in terms of number of animals that died naturally and/or slaughtered respect the total on each individual breeding ranges from a minimum of 0,4% to almost 70% of the animals present in a breeding in which the symtoms were presented as early as August 25, 2000 (figure 46). The variable damage included in the analysis model of regression and represents the dependent variable Y, it is a variable numerical that represents the sum or dead animals

and slaughtered animals during the first wave of the bluetongue epidemic (2000-2001) and was evaluated together with the following explanatory variables:

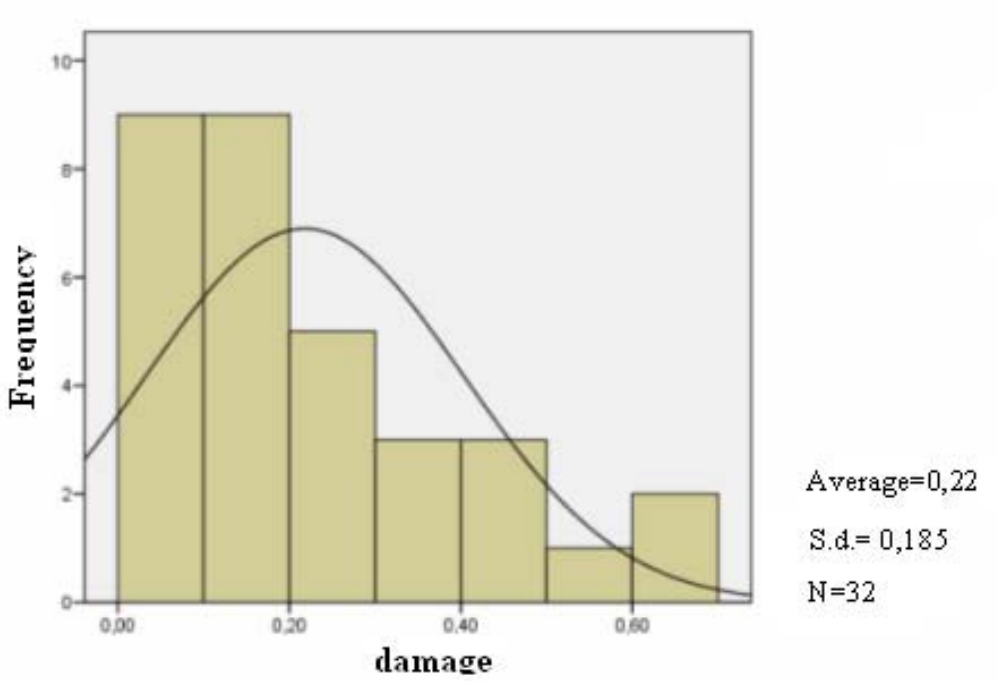
- use of soil (x1)
- average of altimetry (x2)
- presence of water courses (x3)
- soil Ph (x4)
- density of animals within the polygon (x5)
- type of animals present within the polygon (x6).

Information on other variables known to be correlated with the presence/absence and abundance of carrier insects (*Culicoides spp*.) such as:

- NDVI (Normalized Difference Vegetation Index)
- the index of aridity
- the average level of soil temperature

were considered constant throughout the study.

Fig 46. Frequency of breedings respect the suffered damage.



Aspects related to the use of the land (x1).

In each concerned territory that contain species sensible to BT has been georeferenced on GIS. The total territory has been divided into a grid of Voronoi (Figures 47, 48, 49), so that the space around the single breedings was to be precisely restored to each individual breeding. The grid was used in order to match this space to the land in use (Figure 50).

Fig 47. General context of the Island of San Pietro.

Fig 48. Voronoi polygons relative to the breedings on the island.

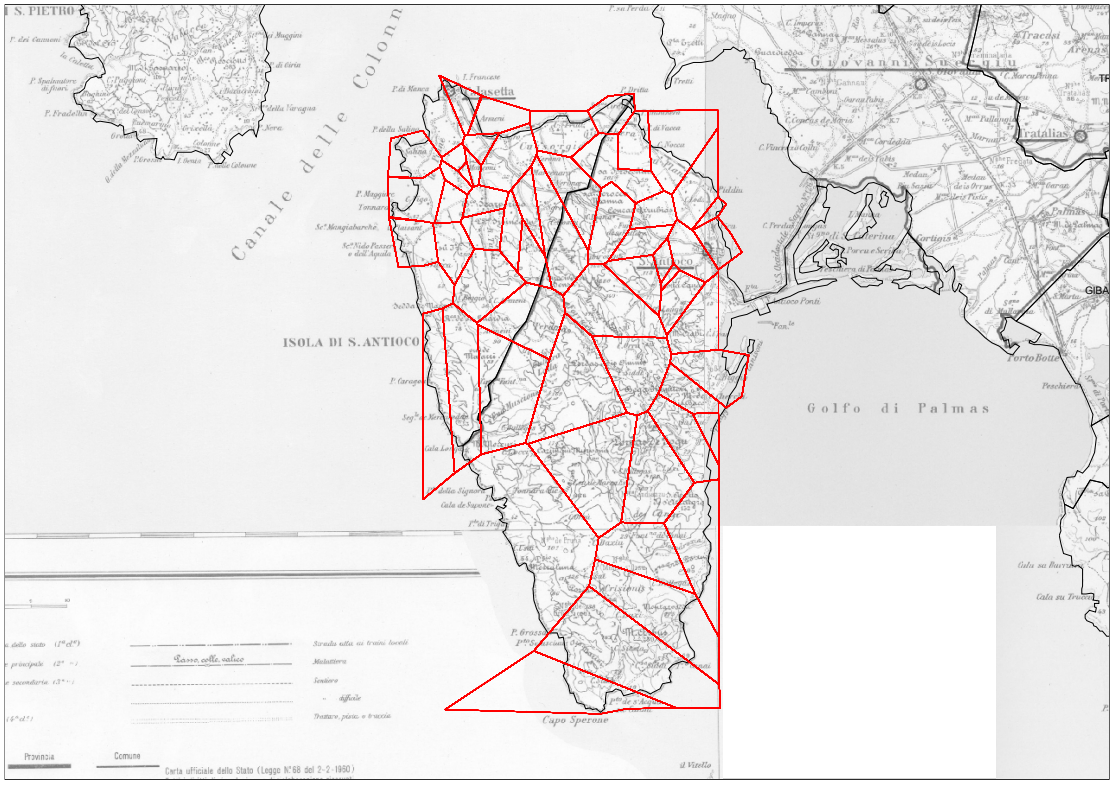


Fig 49. Detail of the pilot farm polygon.

