

## SCIENTIFIC OPINION

### Scientific Opinion on Epizootic Hemorrhagic Disease<sup>1</sup>

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#### ABSTRACT

Following a request from the European Commission, the Panel on Animal Health and Welfare was asked to deliver a scientific opinion on Epizootic Hemorrhagic Disease (EHD). The disease has never been reported in the European Union, however in recent years outbreaks of disease caused by EHD Virus (EHDV) serotype 6 and 7, previously considered to be non-pathogenic were observed in EU neighbouring countries. Clinical signs in cattle are similar to those exhibited by bluetongue affected animals and production losses may be significant. Sheep, although susceptible to infection, do not present clinical signs. EHDV is transmitted between its ruminant hosts by species of *Culicoides* biting midges. There is no scientific evidence to suggest that mosquitoes have a role in the transmission of EHDV. In Europe several suspected EHDV vectors are present, including *C. imicola* and vectors within the *Pulicaris* and *Obsoletus* complex. An assessment of the risk of introduction of EHD virus into the EU was developed by considering 3 possible entrance pathways: i) via imported infectious animals ii) via infectious vectors and iii) other routes e.g. via vaccines or germplasm. The risk estimate for the introduction varied according to the pathway considered and the season of introduction. Vector abundance and climatic conditions could be favourable to sustain EHDV circulation; therefore, the AHAW panel concluded that presence of EHDV in neighbouring countries poses a significant risk for introduction and establishment of EHDV in the EU. A surveillance programme (active and passive) in high risk areas using sensitive diagnostic tests should be established for early detection of disease introduction. In case of an outbreak of EHD in the EU, key actions should include detection of infected animals, epidemiological investigations, restriction of movements and eventually long term surveillance.

#### KEY WORDS

EHD, Epizootic Haemorrhagic disease, EHDV, significance, cattle, Risk assessment, introduction in Europe, monitoring, control

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## SUMMARY

Following a request from the European Commission, the Panel on Animal Health and Welfare was asked to deliver a scientific opinion on Epizootic Hemorrhagic Disease (EHD).

The mandate was composed of four terms of reference : i) the significance of the presence, origin and occurrence of EHD in susceptible species (specially livestock animals) in the European Union (EU) neighbouring countries ii) the possibility of EHD to spread to and within the EU and to persist iii) the role played by different vectors and the means to control them and iv) the possible measures to control and eradicate the disease including surveillance, control of vectors, availability of suitable vaccines and other elements. The disease causative agent is EHD virus (EHDV) a member of the Orbivirus genus. EHDV has been associated with disease in White-tailed deer (*Odocoileus virginianus*) in North America but clinical signs have also been observed in cattle and other wild ungulates. The serotype historically associated with disease in cattle is EHDV-2 (Ibaraki virus). However in recent years outbreaks of disease caused by EHDV-6 and EHDV-7, serotypes normally considered as non pathogenic, were observed in countries neighbouring the EU. Sheep are susceptible to infection but do not present clinical signs. It is likely that other species may also be susceptible to infection but there is a lack of information about the epidemiological role of many domestic and wild ruminant species.

Clinical signs in cattle are similar to those exhibited by Bluetongue (BT) affected animals. In cattle morbidity varies from 1% to 18%, but mortality is usually low. Production losses associated with the disease in cattle may be significant, especially in dairy farms, in the form of lowered milk production. Considerable adverse welfare impact can occur in EHD affected animals. EHDV-2 (Ibaraki strain) is able to cross the placental barrier resulting in abortions and malformations.

Infection with a particular EHDV serotype confers protection against infection by the homologous serotype. Duration of this protection is unknown but indirect field evidence suggests it may last for life. No cross-protection exists between EHDV and BTV.

EHDV is transmitted between its ruminant hosts by species of *Culicoides* biting midges. There is no scientific evidence to suggest that mosquitoes have a role in the transmission of EHDV. In Europe several suspected EHDV vectors are present, including potential vectors such as: *C. imicola* and suspected vectors within the *Pulicaris* and *Obsoletus* complexes. Other species may also act as vectors for EHDV but their competence and capacity has not been investigated.

