SCIENTIFIC REPORT submitted to EFSA

Scientific Review on Epizootic Hemorrhagic Disease[1]

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[1] **Project ID: CFP/EFSA/AHAW/2008/04. Accepted for Publication on 23 November 2009**

Summary

A scientific review of the available knowledge on Epizootic Hemorrhagic Disease (EHD) and *Culicoides* vectors has been conducted. The review collects data on aetiology, pathology, clinical signs, epidemiology with particular attention to the reported host range, diagnosis and, where available, prevention and control tools of Epizootic Hemorrhagic Disease virus (EHDV). An updated atlas summarizes the data of the EHDV geographical distribution worldwide.

Beside the virus, information related to vectors ecology and their distribution has been included. Particularly, aspects of their biology and behaviour have been analysed, together with the existing identification systems including limitations and constraints and the geographical distribution in Europe of all major vector species of *Culicoides* spp. Information on the possible effects of ecological and climatic changes on the geographical spread of vectors and on their potential capacity of transmission of related diseases has also been considered.

The latest part of the review provides the identification of potential hazards in relation to the introduction of such diseases in the EU. The scientific review is based on the available literature, peer-reviewed scientific papers, published documents and scientific reports, including norms and standards of OIE and of other relevant International Organizations.

Key words: Epizootic Hemorrhagic Disease Virus, EHD, Aetiology, Pathogenesis and immunity, diagnosis, *Culicoides*

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Background

Epizootic hemorrhagic disease (EHD) is a disease included within the category of multiple species diseases in Article 1.2.3. of the Terrestrial Animal Health Code of the World Organisation for Animal Health (OIE).

EHD has been considered one of the most important diseases of North American deer and EHD of deer is therefore listed as a notifiable disease in Annex I of Council Directive 92/119/EEC introducing general Community measures for the control of certain animal diseases and specific measures relating to swine vesicular disease.

Epizootic hemorrhagic disease virus (EHDV) is the causal agent of EHD. EHDV is a member of the genus *Orbivirus*, family *Reoviridae,* for which 7 serotypes are recognised and that is closely related to bluetongue virus (BTV).

EHD often causes death in deer and, less frequently, a bluetongue-like illness in cattle. However, certain EHDV serogroups (e.g. Ibaraki virus) may cause more serious epidemics in bovine animals. Sheep can be infected experimentally but rarely develop disease and goats do not seem to show clinical signs of this disease. EHD also complies with the OIE's definition of an emerging disease, namely with the criterion "a known infection spreading to a new geographic area or population."

Epizootic hemorrhagic disease occurs in North America, Australia, Asia and Africa but until recently was not found in Europe or its vicinity. However, EHD has been recorded during 2006 in the Mediterranean basin (Morocco, Algeria, Tunisia and Israel) and also in 2007 in cattle in Turkey (west Anatolia), where it had never been observed before. The outbreaks in Israel during 2006 were attributed to EHDV-7; outbreaks in Morocco and Algeria were similar to EHDV-6 (EHDV- strain 318), while the outbreaks in Turkey are attributed to EHDV-6. The origin(s) of the 2 EHDV serotypes which have affected the Mediterranean countries remain unclear.

EHDV is transmitted by insect vectors, usually midges in the genus *Culicoides*.

It is known that infected deer can remain viraemic for up to 50 days but the length of the viraemia in cattle and sheep remains unclear.

The EHD affected areas in western Anatolia and in Morocco are adjacent or very close to European territories, where BTV has caused serious outbreaks. Following the recently observed pattern of BTV spreading from North Africa to South European regions in Spain, Portugal, France and Italy, EHD remains a matter of concern. In addition, as regards control measures no vaccines are available for most EHDV serotypes.

Therefore, the current distribution of EHD in the vicinity of Europe deserves special consideration as regards i) the epidemiology of EHD and its possible spread into new regions, ii) the surveillance and monitoring activities in the countries at risk, iii) the clinical and laboratory diagnosis of EHD, iv) the possibilities for the development of vaccines against EHD and v) the competent vectors implicated in the spread of EHDV.

The presence of EHD in EU neighbour countries represents a challenge for risk managers. It is therefore necessary to determine the extent of the problem and to decide which surveillance and/or control measures could be put in place to manage the risk. In addition, risk managers have to manage areas of uncertainty, such as: when, how, and how often the incursion may occur, its impact on livestock and whether EHD diseases will become endemic in the EU.

Therefore, the Commission is in need of further scientific advice on risk assessment, significance, vectors, and control measures as regards EHD

Terms of reference

The review on EHD shall include a complete and updated description of disease characteristics and major factors implicated in disease transmission and persistence in both domestic and wild ruminants. Special attention should be given to the following topics:

- Aetiology (virus characteristics including resistance to physical and chemical action);
- Vector ecology;
- Pathobiology (clinical signs, pathology, immunity);
- Diagnosis (differential, laboratory);
- Epidemiology (hosts, transmission, sources of virus, world-wide distribution);
- Available tools for prevention and control of EHD, especially:

New developments on potential vaccines

Measures in response to outbreaks

− Identification of potential risk factors for the introduction and spread of EHD virus in the EU.

Acknowledgements

This contract/grant was awarded by EFSA to:

Contractor: Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise "G. Caporale".

Contract/grant title: Scientific reviews on Crimean Congo Hemorrhagic Fever (CCHF) and Epizootic Hemorrhagic Disease (EHD).

Contract/grant number: CFP/EFSA/AHAW/2008/04

Introduction and Objectives

INTRODUCTION

Epizootic hemorrhagic disease (EHD) is an infectious non contagious vector-borne viral disease affecting ruminants. Wild cervids and particularly white-tailed deer (*Odocoileus virginianus*) are the most susceptible hosts. In these animals the infection might cause severe symptoms characterised by haemorragic lesions. The epizootic hemorrhagic disease virus (EHDV) belongs to the genus *Orbivirus* and the family *Reoviridae*. It is a double stranded RNA virus closely related to the bluetongue virus (BTV). Recent research indicates that 7 are the serotypes to be included in the EHDV serogroup (Anthony et al., 2009). Midges of the *Culicoides* genus act as vectors of EHDV. Being transmitted by species of *Culicoides*, the EHDV distribution reflects that of its vectors and thus is affected by climatic conditions.

In recent years EHDV outbreaks have been reported in countries surrounding the Mediterranean Basin causing clinical disease in cattle farms.

OBJECTIVES

To prepare a review of the latest available knowledge on Epizootic Hemorrhagic Disease (EHD).

To prepare an updated atlas on the worldwide geographical distribution of EHD.

To collect all available information useful for defining the risk of EHD introduction and spread in EU.

To prepare an updated review on vectors of EHD.

AETIOLOGY

Epizootic hemorrhagic disease virus (EHDV) belongs to the family *Reoviridae*, genus *Orbivirus* and shares many morphological and structural characteristics with the other members of the genus such as bluetongue virus (BTV), African horse sickness virus (AHSV) and equine encephalosis virus (EEV) (Maclachlan and Osburn, 2004).

Based on the serological reactivity, 8 serotypes have been historically recognized (Campbell and St George, 1986) (Table 1):

Serotype	**Prototype strain**
EHDV-1	New Jersey
EHDV-2	Alberta, Ibaraki & CSIRO 439
EHDV-3	Ib Ar 22619
EHDV-4	Ib Ar 33853
EHDV-5	CSIRO 157
EHDV-6	CSIRO 753
EHDV-7	CSIRO 775
EHDV-8	DPP 59

Table 1: EHDV serotypes according to Campbell & St George (1986)

Then, in 1992, Gorman added two new serotypes in the EHDV serogroup: XBM 67 and IbAr 4963 (Table 2):

Serotype	Isolation		
	Prototype	Year	Country
1	New Jersey	1955	USA
2	Alberta	1962	Canada
	Ibaraki	1959	Japan
	CSIRO 439	1980	Australia
3	XBM 67	1967	South Africa

4	IbAr 22619	1967	Nigeria
5	IbAr 33853	1968	Nigeria
6	IbAr 4963	1970	Nigeria
7	CSIRO 157	1977	Australia
8	CSIRO 753	1981	Australia
9	CSIRO 775	1981	Australia
10	DPP 59	1982	Australia

Table 2: EHDV serotypes according to Gorman (1992)

But even if XBM/67 is referred to as a serotype, no detailed serologic comparison with other EHDV strains has been reported.

There is no internationally and officially accepted panel of reference strains of EHDV. According to the 8th Report of the International Committee on the Taxonomy of viruses (2005) nine serotypes have been identified worldwide but more recent information indicates that EHDV serotype 3 (IbAr 22619) is in fact the same serotype as EHDV-1 (Anthony et al., 2009).

Widely accepted EHDV serotype reference strains are shown in Table 3.

Reference strains							
EHDV-1 **USA1955/01**	EHDV-2 **CAN1962/01** EHDV-2 (Ibaraki virus) **JAP1959/01**	EHDV-3[M.Dom1] **NIG1967/01**	EHDV-4 **NIG1968/01**	EHDV-5 **AUS1977/01**	EHDV-6 **AUS1981/07**	EHDV-7 **AUS1981/06**	EHDV-8 **AUS1982/06**

Table 3: Commonly accepted reference strains in the ds RNA virus collection at Institute for Animal Health (IAH) Pirbright, UK and at the Arthropod-Borne Animal Diseases Research Laboratory (ABADRL), USA.

Also, difficulties arose in the classification of an untyped strain of EHDV designated as

EHDV-318. This strain was originally identified in the island of Bahrein, and caused an "Ibaraki-like" disease syndrome in cattle and other ruminants in North Africa (Algeria, Morocco and Tunisia) and Turkey. Recent efforts to characterise it have concluded that this strain is serologically identical to EHDV-6 (Anthony et al., 2009). Ibaraki virus is serologically related to the Alberta EHDV-2 strain and has been included in the EHDV serogroup (Sugiyama et al., 1989). The serological correlation was confirmed by the high homology of different gene segments (Uchinuno et al., 2003; Ohashi et al., 1999, 2002; Iwata et al., 2001).

There is a partial antigenic relationship between serotypes, cross-protection has been described between types 1 and 2 (Gaydos et al., 2002) while no cross-protection is reported between serogroups (Quist et al., 1997).

The structure and the composition of EHDV particles are comparable to those of BTV, the prototype virus of the genus. The EHDV virions are non-enveloped particles of approximately 62-80 nm in diameter, structured as a two-layered icosahedral capsid composed of 32 capsomeres (House et al., 1998). The genome of EHDV is composed of 10 double stranded RNA (dsRNA) segments, enclosed within the core particle (Mecham and Dean, 1988). These segments encode for seven structural (VP1 to 7) and four non-structural proteins (NS1, NS2, NS3 and NS3A) (Mecham and Dean, 1988).

The outer capsid composed of VP2 and VP5 is responsible for the serotype antigenic specificity and the neutralizing antibodies.

The core of the virion is formed by two major proteins, VP3 and VP7 and three minor proteins, VP1, VP4 and VP6 (Le Blois et al., 1991).

VP7 is the serogroup specific immunodominant protein. It is highly conserved among the EHDV serotypes showing a high percentage of identity at the amino acid level (Mecham et al., 2003). VP3 contains antigenic determinants common to the members of the bluetongue group (Mecham and Dean, 1988).

Among the non structural proteins (NS), the NS2 forms the virus inclusion bodies (VIB) during replication and is capable of interacting with ssRNA (Theron et al., 1996; 1996a). The smallest RNA genome segment S10, codes for two proteins (NS3 and NS3A) from different in-frame translation initiation codons. Despite the divergence in the nucleic sequence of homologous proteins (64%), BTV and EHDV still share some structural features. The hydrophobic domains of the proteins and the trans-membrane sequence are conserved confirming their potential role as glycosylation sites (Jensen et al., 1994; 1995).

In order to better understand the epidemiology of EHDV, various phylogenetic studies on some isolates have been performed. When sequences of homologous genes within and among serotypes and serogroups (Wilson, 1991, 1994, 2000; van Staden et al., 1991) or sequences of isolates from different geographic regions (Murphy et al., 2005) were compared, it was possible to group EHDV strains depending on the gene segment and/or the deduced aminoacid sequence analyzed: isolates are grouped according to their serotype, serogroup or geographic origin (topotype) (Murphy et al., 2005, 2006; Mecham et al., 2003).

Analysis of the VP2 gene sequence has allowed topotyping isolates within a serotype (Cheney et al., 1996, Wilson, 1994) whereas analysis of the sequence of the VP3 gene (the inner core protein) has allowed topotyping of the isolates according to their geographic origin regardless of serotype (Cheney et al., 1996).

Table 4 summarizes RNA segments and the predicted amino acid sequences of the encoded gene products with their properties and functions. Most of the data are referred to BTV. The contents were adapted from www.iah.bbsrc.ac.uk/dsRNA_virus_proteins/Orbivirus.htm web page (accessed 01/09/2009) and updated according to Anthony et al. (2009). For each genome segment the current accession numbers were reported.

Genome segment (Size: bp)	**Protein nomenclature**	**Location**	**Accession numbers**	**Properties and Functions**
1 (3942)*	VP1 (Pol)	within the sub-core at the 5 fold axis	AM744977* AM744997* AM745007* AM745017* AM745067* AM744987* AM745027* AM745037* AM745047* AM745057* AM745077*	RNA dependent RNA polymerase
2	VP2	outer capsid	L33818 L33821 L33822 L33823 L33824 L33825 L33826 AB078632 AB078628 AB078624 AB030735 AB07862 0D10767	Trimer, controls virus serotype, serotype specific antigen, cell attachment protein, outer layer of the outer capsid, contains neutralising epitopes, most variable protein, involved in determination of virulence cleaved by proteases.
3 (2768)*	VP3 (T2)	sub-core, capsid layer (T=2 symmetry [11])	X61589 K02369 M76616 M79458 L33819 L33820 S68010 AF258621 AB078629 AB078625 AB078621 AB041933 AM744979* AM744999* AM745009* AM745019* AM745069* AM744989* AM745029* AM745039* AM745049* AM745059* AM745079*	Innermost protein capsid shell, sub-core capsid layer, T=2 symmetry, self assembles, retains icosahedral symmetry by itself, controls size and organisation of capsid structure, RNA binding, interacts with internal minor proteins. Highly conserved protein. Physical organisation of genome.

4 (1983)*	VP4 (Cap)	within the sub-core, at the 5 fold axis	AM744980* AM745000* AM745010* AM745020* AM745070* AM744990* AM745030* AM745040* AM745050* AM745060* AM745080*	Dimers, capping enzyme, guanylyltransferase, transmethylase 1, transmethylase 2.
5	NS1 (TuP)	Cytoplasm, forms tubules	X55782 L27647 L27648 M69085	Forms tubules of unknown function in the cell cytoplasm. These tubules are a characteristic of orbivirus replication.
6	VP5	outer capsid	X55782 X59000 AB078633 AB078630 AB078626 AB078622 AB030736	Inner layer of the outer capsid, glycosylated, helps control virus serotype, variable protein, trimer
7 (1162)*	VP7 (T13)	Outer core (T=13 symmetry)	D10766 U43560 AF188643 AB078631 AB078627 AB078623 AF484250 AB041934 AM744983* AM745003* AM745013* AM745023* AM745073* AM744993* AM745033* AM745043* AM745053* AM745063* AM745083*	Trimer, forms outer core surface, T=13 symmetry, in some species (AHSV) it can form flat hexagonal crystals made up of layers of hexameric rings of trimers, involved in cell entry, involved in high core infectivity in vector insect and cells, reacts with "core neutralising" antibodies, Immuno dominant major serogroup specific antigen.
8	NS2 (ViP)	Cytoplasm, viral inclusion bodies (VIB)	L31764 M69091 L31765 AB084285	Important viral inclusion body matrix protein, ssRNA binding, phosphorylated. Can be associated with outer capsid.

9 (1140-1149 western strains, 1073-1074 eastern strains)*	VP6 (Hel)	Within the sub-core at the 5 fold axis	AM744985* AM745005* AM745015* AM745025* AM745075* AM744995* AM745035* AM745045* AM745055* AM745065* AM745085*	ss RNA and ds RNA binding, helicase, NTPase.
10	NS3/ NS3a	Cell Membranes	L29023 L29022	Glycoproteins, membrane proteins, involved in cell exit, in some genera (AHSV) these are variable proteins and may be involved in determination of virulence. Cytotoxic, can disrupt cell membranes

Table 4 : EHDV RNA segments and the predicted amino acid sequences of the encoded gene products with their properties and functions. The contents were adapted from IAH web page. For each genome segment the current accession numbers were reported. * data from Anthony et al. (2009).

As for the structure and composition, the **physio-chemical properties** of EHDV are also similar to those of other members of the genus *Orbivirus*. All the orbiviruses are readily inactivated at pH values below 6.0. Conversely, they are relatively stable at alkaline pH values (7.0–8.5). As non-enveloped virus, EHDV is considered to be relatively resistant to treatment with lipid solvents. However, sodium dodecyl sulphate is able to disrupt the particle and destroy its infectivity. Oxidizing agents such as calcium or sodium hypochlorite (2-3%) or alkalis such as sodium hydroxide at 2% or glutaraldehyde at 2% are considered effective disinfectants. EHDV is also inactivated by heat treatment at 50°C for 3 hours or at 60°C for 15 minutes.

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 a significant loss of titre. This does not occur during lyophilisation or temperature at –70°C or lower (Coetzer and Guthrie, 2004).

In summary:

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

Future researches:

- Full genome sequencing of different serotypes.

PATHOGENESIS AND IMMUNITY

The pathogenesis of the EHDV appeared to be similar to the accepted BTV model with the initial virus replication in the endothelial cells of the lymphatic vessels and in the lymph nodes draining the site of infection. It is then disseminated to the secondary sites of replication such as lymph nodes and spleen. In the viraemic phase the virus is cell-associated. *In vitro* studies indicated that EHDV replication in peripheral blood could be monocyte-dependent (Stallknecht et al., 1997). However it was demonstrated that the circulating viruses are embedded in pits of the erythrocyte surface (Stallknecht et al., 1997; Aradaib et al., 1997). In the red blood cells the virus is present to high titre and for long period of time (Gibbs and Lawman, 1977). This close association protects the virus from the humoral and cell mediated immune responses accounting for the contemporaneous presence of EHDV and neutralizing antibodies in the circulating blood of infected animals.

The ability of the virus to replicate in endothelial cells (McLaughlin et al., 2003) causes a wide spread vascular injury and subsequent disseminated intravascular coagulation that leads to haemorrhages, oedema and tissue necrosis (Tsai and Karstad, 1973).

The present knowledge from experimental trials and natural infections indicates that the incubation period for EHD is from 4 to 10 days. The virus can be detected in the majority of the infected animals from 3 days up to three weeks post infection (pi) with titres sometimes higher than $10^{6.2}TCID_{50}$/ml. (Gibbs and Lawman, 1977; Quist et al., 1997; Aradaib et al., 1994a, Stallknecht et al., 1997, Gaydos et al., 2002a).

However, in a small proportion of deer and cattle, viraemias exceeding 50 days have been reported (Gibbs and Lawman, 1977; Quist et al., 1997). The length of viraemia for 4 different serotypes (EHDV-2, -5, -7 and -8) was monitored in 130 naturally infected sentinel cattle

grazed in northern Australia in an area where EHDV occurred regularly (Gard and Melville, 1992). The data are reported in Table 5.

When infected, animals might show high levels of gamma interferon starting from day 4 post infection (dpi) until the rise of detectable antibodies (10 dpi) (Quist et al., 1997).

Seroconversion to an EHDV-1 strain was first detected on day 15 pi by AGID test and on day 21 pi when the competitive ELISA (cELISA) was used. With EHDV-2, both serological techniques detected antibodies from 22 days post infection (dpi) (Aradaib et al., 1994a) while SN detected antibodies in deer between 10 and 14 dpi (Gibbs and Lawman, 1977). Work et al. (1992) using AGID were able to detect EHDV-1 antibodies in deer from 6 dpi.

Serotype	Duration of viraemia (weeks)				
	<1	1-2	2-3	3-4	4-5
2	46	24	6	4	1
5	31	3	0	1	1
7	0	1			
8	12				

Table 5: Duration of viraemia in sentinel cattle naturally infected with several EHDV serotypes (Gard and Melville, 1992)

In deer, maternal antibodies disappear at 17-18 weeks of age. The presence of passive immunity could not fully prevent infection or viraemia but was able to protect against the severe clinical form of the disease (Gaydos et al., 2002b).