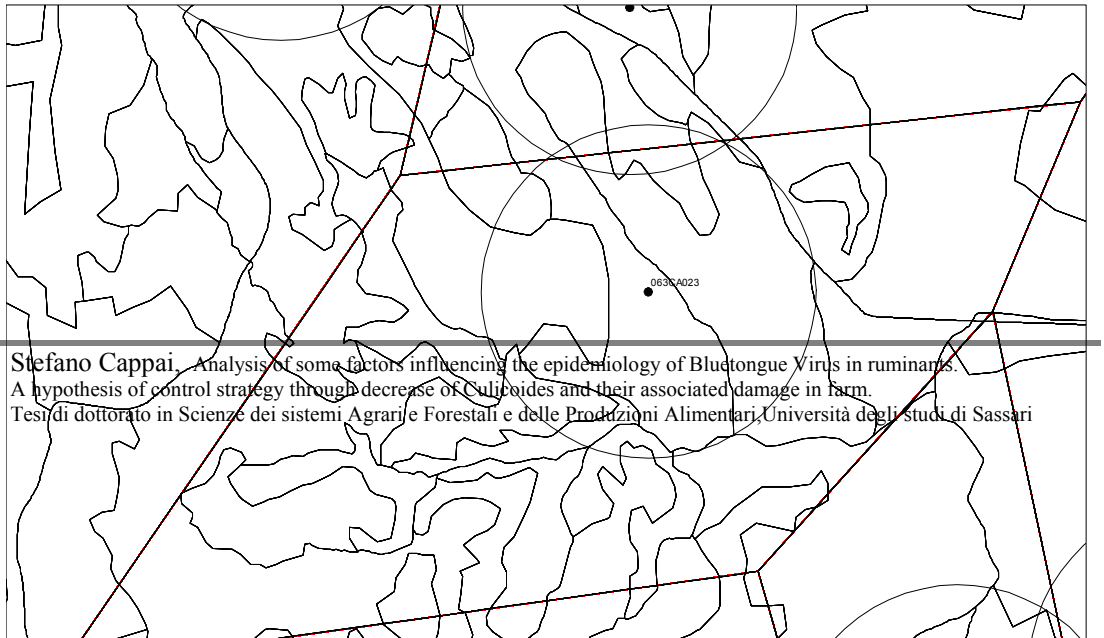
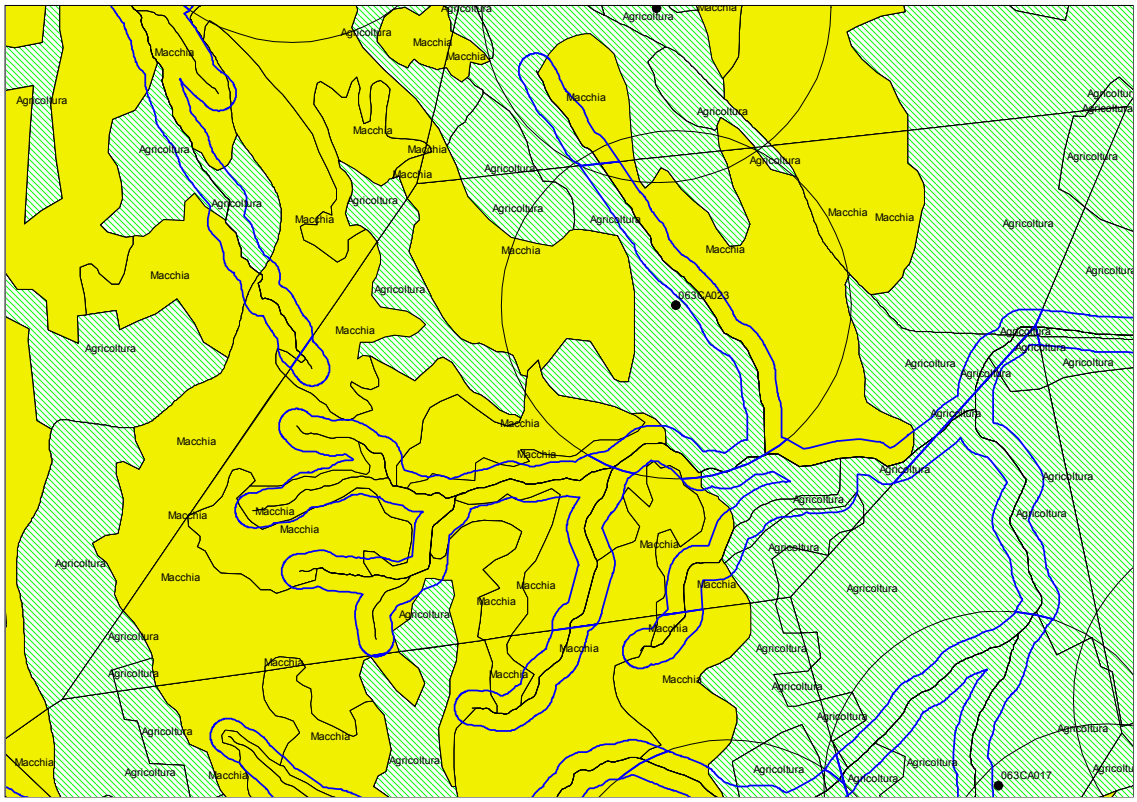


Fig 50. Map of land use in the pilot farm polygon.



Related altimetry issues (x2).

Each Voronoi polygon has been implemented with altimetrical relief (30x30 meters pixel grid), so that in the reference area of each breeding it was possible to identify the average elevation ranges from a minimum of 2.47 to a maximum of 156.56 meters above sea level (figure 51, 52). Subdividing the breedings within the outbreak

according to the median of the damage, we also examined whether there was a significant difference in the average elevation level of the polygons of pertinence in the breedings belonging to different groups.

Fig 51. Map of altimetry relief.