The similarity between the geographical distribution of recent EHDV outbreaks in North Africa and West Asia, and the BTV outbreaks in the same areas at the end of the 90es constitutes a reason for concern. An assessment of the risk of introduction of EHDV into the EU was developed by taking into consideration 3 possible entrance pathways: i) via imported infectious animals ii) via infectious vectors and iii) other routes e.g. via vaccines or germplasm. When quarantine and testing for EHDV are in place, the probability of importing an infectious animal into a EU Member State (MS) was considered to be negligible. The probability of introducing an infectious animal through illegal movement of livestock or transit of wild animals was not considered to be negligible and could be high depending on the origin and the season of movement. Following EHDV introduction by an infectious animal the risk of exposure to EU susceptible animals during periods of vector activity was considered to be high. Depending on the temperature at the time of introduction, the risk of spread was considered to be moderate or high.

The risk of introduction of EHDV into the EU from neighbouring countries by wind dispersal of vectors was rated as high. Vector abundance and climatic conditions could be favourable to sustain EHDV circulation; therefore, presence of EHDV in neighbouring countries poses a significant risk for introduction and establishment of EHDV in the EU.

The risk posed by contaminated vaccines for EHDV introduction was considered to be negligible for authorized medicinal products. The lack of information regarding EHDV presence in germplasm rendered the assessment of the risk of introduction via this pathway not possible.

EHD in deer is a notifiable disease in EU Member states (MS) according to Council directive 92/119/EEC. Active surveillance programmes for EHDV infection are not in place in the EU and are limited in EU neighbour countries. Passive surveillance is complicated by the similarity of clinical signs with BT. Monitoring of disease prevalence (active surveillance schemes) is difficult at present due to the lack of commercial serological diagnostic methods.

As shown for BTV, vaccines may play a primary role for the control of EHDV should the infection enter the EU. However, at this time no inactivated EHD vaccines are commercially available.

A surveillance programme (active and passive) in high risk areas using sensitive diagnostic tests should be established for early detection of disease introduction. In case of an outbreak of EHD in the EU, key actions should include detection of infected animals, epidemiological investigations, restriction of movements, and eventually, long term surveillance. The surveillance should be done by serological testing if such tests are available. If viraemic animals were detected in a previously free area culling or slaughter of these animals should be used as a control measure. In addition, mitigation measures on the *Culicoides* vectors, as used for BTV control (e.g. insecticides and repellents, good farm hygiene to remove vector breeding sites) could be also used for EHDV.

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## BACKGROUND AS PROVIDED BY EUROPEAN COMMISSION

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 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 10 serotypes are recognised (according to Gorman, 1992) 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 outbreaks 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 reported in North America, Australia, Asia and Africa but until recently was not found in Europe or its vicinity. However, EHD has been reported during 2006 in the Mediterranean basin (Morocco, Algeria, Tunisia and Israel) and also in 2007 in cattle in western Anatolia and in Turkey, 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* but it appears that other species of gnats and mosquitoes can also transmit EHDV.

It is known that infected deer can remain viraemic for up to 2 months 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 AS PROVIDED BY EUROPEAN COMMISSION**

In view of the above, and in accordance with Article 29 of Regulation (EC) No 178/2002, the Commission asks EFSA for a scientific opinion on:

- the significance of the presence, origin and occurrence of EHD in susceptible species (specially livestock animals) in the EU neighbour countries for a better understanding of the possible evolution of the disease in the context of the EU epidemiological situation;
- the possibility of EHD to spread to and within the EU and to persist considering epidemiology, vectors, livestock and climatic conditions.
- the role played by different vectors in the spread of EHD and the means to control them.
- the possible measures to control and eradicate the disease including surveillance, control of vectors, availability of suitable vaccines and other elements.

## ASSESSMENT

### 1. Approach to the mandate

The mandate for this scientific assessment focuses on EHDV as a hazard, and the request of the EC is to assess the likelihood of entry, establishment and spread of the hazard in the EU.