The epizootic hemorrhagic disease is a vector borne disease transmitted by certain species of *Culicoides* and little is known about other transmission routes. Oral and fecal shedding of EHDV-1 from white tail deer has been reported (Gaydos et al., 2002c). Similarly EHDV was also recovered from vulvar swabs in a sheep that had recently lambed (Gibbs and Lawman, 1977). The most likely explanation of these findings is blood contamination of the samples. In addition EHDV-2, Ibaraki strain, was isolated from internal organs of infected foetus

demonstrating that transplacental transmission may occur in naturally infected animals (Ohashi et al., 1999).

The isolation of BTV from semen has been reported (Bowen et al 1985) although the presence of the virus in semen appears to be a rare event.

In summary:

- EHD is a systemic disease with vascular endothelial damage that leads to haemorrhages, oedema and tissue necrosis.
- In susceptible hosts, live circulating virus can be detected in the majority of the infected animals from 3 days up to three weeks post infection, rarely longer than 50 days.
- Transplacental transmission occurred in EHDV-2 Ibaraki strain in naturally infected cattle.

Future researches:

- To define the duration of viremia in cattle by using both RT-PCR and virus isolation.
- To define the pathogenesis of the different EHDV serotypes in domestic ruminants.

CLINICAL SIGNS

Epizootic hemorrhagic disease causes a hemorrhagic disease in some North American species of deer (e.g. white tailed deer, black tailed deer), whereas infection is typically asymptomatic in domestic ruminants.

Three clinical forms have been described in these species of deer**,** peracute, acute and chronic. The peracute form is characterized by high fever, anorexia, weakness, respiratory distress, and severe and rapid oedema of the head and neck. Swelling of the tongue and conjunctivae is also common but the haemorrhagic diathesis with bloody diarrhoea and/or haematuria and dehydration are typical findings in dead animals. Deer with the peracute form of disease usually die rapidly, within 8-36 hours sometimes without clinical signs.

In the acute form (or classical form), the above described clinical signs might be accompanied by haemorrhages in many tissue including skin, heart and the gastro intestinal tract. There is often hyperaemia of the conjunctivae and the mucous membranes of the oral cavity with excessive salivation and nasal discharge sometimes blood-tinged. Animals with the acute form might also develop ulcers or erosions of the tongue, dental pad, palate, rumen and abomasum. In both the peracute and acute forms the mortality rate is generally high. In the chronic form deer are ill for several weeks but gradually recover. After recovery, because of the growth interruption, the animals might develop breaks or rings in the hooves and might become lame. In some cases, animals slough the hoof wall or toe and crawl on their knees or chest. Deer with the chronic form might also develop ulcers, scars, or erosions in the rumen (Iowa State Center for Food Security and Public Health)

<http://www.cfsph.iastate.edu/Factsheets/pdfs/epizootic_hemorrhagic_disease.pdf> (accessed 10/06/2009).

Although North American isolates of EHDV when experimentally inoculated in **cattle** were not able to induce clinical disease (Abdy et al., 1999; Aradaib et al., 1994a), in the field, lesions suggestive of vesicular disease have been reported in North American cattle seropositive to EHDV-2 (House et al., 1998).

Before 2006, the only strain of the EHDV serogroup which has historically been demonstrated to be pathogenic for cattle was the Ibaraki virus, a strain serologically related to the Alberta EHDV-2 strain (Iwata et al., 2001). According to the Iowa State Center for Food Security and Public Health: "The disease in Japan is known as Ibaraki disease in cattle and is characterized by eruptions, and the animals may become lame. Animals with the chronic form may also develop ulcers, scars, or erosions in the rumen; extensive damage to the lining of the rumen can cause emaciation. Ibaraki disease in cattle is characterized by fever, anorexia, and difficulty swallowing. The swallowing disorders are caused by damage to the striated muscles of the pharynx, larynx, oesophagus and tongue, and may lead to dehydration, emaciation, and aspiration pneumonia. Oedema, haemorrhages, erosions, and ulcerations may be seen in the mouth, on the lips, and around the coronets. The animals may be stiff and lame, and the skin

may be thickened and oedematous. Abortions and stillbirths have also been reported in some epidemics. Some affected cattle die. Outside Japan, Korea and Taiwan, the majority of the EHDV infections in cattle have been reported as subclinical."

<http://www.cfsph.iastate.edu/Factsheets/pdfs/epizootic_hemorrhagic_disease.pdf> (accessed 10/06/2009).

In 2006 an outbreak affecting cattle caused by EHDV-318 (EHDV-6) (OIE Disease Information, 2 Nov 2006, Vol 19 - No. 44; OIE Disease Information Vol. 19 No. 51) was reported in Morocco, Tunisia and Algeria. This unclassified virus has recently been typed as EHDV-6 (Anthony et al 2009). While not as severe as some reports of the related Ibaraki disease in Japan, these outbreaks were clearly not subclinical. In Morocco almost half of the affected premises had high morbidity; 98 out of the 218 herds had rates of over 33%, but many of these farms were small, with 8 being the largest number of cases on a single farm. The overall morbidity rate was 18% (329 cases out of 1814 susceptible animals), while the mortality rate was 2.2%.

In September of the same year an outbreak of “hemorrhagic disease” was first noticed on over 100 dairy and beef cattle herds in Israel. A strain of EHDV was isolated from samples collected from several herds and was identified by RT-PCR, and typed as EHDV-7. The number of clinical cases per farm varied from several to hundreds, with morbidity rates ranging from 90-100% in the internal area to 1-5% in the coastal area. Duration of acute disease was 7 to 10 days and mortality was about 1.5%.

The main and most prominent clinical signs of the disease in Israel were a sharp reduction in milk production and were highly similar to the signs of Ibaraki. The clinical signs ranged from fever, weakness and stiff gait to serous/purulent nasal discharge, excessive salivation, nasal and lip redness, with cyanosis and erosions of the tongue. Also petechiae on the tips of the lingual and buccal papillae were reported. Other common clinical signs were discoloration, from red to purple, of the udder and hoof, and oedema with haemorrhages (Yadin et al., 2008). Pictures of clinical lesions are available at http://www.icba.org.il/hitpartsut-09-2006.htm. This was the first report of isolation of EHDV

in Israel. Interestingly, this is the first occasion on which EHDV-7 has been shown to cause significant clinical signs in cattle and, most importantly, is the second cases in which an EHDV, which does not belong to the Ibaraki serotype, has had this effect in cattle (Yadin et al., 2008).

In 2007, another outbreak caused by EHDV-318 (EHDV-6) was reported in Turkey. The affected cattle showed stomatitis, swelling of eyelids, respiratory distress, nasal and ocular discharge, redness and scaling of muzzle and lips, lameness and erythema of the udder (Temizel et al., 2009).

In summary:

- EHD can occur in different forms related to the viral serotype and the species involved.
- EHDV serotypes 2, 6 and 7 are responsible for clinical disease in cattle.

GROSS LESIONS

In **white-tailed deer**, EHDV usually causes an acute disease that might be fatal. Body temperature increases for 4 to 8 dpi and lasts 1 to 6 days (Quist et al., 1997).

The lesions vary according to the form of the disease. In the peracute form, there may be severe oedema of the head, neck, eyes and lungs. In the acute form, widespread haemorrhages and oedema can be seen in many parts of the body including the mucous membranes, skin and viscera, particularly the head and gastrointestinal tract. There may also be erosions and ulceration in the rumen and omasum. Dry, grey-white necrotic lesions can sometimes be found in the hard palate, tongue, dental pad, oesophagus, larynx, rumen and abomasum. The lesions of the chronic form may include rings or breaks on the hooves, or sloughing of the tips or walls of the hooves. Deer with chronic disease can also develop ulcers, scars or erosions in the rumen.

Hystopathologic lesions are characterized by haemorrhage and necrosis, with thrombosis of capillaries and small vessels in affected tissues. The widespread vasculitis and the

degenerative changes can be found especially in the tongue, salivary glands, walls of forestomachs and aorta and papillary muscle of the left ventricle of the myocardium (Maclachlan and Osburn, 2004).

In **bighorn sheep** necropsy revealed extensive subcutaneous oedema, presence of yellow fluid in the thorax and pericardial sac, greenish discharge from the nose and blood around the anus. Multifocal haemorrhages in the epicardium and in the papillary muscle of the left ventricle were also reported. Haemorrhages were noted on the conjunctival membranes of the eye and on the serosal surfaces of the rumen and intestine. Scattered grey plaques on the surface of the gall bladder mucosa were also described (Noon et al., 2002a).

Microscopic petechial haemorrhages are consistently present in different organs: myocardium, epicardium, tunica muscolaris and submucosa of the rumen but also in CNS. Areas of myocardial degeneration were also described (Noon et al., 2002a).

In **cattle** affected by Ibaraki virus (EHDV-2) the gross lesions of the mucous membranes, the skin and the vascular system are similar to those reported for EHD in deer. Particularly severe is the degeneration of striated muscular tissue observed in the oesophagus, larynx, pharynx, tongue and the skeletal muscles. Severe lesions affecting the oesophageal and laryngopharyngeal musculature cause difficulty in swallowing which in turn produces dehydration and emaciation, and occasionally the aspiration pneumonia, which constitutes the major cause of death of affected animals (Inaba, 1975). These lesions are hystologically characterized by hyaline degeneration, necrosis and mineralization accompanied by an infiltration of neutrophils, lymphocytes and histiocytes. A marked proliferation of fibroblasts occurs in chronic lesions (Maclachlan and Osburn, 2004).

HOST RANGE

In the past, EHDV has been associated to disease in wild cervids (Nettles et al., 1992), particularly white-tailed deer (*Odocoileus virginianus*) in North America and in cattle in Japan, Korea and Taiwan (Ibaraki virus-EHDV-2). In recent years clinical cases have also been reported in cattle of the countries surrounding the Mediterranean Basin. At least two

EHDV serotypes were responsible for the outbreaks in Morocco, Tunisia, Algeria, Turkey (EHDV-6), Jordan, and Israel (EHDV-7). Other than in North American deer and in cattle, there is scanty and sometimes contradictory information about the susceptibility of domestic and wild ruminant species to EHDV infection.

Experimental data on the EHDV susceptibility of European species of wild and domestic animals by Gibbs and Lawman (1977) showed that red deer (*Cervus elaphus),* fallow deer (*Dama dama*), roe deer (*Capreolus capreolus*), Suffolk cross sheep and Jersey and Friesian cattle developed a detectable viremia. Conversely a serosurvey carried out between 2000 and 2002 in six German national parks (NP) which included free-ranging red deer (*Cervus elaphus*), roe deer (*Capreolus capreolus*) and fallow deer (*Dama dama*) did not detect antibodies against epizootic hemorrhagic disease virus (Frolich et al., 2006).

Although European sheep have been described to be susceptible to EHDV infection although subclinically (Gibbs and Lawman, 1977; Tomori, 1980), in Israel during the recent outbreaks, infection was not reported in this species. Pigs are not susceptible to EHDV infection (Gibbs and Lawman, 1977). Similarly, according to a serological survey conducted in a high EHDV prevalence area where no positive animals were detected (Howerth et al., 1995), dogs seem not to be susceptible to EHDV infection.

The current knowledge over susceptible species is summarized in Tables 6-8. Table 6 displays EHDV infection in cattle. Of the 5 serotypes involved only EHDV-2 (Ibaraki), EHDV-6 and EHDV-7 were able to determine clinical symptoms.

Table 7 summarizes some of the data related to the wild species susceptible of EHDV infection. In these species clinical symptoms were reported in white-tailed deer (*Odocoileus virginianus)* mule deer/ black tailed deer *(Odocoileus hemionus)* and Rocky Mountain bighorn sheep *(Ovis canadensis canadensis).*

Table 8 refers to species in which serological investigation gave inconclusive results. The presence of antibodies was determined by the AGID test which cross-reacts with BTV.

Country	Year	Serological positivity	Virological positivity	References
Northern Australia	1992		EHDV-1-2-5-6-7-8	Weir et al. EHDV-1, a new Australian serotype of epizootic haemorrhagic disease virus isolated from sentinel cattle in the Northern Territory. Vet Microbiol. 1997 Nov;58(2-4):135-43. Gard and Melville. Results of a decade's monitoring for orbiviruses in sentinel cattle pastured in an area of regular arbovirus activity in northern Australia. In: T.E. Walton and B.I. Osburn, Editors, *Bluetongue, African horse sickness and related Orbiviruses*, CRC Press, Boca Raton, FL 1992, pp. 85-89.
British Columbia (Canada)	1987-88		EHDV-2	Dulac et al. British Columbia. Incursion of bluetongue virus type 11 and epizootic hemorrhagic disease of deer type 2 for two consecutive years in the Okanagan Valley. Can Vet J. 1989 Apr;30(4):351.
	1999	EHDV-2		Pasick et al. Incursion of epizootic hemorrhagic disease into the Okanagan Valley, British Columbia in 1999. Can Vet J. 2001 Mar;42(3):207-9.
Japan	1959-60		EHDV-2 (Ibaraki strain)	Omori et al. Ibaraki virus, an agent of Emorrhagic Disease resembling bluetongue. Japan J Microbiol. 1969. 13 (2) 139-157.
	1997	EHDV-2 (Ibaraki strain)		Ohashi et al. Identification and PCR-restriction fragment length polymorphism analysis of a variant of the Ibaraki virus from naturally infected cattle and aborted fetuses in Japan. J Clin Microbiol. 1999 Dec;37(12):3800-3.
Midwestern states (USA)	1996	EHDV-2		House et al. Serological diagnosis of epizootic hemorrhagic disease in cattle in the USA with lesions suggestive of vesicular disease. Ann N Y Acad Sci. 1998 Jun 29;849:497-500.

Indonesia			EHDV-5	Sendow et al. Antibodies against certain bluetongue and epizootic haemorrhagic disease viral serotypes in Indonesian ruminants. Vet Microbiol. 1991 Jun;28(1):111-8. Parsonson and Snowdon. Bluetongue, epizootic haemorrhagic disease of deer and related viruses: current situation in Australia. Prog Clin Biol Res. 1985; 178: 27-35.
Turkey	1986-87	NK (AGID +)		Burgu, I., Akca, Y., Hamblin, C. & Kitching, P. (1991) Epizootic hemorrhagic disease virus-antibodies in Turkey. Tropical Animal Health and Production, 23, 261-262.
	2007		EHDV-6	Temizel et al. Epizootic hemorrhagic disease in cattle, Western Turkey. Emerg Infect Dis. 2009 Feb; 15(2):317-9.
Israel	1950	NK		Komarov and Goldsmith. A disease, similar to BT in cattle and sheep in Israel. Refuah Veterinarith. 1951. Vol 8, no. 3, 96-100
	2001	NK		Promed, archive number 20060925.2739
	2006		EHDV-7	Yadin et al. Epizootic haemorrhagic disease virus type 7 infection in cattle in Israel. Vet Rec. 2008 Jan 12; 162 (2): 53-6.
Colorado (USA)			EHDV-1 EHDV-2	Foster et al. Bluetongue and epizootic hemorrhagic disease virus isolation from vertebrate and invertebrate hosts at a common geographic site. J Am Vet Med Assoc. 1980 Jan 15; 176 (2):126-9.
Texas	1985	EHDV-1 EHDV-2		Fulton et al. Neutralizing antibody responses to bluetongue and epizootic hemorrhagic disease virus serotypes in beef cattle. Am J Vet Res. 1989 May; 50 (5): 651-4.
La Reunion	2003		X**	Bréard et al. Outbreak of epizootic haemorrhagic disease on the island of Réunion. Vet Rec. 2004 Oct 2; 155 (14): 422-3.
	2009		X**	OIE WAHID Disease Information 2009; 22(9).
Indiana (USA)	2000-2002	X		Boyer et al. Exploratory spatial data analysis of regional seroprevalence of antibodies against epizootic hemorrhagic disease virus in cattle from Illinois and Indiana. Am J Vet Res. 2008 Oct; 69 (10): 1286-93.
South Africa	1995-1997	X		Barnard et al. Some epidemiological and economic aspects of a bluetongue-like disease in cattle in South Africa--1995/96 and 1997. Onderstepoort J Vet Res. 1998 Sep; 65 (3): 145-51.
Jordan	2006	NK		Promed, archive number 20061215.3525

Morocco	2004	NK		Promed, archive number 20061214.3513
Morocco	2006		EHDV-6 (previously EHDV-318)	OIE Disease Information, 2 Nov 2006, Vol 19 - No. 44
Tunisia	2006		EHDV-6 (previously EHDV-318)	OIE Disease Information, 28 Dec 2006, Vol 19 - No. 52
Algeria	2006		EHDV-6 (previously EHDV-318)	OIE Disease Information Vol. 19 No. 51
Oman	1987-88		EHDV-2 EHDV-6	Al-Busaidy, S. M. and Mellor, P. S. (1991) Epidemiology of bluetongue and related orbiviruses in the Sultanate of Oman. Epidemiology and Infection, 106, 167-178
Bahrein	1985		EHDV-6 (previously EHDV-318)	Mellor personal communication 1992 (cited in Rabenau et al 1993)

Table 6: EHDV infections reported in **cattle** *(Bos taurus).* For each report the geographical location, the year of occurrence and the source has been given. *Sera tested by AGID: possible cross reaction with BTV antibodies. ** RT-PCR positive

Species	**Country/Year**	**Serological positivity**	**Virological positivity**	**References**
White-tailed deer *(Odocoileus virginianus)****	East coast (USA) 1999		EHDV-1	Murphy et al. Molecular characterization of epizootic hemorrhagic disease virus serotype 1 associated with a 1999 epizootic in white-tailed deer in the eastern United States. J Wildl Dis. 2006 Jul; 42 (3): 616-24.
	West Virginia and Arizona (USA) 1993		EHDV-2	Gaydos et al. Epizootiology of an epizootic hemorrhagic disease outbreak in West Virginia. J Wildl Dis. 2004 Jul; 40 (3): 383-93. Noon et al. Hemorrhagic disease in deer in Arizona. J Wildl Dis. 2002 Jan; 38 (1): 177-81.
	Missouri (USA) 1988 1996		EHDV-2	Fischer et al. An epizootic of hemorrhagic disease in white-tailed deer (*Odocoileus virginianus*) in Missouri: necropsy findings and population impact. J Wildl Dis. 1995 Jan; 31 (1): 30-6. Beringer et al. An epizootic of hemorrhagic disease in white-tailed deer in Missouri. J Wildl Dis. 2000 Jul; 36 (3): 588-91.
	2007-2009		EHDV-6	Allison et al. Detection of a novel reassortant epizootic hemorrhagic disease virus in the United States containing RNA segments derived from both exotic and endemic serotypes. 2009. In press.

	Indiana and Illinois (USA) 2006		EHDV-6	Allison et al. Detection of a novel reassortant epizootic hemorrhagic disease virus in the United States containing RNA segments derived from both exotic and endemic serotypes. 2009. In press.
	Texas and Georgia (USA) 1989-92	EHDV-1 EHDV-2		Stallknecht et al. Hemorrhagic disease in white-tailed deer in Texas: a case for enzootic stability. J Wildl Dis. 1996 Oct; 32 (4): 695-700. Stallknecht et al. Epizootic hemorrhagic disease virus and bluetongue virus serotype distribution in white-tailed deer in Georgia. J Wildl Dis. 1995 Jul; 31 (3): 331-8.
	Texas and Kansas 2008		EHDV-6	Allison et al. Detection of a novel reassortant epizootic hemorrhagic disease virus in the United States containing RNA segments derived from both exotic and endemic serotypes. 2009. In press.
	Mexico 1994	X		Martinez et al. Serosurvey for selected disease agents in white-tailed deer from Mexico. J Wildl Dis. 1999 Oct; 35 (4): 799-803.
	Minnesota (USA) 2000-03	X		Wolf et al. Selenium status and antibodies to selected pathogens in white-tailed deer (*Odocoileus virginianus*) in Southern Minnesota. J Wildl Dis. 2008 Jan; 44 (1): 181-7.
	British Columbia (Canada) 1981-89	EHDV-2		Stallknecht et al. Antibodies to bluetongue and epizootic hemorrhagic disease viruses in a barrier island white-tailed deer population. J Wildl Dis. 1991 Oct; 27 (4): 668-74.
	1988-89	EHDV-2		Dulac et al. British Columbia. Incursion of bluetongue virus type 11 and epizootic hemorrhagic disease of deer type 2 for two consecutive years in the Okanagan Valley. Can Vet J. 1989 Apr; 30 (4): 351.
	1987	EHDV-2		Shapiro et al. A survey of cattle for antibodies against bluetongue and epizootic hemorrhagic disease of deer viruses in British Columbia and southwestern Alberta in 1987. Can J Vet Res. 1991 Apr; 55 (2): 203-4.
Mule deer/ black tailed deer *(Odocoileus hemionus)****	Arizona (USA) 2002	EHDV-1 EHDV-2		Dubay et al. Determining prevalence of bluetongue and epizootic hemorrhagic disease viruses in mule deer in Arizona (USA) using whole blood dried on paper strips compared to serum analyses. J Wildl Dis. 2006 Jan; 42 (1): 159-63.