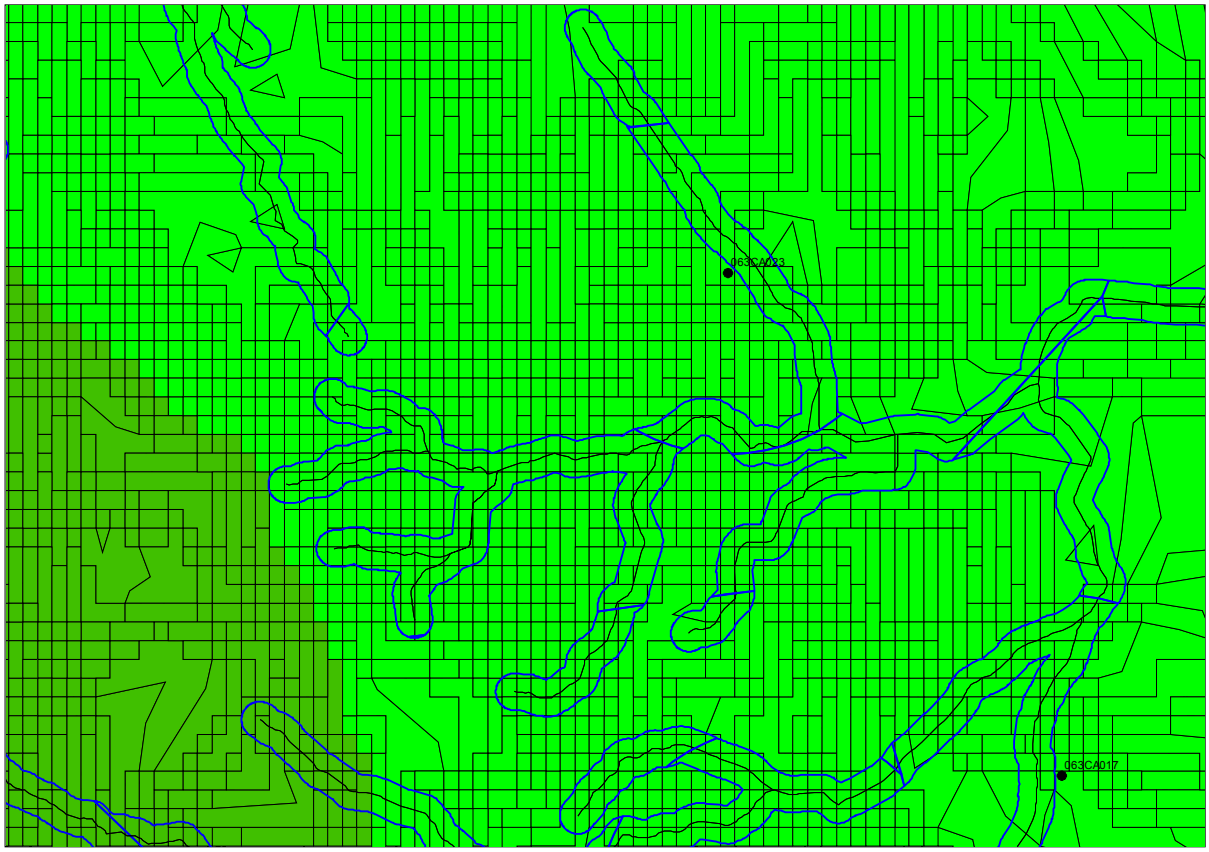
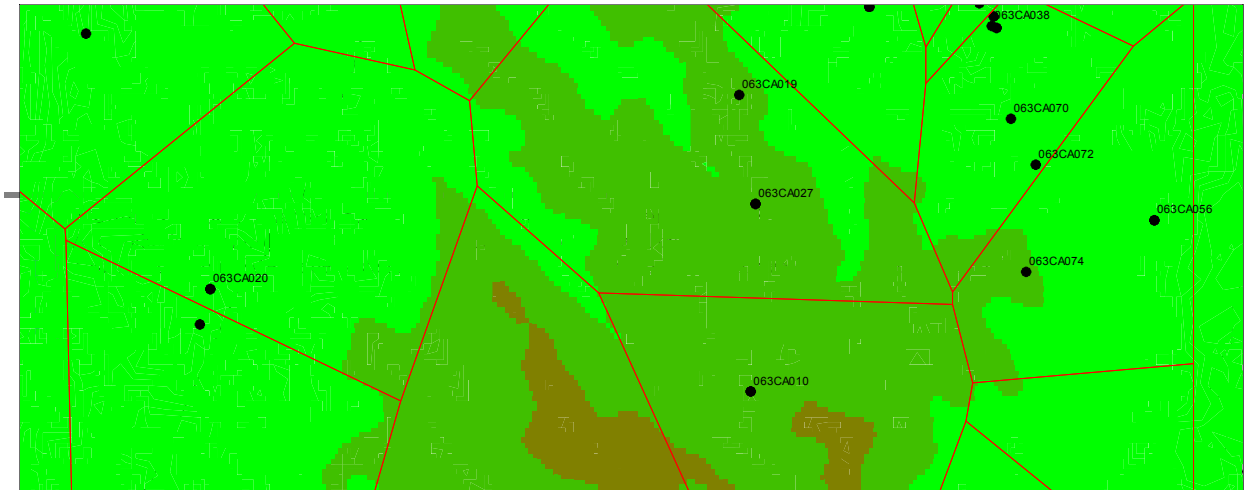


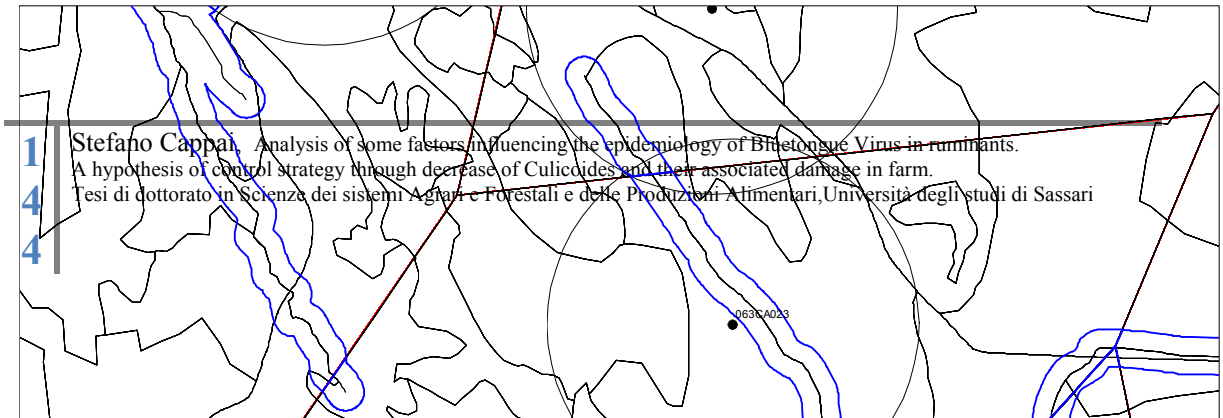
Fig 52. Particular complexity of the altimeter.