The mandate was composed of four terms of reference (ToR):

- ToR 1: The significance of the presence, origin and occurrence of EHD in susceptible species (specially livestock animals) in the EU neighbour countries for a better understanding of the possible evolution of the disease in the context of the EU epidemiological situation;

This ToR was addressed by a comprehensive literature search (Appendix A) in order to provide the necessary information to complete the risk assessment. The review focused on (i) a description of the hazard (EHDV, all known serotypes), (ii) on the identification of host species susceptible to EHDV infection, both wild hosts and livestock, and (iii) on a description of the pathogenesis of EHD, including duration of viraemia, clinical signs and lesions, effects on production and welfare risks, in particular in livestock. This information is contained in Chapter 2 of this opinion.

The geographical occurrence and distribution of EHDV in the world and specifically in EU neighbouring countries was also addressed to understand the significance and the possible evolution of EHD in the context of the EU. This information is included in Chapter 4 of this opinion.

- ToR 2: The role played by different vectors in the spread of EHD and the means to control them.

A considerable amount of scientific information has been already collected on *Culicoides* as vectors of BTV for previous EFSA opinions. Therefore, this ToR was addressed by a literature search with the aim of highlighting the role of *Culicoides* on EHDV transmission. When necessary, cross reference was made to previous EFSA opinions to illustrate data on vector biology, identification, geographic distribution, control and surveillance. This information was included in Chapters 3 and 5 of this opinion.

- ToR 3: The possibility of EHD to spread to and within the EU and to persist considering epidemiology, vectors, livestock and climatic conditions.

The introduction of EHDV into EU, the likelihood of EHDV to spread to and within the EU to persist was assessed following the methodology proposed by the World Animal Health Organization (OIE) for import risk analysis (OIE 2004). The release assessment described the possible virus introduction pathways by commodities. The exposure assessment estimated the probability that a susceptible host from EU is exposed to EHDV and the consequence assessment was performed on assessing the likelihood of spread of EHDV once the hazard has been introduced. The risk assessment model was developed considering as the hazard EHDV of no particular serotype and cattle as the host, however it should be noted that other potential hosts such as sheep or wild ruminants could play an important role. The extrapolation of data originated from the current knowledge of BT was clearly indicated in model description section. The risk assessment is described in section 6.

- ToR 4: The possible measures to control and eradicate the disease including surveillance, control of vectors, availability of suitable vaccines and other elements

Early detection of EHDV infection is crucial for the implementation of any surveillance or control programme. Therefore, the first step to address this ToR, was to perform a literature review on the available diagnostic techniques for EHD and its performance. The possibility of cross-reaction with other orbiviruses was specifically addressed. Information was then provided on monitoring and



control measures (including vaccination) applied in other parts of the world where EHDV is present. This information is contained in Chapter 5 of this opinion.

The conclusions and recommendations emanating from this background information and from the risk assessment performed to answer these ToR are included in Chapter 6 of this opinion.

## 2. Epizootic Hemorrhagic Disease

Epizootic haemorrhagic disease (EHD) is a vector borne disease caused by EHD virus (EHDV), an Orbivirus distinct from bluetongue virus (BTV) (MacLachlan and Osburn, 2004). It is transmitted by *Culicoides* and, like BTV the EHDV group includes a number of distinct serotypes. Sub-clinical EHDV infection of domestic ruminants occurs throughout the temperate and tropical regions of much of the world. In certain circumstances, however, EHDV infection may result in overt disease in infected ruminants, especially cattle and some species of deer, and this probably reflects the pathogenicity of the infecting virus strain and the susceptibility of the animals infected. There is no evidence to date that EHDV infection occurs in Europe.

### 2.1. Epizootic Haemorrhagic 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 BTV, African horse sickness virus (AHSV) and equine encephalosis virus (EEV) (McLachlan and Osburn, 2004) such as being a dsRNA virus with a linear genome of 10 segments. Re-assortment of segments between different virus strains can occur (Allison et al. 2009). No re-assortment between BTV and EHDV has been reported. As for BTV, the primary determinant of serotype specificity is the outer capsid VP2 protein, although VP5, the other external protein, is also involved as a secondary determinant (Schwartz-Cornill, et al., 2008). Historically, based on serological reactivity, a total of 8 EHDV serotypes have been recognised according to Campbell and St George (1986) (Table 1). More recent studies indicate that EHDV serotype 3 (Ib Ar 22619) is in fact the same serotype as EHDV-1 (Anthony et al., 2009a, 2009b) and therefore the total number of EHDV serotypes has been proposed to be 7.