	2001	X	X**	Dubay et al. Epizootiology of hemorrhagic disease in mule deer in central Arizona. J Wildl Dis. 2004 Jan; 40 (1): 119-24..
	1993		EHDV-2	Noon et al. Hemorrhagic disease in deer in Arizona. J Wildl Dis. 2002 Jan; 38 (1): 177-81.
	California (USA) 1987-91	X		Chomel et al. Antibody prevalence of eight ruminant infectious diseases in California mule and black-tailed deer (*Odocoileus hemionus*). J Wildl Dis. 1994 Jan; 30 (1): 51-9.
	Kansas (USA) 1980-2002	EHDV-1 EHDV-2		Flacke et al. Hemorrhagic disease in Kansas: enzootic stability meets epizootic disease. J Wildl Dis. 2004 Apr; 40 (2): 288-93.
Rocky Mountain bighorn sheep *(Ovis canadensis Canadensis)****	Arizona (USA) 1995		EHDV-2	Noon et al. Hemorrhagic disease in bighorn sheep in Arizona. J Wildl Dis. 2002a Jan; 38 (1):172-6.
Pronghorn *(Antilocapra americana)*	Arizona (USA) 2001-03	X*		Dubay et al. Serologic survey for pathogens potentially affecting pronghorn (*Antilocapra americana*) fawn recruitment in Arizona, USA. J Wildl Dis. 2006 Oct; 42 (4): 844-8.
	Oregon (USA) 1996-97	X		Dunbar et al. Health evaluation of a pronghorn antelope population in Oregon. J Wildl Dis. 1999 Jul; 35 (3) :496-510.
Black rhinoceros *(Diceros bicornis)*	South Africa, Namibia and Kenia 1987-89	EHDV-1		Fischer et al. Serosurvey for selected infectious disease agents in free-ranging black and white rhinoceros in Africa. J Wildl Dis. 2000 Apr; 36 (2): 316-23.
White rhinoceros *(Ceratotherium simum)*	South Africa, Namibia and Kenia 1987-89	EHDV-1		Fischer et al. Serosurvey for selected infectious disease agents in free-ranging black and white rhinoceros in Africa. J Wildl Dis. 2000 Apr; 36 (2): 316-23.
Arabian oryx *(Oryx leucoryx)*	Saudi Arabia and the United Arab Emirates 1999-01	X		Frölich et al. Serologic surveillance for selected viral agents in captive and free-ranging populations of Arabian oryx (Oryx leucoryx) from Saudi Arabia and the United Arab Emirates. J Wildl Dis. 2005 Jan; 41 (1): 67-79.

Table 7: List of **susceptible hosts** to EHDV. For each species the serotype involved, the geographical location, the year of occurrence and the source have been reported. *Sera tested by AGID: possible cross reaction with BTV antibodies. ** RT-PCR positive *** Species with clinical symptoms.

Species	**Country/Year**	**Serological positivity**	**Virological positivity**	**References**
Grey brocket deer *(Mazama gouazoubira)*	Bolivia 1999	X*		Deem et al. Disease survey of free-ranging grey brocket deer (*Mazama gouazoubira*) in the Gran Chaco, Bolivia. J Wildl Dis. 2004 Jan; 40 (1): 92-8.
Black bear *(Ursus americanus floridanus)*	Florida (USA) 1993-5	X*		Dunbar et al. Seroprevalence of selected disease agents from free-ranging black bears in Florida. J Wildl Dis. 1998 Jul; 34 (3): 612-9.

Table 8: List of hosts with seroconversion to EHDV or BTV. Sera were tested by AGID with possible cross reaction with BTV antibodies. For each species the serotype involved, the geographical location, the year of occurrence and the source have been reported. *Sera tested by AGID: possible cross reaction with BTV antibodies.

In summary:

- EHDV host range comprises domestic and wild ruminants.
- Pigs are resistant to the EHDV infection.

Future researches:

- To characterize the susceptibility of European domestic and wild ruminants' species to better define their possible role in the epidemiology of EHD.

GEOGRAPHICAL DISTRIBUTION

Epizootic hemorrhagic disease has been reported in North America, Australia, Asia and Africa and, recently, for the first time it was observed in countries surrounding the Mediterranean Basin such as Morocco, Algeria, Tunisia, Israel and Turkey (western Anatolia) (Figure 1).

Figure 1: Map with the localization of the most recent outbreaks (2006-2007) in countries bordering the Mediterranean Basin. Where no geographical details as district/provice etc, are not reported, the whole country has been indicated as infected. More details about the outbreaks are reported in Table 9.

The outbreaks in Israel during 2006 were attributed to a strain belonging to EHDV-7 serotype; outbreaks in Morocco and Algeria were similar to EHDV-6 (previously EHDV-strain 318), while the outbreaks in Turkey were attributed to EHDV-6. The origin(s) of the 2 EHDV serotypes which have affected the Mediterranean countries remain(s) unclear. The geographical distribution of EHDV in EU neighbour regions and worldwide are summarized in Tables 9 and 10 and illustrated in Figure 2.

Geographical Location	Date of occurrence	Infected Host	Status	Identified Serotypes	References

Turkey	07/2007	Cattle	Confirmed clinical infection (RT-PCR from isolate) ELISA (1/6 pos)	EHDV-6 (previous EHDV-318	Temizel et al. Epizootic hemorrhagic disease in cattle, Western Turkey. Emerg Infect Dis. 2009 Feb;15(2):317-9
Turkey	Approx. 1986-87	Cattle and sheep	AGID Low prevalence	NK	Burgu, et al., Epizootic hemorrhagic-disease virus-antibodies in Turkey. Tropical Animal Health and Production 1991, 23, 261-262.
Israel	28/08/2006	Cattle	Confirmed clinical infection	EHDV-7	OIE Disease Information, 28 Sep 2006, Vol 19 - No. 39 http://www.oie.int/eng/info/hebdo/AIS_77.HTM#Sec8 Yadin et al. Epizootic haemorrhagic disease virus type 7 infection in cattle in Israel. Vet Rec. 2008 Jan 12; 162 (2): 53-6.
Israel	2001	Cattle	Confirmed clinical infection only in ELISA	NK	Promed, archive number 20060925.2739
Israel	1950	Cattle	Disease suspected but not confirmed	NK	Komarov and Goldsmith. A disease, similar to BT in cattle and sheep in Israel. Refuah Veterinarith. 1951. Vol 8, no. 3, 96-100.
Jordan	09/2006	Cattle	Disease suspected but not confirmed	NK	Promed, archive number 20061215.3525
Morocco	28/07/2006	Cattle	Confirmed clinical infection (RT-PCR & ELISA)	EHDV-6 (previous EHDV-318)	OIE Disease Information, 2 Nov 2006, Vol 19 - No. 44 http://www.oie.int/eng/info/hebdo/AIS_78.HTM#Sec1
Morocco	2004	Cattle	Confirmed clinical infection	EHDV-6 (previous EHDV-318)	Promed, archive number 20061214.3513
Tunisia	03/09/2006	Cattle	Confirmed clinical	EHDV-6	OIE Disease Information, 28 Dec

			infection AgELISA	(previous EHDV-318)	2006, Vol 19 - No. 52 http://www.oie.int/eng/info/hebdo/A_CURRENT.HTM#Sec4
Algeria	19/07/2006	Cattle	Confirmed clinical infection (RT-PCR)	EHDV-6 (previous EHDV-318)	OIE Disease Information Vol. 19 No. 51 http://www.oie.int/eng/info/hebdo/AIS_65.HTM#Sec11
Bahrein	1985	Cattle	Confirmed clinical infection	EHDV6 (previous EHDV-318)	Mellor personal communication 1992 (cited in Rabenau et al 1993)
Oman	1987-1988	Cattle and goats	Confirmed infection but no clinical disease Seroneutralization	EHDV2 EHDV6 (previous EHDV-318)	Al-Busaidy and Mellor. Epidemiology of bluetongue and related orbiviruses in the Sultanate of Oman. Epidemiology and Infection, 1991. 106, 167-178.
Saudi Arabia	1991	Deer	Confirmed infection	NK	Abuelzein et al. Isolation of a virus serologically related to the bluetongue group from an outbreak of hemorrhagic-disease among exotic deer in Saudi-Arabia. Veterinary Record, 1992. 131, 439-441.
Saudi Arabia and the United Arab Emirate	1999-2001	Arabian oryx	Confirmed infection but no clinical disease AgELISA	NK	Frölich et al. Serologic surveillance for selected viral agents in captive and free-ranging populations of Arabian oryx (*Oryx leucoryx*) from Saudi Arabia and the United Arab Emirates. J Wildl Dis. 2005 Jan;41(1):67-79.

Table 9: EHD geographical distribution in Europe neighbour regions. Status: Disease suspected but not confirmed/Confirmed infection but no clinical disease/Confirmed clinical infection/. NK: Not known

Geographical Location	**Date of occurrence**	**Infected Host**	**Status**	**Identified Serotypes**	**References**
La Reunion	12/01/2009 start date	Cattle	Confirmed clinical infection (RT-PCR)	NK	OIE WAHID Disease Information 2009; 22(9)

La Reunion	2003	Cattle	RT-PCR	NK	Bréard et al. Outbreak of epizootic haemorrhagic disease on the island of Réunion. Vet Rec. 2004 Oct 2; 155 (14): 422-3.
West Virginia (USA)	1993	white-tailed deer (*Odocoileus virginianus*)	Confirmed clinical infection	EHDV-2	Gaydos et al. Epizootiology of an epizootic hemorrhagic disease outbreak in West Virginia. J Wildl Dis. 2004 Jul;40(3):383-93.
Indiana (USA)	2000-2002	Cattle	NK	NK	Boyer et al. Exploratory spatial data analysis of regional seroprevalence of antibodies against epizootic hemorrhagic disease virus in cattle from Illinois and Indiana. Am J Vet Res. 2008 Oct; 69 (10): 1286-93.
Indiana and Illinois (USA)	2006	white-tailed deer	Confirmed clinical infection (virus isolation)	EHDV-6	Allison et al. Detection of a novel reassortant epizootic hemorrhagic disease virus in the United States containing RNA segments derived from both exotic and endemic serotypes. 2009. In press.
Kansas (USA)	1980-2002	white-tailed deer and mule deer	Confirmed clinical infection	EHDV-1; EHDV-2	Flacke et al. Hemorrhagic disease in Kansas: enzootic stability meets epizootic disease. J Wildl Dis. 2004 Apr;40(2):288-93.
Kansas (USA)	2008	white-tailed deer	Confirmed clinical infection (virus isolation)	EHDV-6	Allison et al. Detection of a novel reassortant epizootic hemorrhagic disease virus in the United States containing RNA segments derived from both exotic and endemic serotypes. 2009. In press.
Texas	1985	Beef cattle		EHDV-1 EHDV-2	Fulton et al. Neutralizing antibody responses to bluetongue and epizootic hemorrhagic disease virus serotypes in beef cattle. Am J Vet Res. 1989 May; 50 (5): 651-4.
Texas (USA)	2008	white-tailed deer	Confirmed clinical infection (virus isolation)	EHDV-6	Allison et al. Detection of a novel reassortant epizootic hemorrhagic disease virus in the United States containing RNA segments derived from both exotic and endemic serotypes. 2009. In press.
Colorado (USA)		Dairy cattle		EHDV-1 EHDV-2	Foster et al. Bluetongue and epizootic hemorrhagic disease virus isolation from vertebrate and invertebrate hosts at a common geographic site. J Am Vet Med Assoc. 1980 Jan 15; 176 (2): 126-9.

Missouri (USA)	1988	white-tailed deer	Confirmed clinical infection (virus isolation)	EHDV-2	Beringer J, Hansen LP, Stallknecht DE. An epizootic of hemorrhagic disease in white-tailed deer in Missouri. J Wildl Dis. 2000 Jul; 36 (3): 588-91.
Missouri (USA)	1996	white-tailed deer	Confirmed clinical infection (virus isolation)	EHDV-2	Beringer et al. An epizootic of hemorrhagic disease in white-tailed deer in Missouri. J Wildl Dis. 2000 Jul; 36 (3):5 88-91.
Missouri (USA)	2007-2009	white-tailed deer	Confirmed clinical infection (virus isolation)	EHDV-6	Allison et al. Detection of a novel reassortant epizootic hemorrhagic disease virus in the United States containing RNA segments derived from both exotic and endemic serotypes. 2009. In press.
Florida (USA)	1998	Black bear	Serological survey. BTV/EHDV positive (AGID)	NK	Dunbar et al. Seroprevalence of selected disease agents from free-ranging black bears in Florida. J Wildl Dis. 1998 Jul; 34 (3): 612-9.
Arizona	2002	Mule deer	Serological survey BTV/EHDV positive (AGID/SN)	EHDV-1 EHDV-2	Dubay et al. Determining prevalence of bluetongue and epizootic hemorrhagic disease viruses in mule deer in Arizona (USA) using whole blood dried on paper strips compared to serum analyses. 2006. Journal of Wildlife Diseases, 42, 159-163.
Arizona	2001-2002	Pronghorn (*Antilocapra americana*)	Serological survey BTV/EHDV positive (AGID/SN)	EHDV-1 EHDV-2	Dubay et al. Serologic survey for pathogens potentially affecting pronghorn (Antilocapra americana) fawn recruitment in Arizona, USA. 2006. Journal of Wildlife Diseases, 42, 844-848.
California (USA)	1987-1991	black-tailed and mule deer	Confirmed infection (Retrospective serological study)	NK	Chomel et al. Antibody prevalence of eight ruminant infectious diseases in California mule and black-tailed deer (*Odocoileus hemionus*). J Wildl Dis. 1994 Jan; 30 (1): 51-9.
Midwestern states (USA)	1996	Cattle	Confirmed infection	EHDV-2	House et al. Serological diagnosis of epizootic hemorrhagic disease in cattle in the USA with lesions suggestive of vesicular disease. Ann N Y Acad Sci. 1998 Jun 29; 849:497-500.
British Columbia (Canada)	1987-1988	Cattle	Confirmed infection (Retrospective serological	EHDV-2	Dulac et al. British Columbia. Incursion of bluetongue virus type 11 and epizootic hemorrhagic disease of deer type 2 for two consecutive years in the Okanagan Valley. Can Vet J.

			study)		1989 Apr; 30 (4): 351.
British Columbia (Canada)	1999	white-tailed deer and California bighorn sheep	Confirmed infection (Retrospective serological/RT_PCR study on sentinel cattle)	EHDV-2	Pasick et al. Incursion of epizootic hemorrhagic disease into the Okanagan Valley, British Columbia in 1999. Can Vet J. 2001 Mar; 42 (3): 207-9.
Mexico	1994	white-tailed deer	Confirmed infection but no clinical disease (serological study)	NK	Martinez et al. Serosurvey for selected disease agents in white-tailed deer from Mexico. J Wildl Dis. 1999 Oct; 35 (4): 799-803.
Sudan	1982	*C. shulzei* (sentinel cattle)	Confirmed infection (virus isolation)	EHDV-4 EHDV-6 (previously EHDV-318)	Mohammed and Mellor. Further studies on bluetongue and bluetongue-related orbiviruses in the Sudan. Epidemiol Infect. 1990 Dec; 105 (3): 619-32.
Nigeria	1967-1970	*Culicoides* spp.	Confirmed infection but no clinical disease (virus isolation)	EHDV-3 EHDV-4	Lee. Isolation of viruses from field population of Culicoides (Diptera: Ceratopogonidae) in Nigeria. Journal of Medical Entomology 1979. 16, 76-79.
South Africa	1995-1997	Cattle	Confirmed clinical infection (virus isolation)	NK	Barnard et al. Some epidemiological and economic aspects of a bluetongue-like disease in cattle in South Africa--1995/96 and 1997. Onderstepoort J Vet Res. 1998 Sep; 65 (3): 145-51.
Japan	1959-1960	Cattle	Confirmed clinical infection (virus isolation)	EHDV-2 (Ibaraki strain)	Omori et al. Ibaraki virus, an agent of Emorrhagic Disease resembling bluetongue. Japan J Microbiol. 1969. 13 (2) 139-157.
Japan	1997	Cattle	Confirmed clinical infection (virus isolation)	EHDV-2 (Ibaraki strain)	Ohashi et al. Identification and PCR-restriction fragment length polymorphism analysis of a variant of the Ibaraki virus from naturally infected cattle and aborted fetuses in Japan. J Clin Microbiol. 1999 Dec;37(12):3800-3.
Australia	1992	Cattle	Confirmed clinical infection	EHDV-1-2-5-6-7-8	Weir et al. EHDV-1, a new Australian serotype of epizootic haemorrhagic disease virus isolated from sentinel

			(virus isolation)		cattle in the Northern Territory. Vet Microbiol. 1997 Nov;58(2-4):135-43. Gard and Melville. Results of a decade's monitoring for orbiviruses in sentinel cattle pastured in an area of regular arbovirus activity in northern Australia. In: T.E. Walton and B.I. Osburn, Editors, *Bluetongue, African horse sickness and related Orbiviruses*, CRC Press, Boca Raton, FL 1992, pp. 85-89.
Indonesia	1991	Cattle, Buffaloes, sheep	Confirmed infection (Retrospective serological study)	EHDV-5	Sendow et al. Antibodies against certain bluetongue and epizootic haemorrhagic disease viral serotypes in Indonesian ruminants. Vet Microbiol. 1991 Jun;28(1):111-8.