Aspects related to the presence of water courses (x3).

Each Voronoi polygon has been implemented with the hydrological map of the area, so it was possible to locate the presence of water courses for every breeding. For each localized hydrographic element, a buffer of 30 metres was created, and it was created for each polygon the total surface occupied by the buffer itself (figure 53).

Fig 53. Hydrographic map with a 30 metre buffer.



Correlated aspects of the ph of the soil (x4).

Each Voronoi polygon has been implemented with the map of the soil, properly divided into the type of acidic soil (AC) and alkaline (AK). In this way the reference area of all breedings were unable to determine the extension of different soil types.

Aspects correlated to the density of sensible animal species (x5, x6).

The number of animals within the polygon was calculated using data acquired by the BDN, in use at the IZS. For present animals we considered all animals belonging to the sensible species, such as sheep, goats and cattle.

Results and Discussion

It is known, and has been amply demonstrated that there is a strong correlation between vector density and risk for BT, and that the presence / absence / abundance of vectors is linked to specific environmental parameters, some of which are either fixed (land use, soil type, altitude etc.), others may vary over time (temperature, rainfall, lighting etc..). This correlation is now used to estimate the risk of BT through the mapping of these variables could represent the territory in the absence of direct information on the presence / absence / abundance of vectors. The information is gathered using data Ondestepoort black-light traps that are now the international standard for entomological surveillance of BT. Typically, the area around which are identified on the variables mentioned is about 16 sq. km (Meiswinkel et al., 2008). The territory that we considered is one of the hundred territories in which, in Italy, we observe the highest possible average in terms of vectors caught per night compared to all the territories controlled. We then examined whether there were significant differences in territory belonging to the individual polygons in terms of those variables related to the presence / absence / abundance of *C. imicola*, and the damage found in the first outbreak of BT (2000-2001), in order to transfer into the microcosm that is shown to be valid at the macro level (municipalities).

Development of the model only considers the ovine breedings (the only ones who have suffered measurable)

Model	R	R-square	Corretto R-square	Standard error. Of estimate
1	,771(a)	,594	,162	19,26867

a Stimatori: (Costante), dens_capre, dens_animali, dens_bovini, Abitazioni, Altro, Rocce, Dune, Impianti, fiumi_buffer 30 m Agricoltura, Bosco, area, Macchia, Sistema_Idrico, Media altimetria, Pascolo, SommaDiARTIF

ANOVA(b)

Model		Sum of squares	df	Average of squares	F	Sig.
1	Regression	8682,937	17	510,761	1,376	,264(a)
	Remainder	5940,504	16	371,282		
	Total	14623,441	33			

a Stimatori: (Costante), dens_capre, dens_animali, dens_bovini, Abitazioni, Altro, Rocce, Dune, Impianti, fiumi_buffer 30 m, Agricoltura, Bosco, area, Macchia, Sistema_Idrico, Media altimetria, Pascolo, SommaDiARTIF

b Variabile dipendente: danno

Coefficienti(a)

Model		Coefficienti non standardizzati		Coefficienti standardizzati	t	Sig.
		B	Errore std.	Beta	B	Errore std.
1	(Costante)	1,714	21,439		,080	,937
	Abitazioni	-10,091	18,088	-,118	-,558	,585
	Agricoltura	,393	5,255	,019	,075	,941
	Altro	402,034	514,076	,376	,782	,446
	Bosco	-10,152	16,822	-,210	-,604	,555
	Dune	-11,481	40,976	-,058	-,280	,783
	Impianti	-83,336	62,816	-1,059	-1,327	,203
	Macchia	6,248	5,298	,887	1,179	,256
	Pascolo	21,200	47,530	,277	,446	,662
	Rocce	**75,904**	**37,040**	**,501**	**2,049**	**,057**
	Sistema_Idrico	-36,121	59,794	-,298	-,604	,554
	Media altimetrica	,416	,265	,776	1,574	,135
	SommaDiARTIF	*-85,857*	*51,497*	*-1,515*	*-1,667*	*,115*
	Area totale	3,624	3,520	,311	1,030	,319
	fiumi_buffer30 m	-105,217	244,350	-,139	-,431	,673
	dens_animali	,062	,074	,216	,833	,417

dens_bovini	,470	1,983	,044	,237	,816
dens_capre	5,360	4,175	,260	1,284	,217

a Variabile dipendente: danno

For the verification of normality, we used a nonparametric test: Ks for a sample. Kolmogorov-Smirnov test for a sample (verification of the linearity of the distribution of the variable "damage").

		perdita
Numerosità		34
Parametri normali(a,b)	Media	19,3235
	Deviazione standard	21,05076
Differenze più estreme	Assoluto	,200
	Positivo	,200
	Negativo	-,179
Z di Kolmogorov-Smirnov		1,169
Sig. Asint. a 2 code		,130

a La distribuzione del test è Normale.

b Calcolato dai dati.

