SCIENTIFIC REPORT submitted to EFSA

Scientific review on African Horse Sickness[1]

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Scientific reviews on Classical Swine Fever (CSF), African Swine Fever (ASF) and African Horse Sickness (AHS), and evaluation of the distribution of arthropod vectors and their potential for transmitting exotic or emerging vector-borne animal diseases and zoonoses

Scientific review on African Horse Sickness

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ABBREVATIONS

AHS	African horse sickness
AHSV	African horse sickness virus
BT	Bluetongue
BTV	Bluetongue virus
OVI	Onderstepoort Veterinary Institute
RT-PCR	Real-Time Polymerase Chain Reaction
UV	Ultra Violet Rays

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AHS DEFINITION

African horse sickness (AHS) is an infectious but non contagious viral disease affecting all species of *Equidae* caused by an orbivirus of the family Reoviridae and characterised by alterations in the respiratory and circulatory functions. AHS is transmitted by at least two species of *Culicoides*. Nine different serotypes have been described.Laboratory diagnosis of AHS is essential. Although the clinical signs and lesions are characteristic, they can be confused with those of other equine diseases. As a viral disease, the laboratory diagnosis of AHS can be based on the identification of infectious virus, virus nucleic acid, viral antigens or specific antibodies. (Sánchez-Vizcaíno in the OIE Manual of diagnostic tests and vaccines for terrestrial Animals, 2008)

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AETIOLOGY

African horse sickness virus (AHSV) belongs to the family Reoviridae, genus *Orbivirus* and shares many morphological and structural characteristics with the other members of this genus, bluetongue virus (BTV) and equine encephalosis virus (EEV) (Spence *et al.*, 1984; Coetzer and Guthrie, 2004; ICTV, 2005). AHSV is subdivided into nine serotypes (named AHSV-1 to AHSV-9)(Howell, 1962). No new serotypes have been detected in recent years. There is partial antigenic relationship between these serotypes, with cross-reactivity of homologous antiserum to other subtypes in VN assay (cross-neutralization between types 1 and 2, 3 and 7, 5 and 8, and 6 and 9) (Coetzer and Guthrie, 2004). This cross- protection has been also observed in the field, and is a common practice in South Africa that polyvalent vaccines do omit some serotypes taking into account the cross-protection offered by other types (e.g. 5 and 9 not included, cross-protection by 8 and 6 respectively) (Coetzer and Guthrie, 2004).

Several reviews deal with the morphological, physico-chemical and biological properties of AHS (Alexander, 1935; Bremer *et al.*, 1990; Coetzer and Guthrie, 2004) and very few new data have been elaborated in recent years. The structure and composition of AHSV particles are comparable to those of bluetongue virus (BTV), the prototype virus of the genus. The genome of AHSV is composed of 10 double stranded RNA segments, enclosed within the core particle (Grubman and Lewis, 1992; Roy *et al.*, 1994). The 10 double-stranded (ds) RNA segments are designated by its size and increasing electrophoretic mobility in 1% agarose gels and by molecular weight as L1-L3 (large segments), M4-M6 (medium segments), and S7-S10 (small segments). These segments code for the seven structural proteins (VP1 to 7) and four non-structural proteins (NS1, NS2, NS3 and NS3A). The non-structural proteins are coded by the segments M5, S8 and S10. The two smallest proteins (NS3 and NS3A) are synthesized from the S10 RNA segment, probably from different in-frame translation initiation codons. Nucleotide sequences of all RNA segments and the predicted amino acid sequences of the encoded gene products are available (Grubman and Lewis, 1992; Roy *et al.*, 1994; Vreede and Huismans, 1998).
Virions are non-enveloped particles of approximately 70 nm in diameter, structured as a two-layered icosahedral capsid composed of 32 capsomeres.
The core of the virion is formed by two major proteins, VP3 and VP7, highly conserved among the nine AHSV serotypes and responsible for the group specific typing, and three minor proteins, VP1, VP4 and VP6. (Bremer *et al.*, 1990; Hewat *et al.*, 1992; Grimes *et al.*, 1995).
An outer capsid composed of VP2 and VP5 is mainly responsible for serotype antigenic characteristics; neutralizing epitopes are mainly located in VP2 (Burrage *et al.*, 1993; Vreede and Huismans, 1994; Martinez-Torrecuadrada and Casal, 1995).

The physio-chemical properties of AHSV are similar to other members of the genus *Orbivirus*. The virus is sensible to low pH, being readily inactivated at pH values below 6.0. However, it is relatively stable at alkaline pH values (7.0–8.5). As other orbiviruses, AHSV is considered to be relatively resistant to treatment with solvents, or detergents, although the sensitivity to specific

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detergents varies with virus species. However, sodium dodecyl sulphate will disrupt the particle and destroy its infectivity. AHSV is also relatively heat resistant. Infectivity in blood or tissues is remarkably stable at 4 °C, particularly in the presence of stabilisers. Freezing at temperatures between –20 °C and –30 °C causes significant loss of titre. This does not occur under lyophilisation or temperature at –70 °C or lower (Coetzer and Guthrie, 2004; Mellor and Hamblin, 2004).

Summary as provided by the authors:

- AHSV share basic structural features of other orbiviruses as BTV, although knowledge of AHSV properties is not as deep as for BTV.

Future research identified by the authors

- Sequencing of different serotypes and genomic fragments.

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PATHOGENESIS

General information of AHS pathogenesis proceeds from classical studies (Erasmus, 1973), and there are no major advances in the knowledge of the disease on recent years. A good description of the pathogenesis and clinicopathological findings of AHS can be found in recent reviews and veterinary textbook chapters (Coetzer and Guthrie, 2004; Mellor and Hamblin, 2004).

AHS is a generalised disease of blood and lymphatic vessels, and clinical signs and lesions are related to endothelial damage and increased permeability, varying in severity with the infecting AHSV strain and serotype and the host susceptibility (Laegreid *et al.*, 1993; Coetzer and Guthrie, 2004). After biting of an infected *Culicoides*, the virus multiplies in endothelium of lymph capillary vessels and regional lymph nodes, and a primary viraemia occurs. Dissemination to capillary vessels of many organs then occurs, mainly to the lungs, large intestine, and lymphoid organs, causing a secondary viraemia (Coetzer and Guthrie, 2004; Mellor and Hamblin, 2004).

In susceptible horses, viraemia may last between 4 and 8 days, and rarely is longer than 21 days. A titre of up to $10^{5,0}$TCID$_{50}$ of virus/mL may be observed. The onset of viraemia is usually accompanied by fever, which persists until viraemia disappears (Laegreid *et al.*, 1993; Coetzer and Guthrie, 2004). In blood, AHS virus is closely associated with the erythrocytes, with very few virus in plasma (Laegreid, 1996). In these studies, data on viraemia have been determined using virus isolation. In one study (Sailleau *et al.*, 1997) RT-PCR assay has been compared with virus isolation for detecting AHSV in blood samples form horses experimentally infected with AHSV-4 and AHSV-9. RT-PCR and virus isolation were equally sensitive for detection of AHSV-4, although viraemia was detected more consistently by RT-PCR than by virus isolation from horses infected with the less virulent AHSV-9. Comparisons of virus isolation and molecular techniques for determination of the length of viraemia in horses are not available. As in the case of BTV in ruminants (European Food Safety Authority EFSA, 2007), a much longer RT-PCR viraemia than virus isolation viraemia would be expected.

Several studies have contributed to clarify the pathogenesis of AHS in donkeys and zebras (Barnard *et al.*, 1994; Hamblin *et al.*, 1998). In these species, considered to play a role as reservoirs of AHSV, a subclinical infection occurs in most cases. Donkeys experimentally infected with AHSV-4 develop viraemia which can persist for at least 12 days as determined by virus isolation, albeit at a comparatively lower titre than that recorded for ponies similarly infected. In another study, viral RNA was detected consistently up to day 47 in donkeys (el Hasnaoui *et al.*, 1998).

AHSV infection in zebras is usually subclinical, and the viraemia in this species is significantly longer in experimentally infected zebra than in horses. The virus could be isolated 40 d post-infection from blood and 48 d post-infection from spleen. Due to this subclinical, viraemic status, zebra may act as a vehicle of introduction of AHSV into free areas (Barnard *et al.*, 1994).

Summary as provided by the authors:
- AHS is a systemic disease of blood and lymphatic vessels with endothelial damage as the main basic feature.
- In susceptible horses, viraemia may last between 4 and 8 days, rarely longer than 21 days.

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- RT-PCR and virus isolation seem to be equally sensitive for detection of AHSV, but few studies are available.

Future research identified by the authors

- Detailed comparison of viraemia duration and infectivity using RT-PCR and virus isolation.

CELLULAR TROPISM

Very few studies have dealt with cell and tissue tropism of the virus. Using *in situ* hybridisation on tissues of ponies in the terminal stages of infection with AHSV, virus was found distributed widely in sections of lung and heart, with relatively less amounts in spleen and neck muscle. Infected cells were morphologically identified as endothelium in all organs except spleen, where large mononuclear cells appeared also infected (Brown *et al.*, 1994). In ponies experimentally infected with AHS-4 (Wohlsein *et al.*, 1997), AHSV antigens were detected with inmunohistochemical techniques in formalin fixed, paraffin embedded tissues collected at 8-10 days after inoculation (at terminal stage of the disease). Although AHSV could be found in many organs, cell tropism of the virus appeared also to be restricted to endothelial cells, some cells of the macrophage lineage and reticular cells in lymphoid organs. No AHSV antigen was detected in myocardial muscle cells, or in lymphocytes.
These observations have been confirmed ultrastructurally, with virus replication usually found to occur in endothelial cells (Laegreid *et al.*, 1992; Carrasco *et al.*, 1999). In a recent experimental infection in horses with AHSV-4, virus replication was found also in pulmonary intravascular macrophages, interstitial macrophages, and also fibroblasts (Carrasco *et al.*, 1999). Replication in endothelial cells is followed by cellular damage, with alteration of intercellular junctions, loss of endothelium, increased capillary permeability, and subendothelial deposition of cell debris and fibrin. Oedema, haemorrhages and microthrombi may be seen in many organs, particularly in the myocardium and lung (Gomez-Villamandos *et al.*, 1999).

The tissue tropism of AHSV-4 in the donkey is similar to that of the pony, but the virus appeared to replicate less efficiently in donkey tissues (Hamblin *et al.*, 1998).

Summary as provided by the authors:

- AHSV infects primarily endothelial cells and some cells of the monocyte-macrophage lineage.

Future research identified by the authors

- Detailed organic distribution of cells infected by AHSV by studies with MoAbs.

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CLINICAL SIGNS

AHS in horses usually presents as one of the following four clinical forms: (i) Pulmonary or acute, (ii) cardiac or sub-acute, (iii) mixed or cardio-pulmonary, and (iv) AHS fever (Erasmus, 1973; Coetzer and Guthrie, 2004; Mellor and Hamblin, 2004). The occurrence of one or another form of the disease is determined by innate resistance, acquired heterologous resistant, and virulence of the infecting serotype and strain of AHSV (Coetzer and Guthrie, 2004; Mellor and Hamblin, 2004). In naive horses the form of disease expressed is mainly related to variants of the AHS virus inoculum (Laegreid *et al.*, 1993; Skowronek *et al.*, 1995).

The pulmonary form is a highly fatal acute disease, characterized by fever, depression and severe dyspnoea. The animal extends its head and neck, and frothy nasal discharge from fluid coming from pulmonary alveolar oedema may appear. The prognosis of this AHS form is very poor, and mortality often approaches 95%. Sudden death may occur without previous indication of illness.

The cardiac or sub-acute form of AHS is characterised by fever lasting several weeks. In ponies experimentally infected with AHS-4, fever occurred 4-6 days after infection (Wohlsein *et al.*, 1997).

Subcutaneous oedema of the head and the neck are the most relevant clinical finding. There is also marked supraorbital oedema and congestion of the conjunctivae. Haemorrhages in the conjunctiva may be also observed. Mortality in this form may be around 50%.

Most cases are of the mixed form, a combination of the cardiac and pulmonary forms of disease. This form also causes a high mortality rate, of approximately 70%, with death usually occurring within 3 to 6 days after onset of fever (Coetzer and Guthrie, 2004).

Finally, Horse sickness fever is invariably mild, with very few clinical signs. Animals showing this form usually have only mild to moderate fever and facial oedema, and then recover. This form occurs when horses have partial immunity to the infecting serotype or following infection with less virulent strains.

Endothelial damage induces more or less pronounced haemostatic abnormalities in the different forms of disease. Haematological findings include activation of the coagulation and fibrinolytic systems, with clotting factor consumption (acute and sub-acute forms), as well as leucopoenia, and decreased platelet counts. In AHS fever, some horses show mild thrombocytopenia and leucopoenia (Skowronek *et al.*, 1995).

In resistant species, as the African donkey and zebra, the fever form of AHS is the clinical presentation usually observed (Coetzer and Guthrie, 2004).

The infective dose of AHSV for dogs is high, and the course of the disease is usually similar to the pulmonary form of AHS in horses, with fever and respiratory distress, and high mortality.

Summary as provided by the authors:

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- In susceptible horses, the clinical form of AHS depends largely on the virulence of the infecting strain.
- The mixed form is the most frequently observed presentation in horses.
- Subclinical infection (fever form) is the most frequent form in zebras.