Table 10: EHD worldwide geographical distribution. Status: Disease suspected but not confirmed/Confirmed infection but no clinical disease/Confirmed clinical infection/. NK: Not known

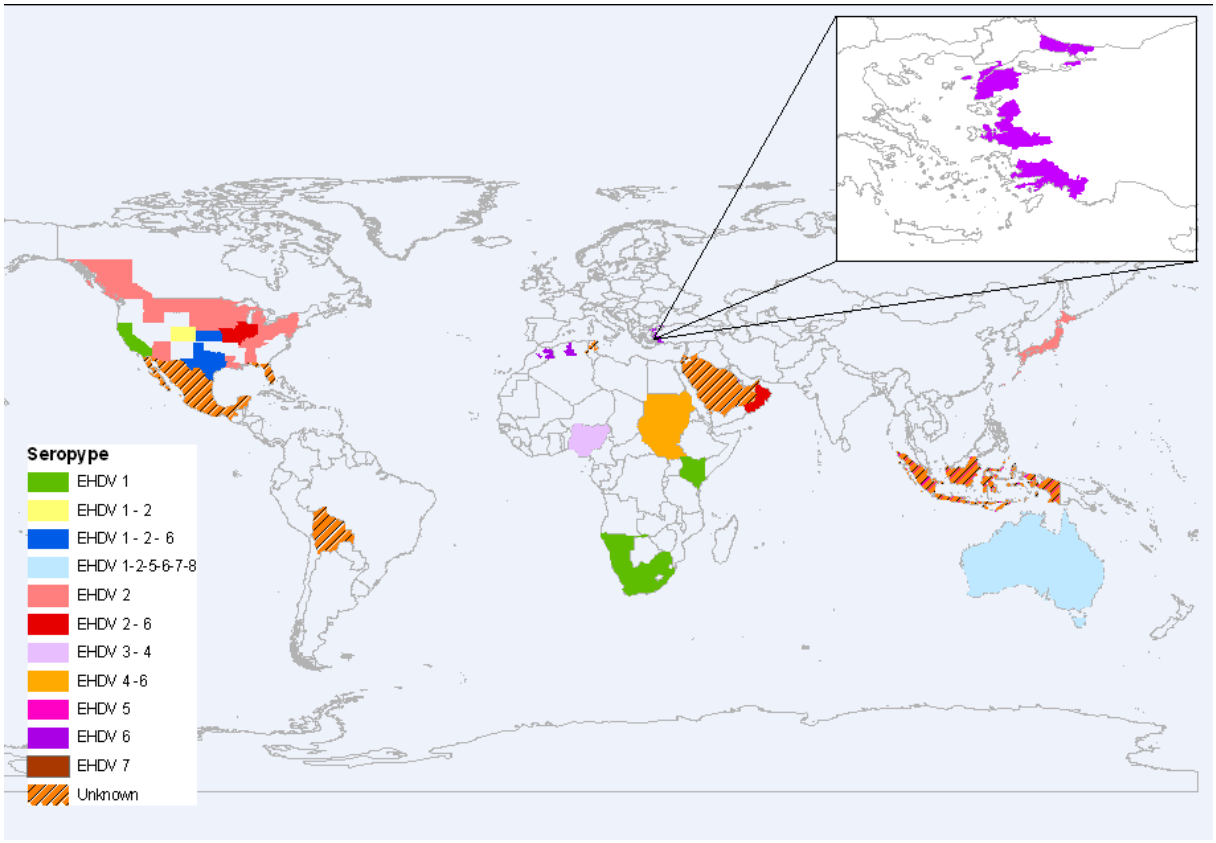

Figure 2: Map with the localization of the EHDV infection worldwide. The map summarizes the data of tables 6, 7, 8, 10 and 12. Where no geographical details as district/provice etc, are not reported, the whole country has been indicated as infected.

USA

The frequency of BT and EHD virus exposure and severity of clinical disease in white-tailed deer, vary greatly in the southeastern U.S. Different epidemiological divisions in this region have been described. Endemic areas include the coastal plains of the south-east, and in this area, most reported cases of HD (haemorrhagic disease) represent the chronic form of the disease. These 'chronic' cases are characterised by hoof and rumen lesions; the disease may affect condition but most infected animals survive. In contrast, in certain areas of the central USA, and in the Piedmont and Appalachian Mountain physiographical regions of the south-east, a pattern of epidemic EHD occurs where high levels of mortality are common. A third pattern exists in Texas and possibly other areas of the south-west and mid-west. In these areas, infections do not result in clinical disease. In Texas, for example, there are very few reported cases of HD even though infection rates, as determined by the presence of antibodies to these viruses, approach 100% (Stallknecht et., 1996).

Based on few clinical reports of EHD and a high antibody prevalence for both the EHDV and BTV (Stallknecht et., 1991a), a similar situation of enzootic stability may occur in southern Florida. This clinical variation relates to variation in herd immunity, specifically the combined effects of maternal antibody transfer (Gaydos et al., 2002b), acquired immunity through previous challenge (Gaydos et al., 2002, Quist et al., 1997), and innate resistance within specific host populations (Gaydos et al., 2002a). There is no evidence to suggest that this observed regional variation is related to variation in EHDV or BTV virulence, either associated with individual EHDV or BTV serotypes or between strains within these serotypes.

In wild ungulates, EHD is seasonal, occurring from mid-summer through to late autumn, and usually peaks in September. From 1990 to 2002, over 220 isolations of EHDV and BTV were made from deer throughout the south-east and mid-west and all have come from clinical submissions within this same seasonal period. The seasonal distribution is most likely to be related to seasonal patterns in vector abundance. The annual cycle of EHD is more difficult to understand. In endemic areas, EHD appears to occur in a two- to three-year cycle (Stallknecht and Howerth, 2004). In epidemic areas, disease occurs in a longer eight- to ten-year cycle (Nettles et al., 1992). These cycles cannot be explained at this time but probably relate to

combined effects of herd immunity and natural or weather-induced fluctuations in vector populations. This is further complicated by the possibility that these short- and long-term cycles may occur concurrently (Stallknecht and Howerth, 2004).

An update on hemorrhagic disease in wild ungulates in the U.S. is reported annually during the Bluetongue Committee meeting of the US Animal Health Association by members of the Southeastern Cooperative Wildlife Disease Study (SCWDS), University of Georgia. The last report included the serotypes isolated during 2007, namely EHDV-1, EHDV-2 and EHDV-6 (Allison et al., 2009). Based on reports of disease that were received from state fish and wildlife agencies during the winter and spring of 2008, there was a major outbreak of EHDV-2 in white-tailed deer in the Eastern United States. The EHDV-2 outbreak probably represented the most extensive Orbivirus outbreak in U.S. history and it affected deer in some areas where EHD does not historically occur (http://www.usaha.org/committees/btbr/btbr.shtml).

To date in 2008, SCWDS has isolated EHDV-1 (Texas), EHDV-2 (Texas, Indiana) and EHDV-6 (Texas, Kansas). Sequence analyses of the 2006-2008 EHDV-6 isolates suggest that this virus may be derived from an EHDV-6/ EHDV-2 reassortment.
(http://www.usaha.org/committees/resolutions/2008/resolution06-2008.pdf) (accessed 10/06/2009)

In summary:

- EHD virus is endemic in North America, Australia and sub-Saharan continent.
- Different EHDV serotypes circulated in EU bordering areas: EHDV-6 in Turkey, Morocco, Tunisia and Algeria and EHDV-7 in Israel.
- According to a serological survey in six German national parks between 2000 and 2002 there is no evidence of EHDV infection among European wild ruminants.

Future researches:

- To define the prevalence of EHD infection in EU bordering areas with ad hoc surveillance plans

VECTORS

The epizootic hemorrhagic disease virus is transmitted by biological vectors. With the exception of a single report in which EHDV was detected in *Anopheles vagus* in Indonesia (Brown et al., 1992) all the species so far identified as vectors of EHDV belong to the *Culicoides* genus. A considerable number of *Culicoides* spp. can simultaneously act as vectors of BTV and EHDV. Furthermore the same vectors can be implicated in the transmission of AHSV and Akabane (AKA) virus, an arbovirus member of the Bunyaviruses (Table 11).

Subgenus	Species Complex	Species	BT	EHD	AHS	AKA
Avaritia Fox, 1955	Imicola	***C. imicola***	***x***	***x***	***X***	***x***
		C. brevitarsis	***x***	***x***		***x***
		C. bolitinos	***x***	***x***	***x***	
	Obsoletus	***C. obsoletus***	***x***	***x***	***x***	
		C. scoticus	***x***			
	Dewulfi	*C. dewulfi*	***x***			
	Orientalis	*C. fulvus*	***x***			
		C. dumdumi	***x***			
		C. orientalis	***x***			
	Grahamii	*C. actoni*	***x***			
	Pusillus	*C. pusillus*	***x***			
	Suzukii	*C. wadai*	***x***			
	Gulbenkiani	*C. brevipalpis*	***x***			
		C. gulbenkiani	***x***	***x***		
		C. tororoensis	***x***			
Culicoides Latreille, 1809	Pulicaris	***C. pulicaris***	***x***		***x***	
		C. magnus	***x***	***x***		
Culicoides	Pulicaris	*C. punctatus*		***x***		
Silvicola Mirzaeva and Isaev, 1990	Cockerellii	Species unknown	***x***			
Monoculicoides Khalaf, 1954	Variipennis	***C. sonorensis***	***x***	***x***	***x***	***x***
	Nubeculosus	*C. nubeculosus*	***x***			
		C. puncticollis	***x***			
Remmia Glukhova, 1977	Schultzei	*C. oxystoma*	***x***			***x***

		C. nevilli	***x***	***x***		
		Species unknown	***x***			
Remmia Glukhova, 1977	Shultzei	*C. kingi*		***x***		
Remmia Glukhova, 1977	Schultzei	*C. schultzei*		***x***		
Oecacta/Remmia	Schultzei	*C. oxystoma*		***x***		
Hoffmania Fox, 1948	Guttatus	***C. insignis***	***x***			
		C. filarifer	***x***			
	Peregrinus	*C. peregrinus*	***x***			
	Peregrinus	lungchiensis = peregrinus		***x***		
	Milnei	*C. milnei*	***x***			
Haematomyidium Goeldi, 1905	Complex unknown	*C. stellifer*	***x***	***x***		
Haematomyidium	Paraensis	*C. lahillei*		***x***		
Haematomyidium	Paraensis	*C. debilipalpis*		***x***		
Haematomyidium	Paraensis	*C. paraensis*		***x***		
Oecacta Poey, 1853	Furens	*C. furens*	***x***			
Meijerehelea Wirth and Hubert, 1961	Complex unknown	*C. pycnostictus*	***x***			
Subgenus unknown	Complex unknown	*C. trilineatus*	***x***			
Hoffmania		*C. venustus*		***x***		
		Anopheles vagus		***x***		
		C. mohave		***x***		

Table 11. Species of *Culicoides* (Latreille, 1809), that have been implicated as confirmed or suspect vectors of BTV and/or EHDV and/or AHSV and/or Akabane virus.

Data as are available suggest that the species of *Culicoides* that transmit EHDV are likely to be similar though not necessarily the same as for BTV (Table 11). Consequently, the information on vector-virus interactions, vector breeding sites, taxonomy, ecology and seasonality, and the effect of climatic variables upon vectors is likely to be the same as has already been provided as part of the EFSA opinion on BT vectors and insecticides (European Food Safety Authority EFSA, 2008). To our knowledge the only potential EHDV vector present in EU is *C. imicola*. For this reason all the information related to its biology will be detailed in the review.

Vector biology

Culicoides imicola is the most easily distinguished by wing pattern from the rest of the European *Avaritia* species. Where doubt in taxonomic identity occurs, a recently developed RT-PCR assay with a specificity of 92% and sensitivity of 95% can be used for quantifying this species from light traps captures without need of classical identification (Cêtre-Sossah et al., 2008).

Culicoides imicola: distribution, vector status and larval habitat

Culicoides imicola has an Afro-Asiatic distribution that was until recently restricted in Europe to the south of the Iberian Peninsula and a few Greek islands located close to Anatolian Turkey. Recently many authors have described an apparent and on-going northward range expansion in this species (Mellor and Wittmann, 2002). In Europe, BTV PCR-based positives have been recorded from pools of *C. imicola* (e.g. Ferrari et al., 2005). In Italy, BTV-4 and BTV-1 have been isolated from parous females of *C. imicola*, respectively in 2003 and 2006 (Goffredo, personal communication). The implication of *C. imicola* as a vector of BTV in Europe is thus based on its distribution and abundance on farms, in outbreak areas, isolation of BTV from parous individuals and historical evidence of its role in transmission elsewhere. Few direct vector competence experiments with European *C. imicola* have been undertaken and results remained limited (Biteau-Coroller, 2006, PhD thesis), due to the difficulties in feeding and maintenance in the laboratory. The breeding sites of *C. imicola* have been clearly identified at the farm level. Moist soil enriched with organic matter appears to be the most suitable habitat for larval development (Braverman et al., 1974).

Adult feeding habits/host preferences

Most knowledge concerning the adult feeding habits was obtained some 20 years ago (e.g. McCall and Trees, 1993; Mellor and McCaig, 1974; Nielsen, 1971). However, it is known that current research on this topic is being conducted by several scientific institutions and universities in Europe. In *C. imicola* examined in Israel, amongst the species considered, horses are the most preferred host, while sheep, dogs, birds respectively are the least preferred hosts (Braverman et al., 1971, 1977; Braverman and Chizov-Ginzburg, 1996). Some of these

findings were based on indirect calculations by comparing the rate of blood engorged females captured inside horse stables with the rate of blood engorged females trapped near other hosts (Braverman and Frish, 1984).

Indoor/outdoor activity

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

Hours of attack and dispersal

In general, the biting activity of the different *Culicoides* spp. in Europe and most of the rest of the world is highest during crepuscular and nocturnal hours. For example, crepuscular and nocturnal activity of *C. imicola* seems to be widely assumed in southern Europe. In a recent study conducted in the Balearic Islands using a light trap equipped with a catch jar rotator (Borràs et al., personal communication), there was continuous activity of *C. imicola* and Obsoletus complex, with nulliparous and parous females detected from sunset to sunrise. In Israel, 43 hourly suction night trappings with black lights showed that *C. imicola* was on the wing throughout the night and the largest numbers were captured during the first 3 hours of night (Braverman et al., 2008).

Overwintering

In warm-temperate areas, the adults of many *Culicoides* species, including *C. imicola,* occur throughout the winter, as demonstrated by the AHS epidemics in Spain (1987-1990) and Morocco (1989-91) 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 additional *Culicoides* species for BTV transmission as well as the ability of

BTV to overwinter at 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 adult vectors 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 through the winter which would permit a continuous very low level transmission of the virus during that period,
- possibility of vertically infection of larvae with transmission of infection to the progeny. The existence of this mechanism, however, has not been confirmed,
- transplacental transmission of the virus to the offspring from ruminants infected while pregnant.

The effects of climate change have also been implied by some authors as a possible mechanism that would increasingly facilitate the overwintering of BTV infection in the northern Europe by increasing the duration of the vector season and hence shortening the vector-free period.

Vector competence is indicated as the ability of the vector to support virus replication and transmission. It is one of the components of vectorial capacity which is defined as "the relative measure of a vector population to transmit a pathogen to a vertebrate population" (Mullens, 1992). When an arbovirus such as EHDV, infects a haematophagous insect, a number of factors including vector species and/or genotype, environment - mainly the environmental temperature and virus species and/or strains might affect the success of the infection. These factors influence the replication and dissemination of the virus in the vector organs including the salivary glands and, in turn, the transmission capability of the vector. In addition to vector competence, other important factors included in vectorial capacity are biting rates, host selection, vector survivorship and the extrinsic incubation period of the virus (Paweska et al., 2005).

Nevertheless, the levels of competence that individual vector species express for EHDV and hence, their vector capacities, are likely to be different than for BTV. It is also likely that the precise temperature requirements for the replication of EHDV in vectors and the number of day-degrees required to attain a transmissible infection in the vectors would also be different than for BTV. Such differences may form the basis of the observed variances in the current regional and global distributions of EHDV and BTV as exemplified, particularly, in Europe and North America.

However, as data on EHDV and its vectors are much scantier than for BTV, currently, such inferences can only be speculative. There is therefore an urgent and pressing need for experimental and other data on the precise spectrum of EHDV vectors in Europe and the requirements for virus transmission by them, before accurate and reliable risk assessments on EHDV spread into and through Europe can be developed.

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 papers (Carpenter et al., 2008, Page et al., 2009; Papadopoulos et al., 2009; Schmahl et al., 2009).

Possible methods for the control of *Culicoides* populations include:

- treating livestock with insecticides, repellents or a 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.

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 breeding sites of *C. imicola*.

However, when using insecticides there are several drawbacks that have to take into account. No insecticidal products are currently authorized specifically against *Culicoides* in the EU. About the efficacy, data concerning the pyrethroid-based compounds are equivocal. While several experimental trials showed some mortality in *Culicoides,* the extent of the effect on virus transmission in 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* or any other insect populations. Treatment of breeding sites remains difficult as habitats are poorly defined for most *Culicoides* species.

Culicoides and climatic changes

The hypothesis of possible correlation between the recent spread of BTV in Europe and climate changes originated from the observation of a spatial and temporal correspondence of these two phenomena (Purse and Rogers, 2009). Two main mechanisms are considered to play a role in the BTV spread (Purse et al., 2005, 2007, 2008; Wilson and Mellor, 2008; Purse and Rogers, 2009):

- the possible influence of temperature and humidity on the biology of vectors, increasing their competence and vectorial capacity,
- the extension northward of *Culicoides imicola*, which is the major vector of the disease in the Mediterranean Basin.

Both BTV transmission cycle and the life cycle of its *Culicoides* vectors are modulated by **temperature** and moisture availability. Increases in both temperature (particularly at night time and in winter) and in precipitation (particularly in summer/autumn) could lead to an increased geographical and seasonal incidence of BTV transmission by 1) increasing the range, abundance and seasonal activity of vectors (Mellor et al., 1998; Wittmann et al., 2002) 2) increasing the proportion of a vector species that is competent 3) increasing the development rates of the virus within vectors (Van Dijk and Huismans, 1982) and extending

transmission ability to additional *Culicoides* species as reported in *C. nubeculosus* (Mellor at al., 1998; Wittmann et al., 2002).

Moisture determine the size and persistence of semi-acquatic breeding sites (Mellor et al., 2000) but also the availability and duration of humid microhabitats in summer/autumn where adults can carry out key activities and shelter from desiccation (Murray, 1991).

The warming of air temperature may also have increased the epidemiological role of Paleartic vectors involved in BTV transmission. The rise in mean temperatures increases their population size and their survival rates during winter season, reducing the extrinsic incubation period throughout an increase of the virus replication, and reducing the gut barrier in insect body allowing to compensate their low competence level (Purse et al., 2005). The increase in vector competence of the "non traditional" vectors and the extended distribution of *C. imicola* provide the opportunity for frequent and wide spread "hand-over" events of the virus between major and novel vector groups.

The hypothesis of an extended distribution of *C. imicola* across the Mediterranean Basin is based on the observation of the current presence of the vector in sites in which previous studies failed to reveal its presence (Wilson and Mellor, 2008; Purse and Rogers, 2009). It was observed in Greece (Mellor et al., 1984; Patakakis, 2004), Italy (Gallo et al., 1984; Scaramozzino et al., 1996; Goffredo et al., 2003), Portugal, Spain (Ortega et al., 1998; Rawlings et al., 1997; Capela et al., 2003; Sarto I Monteys and Saiz-Ardanaz, 2003; Sarto I Monteys et al., 2004, 2005) and France (Baldet et al., 2004; Biteau-Coroller et al., 2006). Those data should be interpreted with precaution since inferring the absence of a species in a location from its absence in previous catches is difficult when the species requires specific sampling methods in order to be caught. Studies before and after the hypothetical vector introduction must be comparable, using the similar methodologies, comparable trapping devices and coincident in time and space.

Even if the spatio-temporal correspondence between changes in European climate and the spread of BTV is undoubted, however other important routes of spread for vector born

diseases should not be underestimated: the role of animal movements for trade or illegally, movements of wild animals or the introduction of infectious vectors (Calistri et al., 2004).

In summary:

- EHDV is transmitted by midges from the *Culicoides* genus. Data as are available suggest that the species of *Culicoides* that transmit EHDV are likely to be similar though not necessarily the same as for BTV. Consequently, the information on vector-virus interactions, vector breeding sites, taxonomy, ecology and seasonality, and the effect of climatic variables upon vectors is likely to be the same as for BT.
- However, as data on EHDV and its vectors are much scantier than for BTV, currently, such inferences can only be speculative.

Future researches:

- Data on the precise spectrum of EHDV vectors in Europe and the requirements for virus transmission by them in order to develop an accurate and reliable risk assessments on EHDV spread into and through Europe.

Geographical distribution of EHDV vectors

The vector geographical distribution and competence differ throughout the world: *Culicoides imicola* is considered to be the main vector in South Africa, *Culicoides brevitarsis* in Australia and *Culicoides sonorensis* in the USA (Wilson, 1991a).

There are four basic requirements to implicate an insect as a vector of a pathogen. These are: **1.** isolate the pathogen from blood-free, field collected insects, **2.** demonstrate in the laboratory that the insect can be infected with the pathogen through a blood meal from an infected host, **3.** demonstrate in the laboratory that the infected insect can transmit the pathogen to a suitable host, and **4.** demonstrate that the insect comes in contact with the host in the field (European Food Safety Authority, EFSA, 2007).

A comprehensive summary of the *Culicoides* species involved in virus transmission is listed in table 12. In Africa, EHDV-4 was isolated from *C. schultzei* in Sudan (Mohammed and Mellor, 1990) and recently a few additional species of *Culicoides*, including *C. bolitinos,*

have been shown to be susceptible to oral infection with 8 serotypes of EHDV (Paweska et al., 2005). In North America *C. lahillei* was the most abundant *Culicoides* species collected during epizootics of hemorrhagic disease among white tailed deer in Georgia (Smith et al., 1996) and its competence as a potential vector was demonstrated (Smith et al., 1996a).