Since the Z K-S test is not significant, the distribution of the variable delay is normal so you can use linear regression. The results of regression analysis described above, the initial hypothesis to exploit a model of harm reduction by BT, through a series of direct actions on any predictor variables associated with it, if on a macroterritorial point of view can have a meaning (eg if the level of analysis is based on the least amount of municipal level), within the limited territory that we analyzed, there appears to be

passable. Supposed explanatory variables (x) of the damage variable (y) the subject of study, none showed significant correlation with the damage variable under study (y). Could not be observed, for example, at the level of land use, no correlation with the various types, either singly or grouped, except for the variable "rocks." Furthermore, no correlation between the amount of territory "naturally predisposed" to provide suitable sites for breeding of the mosquito vector, and the level of damage found in 2000 suggests that many larval outbreaks, at least those that may be responsible of the outbreak, are to be identified mainly in the vicinity of bodies corporate, particularly where animals are most active during the hours of the insect vector. Regarding the altimetry both parametric and non parametric test showed no significant difference in the average level elevation of the territory of relevance of each breeding. By analyzing the variable watercourses analysis of correlation between the level of damage and the area occupied for each polygon, from territories next to water courses, showed no correlation with the level of damage. The analysis was repeated assuming an area around the farm, but inside the polygons already identified, no larger than 300 meters without finding any correlation. Subdividing the farms within the outbreak in 2000, according to the median of the damage and identifying 2 groups like this one below and one above the median level of damage, and measuring any difference in terms of average amount of land held optimal for playback of insect vectors, there was no significant difference in the polygon is complex, both in the 300 meters next to the farms. The analysis on the spatial pH showed that virtually the whole of S. is characterized by acid soils type, except for a small portion of the territory do next isthmus connecting the mainland. Therefore this parameter will be considered a constant and not included in the assessment. The average density of animals of

susceptible species observed was of 81 animals / sq km (0,94-916), with median equal to 38.87 animals / square km. In polygons related to holdings in which damage was found in 2000 the difference between density below and above the median was not significant, and it was not possible to observe the linear correlation between damage and density of sensitive animals.

At this point, we thought it useful to change the working hypothesis, taking into account:

a. the abundance of insect vectors hypothetically responsible for the damage observed in 2000;
b. the relationship between population density per square km of insect vectors and quantity of insects caught by blacklight traps;
c. the relationship between insects caught in blacklight traps and insects emerging from the flickering traps placed near the fixed traps.

We then calculated the relationship between vectors and hosts, for each Voronoi polygon in which we have divided the territory. This report was developed by Harteemink et al. (2009), with the construction of a Next-genaration matrix (NGM).This NGM method is used to 'average' the expected number of hosts infected by one vector and the expected number of vectors infected by one host.

$$k = \begin{bmatrix} k_{11} & k_{12} & k_{13} & k_{14} \\ k_{21} & k_{22} & k_{23} & k_{24} \\ k_{31} & k_{32} & k_{33} & k_{34} \\ k_{41} & k_{42} & k_{43} & k_{44} \end{bmatrix}$$

The array contains 4 elements that are described:

- (1) the vector *C. imicola*;
- (2) the receptive sheep host;
- (3) the receptive goat host;
- (4) the receptive cattle host;

all present and represented in the territory that we considered. The element that we want to change in the assumed model is the density of the insect vector.

To calculate the matrix equal to 0 items can be made:

K11 because it is not been proven the translarval transmission to the insect; K22, K23, K24, K33, K32, K34, K44, K42 and K43 because it can not direct the transfer of BT in sheep, goats and cattle.

$$k = \begin{bmatrix} 0 & k_{12} & k_{13} & k_{14} \\ k_{21} & 0 & 0 & 0 \\ k_{31} & 0 & 0 & 0 \\ k_{41} & 0 & 0 & 0 \end{bmatrix}$$

Therefore, the elements that concern us are related to the transmission vector-susceptible animals and animal susceptible to the transmission-carrier, or in the case of the ovine:

$$k_{12} = \frac{acv}{(h_{ovino} + h_{caprino} + h_{bovino})\gamma_{ovino}}$$

he represents the entity "number of midges infected by one newly infected sheep" and that depends on:

- the average duration of infectiousness, denoted by $\frac{1}{\gamma_{ovino}}$;
- (a) biting rate, which equals the reciprocal of the length of the gonotrophic cycle;
- (c) the number of bites received by the host and the transmission probability per bite;
- (v) local midges density;
- (h) density of sheep.

Our goal at this point is to intervene on the density of the carrier (hypothetically) and thus act on the probability of having secondary cases, at the introduction of primary cases.

From the bibliography we conclude that:

a = 0,17 (Mullens et al., 2004)

c = 0,05 (Carpenter et al., 2006a), (Gerry et al., 2001), (Venter et al., 1998).

h = the data for density in each Voronoi polygons (h ovino, h caprino, h bovino) are detected by BDN (National Data Bank).

γ_{ovno} = 0.125 (Luedke, 1969)

γ_{bovino} = 0.04 (Bonneau et al., 2002), (Luedke et al., 1969), (Singer et al., 2001).

The other element to consider at this point is the number of sheep who become infected for each infected insect vector introduced, represented by:

$$k_{21} = \frac{h_{ovina}}{(h_{ovino} + h_{bovino} + h_{caprino})} * \frac{ab\left(\frac{q}{q+\mu}\right)}{\mu}$$

Where:

- a = biting rate;
- b = transmission efficiency midge to host;
- μ = midge mortality rate;

- q = exponential rate of becoming infect.

When the exponential rates of becoming infectious and the mortality rate are denoted by respectively q and μ, the probability to survive the EIP will be q/(q+μ).

And taking account of k_{12} and k_{21} by virtue of observations on the dynamics of vector insects, it is clear the usefulness of thinking in terms of intervention only in the months immediately preceding those at greater density of insects (May to November) with the intent to reduce this density, acting on breeding sites next to the animals. In our assessment, unlike the work of Hartemink, we take into consideration as insect vector, *C.imicola*, as has been amply demonstrated, in contrast to what happens for example in Northern Europe, that is the carrier involved in the transmission of BT in Sardinia. And catches in the months with higher occurrence of outbreaks in fact, are in fact almost exclusively *C.imicola* (figure 54.55).

Fig 54. Evolution of abundance of *C. imicola* captured from 2003 to 2005.