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LESIONS

Gross lesions at necropsy depend on the clinical form of the disease. In the pulmonary form, alveolar and interstitial oedema of the lungs and hydrothorax are the main findings. Oedema around the loose connective tissue of the trachea and of the aorta, and hyperaemia of the fundic portion of the stomach are also frequently observed. Petechial haemorrhages in the peritoneal, pleural and pericardial serosa may be seen, as well as hydropericardium. Many horses show frothy whitish or pink fluid arousing from the nares.
In the cardiac form, oedematous infiltration of the head and neck musculature is the most prominent lesions, together with subcutaneous oedema of the head. Hydropericardium is consistently present, as well as haemorrhages in epicardium and endocardium. Pale areas of myocardial degeneration have been described. Lung oedema may be also present.
In the mixed form, a combination of these lesions occurs. In fact, most of the fatal cases of AHS may be considered mixed forms, with lesions of the cardiac or the pulmonary form predominating (Coetzer and Guthrie, 2004).
Fatal cases in the dog usually resemble the pulmonary form of horses, with congestion and oedema of the lungs as main findings (Van Rensberg *et al.*, 1981).

Summary as provided by the authors:

- Oedema in subcutaneous and intermuscular tissue and pulmonary oedema are the most striking findings in AHS.
- Haemorrhages are also a frequent finding in pleura, pericardium, and endocardium.

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HOST RANGE

AHS is well adapted to Zebra, which is considered the natural host and also the main reservoir of the virus in Africa. Other equine species and their crossbreeds are susceptible to AHSV infection, and with the exception of Donkey, usually show clinical disease and high mortality. Other known susceptible species are dogs, wild carnivores (hyenas and jackals), elephants and camels (Binepal *et al.*, 1992; Baba *et al.*, 1993; Barnard, 1998; Coetzer and Guthrie, 2004; Mellor and Hamblin, 2004). Dogs get infection probably by the oral route, by consumption of meat from infected animals died form the disease (Van Rensberg *et al.*, 1981). However, the role of dogs in the epidemiology of AHS is considered to be irrelevant, as vectors do not appear to be attracted to them (Braverman and Chizov-Ginzburg, 1996). Serological surveys indicate that natural AHS infection is widespread among a diversity of African wild carnivore species (Alexander *et al.*, 1995). Similarly to dog, it is believed that infection results from ingestion of meat and organs from AHS-infected prey species. The effect of AHSV on these species is largely unknown, as is their role in the maintenance cycle of the disease (Alexander *et al.*, 1995).
Although as much as 10 of 104 (10,4%) camels (*Hyalomma dromedarii*) were seropositive in a survey for AHS in Nigeria (Baba *et al.*, 1993), infection in camels is considered unapparent and rare. Level and duration of viraemia are unknown in this species. Altogether makes unlikely that camels play any role in the epidemiology of AHS (Awad *et al.*, 1981).
The African elephant (*Loxodonta africans*) show also low levels of antibodies to AHSV, and they are regarded as poorly susceptible and unlikely to be a reservoir of AHSV (Barnard *et al.*, 1995).
In a serological survey, sheep and goats did not show antibodies to AHSV, so at least under natural conditions, these species are not susceptible.

Summary as provided by the authors:

- AHSV host range comprises horses and other equines, elephants, camels, dogs and wild carnivores.
- Horses, donkeys and their hybrids, and zebras, are the only species playing a significant role in the epidemiology of AHS.

Future research identified by the authors

- Knowledge on duration of viraemia, infectiosity for vector, and exact role of non-equine species on the epidemiology of AHS.

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TRANSMISSION ROUTE

There are three major routes of transmission of AHS: 1) by spreading of infected vetors, 2) by transportation of carrier wild or domestic animals and/or 3) infected animals. AHS etiological agent was identified as a midge belonging to the *Culicoides* genus, which become infected when feeding on infected horses (Du Toit, 1944). The most significant vector seems to be *Culicoides imicola*, but other species, such as *C. variipennis*, which is common in many parts of the United States and *C. bolitinos*, present in Africa, should also be considered as potential vectors (Boorman et al., 1975). Infection occurs mostly in the warm, rainy season when midges are plentiful, and disappears after frost, when the midges die. Most animals become infected in the period from sunset to sunrise, when the midges are most active. It is important to underline that infected and carrier animals play also an important role in the persistence of the disease: in Africa, in the areas were zebras are present the virus is circulating; when the population of zebras decreases, so does the cases of AHS (Mellor and Hamblin, 2004). In the last decades, a change in the climatic conditions has been reported worldwide. This change has made suitable seasons for Culicoides midges to last for longer time. Therefore, the overwintering period has decreased or even disappeared, favoring the persistence of the vector and its spread to regions over 50ºN (Mellor and Leake, 2000)

Generally, two different scenarios can be found in Europe and the Mediterranean basin:

1) Midges disperse only a few kilometers from their breeding sites, but it has been postulated that they can be borne for longer distances on air currents (Sellers et al., 1977, Cicuéndez, 2007). This phenomenon was studied in the Straits of Gibraltar (South Spain) where it was shown that sand storms dragged Culicoides from the North of Morocco to the South of Spain and, invariably, these storms were followed by bluetongue outbreaks (López-Martínez and Sánchez-Vizcaíno personal communication, 2008). Analysis of field observations on the progression of outbreaks indicates that wind-borne spread of midges may assist the short-distance spread of the disease but that long-distance jumps of the infection are invariably the result of movement of infected Equidae.
2) Ttransportation of infected or carrier wild and domestic animals may provoke outbreaks in places located thousands of kilometers from the origin. This was the case for the outbreak that took place in Spain and Portugal between 1987 and 1990, caused by the importation of infected zebras from Namibia (Sánchez-Vizcaíno, 2004). Zebras were transported to Spain via Lisbon. One group of zebras remained in Madrid province and the other was transported to Alicante, in the West coast of Spain. No explanation was found then to the fact that the disease was not observed in the West coast. Further epidemiological studies showed that the low density of Culicoides present in the West Coast was not enough to spread the disease (Amparo Martínez PhD thesis). However, the zebras that remain in Madrid province got in contact with the Culicoides midges present in the area and the disease spread to three provinces with a total of 27 cases reported and 250 dead horses (Sánchez-Vizcaíno, 2004). The movement of these infected animals provoked an outbreak more than 7000 km from their origin.

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During the 1987–1990 outbreaks of AHS in Spain and Portugal, AHSV isolations were made from mixed pools of Culicoides consisting almost entirely of C. obsoletus and C. pulicaris but excluding C. imicola (Mellor et al., 1990). This finding suggested that one or both of these species might also be involved in the transmission AHSV in Europe (Mellor et al., 1990). C. obsoletus and C. pulicaris group midges were again implicated as vectors of BTV during the 1998–2003 incursions of that virus into Europe and during the outbreaks caused by BTV serotype 8 in the North of Europe (Mehlhorn et al., 2007, Calvete et al., 2008). Since BTV and AHSV utilise the same species of Culicoides as vectors, it is probable that in regions where C. obsoletus and C. pulicaris are abundant future incursions of the equid virus could also extend beyond the distribution of C. imicola (Mellor and Hamblin, 2004).

Summary as provided by the authors:

- AHSV is transmitted by midges from the *Culicoides* genus.
- Midges can cover few kilometres carried by air currents. Long-distance jumps are due to animal movements
- Asymptomatic animals play an important role in the persistence of the disease.

Future research identified by the authors

- Culicoides species involved in AHSV transmission. Role of the climate change.

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GEOGRAPHICAL DISTRIBUTION

African horse sickness (AHS) is endemic in tropical and sub-tropical areas of sub-Saharan Africa. It spreads regularly to Southern Africa and only occasionally to Northern Africa. A few outbreaks have occurred outside in Africa, in the Near and Middle East and Iberian Peninsula.

In the Republic of South Africa outbreaks of AHS were recorded in almost the whole country. The decline in the number of AHS outbreaks over the last decades of the 20th Century, particularly in the southern areas of South Africa, is partly due to the elimination of large free-ranging populations of zebra (*Equus burchellii*), which are considered to be the natural cycling host for the virus (Venter *et al.*, 2006).

In the period from 1959 to 1961 the serotype 9 of the AHS virus (AHSV) spread outside Africa across Saudi Arabia, Lebanon, Syria, Jordan, Iraq, Turkey, Cyprus, Iran, Afghanistan, Pakistan and India. At the end of 1961, following a mass vaccination campaign and the death of over 300,000 equines the disease in Asia came to an end (Mellor and Hamblin, 2004).

During 1965 AHSV-9 spread again beyond the sub-Saharan area into Morocco first, and then Algeria and Tunisia. On October 1966 the disease appeared in Spain, in the area around Gibraltar and a total of 637 animals died or were slaughtered (Rodriguez *et al.*, 1992). The virus was eliminated from Spain within three weeks, following the application of a vaccination and slaughter policy (Mellor and Hamblin, 2004).

After 21 years, in September 1987 the AHSV-4 presence was diagnosed in a Safari Park near Madrid. The outbreak was apparently caused by the importation of a number of sub-clinically infected zebra from Namibia. The disease spread through the Alberche and Perales river basins, affecting a strip of land 100 km long and 50 km wide. One hundred forty six (146) Equidae died or were destroyed until December 1987, when the epidemic apparently ended. In the interim a total of 38,000 animals were vaccinated using a polyvalent attenuated vaccine (Rodriguez *et al.*, 1992).

One year later, at the beginning of October 1988, and almost 600 km away from the previous outbreak, AHS was diagnosed in the province of Cadiz. The disease affected various municipalities in the south of the provinces of Cadiz and Malaga, and was responsible for a total of 156 deaths. The last AHS case was reported in December of 1988. About 18,000 animals were vaccinated, at first with an attenuated polyvalent vaccine and later with a Type 4 monovalent vaccine. On July 1989, the epidemic restarted involving the provinces of Badajoz, Cadiz, Huelva, Cordoba and Sevilla. Cases of AHS were reported in September of 1988 in the Portuguese Algarve and Morocco declared the presence of the disease in the north of the country, near the Straits of Gibraltar, in October 1989. In Spain, 110 animals died directly as a result of the disease and more than 900 were destroyed. Approximately one hundred outbreaks were seen in the five provinces affected before January of 1990. A monovalent, Type 4 modified live vaccine was administered to 242,000 susceptible animals in 12 provinces, thus establishing a buffer zone which in many cases extended over more than 250 km from the most peripheral detected outbreaks (Rodriguez *et al.*, 1992). In Portugal, the control measures applied, based on mass vaccination of all susceptible animals, stamping-out in infected farms ans strict control of animal movements, were able to eradicate the infection within 13 weeks ((Portas *et al.*, 1999). Totally 206 AHS cases in 16 counties were notified in Portugal (Portas *et al.*, 1999).

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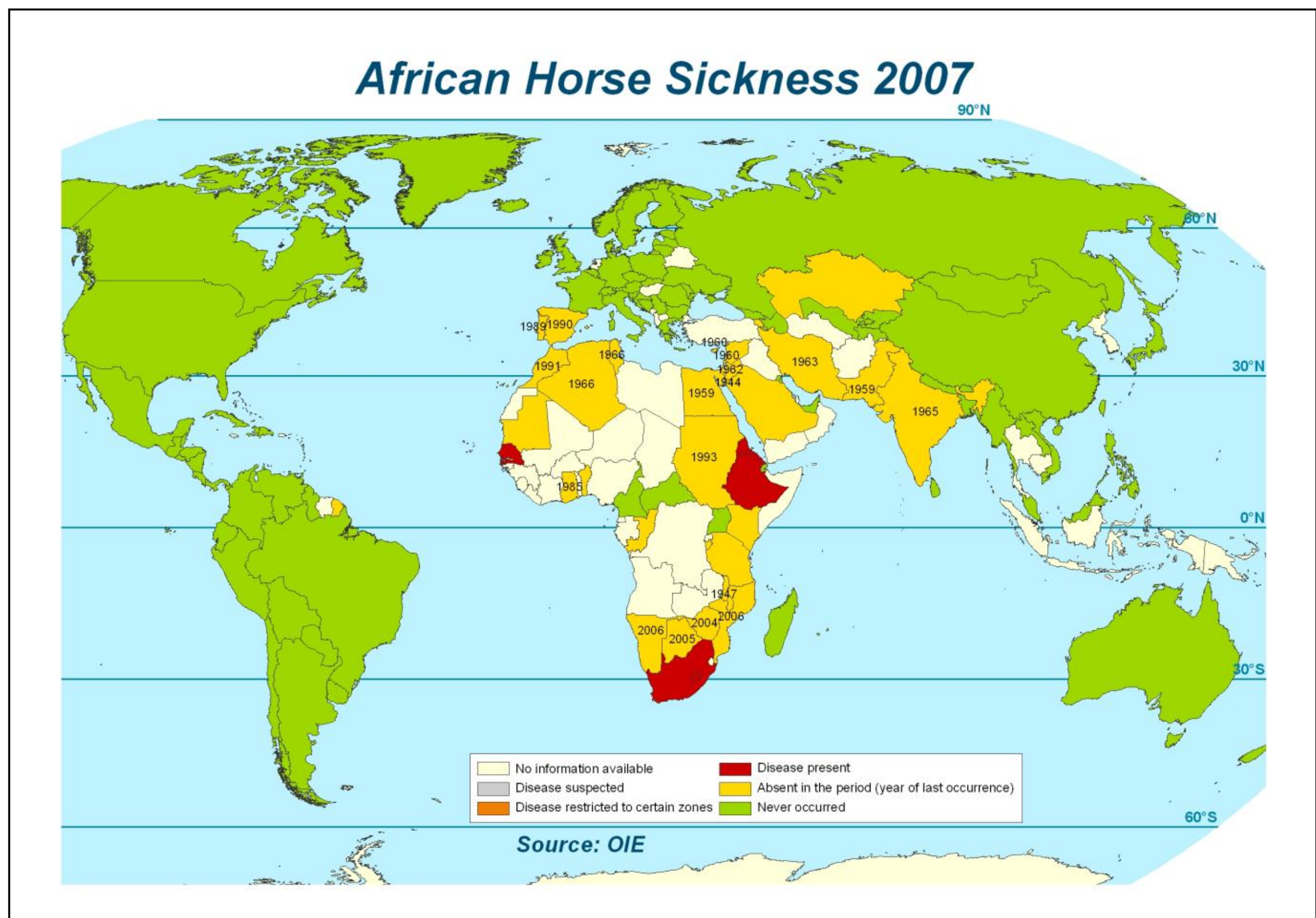
At the beginning of September of 1990, a new case of AHS was confirmed in the province of Malaga. The disease occurred within the territorial limits of 12 towns of this province, including Torremolinos and Mijas, and caused 66 deaths. The last case was diagnosed in November 1990. Mandatory vaccination of the entire equine population of Andalusia was carried out. During the same time-period new cases of AHS were reported in northern Morocco, once again due to the Type 4 AHS virus, but the disease did not reappear in Portugal (Rodriguez *et al.*, 1992).