Subgenus	**Species Complex**	**Species**	**Identified serotypes**	**Geographical Location**	**Vector implication criteria***				**References**
					1	**2**	**3**	**4**	
Avaritia	Imicola	*C. imicola*	2, 3, 5, 6, 7, 8	South Africa		X			Paweska et al. 2005
Avaritia	Imicola	*C. bolitinos*	1, 2, 3, 4, 5, 6, 7	Experimental infection		X			
		C. leucostictus	5, 6, 7			X			
Culicoide*s*	Pulicaris	*C. magnus*	2			X			
		C. nivosus	5, 7			X			
Avaritia	Gulbenkiani	*C. gulbenkiani*	2			X			
		C. zuluensis	7			X			
		C. onderstepoortensis	8			X			
Avaritia	Imicola	*C. imicola*		Sudan	X			X	Aradaib (personal comunication)
Monoculicoides		*C. cornutus*		South Africa	X				Barnard et al. 1998
Remnia				South Africa	X				
Hoffmania		*C. mohave*		Arizona (USA)	X				Rosenstock et al. 2003

Haematomyidium	Paraensis	*C. lahillei*	2	North America (Experimental infection)		X		X	Smith et al. 1996
Monoculicoides	Variipennis	*C. sonorensis*	1, 2	North America	X	X	X	X	Foster et al. 1977 Foster et al. 1980 Smith et al. 1996 Jones et al. 1977 Greiner et al. 1984.
Haematomyidium	Paraensis	*C. debilipalpis*		North America				X	Mullen et al. 1985
Haematomyidium	Paraensis	*C. paraensis*						X	
Haematomyidium	unknown	*C. stellifer*						X	
Avaritia	Obsoletus	*C. obsoletus*		North America					
Hoffmania		*C. venustus*		North America (Experimental infection)		X			Jones et al. 1983.
		Anopheles vagus	JKT 9133 JKT 8312	Indonesia	X				Brown et al. 1992
Avaritia Fox, 1955	Imicola	*C. brevitarsis*	EHDV2, EHDV5, EHDV6, EHDV7, EHDV8	Australia	X			X	St. George et al. 1983 Parsonson and Snowdon. 1985..
Oecacta/Remnia	Shultzei	*C .shultzeii*	EHDV4	Sudan	X			X	Mohammed and Mellor. 1990.
Oecacta/Remnia	Schultzei	*C. oxystoma*			X			X	Yanase et al. 2005
Culicoides	Peregrinus	*C. lungchiensis/* *peregrinus*			X			X	
Culicoides	Pulicaris	*C. punctatus*			X			X	
Oecacta/Remnia	Schultzei	*C. schultzei*	Ibar 49630	Nigeria	X			X	Lee 1979

Table 12. Vectors for EHDV. ***Vector implication criteria:*** There are four basic requirements to implicate an insect as a vector of a pathogen. These are: **1.** isolate the pathogen from blood-free, field collected insects, **2.** demonstrate in the laboratory that the insect can be infected with the pathogen through a blood meal from an infected host, **3.** demonstrate in the laboratory that the infected insect can transmit the pathogen to a suitable host, and **4.** demonstrate that the insect comes in contact with the host in the field (European Food Safety Authority EFSA, 2007).

Data related to vectors potentially able to transmit EHDV in the EU are limited. Other than *C. imicola*, there is no evidence of vectorial capacity for any of the *Culicoides* species identified in European countries. However it has to be kept in mind what occurred in the recent BTV European outbreaks. At the beginning, *C. imicola* was considered the only BTV vector in Europe but, then, when the infection was reported in Northern Europe other species like *C. obsoletus ss, C. scoticus*, *C. pulicaris*, etc have also been recognised as potential vectors. The possible role played by "non traditional" vectors in spreading the EHDV infection, emphasizes the importance of having a detailed map of the potential vector *Culicoides* distribution in EU territory.

Knowing the geographic distributions of the vectors potentially involved in the transmission of EHDV becomes then an essential information for effective disease control. However, as many species of *Culicoides* may occur within a given country, it is also important to evaluate for each species the seasonal patterns of prevalence because, to be classified as a major vector, it is necessary for the seasonal periods of high abundance of a species to coincide with the occurrence of disease. For these reasons, a number of surveys have been conducted over the last 15 years particularly in the western half of the Mediterranean Basin. The result is a detailed mapping of the distribution and seasonality of *C. imicola* and, in some cases, also of the Obsoletus and Pulicaris species complexes.

Several national studies have facilitated the gathering of information on the geographical distribution of the main *Culicoides* vectors of BTV which are also likely to be important in the transmission of EHDV. The countries for which fairly comprehensive data have been accumulated are Morocco (Baylis et al., 1997), Tunisia (Hammami et al., 2008), Spain (Rawlings et al., 1997; Goldarazena et al., 2008), Portugal (Capela et al., 2003), the Balearic Isles (Miranda et al., 2003), Corsica (Baldet et al., 2004), Italy (Goffredo et al., 2004), Greece

(Patakakis, 2004), Germany (Kiel et al., 2009; Mehlhorn et al., 2009), Bosnia and Herzegovina (Omeragić et al., 2009), and Switzerland (Casati et al., 2009). UK seasonal activities of *Culicoides* can be found in the *Culicoides* portal "culicoides.net" (http://www.culicoides.net/culicoides).

The present review will focus on the latest data (2008-2009) on the geographical distribution of the potential EHDV vectors with particular enphasis on EU countries. In the Appendix A a selection of *Culicoides* distribution maps for Europe and bordering countries is presented.

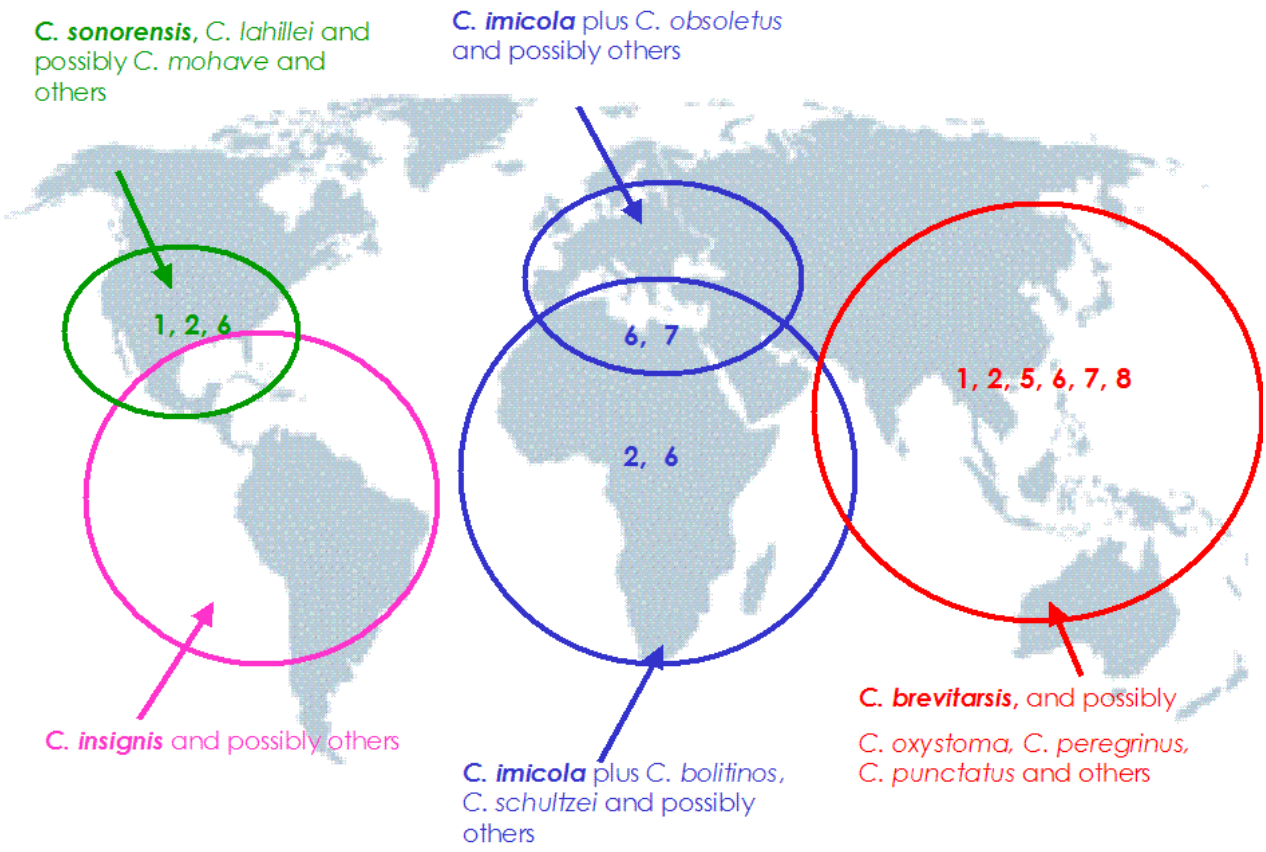

Figure 3: Global distribution of EHDV serotypes and vector species. The *Culicoides* species identified in bold are considered the principal vector of EHDV in each region (if known).

Outside Europe *Culicoides spp.* surveillance in the Southeastern **United States** has been carried out from January to October 2008. Insect were trapped at 69 premises in 6 States. Of

376,500 insects trapped more than 8,000 were classified as *Culicoides*. Twenty two different species of *Culicoides* were identified and the presence of *C. insignis* and *C. sonorensis* was confirmed in endemic BT/EHD areas (Southeastern Cooperative Wildlife Diseases Study (SCWDS), University of Georgia and United States Department of Agriculture, 2008).

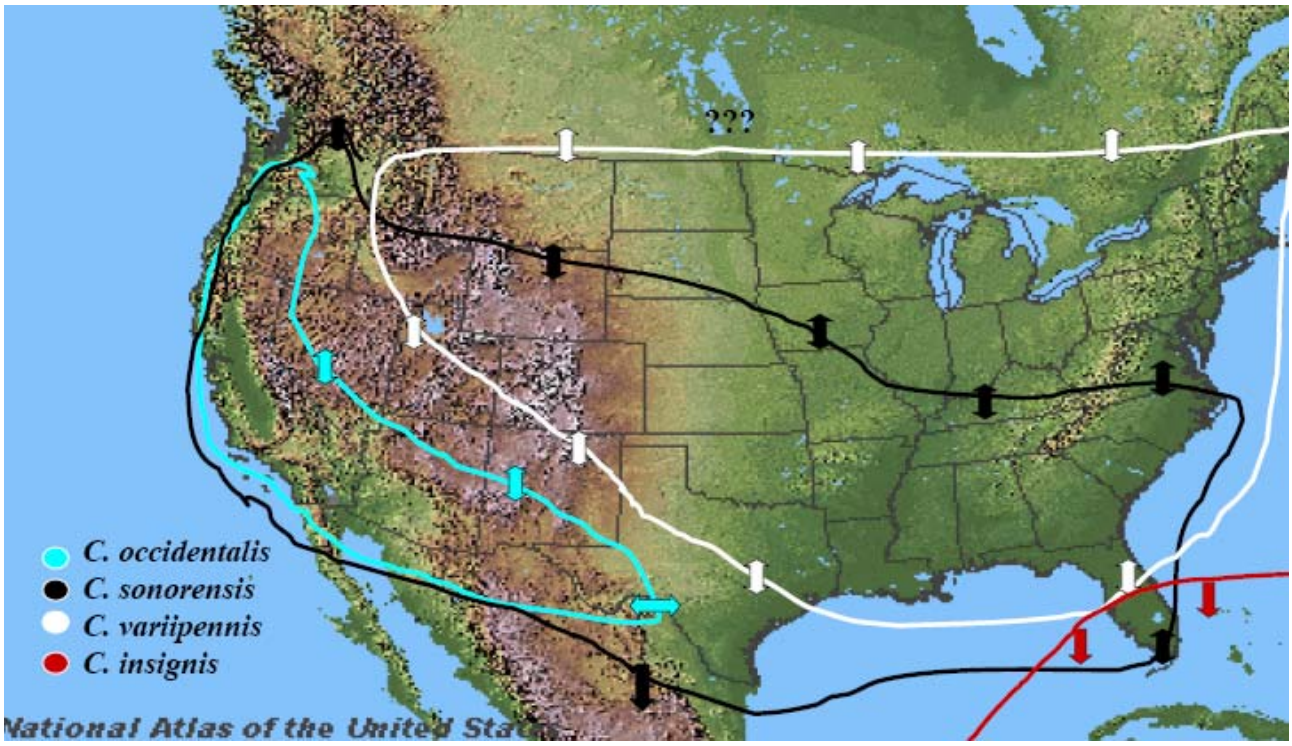

Figure 4: Range distribution of *Culicoides* in the U.S. (Eileen Ostlund, personal communication in *Network of the OIE reference laboratories for Bluetongue. 2nd meeting. Cerrano, Italy 1-2 June 2009)*

To define vector distribution in **Australia**, a National Arbovirus Monitoring Program (NAMP) has been carried out from the seventies together with serological monitoring of cattle located in sentinel herds, and by strategic serological surveys of cattle herds, throughout Australia. Groups of young cattle, previously unexposed to arboviral infection, are blood tested at regular intervals to detect evidence of prior infection or transmission of bluetongue, Akabane and BEF viruses. Insect traps positioned near the monitored herds indicate whether *Culicoides* vectors are present during the period of testing. The presence of vectors is one of the main parameters to define the limits of virus transmission areas which

are shown on the interactive bluetongue zone map. (http://www.animalhealthaustralia.com.au/programs/adsp/namp/namp_home.cfm).

CULICOIDES IDENTIFICATION AND SURVEILLANCE SYSTEMS

The efficiency of virus transmission depends on a combination of factors: efficiency of growth within an insect, vector population numbers and availability of susceptible animals. An understanding of the biology and interactions of the virus, insect vector and animal host is required to develop surveillance and control 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).

Although the European Commission made a great effort for the establishment of a harmonised entomological surveillance scheme across the EU, a unique and integrate picture of *Culicoides* distribution and abundance is not currently available even though a powerful instrument for data collection has been created for the EU Member States: EU-BTNET (http://eubtnet.izs.it/btnet/index.htm). Indeed, data related to the activities performed for the national serological/virological and entomological surveillance plans, should be entered directly by the EU Member States into the EU-BTNET system, according to the provisions included in Commission Regulation (EC) No 1266/2007 of the European Commission laying down additional measures on control, monitoring, surveillance and restrictions of movements of susceptible animals in relation to bluetongue (http://eubtnet.izs.it/btnet/index.htm).

The planning of an appropriate surveillance system for *Culicoides* should be dependent on the objectives of the surveillance itself, which can be schematically summarised as:

- identification of vector species involved in virus transmission in the field,
- definition of seasonal activities to define a vector free/low abundance season,

- detection of virus circulation through insect trapping and analysis.

The objectives of scientific research to determine vector competence, biting rates, host preferences, vector indoor/outdoor, or diurnal activity, should not be confused with the objectives of a surveillance system, which is aimed at the control of the disease, but they provide useful information for the interpretation of field results.

As regards the insect trapping methods to be used in the scientific studies, these should be carefully designed to achieve each specific objective. For example, the use of UV traps is useless when the diurnal activity of the vectors has to be assessed, animal baited traps and/or truck traps should be used for this purpose. For evaluation or determination of vector biting rates animal baited traps using the target species of animal for the disease (e.g. ruminants for BTV and EHDV, equids for African horse sickness) should be used.

Furthermore, trapping methods and protocols should be standardised as much as possible. The OVI UV black-light type trap has already been widely recommended. Protocols to be used in a surveillance plan are fully described in some papers (Goffredo and Meiswinkel, 2004; Meiswinkel et al., 2008).

In summary:

- The major vector of EHDV transmission in the African continent is *C. imicola*, in North America *C.sonorensis* and in Soth East Asia is *C. brevitarsis*.
- In the Mediterranean Basin the occurrence of EHDV could be vectored by *C. imicola*. However, following the large European epidemics of bluetongue also other *Culicoides* species must be considered as possible vectors for EHDV too.
- In the implementation of surveillance systems, trapping methods and protocols should be standardised as much as possible.

DIAGNOSIS

Epizootic hemorrhagic disease has been included in the OIE listed diseases fairly recently (May 2008) and consequently no recommended diagnostic techniques are yet described in the OIE Manual of Diagnostic Tests and Vaccines for terrestrial animals (2008 edition).

When present EHD clinical signs are indistinguishable from those of BT. Consequently, in areas where both viruses are endemic or possible co-circulation of both orbiviruseses can't be excluded the use of highly specific diagnostic tests is strongly required. Some EHD lesions are also similar to those caused by bovine viral diarrhoea/mucosal disease, bovine herpes virus type 1, vesicular stomatitis, malignant catarrhal fever and bovine ephemeral fever.

Virological tests

Virus isolation (VI) can be attempted from the blood of viraemic animals and/or tissue samples namely spleen, lung and lymph nodes of infected animals. The epizootic haemorrhagic disease virus can be isolated by inoculation of embryonated chicken eggs (ECE) or cell cultures. Vero or BHK_{21} are the cell lines most commonly used for growing the virus. *Aedes albopictus* cell lines (e.g. C6/36) have been successfully used for virus isolation as well. Experimental studies have demonstrated that the ECE and *A. albopictus* systems are equally sensitive for recovering EHD viruses (Gard et al., 1988). Cytopathic effect (CPE), which occurs only in mammal cell lines, usually appears between 2 and 7 days after inoculation.

VI techniques are time consuming, expensive, laborious and require skilled personnel; results can take 2-4 weeks to obtain but provide the virus strains which are necessary for further investigations such as molecular characterization.

Ibaraki virus (EHDV-2) can be isolated in bovine cell cultures from both natural and experimentally produced cases of the disease. The virus replicates in and induces CPE in, primary cell lines of bovine, sheep and hamster lung origin, and L cells; but it does not grow neither in primary cultures of horse and swine kidney cells nor in HeLa cell cultures. The virus is readily passaged in 4 to 5-day-old eggs by yolk-sac inoculation and incubation at

33.5° C. It multiplies in the brains of mice of any age after intracerebral inoculation, but younger mice give a better viral growth and develop encephalitis. There is no evidence that rabbits and guinea pigs are susceptible to Ibaraki virus (Inaba, 1975).

Once isolated, the serotype of the virus strain is identified by virus neutralization test (VNT) using known reference antisera (Pearson et al., 1992) or by fluorescence inhibition test (Blacksell et al., 1994a).

The antigen-capture c-ELISA (Mecham and Wilson, 2004) and the sandwich ELISA (Thevasagayam et al., 1996a) can be used for EHDV detection/identification from both field samples and tissue cultures.

Several group specific reverse-transcriptase polymerase chain reaction (RT-PCR) methods for the detection of EHDV nucleic RNA have been developed (Aradaib et al., 1998, 2003). A brief summary of the main assays is reported in the Table 13.

Genome target	Real-Time/Gel based	Specificity	Limit of detection	References	Annotations
Segment 10	Real time	Tested with 24 BTV reference strains	------	Wilson et al., 2009	TaqMan. 7 primer sets and 3 probes
Segment 6	Nested; gel based	Tested with North American BTV strains (2,10,11,13,17)	0.1 fg of viral RNA	Aradaib et al., 2003	Tested with EHDV-1 and 2
Segment 3	Gel based	------	-----	Ohashi et al., 1999	Tested with EHDV-1, 2, 7, 8, 9, 10
Segment 10	Gel based	------	1 pg RNA	Aradaib et al., 1998	Sensitivity increases to 10 fg with a chemiluminescent step
Segment 6	Capture nested gel based	Tested with BTV and AHSV strains	------	Wilson, 1994	Used also on semen (Wilson, 1999)

Table 13: summary of the group specific RT-PCR assays developed for EHDV diagnosis.

There are also serotype specific RT-PCR based on partial amplification of segment 2 (Brodie et al., 1998; Aradaib et al., 1995, 1995a). However the duration of EHDV positivity by RT-PCR in blood is largely unknown, there is evidence that it lasts longer than the period over which infectious virus can be isolated (Aradaib et al. 1994). Amongst the diagnostic molecular techniques a dot blot hybridization (Mohammed et al., 1996) and RT-PCR followed by restriction analysis and sequencing (Harding at al., 1996) have been reported for the characterization of field isolates.