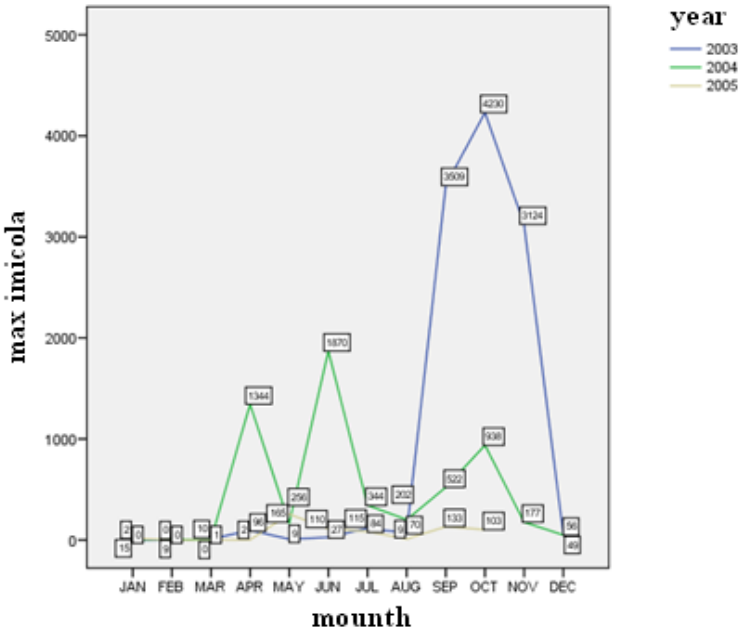
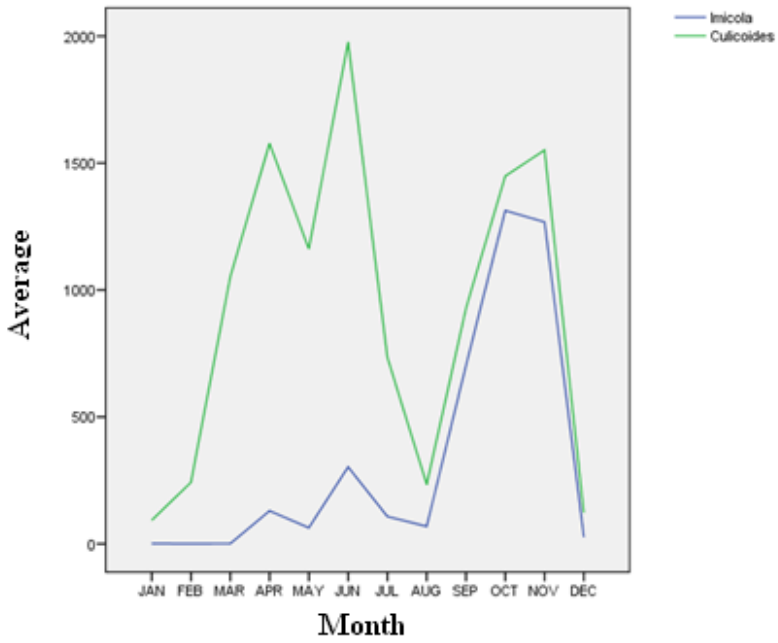


Fig 55. Difference between the average catch of *C. imicola* and *Culicoides spp.* performed in the fixed trap during the experiment.



Based on the above, we find the following the largest eigenvalue of this matrix, equivalente al valore R_0 (basic reprodution number), epidemiological indicators useful for measuring the evolvability of an epidemic (Diekmnan and Heesterbeek, 2000).

Largest eigenvalue = $R_0 = \sqrt{\frac{a^2 bcqvh_c}{\gamma_c (h_c + h_s)^2 \mu(q+\mu)} + \frac{a^2 bcqvh_s}{\gamma_s (h_c + h_s)^2 \mu(q+\mu)}}$

Thus, by decreasing the density of insect vectors, the idea is to indirectly maintain the value of R0 <1. The graph of Figure 56 shows the variation dl value R0, depending on the number of insects captured by the fixed trap. The threshold value which in theory should not be exceeded is 60 *C. imicola*. It is interesting to note that catches during the period at risk in 2003-2005, relating to the trap sets of S. Antioco are very different over the years, particularly in 2003, the density of *C. imicola* has far exceeded this threshold. Since the model described is related to a supposed virgin population, it is conceivable only in the case of introduction of new serotypes. But when we had to calculate the value R0 in partially immune populations, the model should be corrected, and may be subject to further developments.

Fig 56. Variation of the Basic Reproduction Number in accordance with the catch of the fixed traps.

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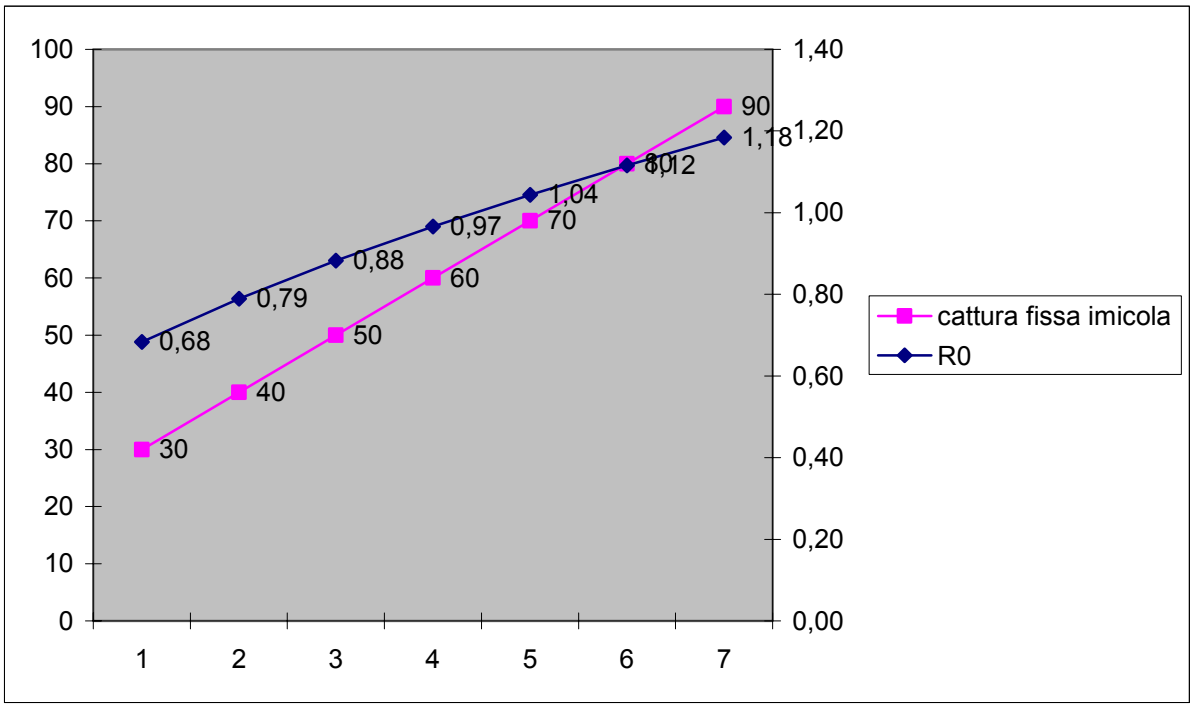