**Table 1:** Currently recognised EHDV serotypes (adapted from Campbell and St George, 1986)

Serotypes
EHDV-1 New Jersey
EHDV-2 Alberta, Ibaraki and CSIRO 439
EHDV-3 Ib Ar 22619a
EHDV-4 Ib Ar 33853
EHDV-5 CSIRO 157
EHDV-6 CSIRO 753
EHDV-7 CSIRO 775
EHDV-8 DPP 59

a: EHDV serotype 3 (Ib Ar 22619) has been found to be similar to EHDV-1

Also, recent efforts to characterise an un-typed strain of EHDV designated as EHD-318 have concluded that this strain is serologically identical to EHDV-6 (Anthony et al., 2009a, 2009b). EHDV-318 was responsible for the recent outbreaks occurring in North Africa and Turkey. A panel of reference strains of EHDV is not yet officially recognised. In Table 2, however, a widely accepted list of reference strains is displayed.

**Table 2:** 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.

Reference strains							
	EHDV-2 <u>CAN1962/01</u>						
EHDV-1 <u>USA1955/01</u>	EHDV-2 (Ibaraki virus) <u>JAP1959/01</u>	EHDV-3 <sup>a</sup> <u>NIG1967/01</u>	EHDV-4 <u>NIG1968/01</u>	EHDV-5 <u>AUS1977/01</u>	EHDV-6 <u>AUS1981/07</u>	EHDV-7 <u>AUS1981/06</u>	EHDV-8 <u>AUS1982/06</u>

a: EHDV serotype 3 (Ib Ar 22619) is proposed to be the same serotype as EHDV-1

Genomic studies and phylogenetic analyses have also been used to better understand the epidemiology of EHDV (Wilson, 1994b; Wilson, 1991b; van Staden et al., 1991; Cheney et al., 1996; Mecham et al., 2003; Murphy et al., 2005; Murphy et al., 2006). According to these studies it was possible to group the North American EHDV strains on the basis of their serotype or topotype. More recent studies comparing the whole genome of typed and untyped EHDV strains revealed that the internal proteins can be used to group EHDV into “eastern” (i.e. Asia and Australia) and “western” (i.e. Americas, Africa and Middle East) strains (Anthony et al., 2009b)

It should be noted that there are genetic variations within serotypes, thus strain designations should be based on serological and molecular characteristics. However, genetic differences in the VP5 gene do not change the serotype designation (Anthony, et al., 2009a). Analysis of the VP2 gene sequence showed a 97% identity within North American strains of EHDV-2 allowing the determination of the geographic origin (topotypes) of isolates within this serotype (Cheney et al., 1996). EHDV-3 (NIG1967/-1) has been shown to be serologically similar to EHDV-1 and has an 88.7% identity with EHDV-1 (USA1955/01) for the VP2 gene sequence (Anthony et al., 2009a) but, there are distinct genetic differences between other gene sequences for these viruses (Wilson et al., 2009b; Anthony et al., 2009b). Ibaraki virus has long been known to be a strain of EHDV-2 (Sugiyama et al., 1989) and has a high homology of the gene segments of other strains into this serotype (Uchinuno et al., 2003; Ohashi et al., 1999; Iwata et al., 2001). However, EHDV-2 (Ibaraki strain) has been shown to cause disease in cattle (Omori et al., 1969) while the North American strains of EHDV-2 have not. Also EHDV-318 (now classified as EHDV-6) and EHDV-7 caused disease in cattle in North African, Turkey and Israeli outbreaks, while the same serotypes were reported as non pathogenic in Sudan and Australia.