Serological test

The gold standard for the identification and quantification of antibodies against EHDV serotypes present in test samples is the serum neutralization (SN) test. With this technique, serotype-specific antibodies are detected and quantified. The disadvantage is that all suspected virus serotypes must be included in the assay, consequently, it can be a very time consuming and labour intensive test to perform. A titre > or equal 1:10 is usually considered specific for EHDV. The SN test requires at least 3 to 5 days to be completed (Pearson et al., 1992).

The AGID has been widely used to detect EHDV antibodies (Stallknecht et al., 1991; Dunbar et al., 1998; Deem et al., 2004; Dubay et al., 2004) from sera of infected animals. In the past this test has been used for animal export. It is simple, economical and reliable but is less sensitive and specific than the c-ELISA. The AGID can detect antibodies from 5-15 days after infection to 2 years or more (Pearson, 1992). Unfortunately cross-reactions amongst related viruses belonging to different Orbivirus serogroups have been observed making this test inadequate when BTV and EHDV may be co-circulating in the same areas.

The complement fixation test (CFT) is sensitive and specific for EHDV diagnosis and has been used until 1980 for diagnosis and qualification of animals for export. The test is group-specific and cheap, with a sensitivity similar to the SN and AGID tests. CFT allows detection and quantification of antibodies for 4-12 months after infection but is less reliable after this period (Pearson, 1992). For these reasons it is particularly useful in detecting recent EHDV infections but not for detecting older infections.

Several ELISAs have been developed using both whole virus particles and recombinant proteins (Afshar et al., 1992a, Thevasagayam et al., 1996). The group specific antigen is VP7: it has been cloned in Baculovirus, expressed in insect cells and, once purified, used to set up different ELISAs: competitive assays (Luo and Sabara, 2005), capture assays (Mecham and Wilson, 2004) or blocking assays ELISA (Mecham and Jochim, 2000). A brief summary of the main assays is shown in Table 14.

ELISA	Antigen	Monoclonal antibody	Notes	Specificity (Sp)	Sensitivity (Se)	References
Competitive	VP7 recombinant	VP7 MAb 18B2	Tested with EHDV-1 and BT (serotype unknown)	------	------	Luo and Sabara, 2005
Capture	VP7 recombinant	VP7 MAb 4F4.H1	Tested with: EHDV-1, EHDV-2, and BTV	------	------	Mecham and Wilson, 2004
Blocking and competitive	Purified EHDV-1 and EHDV-2	VP7 MAb 4F4.H1	Tested with: EHDV-1, EHDV-2, and field samples tested with AGID	------	------	Mecham and Jochim, 2000
Competitive	Purified EHDV-1	VP7 MAb C.31	Tested with: EHDV-1, EHDV-2, and field samples tested with AGID	95.1%*	90.0%*	Afshar et al., 1997
Competitive	Purified EHDV-1	------	Tested with: EHDV, AHS and BTV	------	------	Thevasagayam et al., 1995
Competitive	Purified EHDV	Ig G1 bovine MAb	Tested with: EHDV-1-4 and field sera	99.3%*	91.5%*	Afshar et al., 1992

Table 14: summary of the ELISAs developed for EHDV diagnosis. * Sp and Se are relative to the AGID test.

In summary:

- The gold standard for serotype identification is Virus Neutralization test
- RT-PCR based assays allow the identification of specific serotypes.
- Real-time RT-PCR allows the detection of EHDV with an increased sensitivity compared to gel based RT-PCR lowering the risk of contamination.

Future researches:

- Definition of reference strains and antisera for armonization of diagnostic techniques. In such context the organization/participation of laboratories to ring trials should be encouraged.
- Development of ELISA tests commercially available.

PREVENTION AND CONTROL MEASURES

Recommended control measures for EHD

Although listed in the OIE diseases,, EHD is not yet included in the Terrestrial Animal Health Code nor in the Manual of Diagnostic Test and Vaccines where it is cited in the BT chapter (2008 edition) There should be an upcoming revision of the OIE Manual chapter on Bluetongue that will include some basic EHDV content.

The Council Directive 92/119 /EEC, of 17 December 1992 include EHD amongst the compulsorily notifiable diseases. The Directive defines the general measures to be applied once an outbreak of the EHDV is suspected. If one or more animals on a holding are suspected of being infected with EHD, 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 animals, keeping isolated the suspected cases taking into account the possible role of vectors, and banning the movement of animals 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 EHDV is no longer suspected.

Once an outbreak is confirmed, the veterinarian must have all susceptible species slaughtered and their carcases destroyed, where possible, on the spot. Appropriate treatments should be used also on substances or waste likely to be contaminated as well as buildings and vehicles should be cleaned and disinfected.

An epidemiological enquiry has to be carried on covering 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 3

kilometres around the infected holding) and a surveillance zone (of at least 10 kilometres beyond the protection zone), in which certain specific measures are applied. These measures include the identification of all the holdings breeding susceptible species, 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.

Each Member State must draw up a contingency plan meeting the criteria laid down in the Directive and submit it to the Commission no later than six months after the implementation of the specific measures for EHD. The Standing Committee on the Food Chain and Animal Health assists the Commission in the management of the measures taken against EHD.

Directive 2004/68/EC, amended by Commission Decision 2008/752/EC, describes the animal health principles on which importation of live animals is based and the requirements to be fulfilled by a third country, including the contents of the veterinary certificate in origin. Terrestrial mammals of species belonging to the taxa Proboscidea and Artiodactyla and their cross breeds can be imported to the EU only from countries or parts of countries listed in Annex I of this Commission Decision. During their transport to the European Community, the animals shall not be unloaded in the territory of a country or part of a country that is not approved for imports into the Community of these animals. For part of Canada, additional guarantees regarding BT and EHD diagnostic tests are required in the veterinary certificate.

The animals have to be inspected at an approved EU Border Inspection Post (BIP) and the relevant information has to be sent via TRACES. The veterinary certificate includes the following data:

- Free from EHD for 12 months
- Vaccination is not performed
- The import of domestic cloven-hoofed animals vaccinated against EHD is not permitted
- Remaining in that territory at least 3 months before dispatch to EC
- Without contact with imported cloven-hoofed animals at least 30 days

- The animals have remained since birth or at least 40 days before dispatch in the holding free from official prohibition on health grounds (40 days are estimated as maximum incubation period in Council Directive 92/119/EC)
- in and around the territory where the animals remaining before dispatch 40 days, there has been no case/ outbreak of EHD, during the previous 60 days
- they are not animals to be killed under a national programme for the eradication of diseases, nor have they been vaccinated against the disease
- they have reacted negatively to a serological test for detection of antibody for EHD, carried out two occasions on samples of blood taken at the beginning of the isolation/ quarantine period and at least 28 days later, on …….. (date) and on ……(date), the second of which must have been taken within 10 days of export
- The animals have to be examined within 24 hours of loading by the official veterinarian and show no clinical sign of disease

'ANNEX I

LIVE ANIMALS

PART 1

List of third countries or parts thereof (*)

Country (a)	Code of territory	Description of territory	Veterinary certificate		Specific conditions
			Model(s)	SG	
1	2	3	4	5	6
CA — Canada	CA-0	Whole country	POR-X		IVb IX
	CA-1	Whole country except the Okanagan Valley region of British Columbia described as follows: — From a point on the Canada/United States border 120° 15' longitude, 49° latitude — Northerly to a point 119°35' longitude, 50° 30' latitude — North-easterly to a point 119° longitude, 50° 45' latitude — Southerly to a point on the Canada/United States border 118° 15' longitude, 49° latitude	BOV-X, OVI-X, OVI-Y RUM (**)	A	
CH — Switzerland	CH-0	Whole country	(***)		
CL — Chile	CL-0	Whole country	BOV-X, OVI-X, RUM		
			POR-X, SUI	B	
GL — Greenland	GL-0	Whole country	OVI-X, RUM		V
HR — Croatia	HR-0	Whole country	BOV-X, BOV-Y, RUM, OVI-X, OVI-Y		
IS — Iceland	IS-0	Whole country	BOV-X, BOV-Y RUM, OVI-X, OVI-Y		
			POR-X, POR-Y	B	
ME — Montenegro	ME-0	Whole country			I
MK — The former Yugoslav Republic of Macedonia (****)	MK-0	Whole country			I
NZ — New Zealand	NZ-0	Whole country	BOV-X, BOV-Y, RUM, POR-X, POR-Y OVI-X, OVI-Y		III V

Country (a)	Code of territory	Description of territory	Veterinary certificate		Specific conditions
			Model(s)	SG	
1	2	3	4	5	6
PM — St Pierre Miquelon	PM-0	Whole country	BOV-X, BOV-Y, RUM, OVI-X, OVI-Y CAM		
RS — Serbia (*****)	RS-0	Whole country			I

(*) Without prejudice to specific certification requirements provided for by any relevant Community agreement with third countries.
(**) Exclusively for live animals other than animals belonging to the cervidae species.
(***) Certificates in accordance with the agreement between the European Community and the Swiss Confederation on trade in agricultural products (OJ L 114, 30.4.2002, p. 132).
(****) The former Yugoslav Republic of Macedonia; provisional code that does not prejudge in any way the definitive nomenclature for this country, which will be agreed following the conclusion of negotiations currently taking place on this subject in the United Nations.
(*****) Not including Kosovo as defined by United Nations Security Council Resolution 1244 of 10 June 1999.

Annex I: List of third countries allowed to import into the Community certain live ungulate animals and their fresh meat. Commission Decision 2008/752/EC.

Control and surveillance measures of EHD applied by region and/or country

To date, there are no detailed studies describing the effects of the control measures undertaken in the countries where the disease has affected domestic and/or wild ruminants.

The serological and entomological data are available on areas with virus circulation; they have been mostly obtained from samples collected for BTV surveillance (Israel, USA and Australia).

America

In the United States there are no official control measures for EHDV. Samples are tested at the National Veterinary Services Laboratories (NVSL) which annually reports the results from domestic animals at the BT Committee meeting of the US Animal Health Association (http://www.usaha.org/committees/btbr/btbr.shtml). EHDV is considered enzootic in some of the southern States. The lack of surveillance measures is explained by the fact that although the disease can apparently have a large impact on captive (farmed) deer as well as wild deer it doesn't have a significant economic impact on the cattle industry (beef or dairy). Clinical signs of EHDV in sheep or goats have never been reported.

The USA Veterinary Services has been supporting BT and EHDV surveillance since 1980 through cooperative agreements with the Southeastern Cooperative Wildlife Disease Study (SCWDS) at the University of Georgia. The SCWDS provides annual nationwide data on the occurrence of Orbivirus haemorrhagic disease in wild ruminants. Further, the SCWDS is assisting the VS with vector concerns by determining the species of *Culicoides* present in the southeastern United States and Puerto Rico.

There are no licensed vaccines available in the USA although one of the vaccine producers in the United States is working towards licensing such a vaccine. The only available vaccine in the USA is an "autogenous" vaccine which is a killed virus vaccine, prepared from an isolate from the affected premises. For several years the NVSL has been requested to provide EHDV strains isolated from samples submitted from farmed deer to a company so that an autogenous vaccine can be prepared. But there are limits that apply to autogenous vaccines. They include:

- Requests must be approved by USDA;
- Must have veterinarian-patient-client relationship;
- Organisms isolated from the ill or dead animals in herd of origin;
- Organism must be considered likely cause of illness in herd of origin;
- Must be inactivated (killed) virus;
- Used in herd of origin (sometimes in adjacent herds);
- Tested for purity and safety prior to release – expedited testing; and
- Not tested for efficacy.

In particular the EHD/BT vaccine contains killed viruses of EHDV types 1 & 2 and BTV type 17. The vaccine is an autogenous vaccine produced for MWHBRA Members to use on their deer in Missouri. The vaccine is new and not proven to work but it is a start.

The vaccine can be given at any time followed by a booster shot 2 to 4 weeks later. The vaccine can be administered by dart or syringe. A yearly booster shot is also required. Dosage is 2cc s.c. or i.m. One bottle of vaccine = 100 ml = 50 doses. The cost is $4.00 per dose or $200.00 a bottle for in-state and $250.00 for out of state.

More information on the autogenous vaccine can be found on the web site http://mwbhra.org/blog/missouri-vaccine/.

Asia

To date, there are few studies describing the control measures undertaken in the Asiatic countries where the disease affected domestic ruminants.

In **Israel**, following the confirmation of the EHDV outbreak some control measures were undertaken in order to control the spread of the disease: control of arthropods; disinfection of

infected premises, culling of the affected animals, quarantine and animal restriction movements inside the country. Some serological surveillance has been carried out

(http://www.oie.int/eng/info/hebdo/AIS_77.HTM#Sec8).

In **Japan** vaccines have been used following Ibaraki outbreaks (Kim, Lyoo, Y. S., Chang, C. H., Rhee, J. C. Development of an inactivated vaccine against Ibaraki disease in cattle. In Research Reports of the Rural Development Administration, Veterinary available at http://www.cababstractsplus.org/abstracts/Abstract.aspx?AcNo=19942201255).

A product produced by Kyotobiken is commercially available. It is a bivalent vaccine against bovine ephemeral fever virus and Ibaraki virus. Viruses are propagated in cell culture, formalin-inactivated and aluminium-gel adjuvated.

(http://www.kyotobiken.jp/english/product/bovine/index.html).

Monitoring of sentinel cattle and sheep in the northern part of **Australia** for a number of arboviruses has been carried out since 1975. The area has been selected since contains an important interface between the southern limit of known *Culicoides* vectors and large populations of susceptible hosts. The sentinel herds have been used to assist with the definition of arbovirus distribution and to predict disease outbreaks when variations in transmission patterns have been noticed. Serial sampling of sentinel animals and serological testing of their blood samples has allowed the Australians to define the spread of different viruses and, indirectly, define their vector distributions. In fact monitoring for one or two agents is unlikely to provide a reliable indication of the distribution of a vector over a series of years, but an accurate distribution has been obtained by monitoring for a number of agents simultaneously. The contemporaneous use of different species of sentinels has been useful to assess differences in vector preferences for sheep and cattle (Kirkland et al., 1992). In Australia EHDV is generally regarded to be present within the distribution areas of BTV-vectors, even if it is not specifically mentioned in Australia's National Arbovirus Monitoring Programme (NAMP). Australia's BT-zone map which, in broad lines, covers the areas where the vector *Culicoides brevitarsis* is present, may be accessed at the web-site of NAMP

(http://www.animalhealthaustralia.com.au/programs/adsp/namp/namp_home.cfm). In addition to BTV, NAMP includes data related to 2 other vector-borne, bovine viral diseases: Akabane and Bovine ephemeral fever.

Africa

In **Morocco** the control measures undertaken against EHD were the control of arthropods; control of wildlife reservoirs; quarantine; vector control of infected premises/establishment(s); awareness campaign for veterinarians and farmers; reinforced surveillance for clinical signs of the disease. A serological screening will be undertaken (OIE Disease Information, 2 Nov 2006, Vol 19 - No. 44).

In **Algeria** and **Tunisia** the control measures have been limited to the control of arthropods, quarantine, disinfection of infected premises/establishment(s) and dipping/spraying (OIE Disease Information, 19 No. 51, OIE Disease Information, 28 Dec 2006, Vol 19 - No. 52).

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

The major routes for the introduction/spread of EHD in EU are likely to be the same as for BTV. Introduction of infected viraemic animals must coincide with the presence of the adult vectors in the region. The introduction though wildlife animals should be considered as a potential source of infection even if the list of known wild susceptible species and the possibility of transboundary movements should be assessed. The risk of introduction would depend on the prevalence of EHDV in wild hosts as well as their geographical distribution and range of movements. In addition the evidence of transplacental transmission of the Ibaraki strain (EHDV-2) in Japan (Ohashi et al., 1999) should be regarded as a critical point in bovine trade.

The possibility of the introduction of infected *Culicoides* on the wind from the north of Africa should also be considered. Although vectors are considered as the principal route of transmission of EHDV, the faecal and oral shedding of EHDV serotypes 1 and 2 has been experimentally demonstrated as potential source of infection in white-tailed deer (Gaydos et

al., 2002c). The epidemiological significance of these routes still has to be determined and the presence of the virus in faeces and oral swabs is probably due to the presence of viraemic blood in the gastrointestinal lumen or oral cavity.

To our knowledge there are no data on the role of semen in EHDV transmission.

The contamination of genetically-engineered Chinese hamster ovary (CHO) cells during the production of biologicals with EHDV (Rabenau et al., 1993) can also be a potential source of infection.

***Culicoides* transportation**

Vector presence is bound to climatic factors like temperature, rain and relative humidity (Sellers and Maarouf, 1991). Furthermore *Culicoides* are highly adapted to a wide range of temperatures and moisture and this is reflected in the distribution of the orbiviruses transmitted by them.

Culicoides can spread the virus through active, unaided flight. In absence of wind or at low wind speeds, *Culicoides* can fly only short distances in any direction, downwind or upwind (Murray, 1987). Such short distance progress of the Orbivirus infection is due to local movement of hosts or active vector flight in affected areas - which are the basis of the EHDV cycle in host and vector.

However, infected vectors may also be transported from their areas of origin to disease-free countries, contributing to the spread of the disease as for BTV spread from North Africa to Sardinia (Calistri et al., 2004). *Culicoides* can also fly over long distances carried by the wind (Sellers and Maarouf, 1991), for distances of 1-700 km. The temperatures at which flight can occur are from 12°C to 35°C and such temperatures can be found at heights up to 1.5 km. *Culicoides* have been caught at heights up to 1.7 km from airplanes. Such suitable conditions have occurred several times as reported by Sellers (1992). The following table, adapted from Sellers (1992), shows the cases where the wind-borne spread of EHDV infected *Culicoides* has been postulated:

Winds	*Countries*
Intertropical convergence zone	Sudan, Oman
Tropical storm	Japan
Warm winds from subtropical areas to higher latitudes	Australia

Evidence of correlation between the *Culicoides* transportation by wind and outbreaks of the related BTV is supported by the appearance of BTV-2 in Mediterranean islands (Sardinia, Balearic and Corsica) subsequent to the occurrence of dust storms originating in North Africa and moving through the western Mediterranean Basin (Calistri et al., 2004). The sand storms have been documented by satellite imaging and photographs. The wind-borne origin of the outbreaks was supported by molecular epidemiological data which demonstrated a high degree of homology between the North African, Italian, Balearic and Corsican strains.

Animal trade

The history of Orbiviruses in Europe highlights the importance of the animal movement as a mean of introduction and spread as for EHDV in EU.

The introduction of susceptible species into the EU is strictly regulated as reported in paragraph “Recommended control measures for EHD”.

The official data for bovine and ovine trade into EU has been taken from Eurostat which probably maintains the most complete database on animal trade in Europe. Tables 15 and 16 show the number of bovines and ovines introduced by each EU country from 2005 to 2008; the origins of these animals are also reported. Among the third countries we take into consideration only the ones in which disease has been reported (Tables 9-10) and the countries listed in the Annex I of the Commission Decision 2008/752/EC containing the list of third countries are allowed to import into the Community certain live ungulate animals and their fresh meat. Unfortunately there are no official data referred to the trade into EU of wild ungulates.