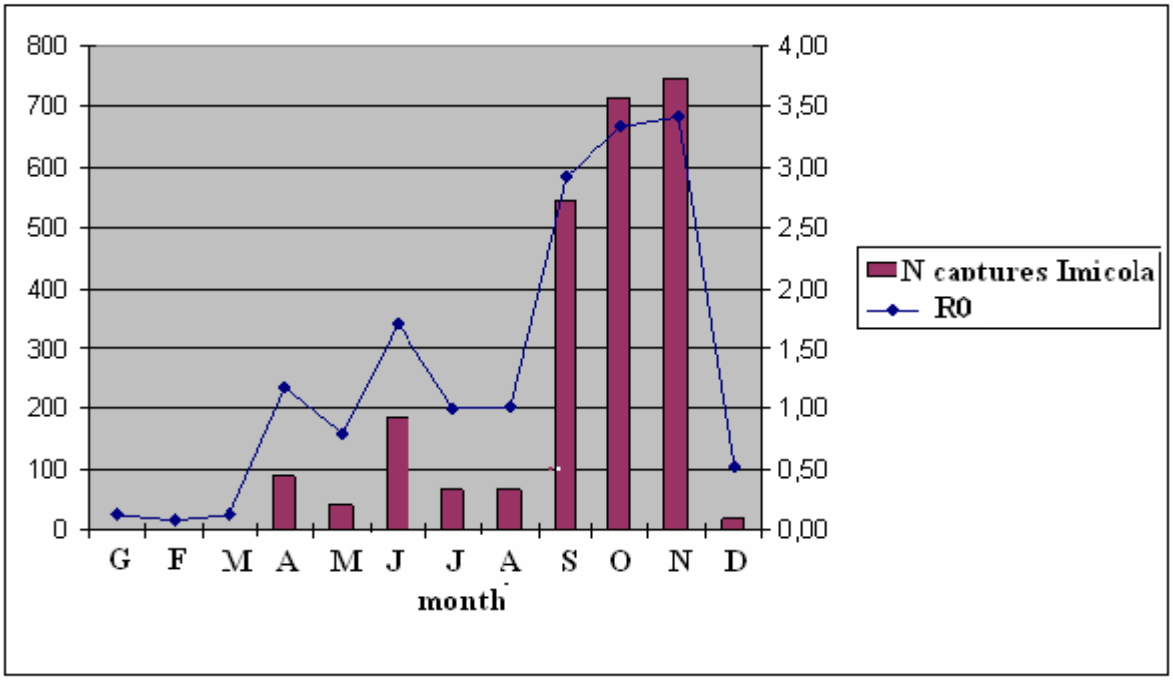
To solve the matrix and to identify the values of R0, (maximum eigenvalues - largest eigenvalue) for the months mentioned above, we used as an estimate of the density of insect vectors, the average monthly range those caught in the trap functioning in pilot companies indicated in Table 7.

Tab 7. Average estimates of R0 in the year.

	G	F	M	A	M	G	L	A	S	O	N	D
K_{12}	0,0545	0,0212	0,0575	4,5135	2,0480	9,3800	3,2976	3,3728	27,4123	36,0530	37,6668	0,9153
K_{13}	0,0003	0,0001	0,0003	0,0227	0,0103	0,0471	0,0166	0,0169	0,1376	0,1810	0,1891	0,0046
K_{21}	0,00064	0,00064	0,00064	0,00064	0,00064	0,00064	0,00064	0,00064	0,00064	0,00064	0,00064	0,00064
K_{31}	0,00004	0,00004	0,00004	0,00004	0,00004	0,00004	0,00004	0,00004	0,00004	0,00004	0,00004	0,00004
R_0	0,13	0,08	0,13	1,18	0,80	1,70	1,01	1,02	2,91	3,34	3,41	0,53

It is possible to notice that the density values found in the months of July, you will get the development of an outbreak, since theoretically, with this average density of vectors and the density of animals of susceptible species, there are always values for R0> 1 (Figure 57). In the table it is interesting to note that the value of R0 is extremely interesting at the time of introduction of new serotypes, while it is clearly less interesting in the case of movement of serotypes already introduced (Table 8).

Fig 57. Relationship between the values of R0 and density of *C. imicola* per month.



Tab 8. R0 values and estimated values of *C. imicola* found in the fixed traps.

year		july	august	september	october	november	december
2003 (BTV4)	Imicola	49	70	2479	2558	3124	56
	R_0	0.87	1.04	6.20	6.30	6.96	0.93
	outbreaks	0	2	12	5	0	0
2004	Imicola	208	130	206	380	177	49
	R_0	1.79	1.42	1.78	2.42	1.65	0.87
	outbreaks	0	0	0	0	0	0
2005	Imicola	25	7	41	62	40	2
	R_0	0.63	0.32	0.41	0.98	0.78	0.17
	outbreaks	0	0	0	0	0	0
2006 (BTV1)	Imicola	14	168	520	699	1609	134
	R_0	0.46	1.61	2.84	3.29	5.00	1.44
	outbreaks	0	0	0	0	12	8