In terms of diagnostic development it is important to note that VP7 is the serogroup specific immunodominant protein and is highly conserved among the EHDV serotypes, showing a high percentage of identity at the amino acid level (Mecham et al., 2003). The genes encoding the non-structural proteins NS 1-3 are highly conserved within the serogroup but distinct from BTV (Wilson, 1994a and b; Jensen et al., 1994; Jensen and Wilson, 1995; Anthony et al., 2009a). This genetic conservation makes the NS proteins good targets for nucleic acid detection methods.

More details on the available genetic information can be found at: ([http://www.reoviridae.org/dsrna\\_virus\\_proteins//Accession%20numbers%20for%20EHDV%20sequences.htm](http://www.reoviridae.org/dsrna_virus_proteins//Accession%20numbers%20for%20EHDV%20sequences.htm)).

The physio-chemical properties of EHDV are similar to those of other members of the genus Orbivirus (e.g. BTV, AHSV and EEV) (Coetzer and Guthrie, 2004).

## 2.2. Susceptible hosts

EHDV has been historically associated with disease in wild cervids, particularly white-tailed deer (*Odocoileus virginianus*) in North America. Ibaraki virus, now classified as EHDV-2, was found to cause disease in cattle in Japan (Omori et al., 1969). EHDV-6 and EHDV-7 have recently been shown to be pathogenic for cattle in some Mediterranean countries (Temizel et al., 2009, Yadin et al., 2008). European sheep have also been found to be susceptible to infection with EHDV but although viraemia has been detected the infection was subclinical (Tomori 1980, Gibbs et al., 1977, Thompson et al., 1988). The role of goats as a host for EHDV is uncertain. EHDV antibodies have been detected by AGID test in goats from regions where EHDV occurs in the field and it may be that a proportion of these, like sheep, develop a low level viraemia (Al Busaidy and Mellor 1991). In a field study in Indonesia using seroneutralization, only 1 out of 88 goats showed antibodies against EHDV-5. Additional studies need to be done to clarify the role of goats as host for EHDV. Pigs are not susceptible to EHDV infection (Gibbs et al., 1977) and little is known about susceptibility of dogs. In a serological survey conducted in an area having a high prevalence of EHD in white-tailed deer no EHDV positive dogs were detected (Howerth et al., 1995). There is still a considerable lack of information about susceptibility to EHDV infection and the epidemiological role of many domestic and wild ruminant species. The current knowledge on susceptible species to EHDV is summarised in Table 3.

**Table 3:** EHDV Susceptible species

Latin name	Common name	Infection	Clinical disease	Serotype/detection	Reference <sup>a</sup>
<i>Bos taurus</i>	Cattle	Nat	yes	2 (Serol)	House et al., 1998
			no	2(Serol)	Pasick et al., 2001
			no	2(VI)	Dulac et al., 1989
		Nat	yes	2 (Ibaraki)	Omori et al., 1969
				(VI)	Ohashi et al.,1999
				(Serol)	Temizel et al.,2009
			yes	6 (VI)	Yadin et al., 2008
		Nat	yes	7(VI)	Sendow et al., 1991
			no	5 (Serol)	Gard and Melville, 1992
			no	2, 5, 7, 8 (VI)	Weir et al., 1997
<i>Odocoileus virginianus</i>	White-tailed deer	Nat	yes	1 (VI)	Gaydos et al., 2004
			yes	6 (VI)	Murphy et al., 2006
			yes	1 (VI)	Allison et al., 2009
<i>Antilocapra americana</i>	Pronghorn antelope	Nat	?	Unknown *	Dubay et al., 2006a Dunbar et al.,1999
<i>Odocoileus hemionus</i>	Mule deer	Nat	yes	1 (Serol)	Dubay et al., 2004
			yes	2 (VI)	Noon et al., 2002a
<i>Oryx leucoryx</i>	Arabian oryx	Nat	no	Unknown*	Frolich et al., 2005
<i>Mazama gouazoubira</i>	Grey brocket deer	Nat	?	Unknown *	Deem et al., 2004
<i>Ovis canadensis canadensis</i>	Rocky Mountain bighorn sheep	Nat	yes	2 (VI)	Noon et al., 2002b
<i>Ursus americanus floridanus</i>	Black bear	Nat	no	Unknown*	Dunbar et al., 1998
<i>Cervus elaphus</i>	Red deer	Exp	no	1 (VI)	Gibbs and Lawman 1977
<i>Dama dama</i>	Fallow deer				
<i>Capreolus capreolus</i>	Roe deer				
<i>Muntiacus muntjac</i>	Muntjac deer				
<i>Ovis aries</i>	Sheep				
<i>Capra hircus</i>	Goat	Nat	no	2 and 6 *(Serol)	Al-Busaidy and Mellor, 1991