	2005		2006			2007		2008	
	UNITED STATES	SWITZERLAND	CANADA	CROATIA	SWITZERLAND	CANADA	SWITZERLAND	CANADA	SWITZERLAND
AUSTRIA	0	32	0	0	68	0	84	0	95
BELGIUM	0	0	0	0	0	0	0	0	0
BULGARIA	0	0	0	0	0	0	0	0	0
CYPRUS	0	0	0	0	0	0	0	0	0
CZECH REPUBLIC	8	0	0	0	0	0	0	0	0
GERMANY	0	440	0	0	301	0	236	0	65
DENMARK	0	0	0	0	0	0	0	1	0
ESTONIA	0	44	0	0	64	0	52	0	20
SPAIN	0	0	0	0	28	0	401	0	885
FINLAND	0	0	0	0	0	0	0	0	0
FRANCE	0	54	2	0	22	0	73	0	57
UNITED KINGDOM	0	0	0	0	0	0	0	0	0
GREECE	0	0	0	0	0	0	0	0	35
HUNGARY	0	0	0	0	0	0	0	0	0
IRELAND	0	0	0	0	0	1	0	0	0
ITALY	0	3528	0	0	3222	0	3056	0	2251
LITHUANIA	0	0	0	0	0	0	0	0	0
LUXEMBOURG	0	0	0	0	0	0	0	0	0
LATVIA	0	0	0	0	48	0	278	0	33
MALTA	0	0	0	0	0	0	0	0	0
NETHERLANDS	1	0	0	0	0	0	0	0	0
POLAND	44	0	0	0	0	0	0	0	0
PORTUGAL	0	0	0	0	136	0	177	0	216
ROMANIA	0	0	0	0	61	0	0	0	21
SWEDEN	0	0	24	0	0	0	0	0	0
SLOVENIA	0	0	0	7	0	0	0	0	0
SLOVAKIA	0	0	0	0	0	0	0	0	0
Total	4151		3983			4358		3679	

Table 15: Number of bovines introduced in the EU countries from 2005 to 2008 (source: Eurostat, DS-016890-EU27 Trade Since 1995 By CN8. Extracted 30/07/2009 16.27.46).

	2005	2006	2007		2008
	SWITZERLAND	SWITZERLAND	SWITZERLAND	CROATIA	SWITZERLAND
AUSTRIA	4	5	102	0	3
BELGIUM	0	0	0	0	0
BULGARIA	0	0	0	20	0
CYPRUS	0	0	0	0	0
CZECH REPUBLIC	0	0	0	0	0
GERMANY	59	55	74	0	109
DENMARK	0	0	0	0	0
ESTONIA	0	0	0	0	0
SPAIN	0	0	0	0	0
FINLAND	0	0	0	0	0
FRANCE	0	0	0	0	0
UNITED KINGDOM	0	0	0	0	0
GREECE	0	0	0	0	0
HUNGARY	0	0	0	0	0
IRELAND	0	0	0	0	0
ITALY	13	17	22	0	0
LITHUANIA	0	0	0	0	0
LUXEMBOURG	0	0	0	0	0
LATVIA	0	0	0	0	0
MALTA	0	0	0	0	0
NETHERLANDS	0	0	0	0	0
POLAND	0	0	0	0	0
PORTUGAL	0	0	0	0	0
ROMANIA	0	0	0	0	0
SWEDEN	0	0	0	0	0
SLOVENIA	0	0	0	0	0
SLOVAKIA	0	0	0	0	0
Total	76	77	218		112

Table 16: Number of ovines introduced in the EU countries from 2005 to 2008 (source: Eurostat, DS-016890-EU27 Trade Since 1995 By CN8. Extracted 05/11/2009 17.23.23).

However, the possible illegal introductions of EHDV-susceptible animals from infected countries of uncertain disease status or of sub-clinically infected animals, from areas where the presence of infection is not yet detected, cannot be excluded.

Trans boundary movements of wild species needs to be analyzed in order to quantify the risk of introduction of the disease from the third countries bordering the EU.

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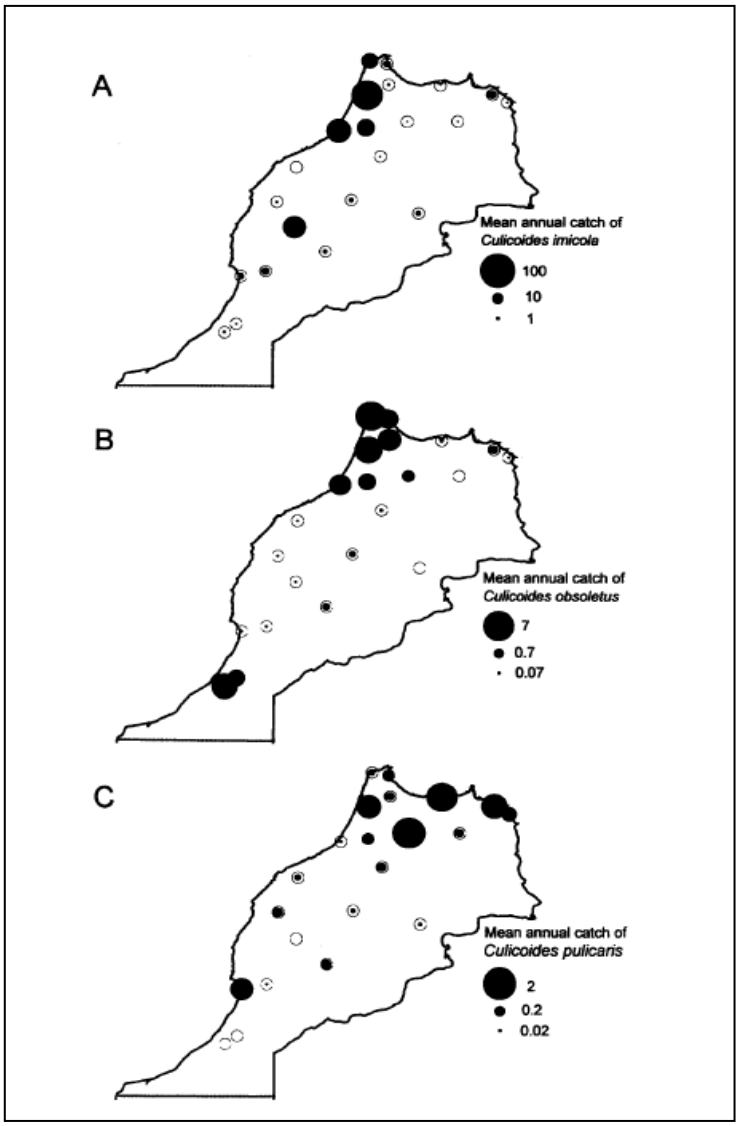
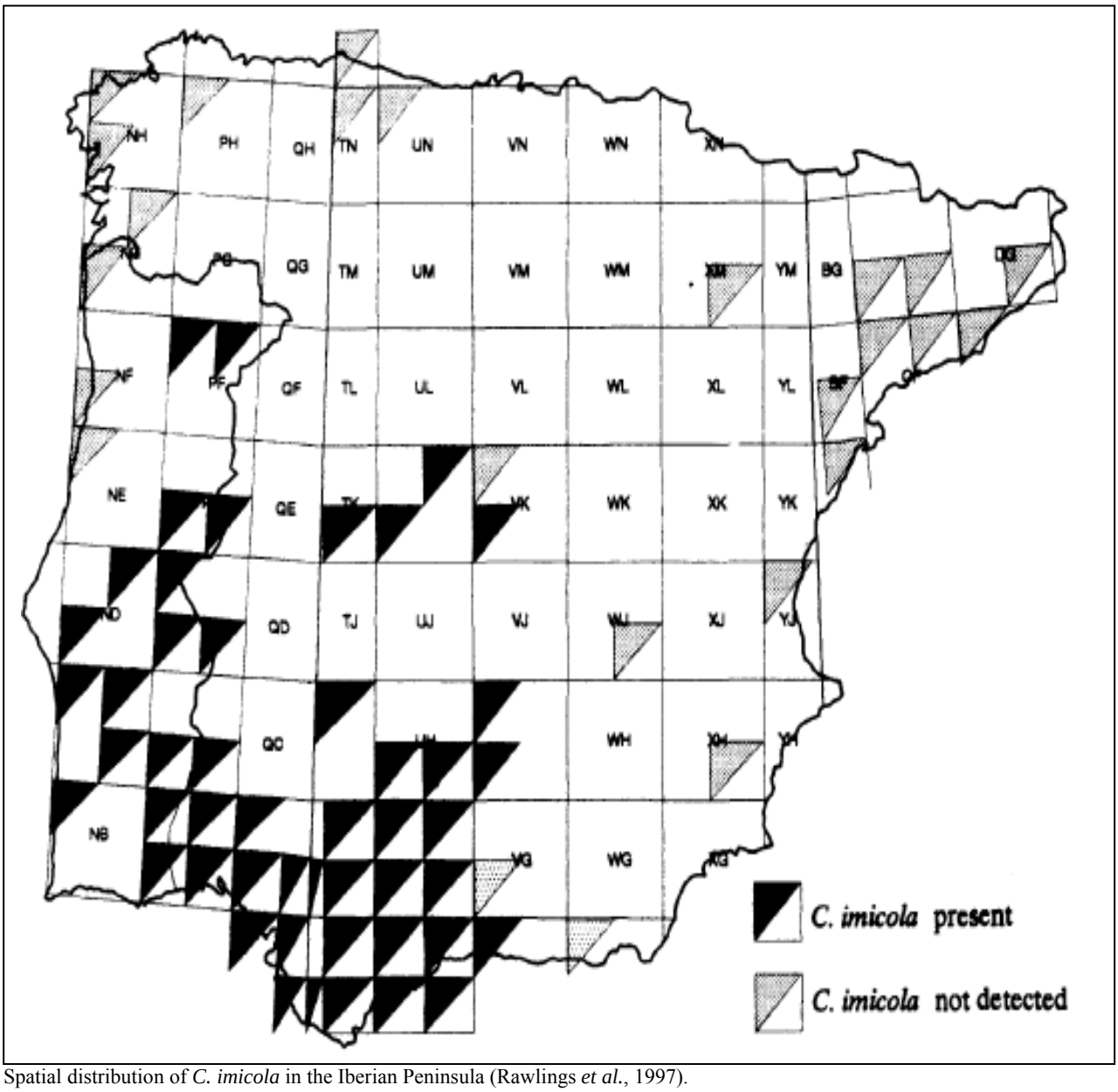
Appendices

Appendix A

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



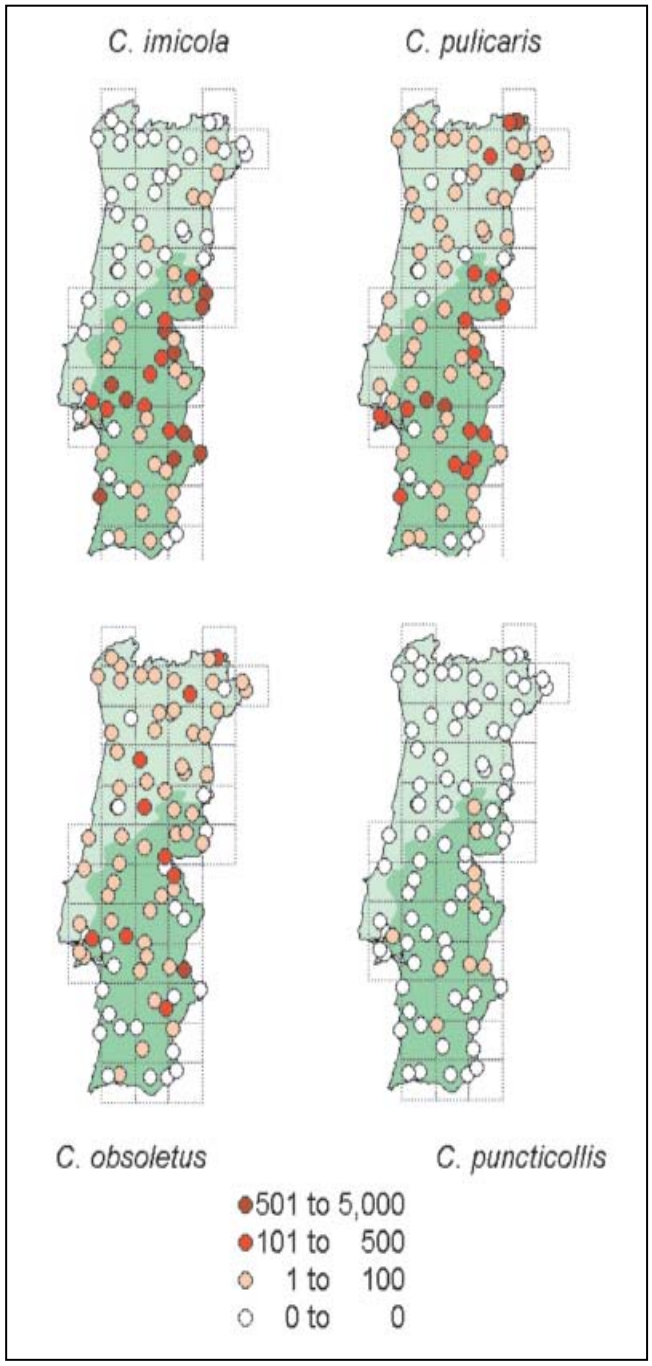


Spatial distribution of *C. imicola* in the Iberian Peninsula (Rawlings *et al.*, 1997).

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)

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).



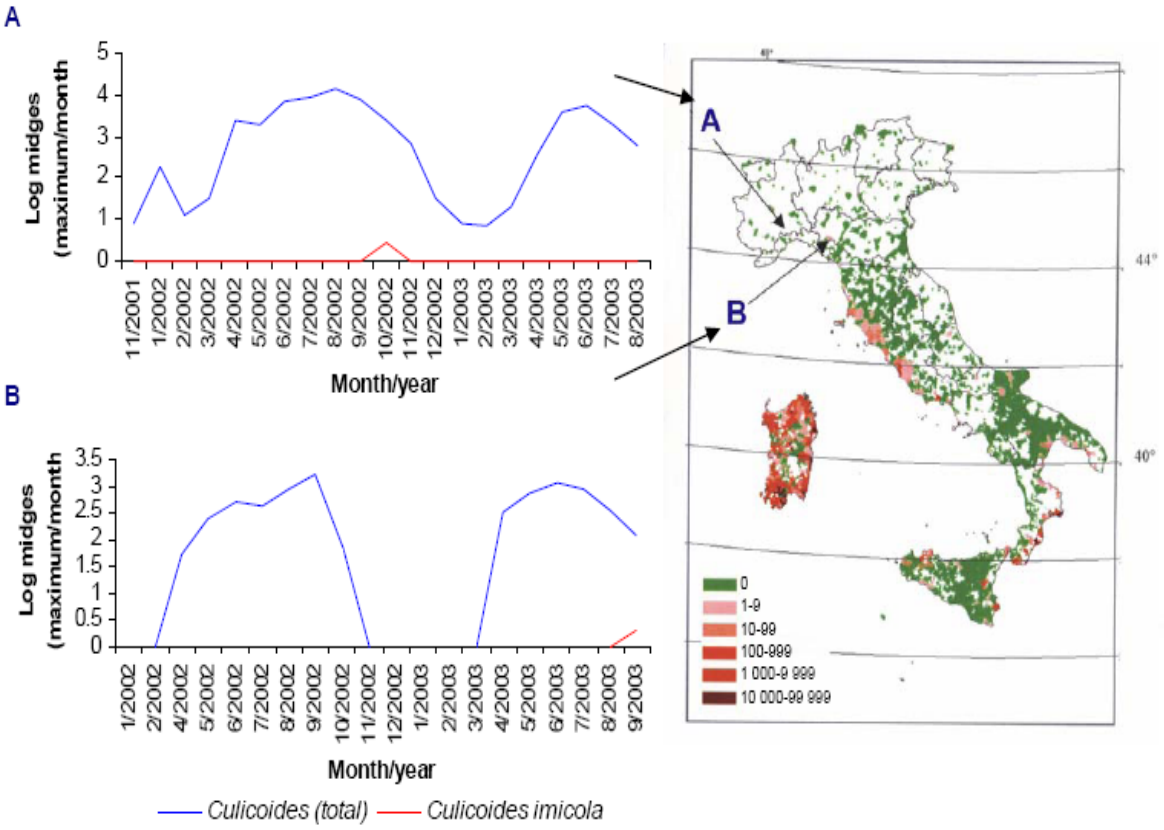


Figure 3
Maximum light-trap abundances of *Culicoides imicola*; collections from 1 533 municipalities in all regions of Italy (2000-2003) and seasonal abundances of total *Culicoides* in the two northernmost collection sites where *C. imicola* was found in the Provinces of Genoa (A) and Parma (B)