In Morocco the disease involved 3 provinces in 1989 (Larache, Tanger and Tetouan) with a total of 512 AHS cases. In 1990 the diseases spread into other 4 provinces (Chéfchaouen, Kénitra, Sidi Kacem and Taounate) with a total number of 555 cases (Lhafi *et al.*, 1992). After the epidemic of 1989-1991 involving the Iberian Peninsula and Morocco, the presence of the disease was not notified outside its endemic sub-Saharan area, with the exception of Yemen, where the disease was sporadically detected.

In **Figure 1** is presented the last available picture of AHS global distribution, based on the 2007 six-monthly reports to OIE by National Veterinary Authorities.

Figure 1. African horse sickness geographical distribution (source: OIE).



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In **Table 1** the number of outbreaks notified to OIE in the period from 1996 to 2004 is presented. During the last 3 years it is noteworthy the situation in Senegal (58 outbreaks notified in 2007) and in South Africa, where the disease is appeared also in the southern part of the country, in the Western Cape region (**Figure 2**).

Table 1. Number of outbreaks notified to OIE (1996-2004).

Country	1996	1997	1998	1999	2000	2001	2002	2003	2004
Botswana	1	1		6	3	11	1	1	2
Burkina Faso		40		3		1	1		
Cape Verde				23					
Eritrea			7	1	1	1	1	1	2
Ethiopia	54	30	110	35	14	63	28	101	45
Gambia	2	7							
Lesotho			1					2	
Namibia		1		2	2	7	1		
Senegal	1	17	4	13	5	13	10	6	5
South Africa	91	22	46	100	186	88	123	89	196
Swaziland									1
Yemen		1						14	
Zambia									2
Zimbabwe	8	1	1	12	3		1	2	1

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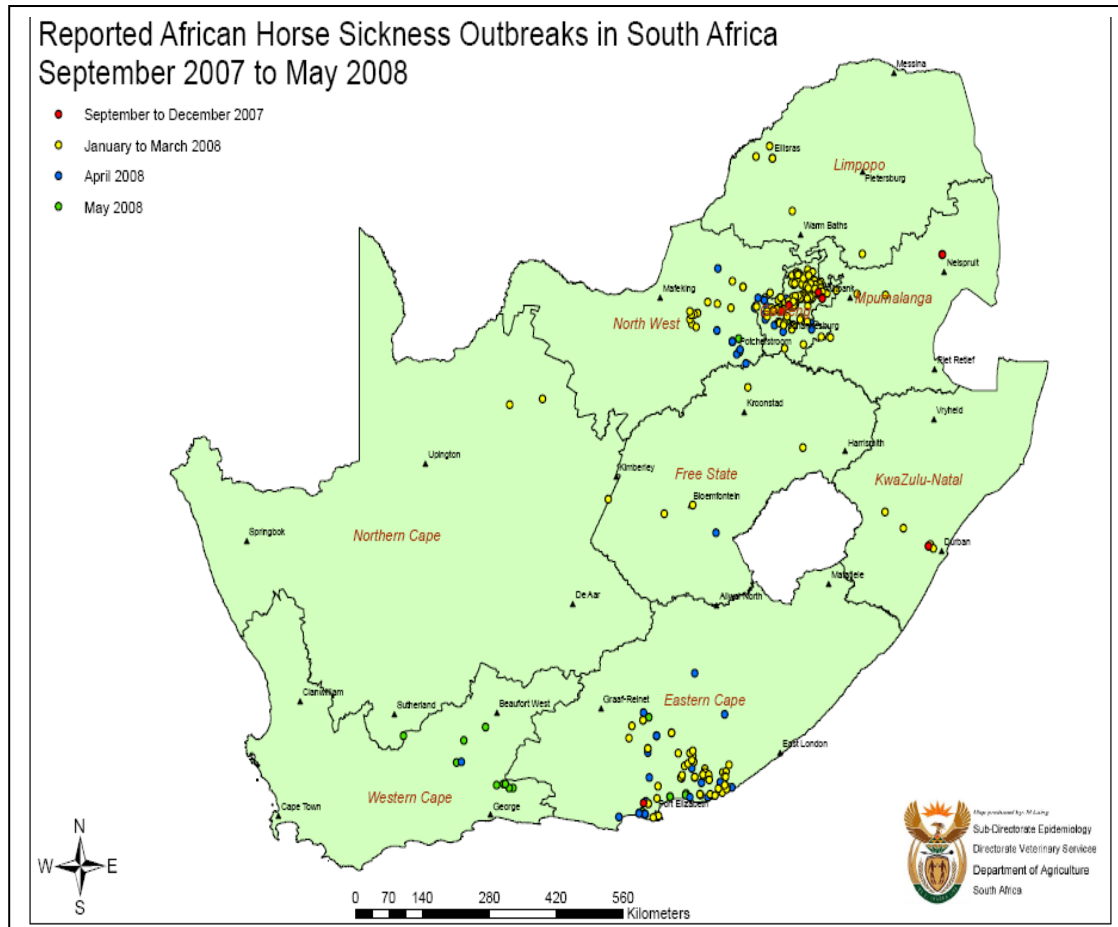
Figure 2. geographical distribution of 2007-2008 outbreaks (until May 2008) in South Africa (source: Directorate Veterinary Services of South Africa, http://www.nda.agric.za/vetweb/Epidemiology/Disease%20Maps/AHSSept07toMay08.pdf)



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Summary as provided by the authors:

- AHS virus is mainly confined in the sub-Saharan continent with rare raids to North Africa. Yemen, where the infection seems to be endemic, represents, however, an important exception.
- One large incursion of AHS in the Mediterranean Basin occurred in the period 1959-1961.
- Spain experienced two epidemic episodes: in 1966 and in the period 1987-1990. In the latter Portugal also was involved. In both epidemics Morocco was infected.

GEOGRAPHICAL DISTRIBUTION OF AHS VECTORS

The major vector of AHSV in the African continent is *C. imicola*, and for many years this species was considered to be the only *Culicoides* involved in AHSV transmission in the field (Mellor and Hamblin, 2004). A second vector was involved in the AHS transmission in South Africa. In year 2000, *C. bolitinos* was indicated as possible AHS vector in South Africa (Venter *et al.*, 2000; Meiswinkel and Paweska, 2003). However, as long ago as 1975 it was shown that *C. sonorensis* (*C. variipennis*) the North American vector of BTV is also an efficient laboratory vector of AHSV (Boorman *et al.*, 1975).

In the Mediterranean Basin the occurrence of AHS in the past was always linked to the presence of *C. imicola*. Nowadays, following the large European epidemics of bluetongue in the Mediterranean and Continental European countries, where also *Culicoides* species other than *C. imicola* played a significant role in the virus transmission and in the overwintering mechanism, the area at risk for AHS spreading should be extended far beyond the *C. imicola* area. In fact, other *Culicoides* species must be taken into account as potential vectors for AHS: species belonging to Obsoletus, a Pulicaris Complexes. However, the role of some European bluetongue vector species, namely *C. dewulfi* and *C. chiopterus*, as possible vectors of AHS need to be carefully assessed in relation to the possibility of their reproduction on horse dung.

Although the European Commission made a great effort for the establishment of a harmonised entomological surveillance scheme across all the EU, an unique and integrate picture of *Culicoides* distribution and abundance is not currently available. Several national studies, however, permitted to obtain useful information on the geographical distribution of the main *Culicoides* vectors.

In the following pages a selection of *Culicoides* distribution maps for Europe are presented.

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Summary as provided by the authors:

- The major vector of AHSV in the African continent is *C. imicola*.
- *C. bolitinos* is also implicated in AHSV transmission in South Africa.
- In the Mediterranean Basin the occurrence of AHS in the past was always linked to the presence of *C. imicola*. However, following the large European epidemics of bluetongue also other *Culicoides* species must be considered as possible vectors for AHS too.

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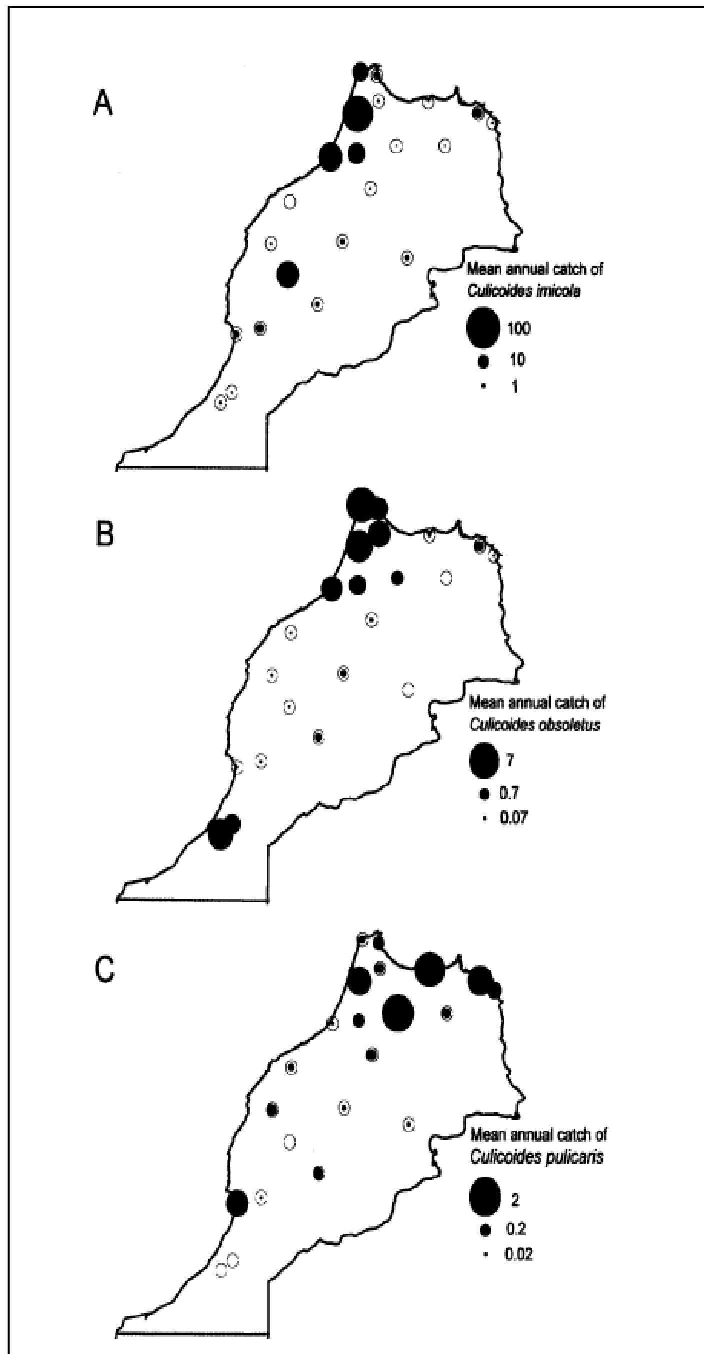
Spatial distribution of C. imicola (A), Obsoletus complex (B) and Pulicaris comples (C) in Morocco (Baylis *et al.*, 1997).



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Spatial distribution of C. imicola in the Iberian Peninsula (Rawlings *et al.*, 1997).