From the experimental data collected during the years 2007 and 2008 in the pilot company, a correlation was observed between the number of insects caught by flickering traps, and the number of insects captured at the same time by the black-light traps. (R2 = 0.39) in the vicinity of the animals. The average ratio observed between vector density per square km and square meter of land useful for reproduction (larval outbreak) was 1 / 668, so it is conceivable that by removing a quantity proportional to the outbreaks should reduce the risk of infection and therefore of the damage found, keeping the level density below the threshold value. The intervention should be restricted to areas close to sites of shelter animals, and performed when the traps were still important signal resumption of activity, because, at least in the island of Sant' Antioco, there is a relationship between the damage suffered and the territory taken in its entirety. Therefore if it is confirmed on a larger scale that the modification work on larval habitat (eg on all herds of susceptible species of Sant'Antioco), would lead to a real reduction in the density of insect vectors (recordable the average data of captures of the trap sets), as much of the report for 1 larvae in the trap = 668 insects captured by the trap sets (blacklight trap), we provide a powerful tool that may contribute, either by reducing the value R0 and together with all other weapons in our possession, the limitation of damages. In particular, we estimated the extension in terms of land suitable for larval outbreaks, can produce a quantity of insect vectors of damages for each farm. To achieve the goal of maintaining the density of *C. imicola*, below the

threshold value suggested we propose a modification work on the habitat as described in our experiments. In particular, we estimated the extension in terms of land suitable for larval outbreaks, can produce a quantity of insect vectors of damages for each farm. Since there seems to be no correlation between the extent of areas suitable for breeding throughout the polygon Voronoi attributable to individual farmers, and the amount of damage found, we deem it useful to act locally, exclusively on breeding sites identified in the vicinity of . The extension of the treatment that we estimated using as benchmarks the average catches of the fixed traps of the pilot company in the following way:

surface to be treated = RS

R = average ratio of insects captured by the fixed traps / insects per flickering untreated traps (668 / 1)

S = surface trap throttle = 0.21 m2

Inserting the values observed we obtain an average value of 140.28 m2 of land to be covered for business. To evaluate a benefit cost compared to what in effect happened in the year 2000 without any intervention, we estimated the total cost of intervention for each polygon, intending to cover all the farms where animals are susceptible, and 55 companies husbandry (Table 9).

The main cost items are:

Assessment and identification of sites to be amended

For the evaluation and identification of sites suitable for larval production a daily inspection is required, assuming an average of 2 farms per day, it would be sufficient work of specialized professional figure 1 (veterinarians, agronomists) for 1 month. Agronomic interventions may be entrusted to technicians who operate on the basis of information received and collected during the inspection. Therefore, it is estimated the work of 1 technician for 2 months. Furthermore, it is crucial the work of the same owners for all actions of the Council for management possibly prevent the formation of these sites (such as rehabilitation of water losses by drinking, the rational disposal of sewage, etc. if any ploughing channelling etc.).

Agronomic interventions

Assuming the use of lime for all breeding sites identified in the companies and estimating a quantity of 80 grams (0.08 Kg) per square meter, this would require a quantity of 22.24 kg (0.08lbs x 278 m2), equivalent to about 1 bag 25 kg per farm. Throughout the territory would be sufficient then 55 sacks.

Tab 9. Estimation of intervention costs.

Operating cost	**Euro**
Vet / agronomist	3000
technician	4000

material	1000
Total	8000

Analyzing data on the damage found during the first outbreak that any sheep killed was refunded to farmers at an average price of 100 euros, to which was added an amount of further 120 euros for the lost income. A similar figure has been recognized for each sheep that died. Therefore, the total damage in euro (Table 10) calculated for the territory of the Island of Sant' Antioco was 144.540 euros.

Tab 10. Total economic damage in the town of S. Antioco that was compensated.

Municipality	Average for one herd	N	Standard deviation	Amount	Min	Max
CALASETTA	2965.6000	25	4884.01	74140.00	.00	19360.00
SANT'ANTIOCO	2346.6667	30	3546.13	70400.00	.00	13640.00
Totale	2628.0000	55	4177.51	144540.00	.00	19360.00

Conclusion

The difficult struggle to BT should be based on appropriate prophylaxis measures addressed to the direct health integrated pest insect vector. For this, assistance must be provided for bio-security, implemented in the field with:

- The structural adjustment of enterprises, so the farmers can also benefit from measures contributory;
- The reclamation of wetlands in the fields and farms, where they develop the larvae of the insect;
- Anti direct larvicidal with organic products (not synthetic) according to new findings of scientific research, which is addressed specifically in this work;
- Direct protection of animals;
- The preparation of an adequate system of control and monitoring for animals brought in Sardinia as to avoid new infectious agents;

- The training and health education of the farmers, who, appropriately sensitized take those precautions that health standards by reducing risk factors, are critical to the success of the struggle.

The results of this work have shown that it is possible through a targeted intervention to make it inhospitable to larval breeding sites, reducing significantly the development of the insect vector of BT. The alkalinization of the larval breeding sites proved in all respects an effective procedure for the reduction of the insect vector providing positive outcome to the hypothesis tested. The processing of data and observations collected during the experiment conducted by us we have also provided information on various "lifestyle" of the carrier, and how his presence can be interfaced with the density and location of species susceptible to the disease . The fight the insect vector and study of its interaction with our habitat appears to be an important phase of the strategy of struggle. The difficult realities of the island, due either to a particular aspect of geo-pedological deep ties to both the traditions and cultures must therefore make use of these alternative methods of struggle to counter one of the worst diseases ever to hit the island's livestock.

Future prospect

It seems important a future study based on enlargement of the sites most tractable to measure a decrease of *Culicoides* responsible for future epidemic waves, using the data developed by us in an optical reduction of direct damages and indirect holdings of sheep, goats and cattle in Sardinia. Reinventing our experiment with a range encompassing several farms grew considerably the inhospitable surface for the carrier,

and contains many suggestions on the actual effectiveness and future development of the hypothesis experimentally. The state of alert to the possible arrival of new serotypes already present in areas close to us is the basis of the best possible approach to the damage caused by the disease. It also appears to need a greater interaction with the farmer and specialized professionals who can provide agricultural guidance, structural and management of land and crops combined with farming, which could disadvantage the presence of larval breeding sites. It would be desirable therefore, a profound awareness campaign and information on a large scale.

ABBREVIATION

BT -Bluetongue

BTV -Bluetongue virus

CE -European Concil

CESME -Centro Studi Malattie Esotiche

EFSA - European Food Safety Authority

EU -European Union

MRL - Maximal residual limits

NAMP -Arbovirus Monitoring Program

NDVI - Normalized Difference Vegetation Index

ULV -Ultra Low Volume

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