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

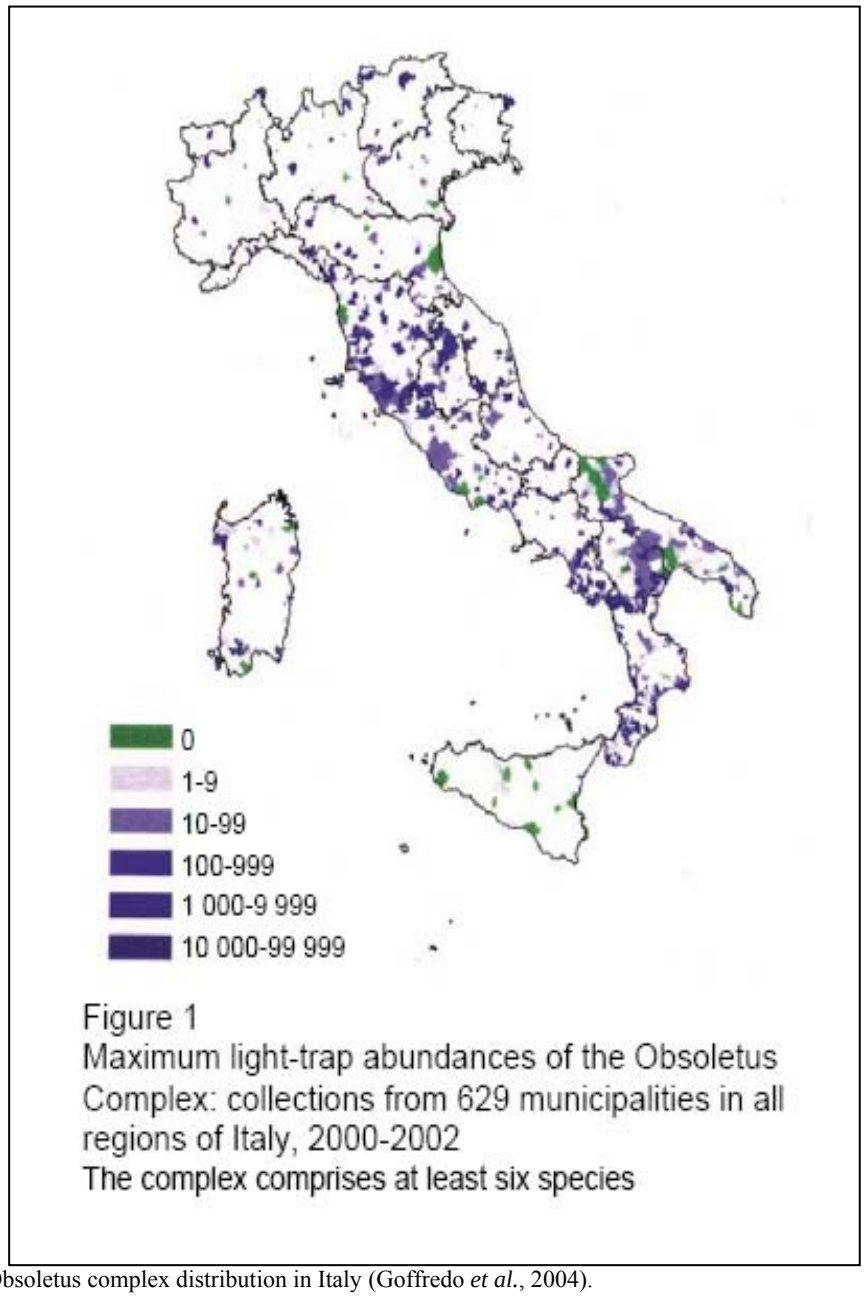
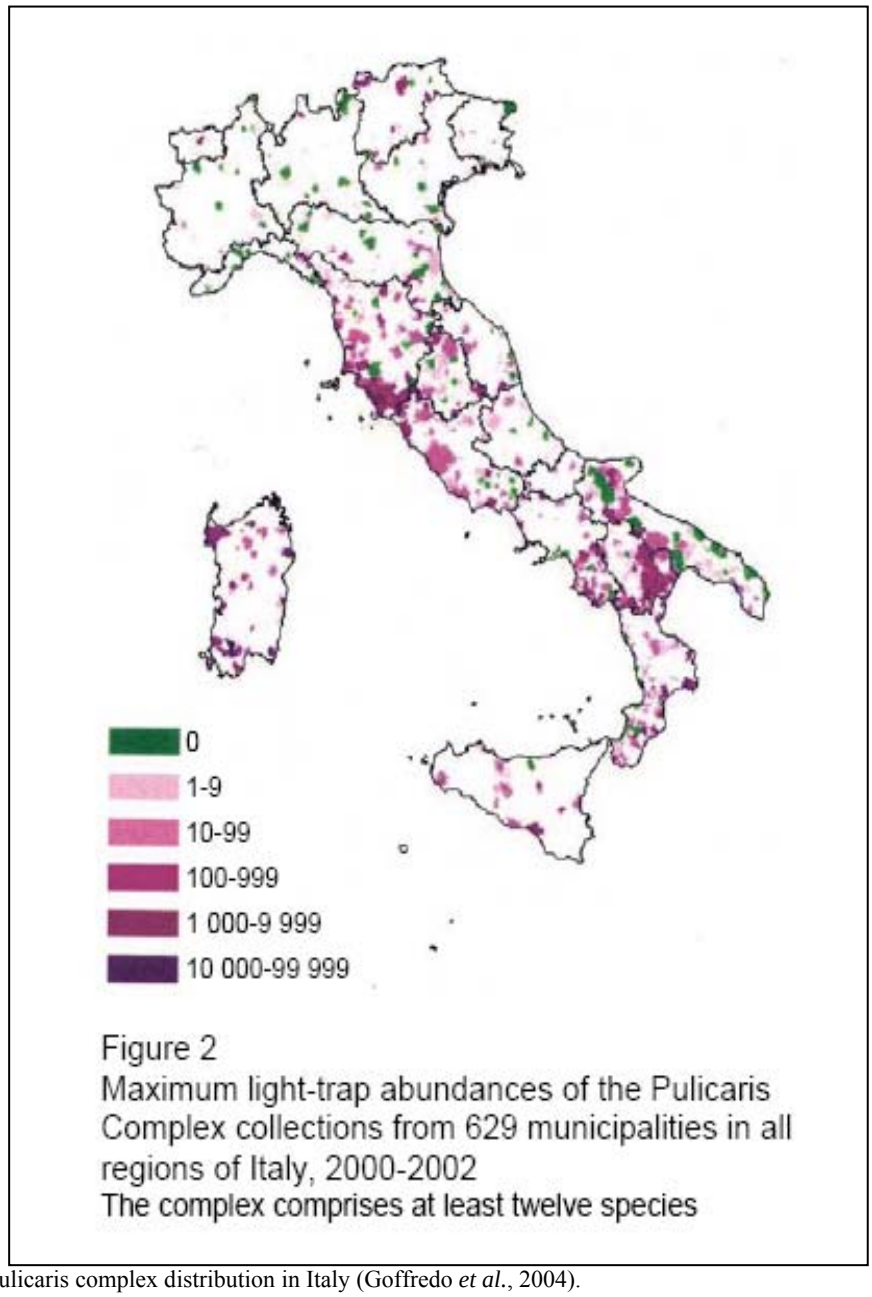
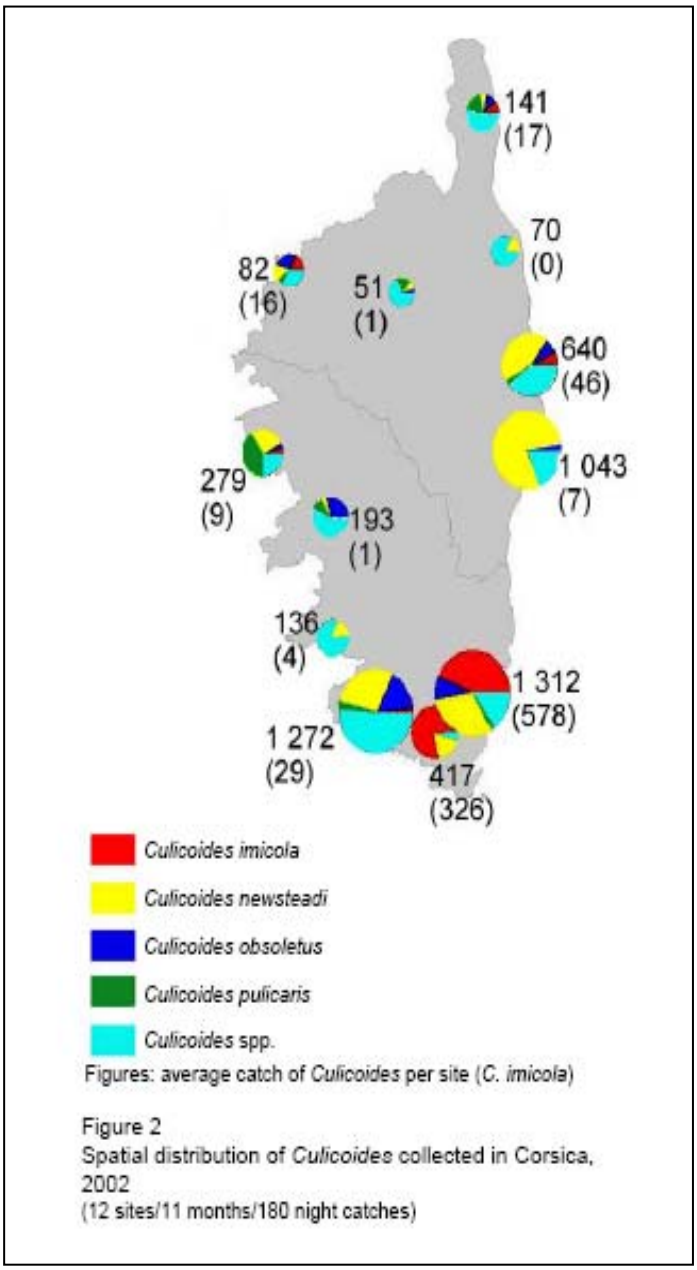


Figure 1
Maximum light-trap abundances of the Obsoletus Complex: collections from 629 municipalities in all regions of Italy, 2000-2002
The complex comprises at least six species

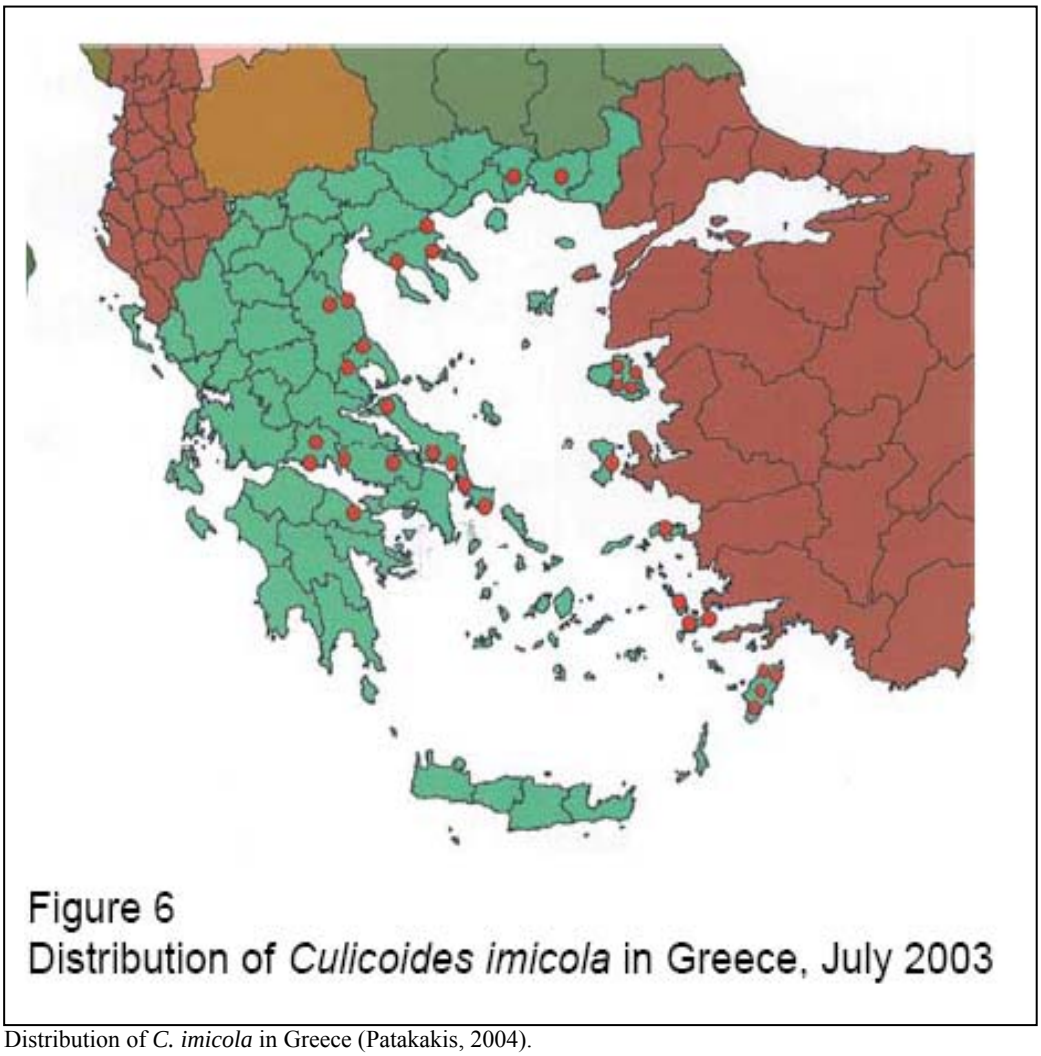
Obsoletus complex distribution in Italy (Goffredo *et al.*, 2004).



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

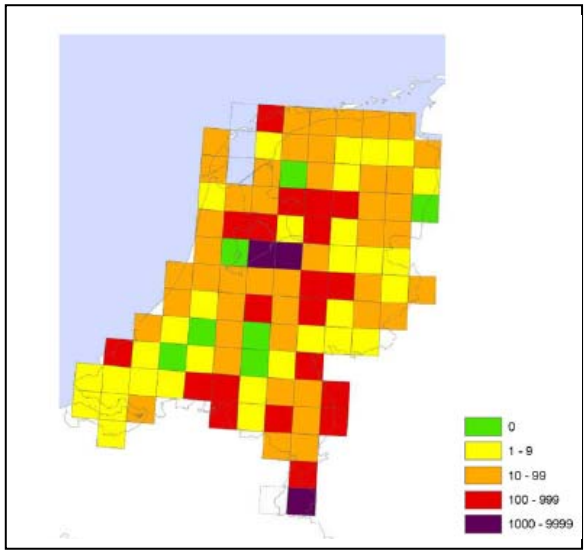


Distribution of *Culicoides* species in Corsica (Baldet *et al.*, 2004).

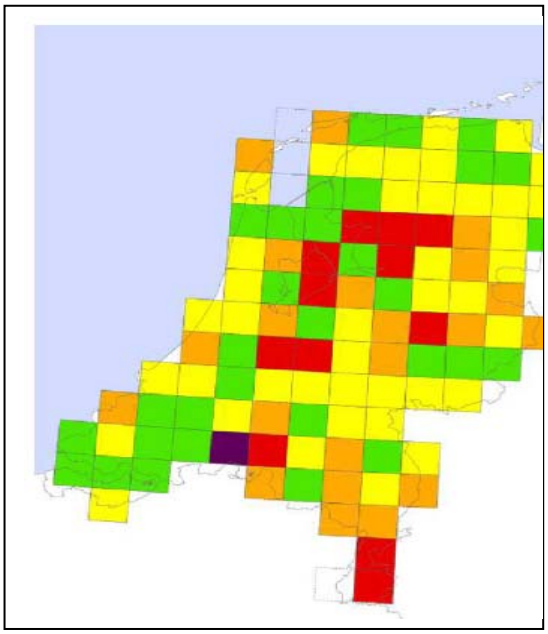


Distribution of *C. imicola* in Greece (Patakakis, 2004).

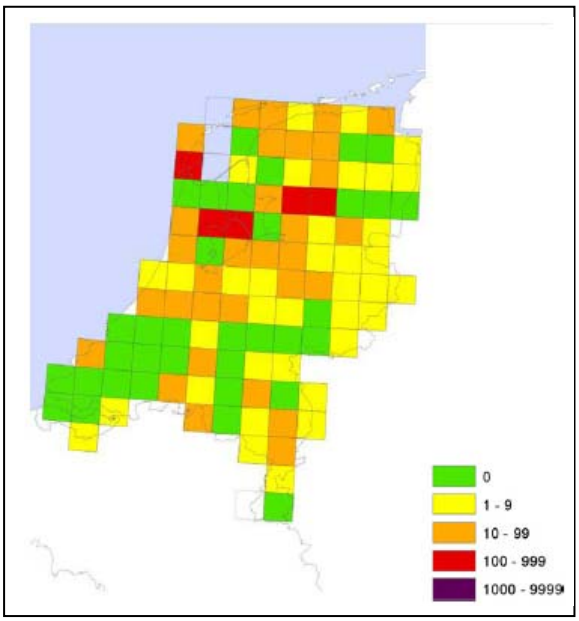
Geographical distribution of Obsoletus complex in The Netherlands (Meiswinkel *et al.*, 2008).



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



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



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.

APPENDIX B

To prepare the scientific review on Epizootic hemorrhagic disease (EHD) including: aetiology, epidemiology, pathology, diagnosis and geographical distribution.

*Search **methods** for identification of proper references*

We searched the **electronic databases** (EDB) PubMed, OVID, Elsevier. The research also included the use of **MeSH Thesaurus** –with the Subject heading combination. The search was limited to words in the title or the abstract and no language restrition was imposed. The search was conducted in 10th February 2009.

We integrated the search by the **bibliographies of references reviewed** choosing primary original research papers with data relevant to the contents of the EHD scientific review without restrictictions on publication year. References from two Orbiviruses conference proceedings specifically **handsearched** for the objectives of the review were also included: Bluetongue, African Horse Sickness and related Orbiviruses. *1992. Proceedings of the second International Symposium* edited by *T.E. Walton, B.I. Osburn* and *Bluetongue. 2003. Third International Symposium. Veterinaria italiana, vol. 40 (3)/(4).*

We also searched the following **editorial sites** for book contents: Blackwell, SAGE publisher, Cochrane Library, Elsevier, Biomedcentral.

The terms included in the search are reported in the table included in the appendix.

All the titles and, where available, abstracts collected in the EDBs were reviewed and accepted only if both reviewers agreed. The criteria for exclusion were: a) contents not relevant to or specifically addressing the objectives of the review; b) insufficient details (only title available or not comprehensive abstract); c) not original research papers. When possible, complete papers were collected.

Grey literature sources were searched by using the Scirus and Google scholar searching engines. To ensure the quality of the results different criteria of selection were applied: firstly the results were screened according to the specificity of the contents, the second level of

control was based on the source of data: academic web sites (url-extension ".edu") and official institution (url-extension ".org") were considered as additional value to guarantee the quality of the contents. The following step for the screening of the results is the Pdf format of the document searched to prevent changes of the text. The quality of the sources was further assessed according to classical criteria as the presence of the author name, the year of publication, the source and the references.

All searches included items published from January 1989 to June 2009.

All citations were uploaded in End note. Duplicate abstracts were removed using the internal software and manually de-duplicated.

***Results** of the search*

The numbers of items retrieved by searching in Pubmed and MeSH are reported in the following table:

Queries	**Pubmed**	**MeSH**
Search **Epizootic hemorrhagic disease**	296	91
Search **EHDV** or **EHD**	307	--
Search **Epizootic hemorrhagic disease, epidemiology**	98	0
Search **Epizootic hemorrhagic disease, diagnosis**	176	52
Search **Epizootic hemorrhagic disease, diagnostic tests**	10	0
Search **Epizootic hemorrhagic disease, Culicoides***	27	9
Search **Epizootic hemorrhagic disease, aetiology**	0	83
Search **Ibaraki, Epizootic hemorrhagic disease or EHDV**	10	--

*In MeSH *Ceratopogonidae* is the subject heading which include C*ulicoides*

The search in **OVID** EDB (Medline not included to avoid duplication of data) produced 208 items when the searching terms Epizootic hemorrhagic disease were used as "Keywords" and

91 when searched in the "Title" field. The search by using EHDV in "keywords" or "Title" fields produced 7 and 3 items respectively.

The handsearch in conference **proceedings** collected 18 references while 8 references were from bibliographies of references reviewed.

Editorial sites provided 6 **book** chapters and among the **grey literature** 6 citations were found.

A total of 196 references passed relevance screening and are included in the reference list.

Further quality assessment of the references included in the review was done during the 1st and 2nd meeting of the Experts of the Panel on Animal Health and Welfare for the Scientific Opinion on Epizootic Hemorrhagic Disease.

GLOSSARY/ABBREVIATIONS

EHD	Epizootic hemorrhagic disease
EHDV	Epizootic hemorrhagic disease virus
BT	Bluetongue
BTV	Bluetongue virus
AHS	African horse sickness
AHSV	African horse sickness virus
EEV	equine encephalosis virus
AKA	Akabane virus
OIE	World Organisation for Animal Health
UV	Ultra Violet Rays
dpi	Days post infection
RT-PCR	Real-Time Polymerase Chain Reaction
ELISA	Enzyme Linked immunoSorbed Assay
AGID	Agar Gel Immuno Diffusion
CFT	Complement fixation test
SN	Serum neutralization
VNT	Virus neutralization test
IAH	Institute of Animal Health, Pirbright

DEFINITIONS

Serogroup: group of viruses which share common epitopes. These epitopes are usually located in the inner part of the capsid and react against the same in immunological tests. All viruses that react to antibodies directed against the inner protein are said to belong to the same group. The inner protein is called the group specific antigen while the external proteins are called the type-specific antigens.

Serotype: all isolates of a virus that can be neutralized by a common antiserum are said to belong to the same serotype. Since "neutralization" involves the external proteins of a virion the serotype is dictated by these proteins. The term "serological property" refers to the antigenic characterization of a virus

Viral isolation: isolation of the virus from clinical specimens collected from either mammalian or insect samples and typically done in either embryonnated chicken eggs or in cell culture.

RT-PCR evidence: detection of nucleic acids of the virus regardless of whether the virus is infectious or not.

L cells: a series of mouse methylcholanthrene induced sarcoma cell line maintained in culture since the 1950s. The cells were isolated from a strain (c3h) of mouse fibroblasts grown in tissue culture. They are employed for their ability to support replication of many types of viruses.

Species Complex: it is a term employed in the strict cladistic sense i.e. to group two or more closely related terminal taxa, presumably recently evolved, and united phylogentically in that they share one or more synapomorphic features (for e.g. all 12 species of the Imicola Complex have the apices of the male parameres erect and simple, and the female abdomen is adorned dorsally on segments 2-4 with small, round, pigmented terga).

Vectorial capacity: is a relative measure of the ability of a vector population to transmit a disease pathogen such as BTV among members of a vertebrate population. It is defined as follows: $C = ma^2Vp^n/(-\log_c p)$ where C = number of new infectious particles per case per day, m = number of vectors per host, a = number of blood meals taken by a vector per host per day, V = vector competence, p = daily survival probability of the vector, and n = extrinsic incubation period in days.

Vector competence: refers to the suitability of a vector for pathogen replication/development. This competence is dependent upon the genetic makeup of the midge and external environmental influences.

Vector implication: there are four basic requirements to implicate an insect as a vector of a pathogen. These are: 1. isolate the pathogen from field collected insects, 2. demonstrate in the laboratory that the insect can be infected with the pathogen through a blood meal from an infected host, 3. demonstrate in the laboratory that the infected insect can transmit the pathogen to a suitable host, and 4. demonstrate that the insect comes in contact with the host in the field.

Viraemia: circulation and presence of infectious virus in the blood of the mammalian host as detected by virus isolation. Importantly, EHDV nucleic acid can be detected by RT- PCR assay in the absence of infectious virus in the blood of ruminants following infection especially as the time interval, subsequent to infection, increases.

Episystem: the species (vector, host and pathogen) and environmental aspects of an epidemiological system within a particular ecosystem.

Extrinsic incubation period: the time interval between oral infection of a vector and pathogen transmission, involving entry of virus into midgut, replication, dissemination through the haemocoel and infection of the salivary glands.

Palaearctic: one of the eight ecozones into which the world is divided, which extends across Europe, North Africa and North Asia, north of the tropics.

Endophilic: *Culicoides* which feed only on animals indoor.

Exophilic: *Culicoides* which feed only on animals outdoor.