C. imicola present

C. imicola not detected

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Observed occurrence (red, presence; green, absence) of *Culicoides imicola* (Calvete *et al.*, 2008)

Observed occurrence (red, presence; green, absence) of *Culicoides obsoletus* (Calvete *et al.*, 2008)

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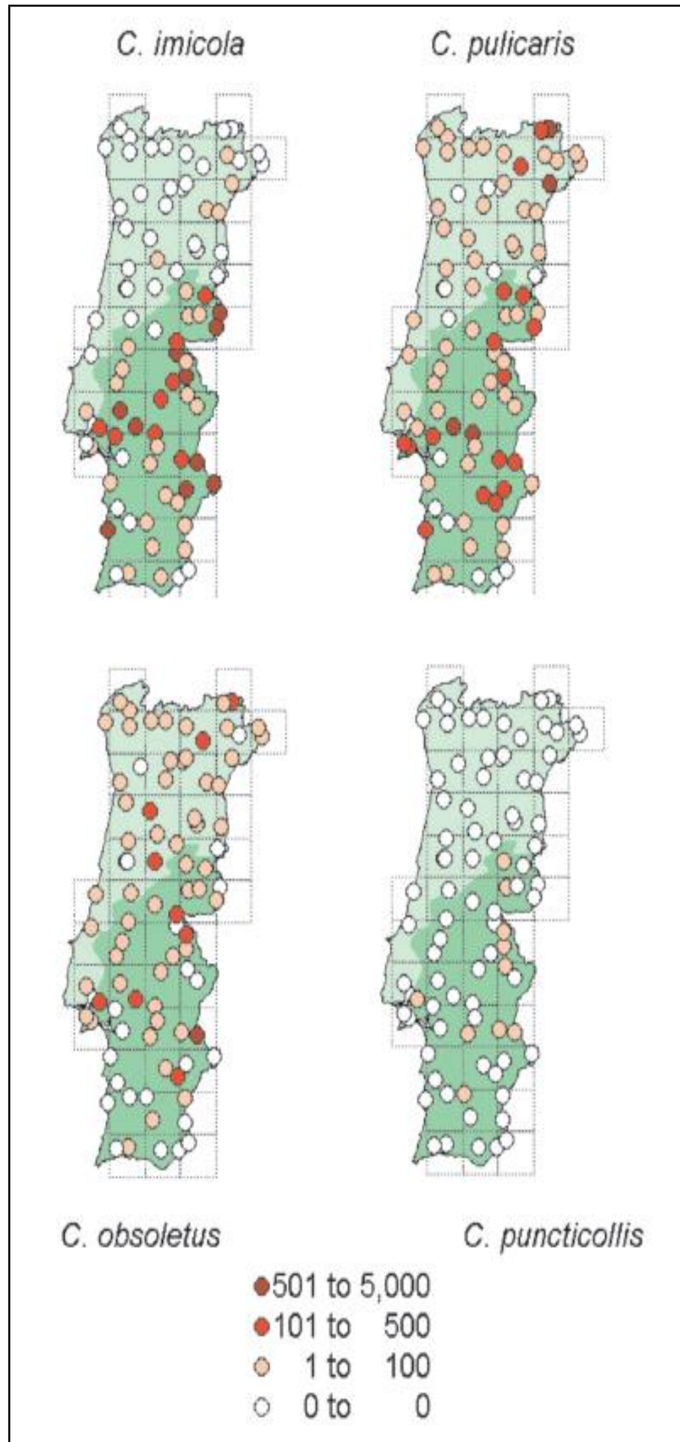
Maximum catches of *C. imicola*, *C. obsoletus*, *C. pulicaris* and *C. puncticollis* complexes at each site in Portugal in summer over two nights trapping (2000–2001) (Capela *et al.*, 2003).



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C. imicola distribution in Italy (Goffredo *et al.*, 2004).

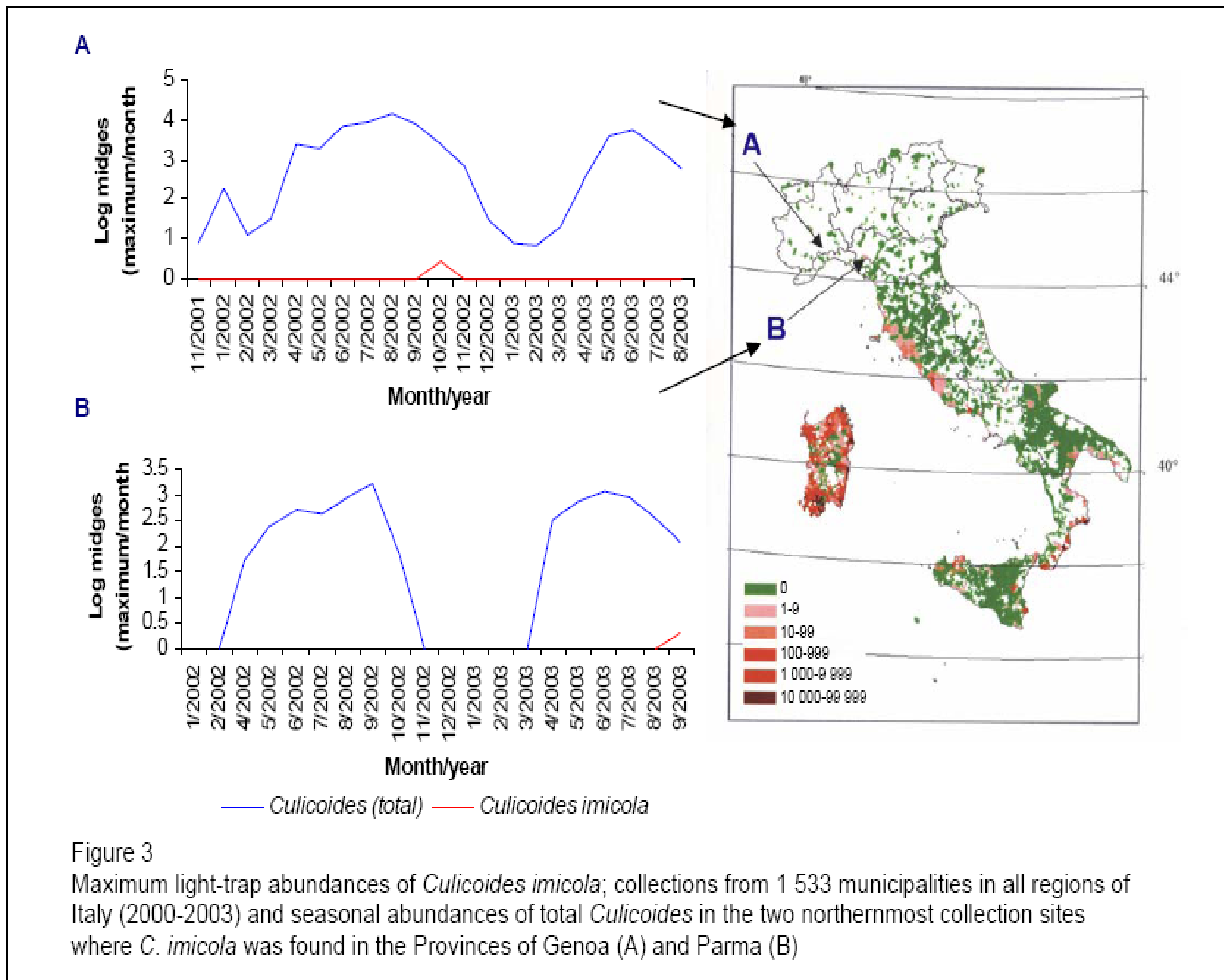

Figure 3
Maximum light-trap abundances of *Culicoides imicola*; collections from 1 533 municipalities in all regions of Italy (2000-2003) and seasonal abundances of total *Culicoides* in the two northernmost collection sites where *C. imicola* was found in the Provinces of Genoa (A) and Parma (B)

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Obsoletus complex distribution in Italy (Goffredo *et al.*, 2004).

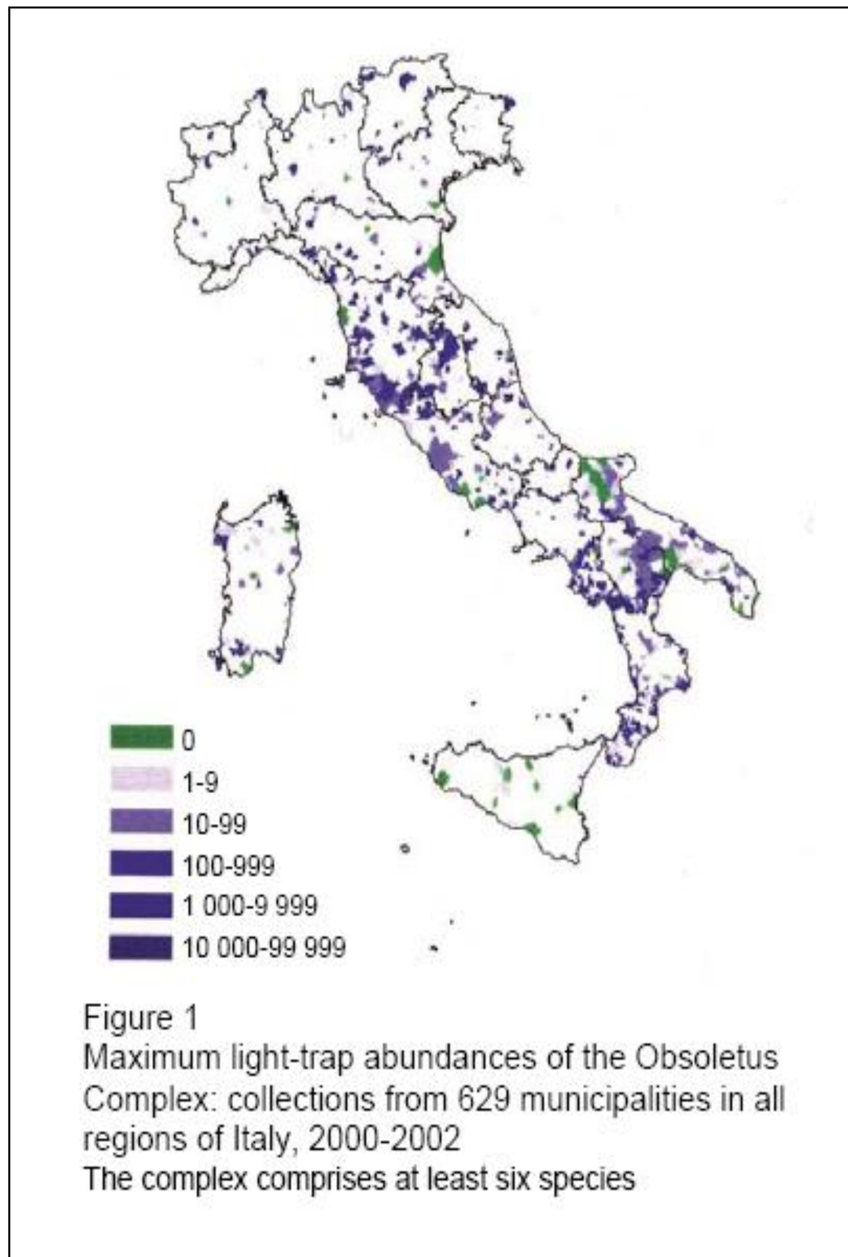

Figure 1
Maximum light-trap abundances of the Obsoletus Complex: collections from 629 municipalities in all regions of Italy, 2000-2002
The complex comprises at least six species

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Pulicaris complex distribution in Italy (Goffredo *et al.*, 2004).

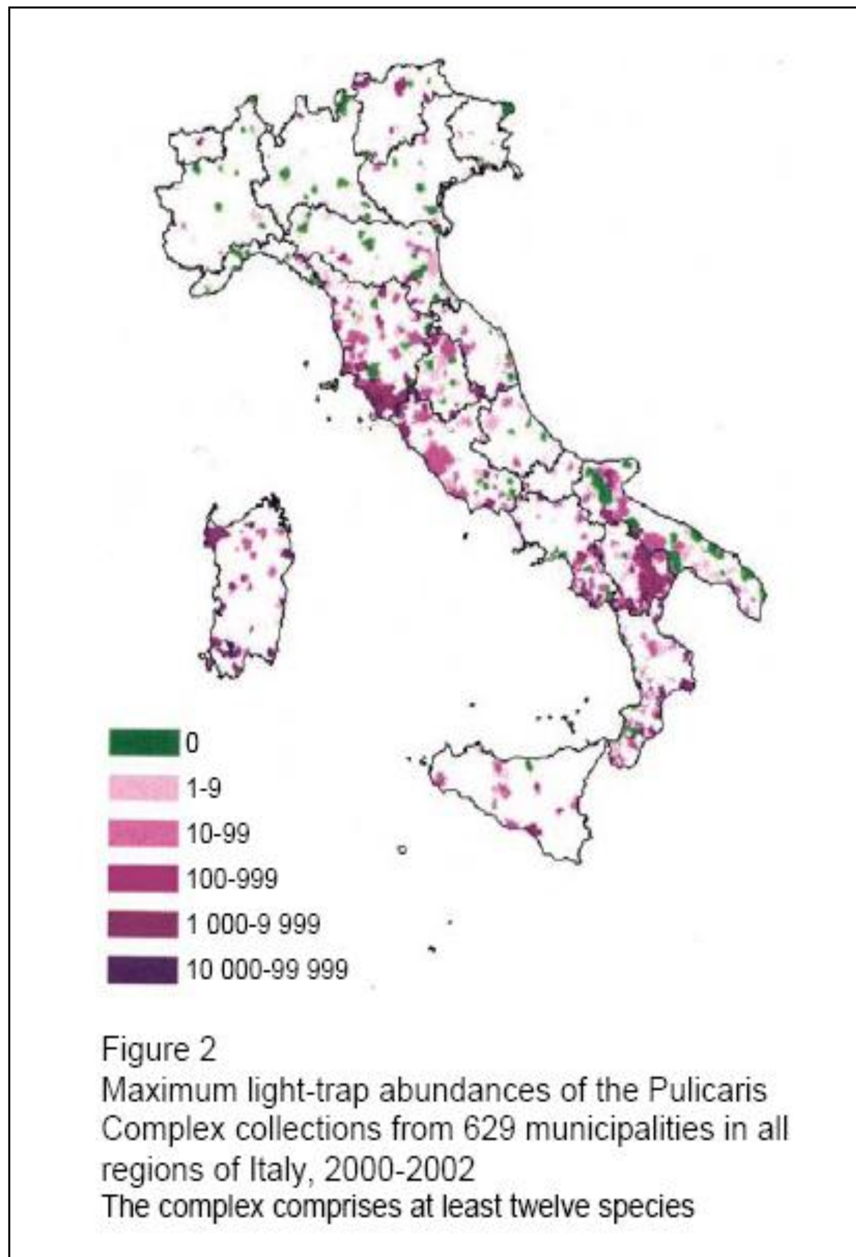

Figure 2
Maximum light-trap abundances of the Pulicaris Complex collections from 629 municipalities in all regions of Italy, 2000-2002
The complex comprises at least twelve species

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Distribution of *Culicoides* species in Corsica (Baldet *et al.*, 2004).

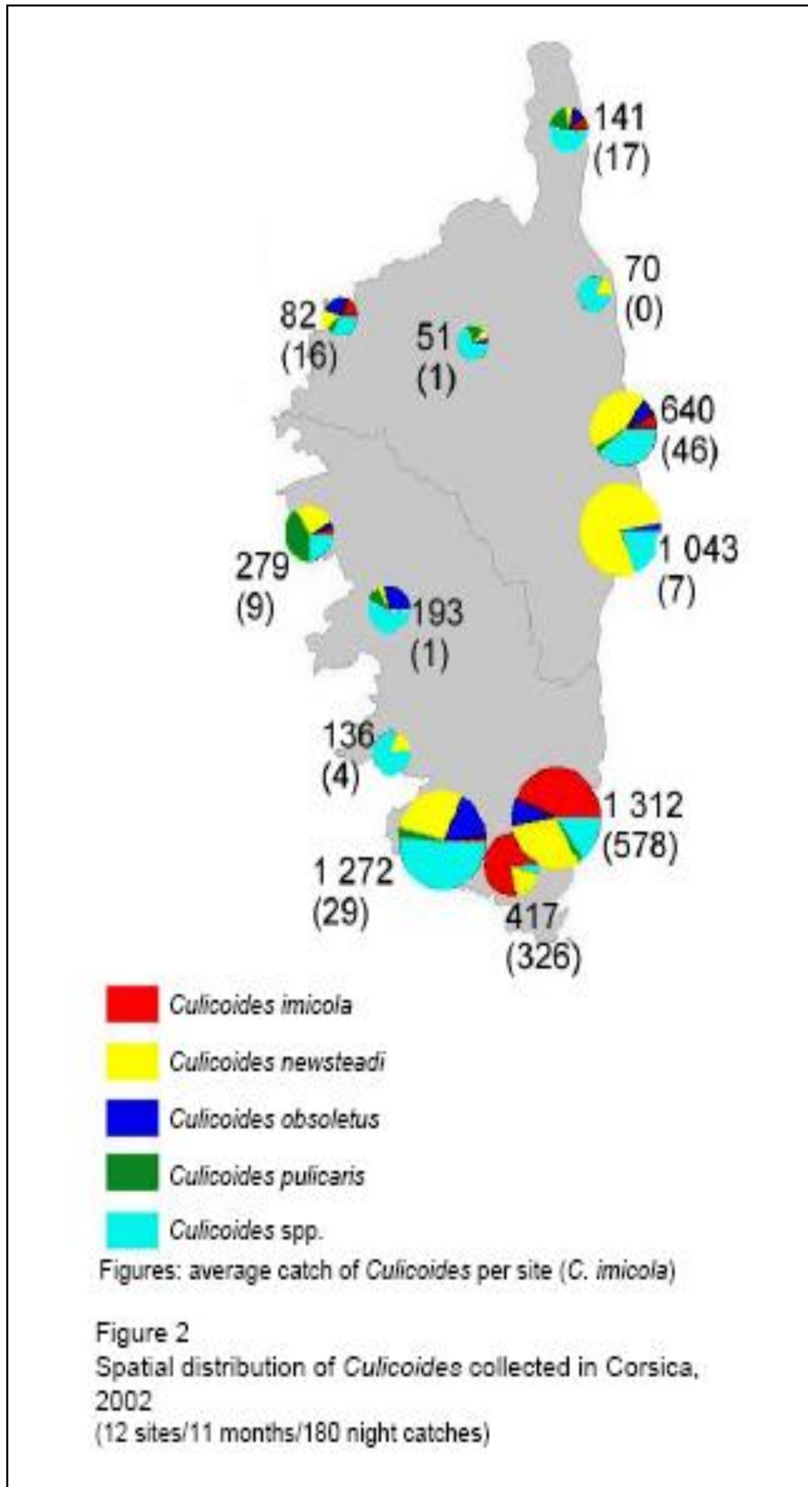

Figure 2
Spatial distribution of *Culicoides* collected in Corsica, 2002
(12 sites/11 months/180 night catches)

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Distribution of *C. imicola* in Greece (Patakakis, 2004).

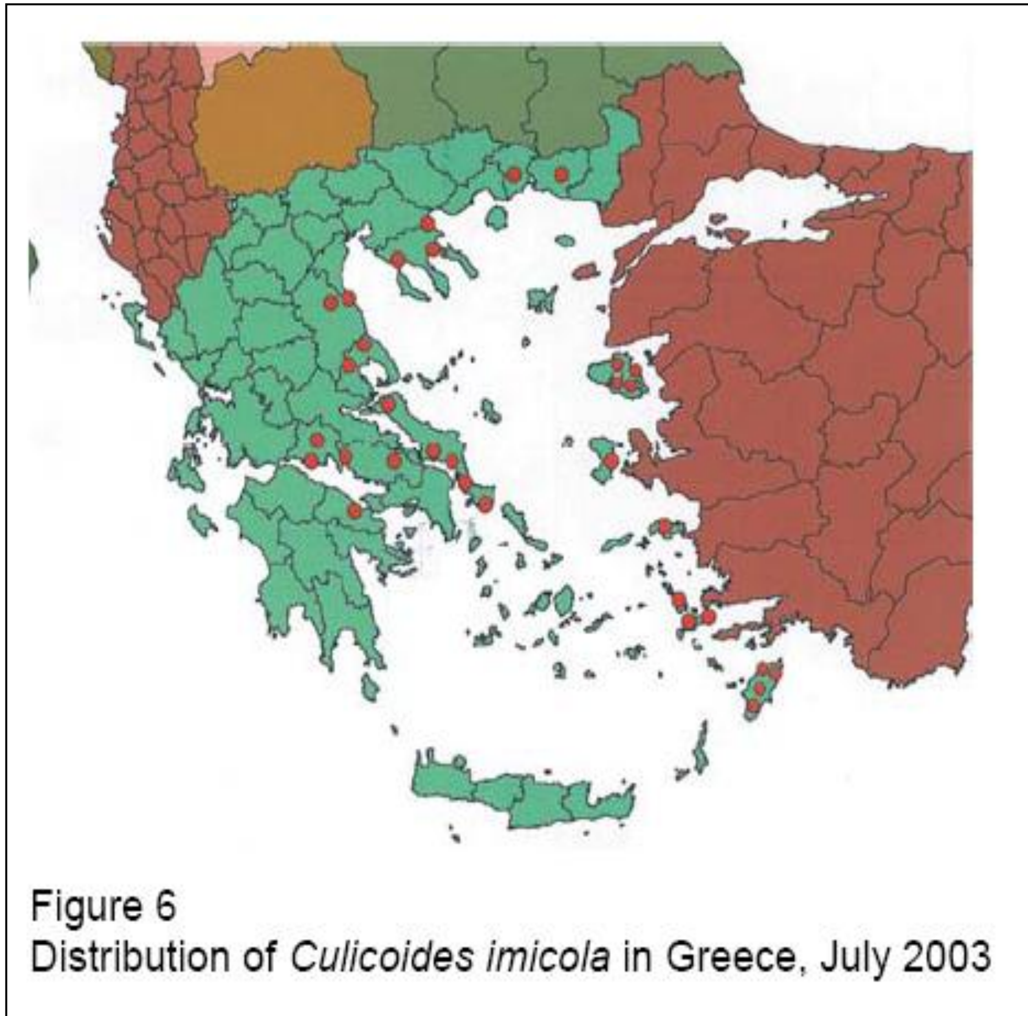

Figure 6
Distribution of *Culicoides imicola* in Greece, July 2003

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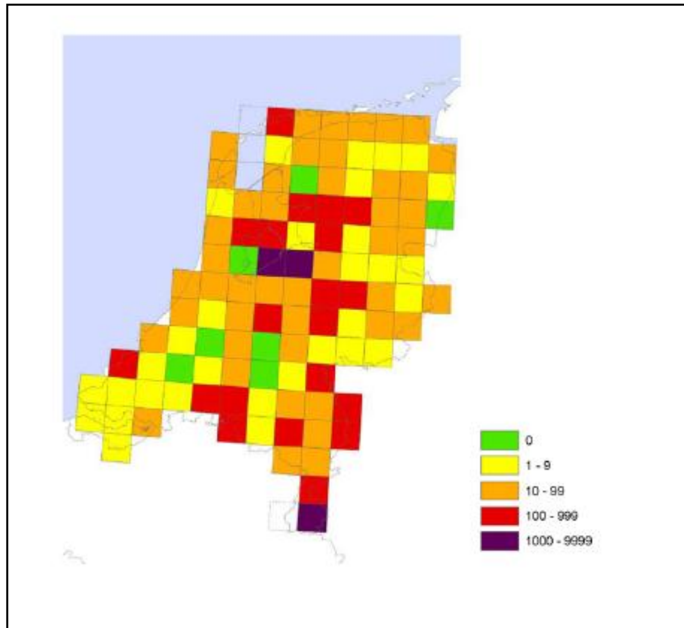
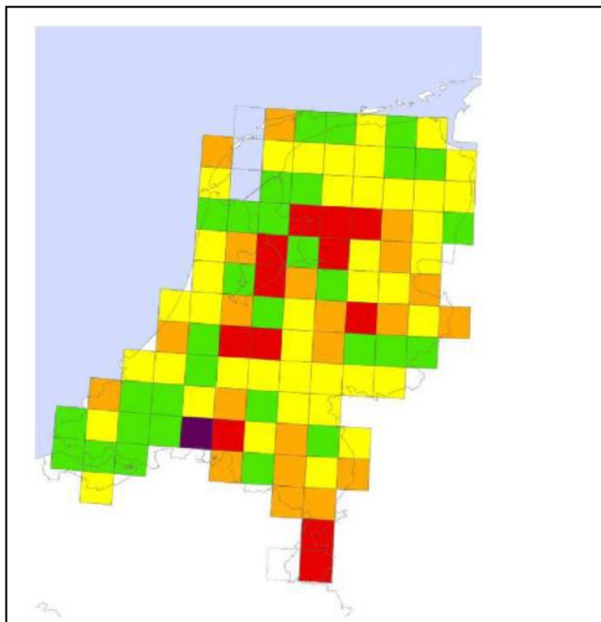
Geographical distribution of Obsoletus complex in The Netherlands (Meiswinkel *et al.*, 2008b).



Geographical distribution a of *C. dewulfi* in The Netherlands (Meiswinkel *et al.*, 2008b).



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Geographical distribution of *C. chiopterus* in The Netherlands (Meiswinkel *et al.*, 2008b).

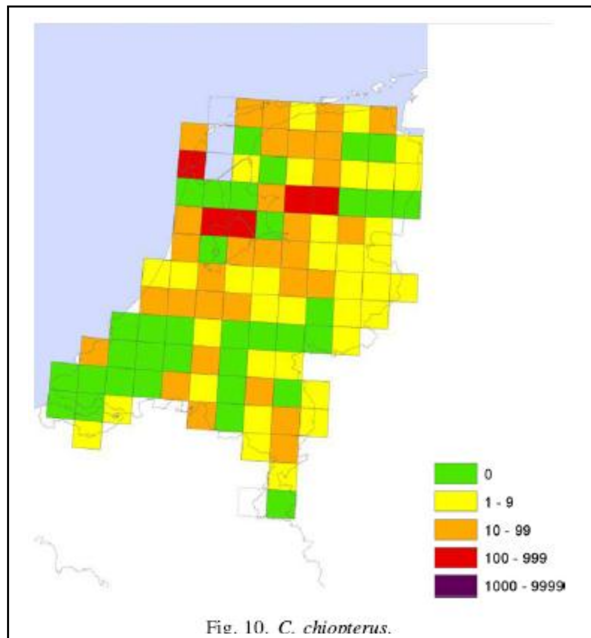
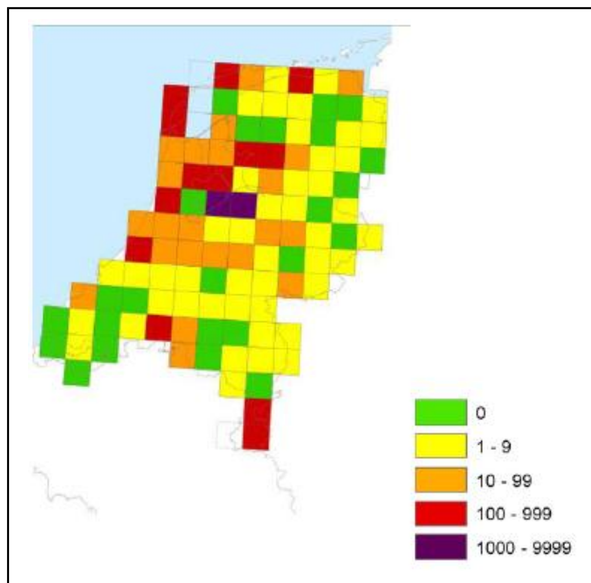


Fig. 10. *C. chiopterus*.

Geographical distribution of Pulicaris complex in The Netherlands (Meiswinkel *et al.*, 2008b).



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Abundance of *Culicoides* species trapped in Belgium (De Deken *et al.*, 2008).

Summary of the most abundant *Culicoides* species trapped near Belgian outbreak sites in 2006

Culicoides species	Number[a]		%[b]		Number of locations[c]	% of locations[d]
	♂	♀	♂	♀		
C. obsoletus	184		9.4		19	65.5
C. scoticus	25		1.2		3	10.3
C. dewulfi	68		3.5		12	41.4
C. chiopterus	42		2.1		8	27.6
C. obsoletus complex		1322		67.5	29	100
C. pulicaris	57	54	2.9	2.8	13	44.8
C. punctatus	9	66	0.5	3.4	19	65.5
C. festivipennis	30	79	1.5	4.0	8	27.6
C. nubeculosus	5	6	0.3	0.3	4	13.8

[a] Number of specimens trapped during the whole survey.
[b] Percentage of total catch.
[c] Number of locations where a certain species was trapped.
[d] Percentage as regards to the total of positive trapping sites.

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LATEST AVAILABLE KNOWLEDGE ON THE BIOLOGY AND ECOLOGY OF *CULICOIDES* SPP

A recent review on the latest knowledge on the biology and ecology of *Culicoides* spp. in relation to bluetongue transmission in Europe and possible control methods has been produced by EFSA ((European Food Safety Authority EFSA, 2008). The great majority of conclusions made in the EFSA opinion may be valid for the transmission of AHS.

In particular, the following aspects of the biology and ecology of *Culicoides* vectors should be considered:

- adult feeding habits and host preferences,
- indoor and outdoor activity,
- vector seasonality and possibility of AHS endemisation (overwintering) in European climatic conditions.

ADULT FEEDING HABITS AND HOST PREFERENCES

In EFSA opinion is clearly stated that from a practical point of view the *Culicoides* species that transmit BTV are considered to be opportunistic blood feeders upon birds and mammals, but they are strongly attracted to large mammals. Past studies in Israel on *C. imicola* indicate that amongst the species considered, horses are the most preferred host, while sheep, dogs, birds respectively are the least preferred hosts (Braverman *et al.*, 1971; Braverman and Chizov-Ginzburg, 1996).

The possible role in AHSV transmission of two BTV vectors species implicated in the spread of BTV in Northern Europe, namely *C. dewulfi* and *C. chiopterus*, should be carefully assessed in consideration of the capacity of horses dung to be a suitable breeding site for such vector species.

INDOOR AND OUTDOOR ACTIVITY

Studies conducted in South Africa (Meiswinkel *et al.*, 2000) demonstrated that the stabling of horses at night offers a significant protection from AHS infection, since the most important vector of the disease, *Culicoides imicola* has been shown to be exophilic. The other important AHS vector in South Africa *C. bolitinos* showed a more endophilic behaviour. The closing of doors and the gauzing of windows, however, led to a 14-fold reduction in numbers of *C. bolitinos* and *C. imicola* entering stables (Meiswinkel *et al.*, 2000).

Following the BTV epidemic in the Northern Europe, the studies conducted showed a certain degree of indoor activity by Obsoletus complex in France (Baldet *et al.*, 2008), in The Netherlands (Meiswinkel *et al.*, 2008a), Belgium and the UK (European Food Safety Authority EFSA, 2008). The role of such behaviour in BTV transmission is still unknown.

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VECTOR SEASONALITY

In temperate areas, most *Culicoides* species overwinter, including *C. imicola*, as demonstrated by the AHS subsequent epidemics in Spain in 1987-1991 as well as by the spread and constant presence of various BTV serotypes in Spain, Italy and other Mediterranean countries.
The recent BTV epidemic in Northern Europe, however, demonstrated the vector competence and capacity of other *Culicoides* species for BTV transmission as well as their capacity of enduring the overwintering of the infection even on latitudes traditionally considered not suitable for the endemisation of such vector-borne diseases.
Many mechanisms have been considered to explain this phenomenon:

- survival of non active infected adults during winter at a sufficient rate to be able to induce a restart of the transmission cycle,
- feeding activity of a small number of adults all around the year, which would permit a continuous transmission of the virus during the year, but at very low level during the winter,
- possibility of vertically infection of larvae with transmission of infection to the progeny. This mechanism, however, has been never observed until today.

The effects of the climate changes also were taken into consideration by some Authors as possible mechanism that would facilitate the overwintering of BTV infection in the Northern Europe.

Summary as provided by the authors:

- the great majority of conclusions taken in a recent published EFSA opinion on bluetongue may be extended also for AHS vectors.
- The following aspects of the biology and ecology of *Culicoides* vectors should be considered: (a) adult feeding habits and host preferences, (b) indoor and outdoor activity, (c) vector seasonality and possibility of AHS endemisation (overwintering) in European climatic conditions.

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CULICOIDES IDENTIFICATION AND SURVEILLANCE SYSTEMS

The recently published EFSA opinion on bluetongue (European Food Safety Authority EFSA, 2008) includes a clear and well structured review of the main vectors species identified in Europe, their nomenclature, characteristics and criteria for evaluating their potential vector role. Recently several RT-PCR have been also developed for the identification of *Culicoides* species (Gomulski *et al.*, 2006; Mathieu *et al.*, 2007; Nolan *et al.*, 2007; Cêtre-Sossah *et al.*, 2008).

More complex is the choice of an appropriate surveillance system for *Culicoides*. It should be strictly dependent of the objectives of the surveillance itself, which can be schematically summarised as follows:

- definition of seasonal patterns with the aim of possibly defining a vector low abundance season,
- identification of vector species involved in virus transmission in the field,
- detection of virus through insect trapping.

The objectives of scientific studies and researches, for examples for determining vector competence, biting rates, host preferences, vector indoor/outdoor or diurnal activity, must be not confused with the possible objectives of a surveillance system, which should be basically aimed at the control of the disease.

Regarding the trapping methods to be used in the scientific studies, they are strictly linked to each objective. For example, the use of UV traps is not of benefit when the diurnal activity of the vectors is intended to be assessed, and animal baited traps might be recommended in case the biting rates must be evaluated.

On the contrary, in case of implementation of surveillance systems, trapping methods and protocols should be standardised as much as possible. Actually the OVI UV black-light type traps is recommended. Protocols to be used in a surveillance plan are presented in some papers (Goffredo and Meiswinkel, 2004; Meiswinkel *et al.*, 2008b).

Summary as provided by the authors:

- the possible objectives of entomological surveillance activities may be summarised as follows: (a) definition of seasonal patterns with the aim of possibly defining a vector low abundance season, (b) identification of vector species involved in virus transmission in the field, (c) detection of virus through insect trapping.
- In the implementation of surveillance systems, trapping methods and protocols should be standardised as much as possible.

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CULICOIDES CONTROL METHODS

A comprehensive review of the possible chemical and physical control methods is included in the recently published EFSA opinion (European Food Safety Authority EFSA, 2008) and scientific paper (Carpenter *et al.*, 2008).

Possible methods for the control of *Culicoides* population 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,
- removal or reduction of larval breeding sites on farm holdings.

However, no insecticidal products are currently authorised specifically against *Culicoides* in the EU. Data concerning the efficacy of pyrethroid-based compounds are equivocal. While several experimental trials showed some mortality in *Culicoides* the extent of the effect on infection transmission on the field is unclear.

Environmental consequences as well as the risk of residues in food for human consumption must be carefully evaluated when insecticides are widely used for controlling *Culicoides* populations.

Treatment of breeding sites remains difficult as habitats are poorly defined for most species.

Dung removal/treatment especially in countries with Mediterranean climate when feasible and practical has the potential to reduce populations of *C. imicola*. Additionally, preventing overflow of water from water troughs and leaking water installations and keeping the animal premises as dry as possible would prevent/reduce creation of developmental sites of *C. imicola*.

Summary as provided by the authors:

- *Culicoides* control strategies based on the used of chemical compound are controversial.
- Effects on insect mortality were experimentally observed with the use of pyrethroid based repellents, but the real impact on infection transmission is unclear.
- Environmental and food safety aspects should be taken into consideration before the use of insecticides on food producing animals and/or in their stable is authorised.
- Treatment of breeding sites remains difficult due to the difficulties in identifying such habitats.
- Dung removal or treatment as well as measures for preventing the presence of mud water pools and other micro-habitats suitable for *C. imicola* breeding should be recommended all time it is feasible.

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DIAGNOSIS

The spread of AHS to the South of Europe in the late 80s improved greatly the diagnostic assays available (OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals, 2008). The first tests developed were based on the inoculation of infected blood on stable cell lines (BHK1, MS or Vero). Cytopathic effect may appear between 2 and 10 days after inoculation. This technique was time consuming and results were obtained only after a long period of time, which delayed control measures.

Sandwich ELISA tests meant a great improvement in the diagnosis of AHS since results were obtained within 2-4 hours (Hamblin et al., 1990, Laviada et al., 1992). These ELISA tests use either polyclonal –Hamblin et al., 1990- or monoclonal antibodies –Laviada et al., 1992- against one of the proteins that is more conserved among serotypes – protein VP7. Both methods have been demonstrated to be adequate for the diagnosis of AHS, due to their high sensitivity and specificity and the availability of results within a working day.

With the development, improvement and implementation of PCR (Polymerase Chain Reaction) in all the diagnosis laboratories, this extremely useful technique was available for the detection of AHSV. Combined with DNA sequencing, provided the first sequences of AHSV segment 5 (Mizukosi et al., 1992). The tandem PCR-endonuclease cleaving was further developed in order to differentiate field viruses from vaccine strains (Zientara et al., 1993; Laviada et al., 1997). PCR evolved with the development of new reagents that contained a mix of enzymes working in a step-wise manner. Thus, RT-PCR could be performed in one step, avoiding manipulation of tubes containing amplified product and the subsequent risk of contamination (Zientara et al., 1994). Real-time RT-PCR has been recently applied to AHSV detection. In 2008, three studies have reported the standardization of real-time RT-PCR for the detection of AHSV VP7 (Fernandez-Pinero et al., 2008, Agüero et al., 2008) and AHSV NS1 (Rodriguez-Sanchez et al., 2008). All these assays allow a rapid and reliable detection of the virus within hours.

A panel of serological tests has been designed in order to detect the presence of AHS antibodies in suspicious sera samples:

1) Indirect ELISA: Chuma et al. in 1992 succeed in expressing AHSV VP7 protein in order to use it in a group-specific ELISA test. Nowadays, a recombinant VP7 protein has been used as antigen for AHSV antibody determination with a high degree of sensitivity and specificity. Other advantages of this antigen are its stability and its lack of infectivity

2) Immunoblotting assay was also a useful tool to identify AHSV proteins which provided high sensitivity and easy adaptation to low number of samples. The finding that the attenuated vaccines against AHSV did not contain NS3 protein provided one of the first DIVA assays (Differentiate Infected from Vaccinated Animals) for AHSV (Laviada et al., 1993).

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3) NS3 ELISA: An indirect ELISA to distinguish between infected horses and horses vaccinated with an inactivated purified AHSV serotype 4 vaccine, using a recombinant NS3 protein as antigen, was described in 1995 by Laviada et al. The importance of this test is due to the fact that it allows differentiation between infected animals and those vaccinated animals vaccinated with an inactivated vaccine. Therefore, this technique is an important diagnostic reagent that could allow the transportation of vaccinated horses.

4) Complement fixation is another OIE prescribed test, especially for international trade permission. However, the use of this test has decreased due to the good results and wider information provided by ELISA.

5) The gold standard for the identification of AHS serotypes present in suspicious samples is Virus Neutralization (VN). With this technique, serotype-specific antibodies are detected (Hazrati and Ozawa, 1965; House et al., 1990). The VN test may have additional value in epidemiological surveillance and transmission studies, mainly in endemic areas where multiple serotypes are likely to be present (Mellor and Hamblin, 2004).

As a method for direct detection of AHS, the OIE also recommends intracerebral inoculation in 1 to 3 days old mice. In positive cases, animals develop nervous signs between 3 and 15 days post-inoculation. Brains from infected animals are collected, homogenised and re-inoculated intracerebrally into at least six 1–3-day-old mice. This second passage should present a shortened incubation period (2–5 days) and 100% infectivity. Virus isolates are then typed directly from mouse brain by conventional neutralisation (VN) or by RNA extraction and sequencing.

Summary as provided by the authors:

- The gold standard for serotype identification is Virus Neutralization
- Reliable detection tests based on antigen-antibody binding and PCR techniques allow the specific detection of AHSV.
- New RT-PCR based assays allow the identification of specific serotypes. They amplify common sequences but the subsequent use of restriction assays allow the detection of specific nucleotide sequences, present only in one serotype.
- Real-time RT-PCR allows the detection of AHSV with an increased sensitivity.

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PREVENTION AND CONTROL MEASURES

Control and eradication of AHS has been achieved, so far, in Portugal, Morocco and Spain (Sánchez-Vizcaíno, 2004; Portas et al., 1999). The key to the success in the control of AHS in Spain was the use of a polyvalent attenuated vaccine produced by Ondersteport and an attenuated monovalent type 4 vaccine from the same source, together with the establishment of protection areas, control of animal movements, slaughter of affected animals, control of *Culicoides* and serological surveillance.

AHSV serotype 4 was detected in Spain in September 1987. The origin of the outbreak was the importation of 10 zebras from Namibia in June 1987 (Sánchez-Vizcaíno et al., 2004). The disease was detected in the central part of the country and eradicated in January 1988 using the above mentioned control measures. However, a new case was reported in the South of Spain in October 1988. This time, the virus spread into the provinces of Cadiz, Huelva, Cordoba and Sevilla, where a total of 157 horses died and 845 were culled. The last outbreak in the South of Spain took place in October 1990. Enforcement of the control measures was necessary to eradicate the disease was necessary. These measures included the identification of vaccinated animals, increase of the surveillance, use of sentinel animals and more control of the vector. The efficacy of these measures was confirmed when AHS was finally eradicated from Spain in November 1993.

CHALLENGES OF THE CONTROL OF AHS

Since AHS vaccines are sparse, a bank of vaccines is required in order to provide the required vaccination in areas in need. These measures should be accompanied by epidemiological studies, risk analysis of the spread and introduction of the disease in disease-free areas and follow-up studies on the efficacy of the control measures taken. Previous experience acquired in past outbreaks (like the one that took place in Spain between 1987-1991) should be taken into account for the design of control strategies in other countries.

POTENTIAL SOURCES OF INTRODUCTION/SPREAD OF AHS

No so much data found. The reasons for the introduction/spread of the disease may be the same as for BTV. Introduction of infected animals must coincide with the presence of the vector in the region. The introduction though wildlife animals could be a potential source of infection (i.e. the introduction of infected but asymptomatic zebras into a zoo was the source of infection in the Spanish epidemic 1987-1991). The possibility of potential introduction of infected *Culicoides* though the wind from the North of Africa should also be considered. Lord et al. (1996) developed a simulation model of AHS in Spain to study what factors affect the likelihood of an epidemic after the introduction of AHSV. Their study concluded that midge population size, the recovery rate in horses, and the time of year when the virus was introduced were the most significant factors in determining whether or not an epidemic occurred.

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RECOMMENDED CONTROL MEASURES FOR AHS

AHS is included in the Infectious Diseases of Horses Order 1987 and the Specified Diseases (Notification and Slaughter) Order 1992.This means that any suspicion of the disease must be reported by the horse's owner or vet to the competent authority, which will implement control regulations. Current European legislation specifies that AHS must be controlled by the slaughter of infected animals, destruction of the carcasses, and the establishment of a protection zone of at least 100 kilometres radius around infected premises. This, together with a further surveillance zone of at least a further 50 km, remains in force for at least 12 months. The scale of the zone is because virus movement over long distances via windborne infected *Culicoides* midges has been suggested. Its appearance in the UK could spell the death knell for horse racing and all other forms of equestrian sport and leisure riding. All horses imported from outside the European Union must either come from countries free of African Horse Sickness, be vaccinated or non-vaccinated and, if imported from the AHS-free zone in South Africa, must be tested for AHS before being allowed into the country. If there is an outbreak in the UK, the international trade of UK horses will cease immediately. Therefore, horses will not be able to be sold for export or be able to participate in equestrian events in other countries.

CONTROL AND SURVEILLANCE MEASURES OF AHS APPLIED BY REGION AND/OR COUNTRY

Europe

Two studies have collected the experience to control AHS after the outbreaks that occurred in Portugal between 1989-1991 (Portas *et al.*, 1999) and Spain between 1987 and 1991 (Sanchez-Vizcaino, 2004). Both studies described how a combination of efficient vaccination programs, animal restriction movements and culling of the affected animals proved to be a good combination of control measures which lead to the eradication of AHS in Europe. Polyvalent and serotype 4 monovalent attenuated vaccines were used to control these outbreaks with excellent results, since these vaccines provided a good protection (Sánchez-Vizcaíno, 2004).

An inactivated vaccine was developed by Merial and its efficacy was tried under laboratory (House et al., 1992) and field conditions (Sánchez-Vizcaíno 2008, personal communication). Despite the good results obtained with this vaccine, it is no longer available. The main advantage of this vaccine was that it allows to differentiate between infected and vaccinated animals. This was based in the lack of NS3 protein in the vaccine strain (Laviada et al., 1995).

In other study carried out in 1996 (Martinez-Torrecuadrada *et al.*, 1996), AHS virus-like particles were successfully assayed in a small group of horses. These particles provide a stable vaccine, unable to revert or recombine. The development of safer vaccines based on this technology is a step forward to the achievement of better tools for the control of AHS.
The Council Directive 92/35/EEC, of 29 April 1992, established the control measures to combat AHS once an outbreak of the disease is suspected. If one or more equidae (horses) on a holding are

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suspected of being infected with AHS, the official veterinarian must notify the competent authority and take a number of measures, including placing the suspect holdings under surveillance, carrying out a census of the dead and infected equidae and the places likely to facilitate the survival of the vector, carrying out an autopsy on suspect animals and banning the movement of equidae to or from the suspect holdings. These measures may only be discontinued by the veterinarian when the competent authority has confirmed that the presence of AHS is no longer suspected. If an outbreak of AHS is confirmed, the veterinarian must have all infected equidae slaughtered and their carcases disposed of. Measures may also extend to all holdings situated within a 20 km radius, have all animals in this area vaccinated and carry out an epidemiological survey. The survey must cover the various aspects of the disease, in particular the length of time of its presence, its origin and the presence and distribution of the disease's vectors. The competent authority must establish a protection zone (of at least 100 kilometres around the infected holding) and a surveillance zone (of at least 50 kilometres beyond the protection zone), in which certain specific measures are applied. These measures include the identification of all holdings containing equidae, visits and examinations by the official veterinarian and restriction of movements of the animals concerned. People living in these zones must be informed of all the restrictions in force. Systematic vaccination of the animals may be carried out in the protection zone. Annex I of the Directive contains a list of the laboratories designated by each Member State for carrying out the tests laid down in the Directive. These laboratories are required to liaise with the Community Reference Laboratory located in Algete (Spain), whose duties include coordination and providing assistance. In certain cases Commission experts may carry out examinations on a number of the holdings concerned. Each Member State must draw up a contingency plan meeting the criteria laid down in the Directive. For example, they must set up a crisis centre and provide a list of local disease control centres and detailed information on the staff involved in control measures. The Standing Committee on the Food Chain and Animal Health assists the Commission in the management of the measures taken against AHS. Its remit includes laying down the duration of the measures and taking additional action. Other regulations related with AHS are shown in **Table 2**.

Table 2. Current regulations related with the control of AHS

Act	Day of entry into force	Official Journal
Directive 92/35/EEC	18.5.1992	OJ L 157 of 10.6.1992
Regulation (EC) No 806/2003	5.6.2003	OJ L 122 of 16.5.2003
Decision 2006/911/EC	12.12.2006	OJ L 346 of 9.12.2006
Directive 2006/104/EC	1.1.2007	OJ L 363 of 20.12.2006
Decision 2007/729/EC	-	OJ L 294 of 13.11.2007

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Asia

Historically, serotypes -3 and 9- have been detected in several countries of Middle and Far East (**Table 3**).

The control measures taken in these countries were based on the restriction of animal movements, precautions at the border, screening of sentinel animals and stamping out. Vaccination was not included as a control measure in most of the affected countries. Nevertheless, none of them is affected nowadays.

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Table 3. Outbreak of AHS in Asia from 1944 to 1981

Source: CIDRAP, adapted from (House, 1993; Erasmus, 1998; Martínez-Torrecuadrada, 2001).

Outbreak location	Year	AHS serotype
Egypt, Palestine, Syria, Jordan	1944	3
Israel, Iran, Pakistan, Afganistan	1959	9
Israel, Iran, Pakistan, Afganistan, India, Turkey, Iraq, Syria, Lebanon, Jordan, Cyprus	1960	9
India, Pakistan, Turkey, Iran, Jordan, Iraq	1961	9
Pakistan	1974	Undetermined
Saudi Arabia	1975	Undetermined
Yemen	1980-1981	Undetermined

Africa

In most of the African countries the measures taken have been vaccination, restriction of animal movements, surveillance, controls at the borders and stamping out. However, countries in the North of the continent (Egypt, Morocco and Algeria) managed to control their outbreaks without vaccination (**Table 4**). Vaccination is ongoing in Eritrea, Ethiopia, Namibia, Senegal and South Africa. Despite the efforts to control the disease in the continent, Ethiopia, Senegal and South Africa are affected nowadays by the disease. The situation in South Africa has been controlled in some areas, and the outbreaks are now restricted to the provinces of Gauteng, Mpumalanga, North-West Province, Free State, Limpopo, KwaZulu Natal and Eastern Cape (www.**africanhorsesickness**.co.za).

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Table 4. Control and surveillance measures of AHS applied by country

Country (Period)	Measures taken [Source]
Botswana (2005)	Movement restrictions were imposed on horses in the affected districts to limit disease spreading. There is no free vaccination against this disease and farmers were advised to buy vaccine from the Livestock Advisory Centres (LAC) and vaccinate their animals. Eleven outbreaks were recorded during the reporting period (2002) [OIE]
Eritrea (2006-Jun 2007)	Any suspicion of the disease must be reported by the horse's owner or vet to the Department of Environment Food and Rural Affairs (Defra), which will implement control regulations. Current European legislation specifies that AHS. All horses imported from outside the European Union must either come from countries free of African Horse Sickness, be vaccinated or non-vaccinated and, if imported from the AHS-free zone in South Africa, must be tested for AHS before being allowed into the country [www.horsetrust.org.uk]
Etiopía (2005-2007)	Vaccination of all equine in the area and a broad buffer zone is recommended to restrict spread by infested wind-blown midges. Also, care should be exercised to quarantine animals in infected areas until the passage of the rainy season. The disease occurs over a large area of Ethiopia and use of vaccination has started [http://www.africa.upenn.edu/eue_web/diseas94.htm]
Senegal (2005-2007)	Disease control measures have been applied (Supposely the same measures that are in force in Europe) [DEFRA].
South África (2005-2007)	They follow the EU Decision 2001/622/EC of 27th July 2001 and Animal Diseases Regulations No. R885 of 21st September 2001, and the amendments made to the vaccination protocol, the requirements for movements and the boundary changes [www.africanhorsesickness.co.za].

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IDENTIFICATION OF POTENTIAL HAZARDS IN RELATION TO THE INTRODUCTION AND SPREAD OF AHS IN THE EU

CULICOIDES TRANSPORTATION

Vector presence is bound to climatic factors like temperature, rain and relative humidity (Sellers and Maarouf, 1991). Wind constitutes the most crucial factor for the spread of *Culicoides* midges (Cicuéndez et al., 2007. Due to the small size of the midges (1-3mm), the wind can transport them over more than 700 km when the temperature and speed conditions are suitable (Sellers and Maarouf, 1991). Therefore, infected vectors may be transported from their origin areas to disease-free countries, contributing to the spread of the disease.

Studies relating *Culicoides* transportation by wind and Bluetongue (BT) outbreaks reported the appearance of BT serotype 4 in the South of Spain subsequently to the occurrence of sand storms in the Straits of Gibraltar (Cicuéndez, 2007). The sand storms could be followed by satellite imaging. The fact that *Culicoides* midges were dragged within the storm was corroborated when the viruses present in the South of Spain were sequenced and compared with BTV serotype 4 from the North of Morocco and the homology was 100%. No animal movements were register between Spain and Morocco at that time.

ANIMAL TRADE

The history of the AHS in Europe clearly indicates the importance of the animal movement as a mean of infection introduction and spread. Zebras and donkeys are considered more at risk than horses for the introduction of the virus into free areas, given their longest viraemia and the attitude to rarely exhibit the clinical signs of infection (Mellor and Hamblin, 2004).
The introduction of horses and other equidae into the EU is strictly regulated and the importation of susceptible animals from endemic countries or areas is strictly forbidden. Several legislative acts regulate the imports of these animals from Third Countries:

- Council Decision 79/542/EEC (OJ No L 146, 14. 6. 1979, p. 15) establishing the list of third countries from which Member States authorize imports of bovine animals, swine, equidae, sheep and goats, fresh meat and meat products, and its amendments,
- Council Directive 90/426/EEC of 26 June 1990 on animal health conditions governing the movement and imports from third countries of equidae (OJ No L 224, 18. 8. 1990, p. 42),
- Commission Decision 92/160/EEC (OJ No L 71, 18. 3. 1992, p. 27established the regionalization of certain third countries for imports of equidae, and its amendments,

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- Commission Decision 92/260/EEC (OJ No L 130, 15. 5. 1992, p. 67) established the animal health conditions and veterinary certification for temporary admission of registered horses, and its amendments,
- Commission Decision 93/197/EEC (OJ No L 86, 6. 4. 1993, p. 16) established the animal health conditions and veterinary certification for imports of registered equidae and equidae for breeding and production, and its amendments,
- Commission Decision 2001/622/EC of 27 July 2001 amending Decisions 92/160/EEC and 97/10/EC with regard to the regionalisation of South Africa and repealing Decision 1999/334/EC on protection measures with regard to registered horses coming from South Africa (OJ No L 216, 10. 8. 2001, p. 26).

However, the possibility of illegal introductions of AHS susceptible animals from infected countries or of sub-clinically infected animals from areas where the presence of infection is not yet detected, may be not excluded.

Few official data are available on the international trade of equidae. Eurostat keeps probably the most complete database on animal trade in Europe. In the analysis of data on horses trade in relation to the risk of introduction of AHS a distinction should be made by the animal introduced for slaughtering and those not intended for slaughter (for breeding, recreation, competitions, etc.). Eurostat permits to make this kind of distinction, although the data should be carefully interpreted, given the possibility of mistakes in their registration and notification.

Table 5 shows the number of slaughter horses introduced by each EU country from 2000 to 2005, and in **Table 6** the origins of these animals are reported. It is noteworthy that over the 80% of slaughter horses are introduced by Italy as well as over the 90% of movements are confined within the EU borders. The introduction of this type of animals from Third Countries is mainly from the Eastern Europe (**Figure 3**).

On the contrary, in the case of horses not intended for slaughter, the origin of the animals is more diversified. (**Table 7**), with a significant number of animals coming from other Continents, including the Northern Africa and the Arabian Peninsula.

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Table 5. Number of slaughter horses introduced in the EU countries from 2000 to 2005 (source: Eurostat).

Country	2000	2001	2002	2003	2004	2005
Austria	34	0	182	134	86	41
Belgium	8535	10441	7639	5112	3266	4682
Bulgaria	0	0	0	0	0	0
Cyprus	0	0	0	0	0	0
Czech Republic	0	0	0	0	0	0
Denmark	2	0	0	0	0	3
Estonia	0	0	0	0	0	0
Finland	0	0	0	0	0	0
France	7685	10870	7405	7847	7490	6240
Germany	326	587	340	23	26	0
Greece	0	0	0	0	0	0
Hungary	0	1 046	566	0	1	0
Ireland	0	0	9835	1414	5005	55
Italy	124600	145210	92741	60713	78642	66162
Latvia	0	0	0	0	10	0
Lithuania	102	136	268	79	22	36
Luxembourg	0	0	0	13	576	87
Malta	0	0	0	0	0	2
Netherlands	0	1	1	131	87	4
Poland	0	0	0	0	4609	3313
Portugal	0	0	0	0	0	0
Romania	0	0	0	0	0	0
Slovakia	0	0	0	0	0	0
Slovenia	17	60	0	0	5	0
Spain	258	757	1396	701	275	907
Sweden	0	2	1	2	2	6
United Kingdom	0	0	0	0	370	47

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Table 6. Origins of the slaughter horses introduced in the EU countries from 2000 to 2005 (source: Eurostat).

Countries	2000	2001	2002	2003	2004	2005
Poland	50545	40408	30932	15038	40668	32055
Romania	28089	62742	28458	22841	15709	1392
France	13225	8543	6575	6787	6818	8743
Hungary	9843	14653	3840	45	1938	3735
Lithuania	8913	9043	3411	124	4088	6324
Yugoslavia	6735	1918	72	0	0	0
Spain	4637	8320	8945	8762	9885	11282
Bosnia And Herzegovina	3736	0	0	0	0	0
Germany	3633	4642	4282	3756	3091	3481
Netherlands	3490	3826	5093	2250	1063	2420
Belarus	2532	3509	3783	5917	6783	5305
Croatia	1435	0	0	0	0	30
Slovenia	1262	846	501	179	19	110
Denmark	1194	2215	1149	582	444	434
Austria	855	1924	1618	1758	1106	802
Belgium	422	1348	1825	1716	1520	2381
Bulgaria	276	4054	8340	3526	1266	2552
Italy	217	239	147	206	85	16
Greece	124	12	11	0	0	0
Estonia	100	39	84	22	0	0
Czech Republic	76	545	127	0	0	0
Portugal	35	0	141	118	156	171
Andorra	35	0	33	24	5	51
Sweden	29	14	1	2	0	4
Russian Federation (Russia)	26	0	6	0	10	0
United Kingdom	25	57	5420	1415	5005	0
Luxembourg	24	18	7	0	2	0
Slovakia	23	49	57	0	130	0
Ireland	19	22	0	0	303	93
Norway	2	2	1	2	2	9
Switzerland	2	1	26	0	0	0
Ukraine	0	72	1014	1078	371	195
United States	0	26	4390	1	0	0
Morocco	0	23	0	0	0	0
Sierra Leone	0	0	70	0	0	0
Argentina	0	0	15	3	0	0

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Figure 3. Main trade flows of slaughter horses from 2000 to 2005 (source: Eurostat).

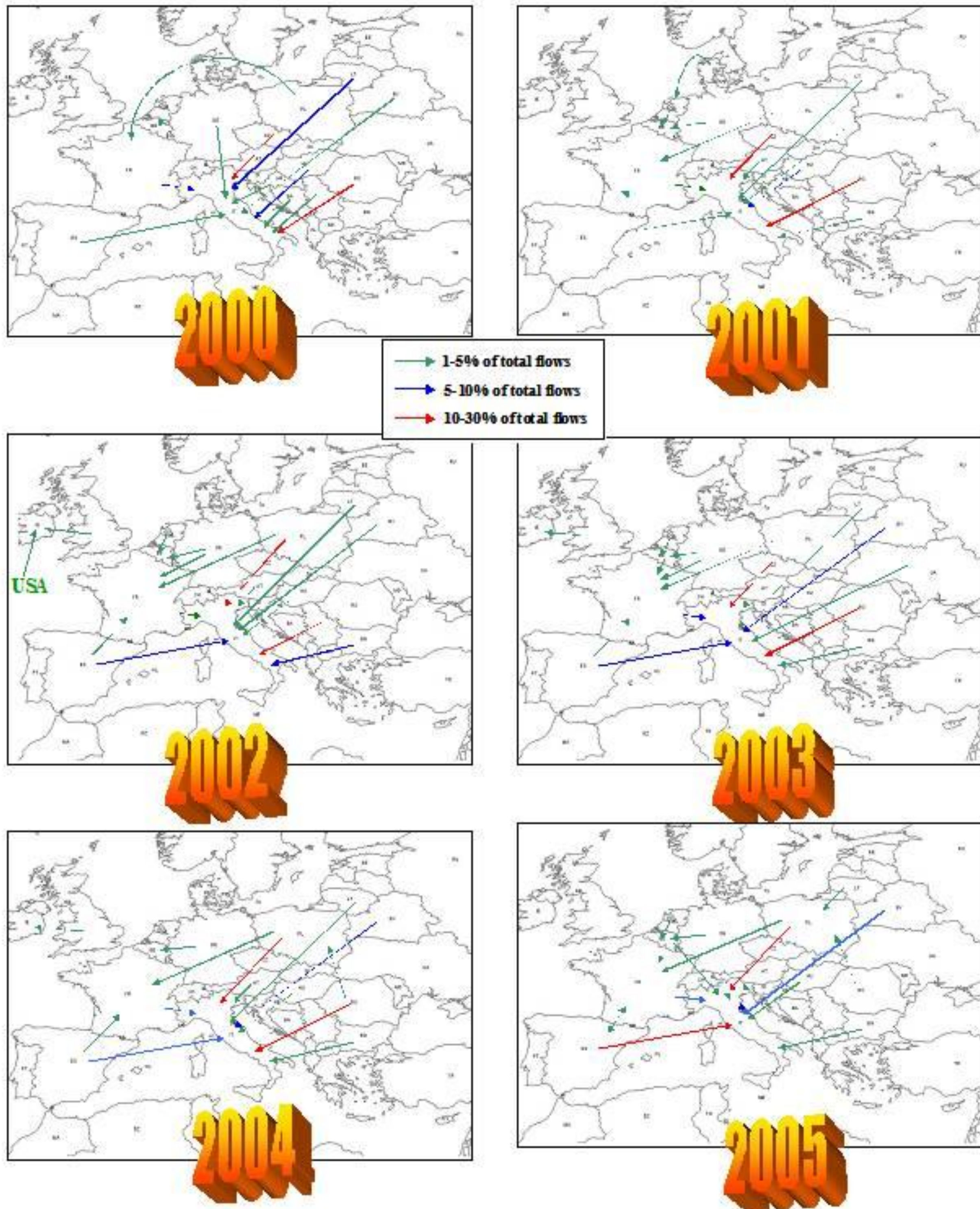

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Table 7. Number of horses not intended for slaughter introduced in the EU countries from 2000 to 2005 (source: Eurostat).

Countries	2000	2001	2002	2003	2004	2005
Austria	757	782	546	867	567	164
Belgium	8225	7387	5912	5538	6325	5214
Bulgaria	24	24	66	49	143	124
Cyprus	8	41	60	21	1	9
Czech Republic	404	456	660	485	188034	13500
Denmark	895	779	1306	1546	651	369
Estonia	30	62	29	33	13	8
Finland	515	441	410	418	284	172
France	12211	5563	4319	2610	2347	2497
Germany	3732	3137	3095	3492	3382	1889
Greece	113	221	602	310	144	226
Hungary	2691	191	379	272	82	137
Ireland	4594	3399	3504	4288	3491	5318
Italy	20104	39185	32904	67937	48952	25143
Latvia	37	19	68	10	23	2
Lithuania	23	41	113	38	74	73
Luxembourg	402	165	459	57	951	206
Malta	309	500	288	468	508	349
Netherlands	1407	2387	1527	1293	1406	1542
Poland	0	0	0	0	716	369
Portugal	11	15	19	189	89	145
Romania	0	0	13	32	30	0
Slovakia	0	0	0	0	40	7
Slovenia	181	203	342	263	439	27
Spain	2532	3626	20210	22193	15391	2248
Sweden	1378	1730	1097	1263	1309	960
United Kingdom	6580	3590	3825	3931	4807	15444

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Table 7. Origins of the horses not intended for slaughter introduced in the EU countries from 2000 to 2005 (source: Eurostat).

Countries	2000	2001	2002	2003	2004	2005
Netherlands	13013	7197	6535	7324	8220	5955
Spain	8886	4829	4130	812	274	713
Romania	8826	15551	11844	19165	17940	11314
France	7404	9938	12661	23832	17339	13784
United Kingdom	4105	3876	3170	4022	3201	4547
United States	3871	2533	2282	2614	2463	2832
Belgium	3076	2120	1799	1933	1336	1932
Ireland	2041	1907	1932	2024	2102	2129
Germany	1921	1843	2291	1490	3437	2663
Poland	1724	1305	1271	27500	16881	1093
Argentina	1621	1702	1924	1848	2423	2489
Iceland	1430	1132	961	1037	1239	1073
Austria	1224	1612	1009	616	1061	14329
Switzerland	966	984	1145	889	1266	1029
Bosnia And Herzegovina	522	0	1	0	22	0
Hungary	503	626	2599	5481	189040	418
Andorra	453	360	242	217	240	252
Czech Republic	418	392	506	514	189	68
Latvia	416	425	358	371	104	1
Croatia	348	1642	945	962	734	724
Norway	336	869	291	270	249	334
Sweden	318	193	249	169	201	138
Russian Federation (Russia)	311	197	215	180	207	195
Italy	301	525	464	338	483	332
United Arab Emirates	298	427	432	551	477	798
Canada	287	406	349	235	380	289
Denmark	283	116	117	58	141	110
Estonia	264	228	225	215	75	0
Lithuania	255	223	1954	4374	1609	193
Uruguay	235	523	344	314	384	446
Japan	233	10	33	22	9	16
New Zealand	175	97	212	312	221	188
Australia	151	70	168	82	146	110
Yugoslavia	144	7893	5374	3856	0	0
Slovakia	138	135	94	47	390	4
Belarus	135	110	118	148	192	143
Bulgaria	90	819	451	2299	2510	2468
Former Yugoslav Republic of Macedonia	63	205	53	92	19	18
Brazil	53	280	48	56	72	53
Chile	51	47	77	65	87	73
Finland	50	32	12	19	12	4
Ukraine	45	112	290	736	210	53
Morocco	39	40	36	30	21	22
Mexico	27	40	46	42	41	24
Turkey	21	18	8	15	15	28
Saudi Arabia	16	3	7	10	8	7
Luxembourg	15	23	89	64	83	5
Slovenia	9	39	30	82	186	0
Hong Kong	8	13	20	19	15	21
Qatar	7	3	13	1	9	27
Greece	6	43	28	5	0	5
Portugal	5	146	12181	26	19	225
Malaysia	5	21	10	45	56	2
Tunisia	4	4	36	80	1	1
Egypt	4	4	2	10	11	9
Israel	3	5	11	22	16	12
Bahrain	3	0	18	2	0	4
Moldova, Republic of	3	0	0	0	0	0
Cyprus	2	0	0	3	0	0
Jordan	1	12	2	11	28	13
China (People's Republic of)	1	3	1	0	2	1

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Summary as provided by the authors:

- The introduction of infected animals (zebras from Namibia) in 1987 caused in the past an AHS epidemic in the Iberian Peninsula which last to 1990.
- Animal trade must be considered a potential source for virus introduction and spread in EU.
- Although the EU legislation is very strict in relation to the introduction of horses and other Equidae from Third Countries and does not permit the import of susceptible animals from infected countries, the analysis of data on trade flows of live horses introduced into EU not for slaughter (for breeding, competitions, etc.), shows the existence of a constant introduction of animals from countries of the Northern Africa and of the Arabian Peninsula. This could represent a not negligible risk in case of AHS spread out of its sub-Saharan niche.

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