<i>Diceros bicornis</i>	Black rhinoceros	Nat	no	Unknown	Fischer-Tenhagen et al., 2000
<i>Ceratotherium simum</i>	White rhinoceros	Nat	no	Unknown	Fischer-Tenhagen et al., 2000

\*Sera tested by AGID: possible cross-reaction with BTV antibodies.

Infection: (Nat) Natural or (Exp) Experimental

Detection: (Serol) Serological detection or (VI) viral isolation

a) The list of references is not exhaustive. The references selection criteria were the completeness of information instead of first occurrence.

### 2.3. Pathogenesis of EHDV infection

The pathogenesis of the EHDV is similar to that of BT, with initial virus replication in endothelial cells of the lymphatic vessels and in the lymph nodes draining the site of infection (Sohn and Yuill, 1991). EHDV is then disseminated to the secondary sites of replication such as other lymph nodes and spleen. In the viraemic phase, the virus is cell-associated and in particular to red blood cells where the virus is present in higher titre for longer period of time (Gibbs and Lawman, 1977, Aradaib et al., 1997). In vitro studies have indicated that replication of the EHDV could be monocyte-dependent (Stallknecht et al., 1997)

The duration of viraemia has been investigated in natural or experimental studies in deer and cattle. Even though in some cases it was possible to isolate EHDV from animals for more than 50 days, usually infectious virus could not be detected beyond 3 weeks from infection.

Of 11 white-tailed deer experimentally infected with EHDV-2, five were viraemic at day 2 p.i., and by day 4 all animals were viraemic. Viraemia peaked at day 6 with titres exceeding 104.9 TCID<sub>50</sub>/ml of blood. Two deer were still viraemic at day 59, although with a very low titre (<102.3 TCID<sub>50</sub>/ml). Clinical disease was observed between days 6 and 14, but no deaths were reported (Gaydos et al., 2002b). In another experimental study using EHDV-2, viraemia was detected in 3 of 16 inoculated deer at PID 2, and all the animals were viraemic by PID 4. Peak of viraemias occurred between PID 4 and PID 10 (Table 4). Two deer remained viraemic until PID 56, when the study was concluded (Quist et al., 1997).

**Table 4:** Viraemia in white-tailed deer experimentally infected with EHDV-2 (adapted from Quist et al., 1997)

Days Post infection	2	4	6	8	10	12	14	17	21	24
Number of viraemic deer /total	3/16	16/16	16/16	14/14	11/13	10/12	10/12	10/11	8/11	6/11

Days Post infection	28	30	32	36	42	49	56
Number of viraemic deer /total	6/11	6/11	3/11	2/10	2/10	2/10	2/10

In cattle experimentally infected with EHDV-2 the virus was isolated from 4 of 6 animals on PID 28 and from one animal at PID 50, but from 0 out of 6 at 2 and 3 months (Gibbs and Lawman, 1977). Calves inoculated with EHDV-1 and EHDV-2 had a detectable viraemia from 9 to 23 dpi and from 8 to 16 dpi, respectively (Aradaib et al., 1994b)

A strain of EHDV-2 isolated from a white-tailed deer that died of a hemorrhagic disease was passaged twice in deer and used to inoculate calves. Calves inoculated became infected, as evidenced by development of viraemia and seroconversion. The results of viraemia detection are summarized in Table 5 (Abdy et al., 1999)

**Table 5:** Viraemia in calves experimentally infected with EHDV-2 (adapted from Abdy et al., 1999)

Days Post Infection	6	8	10	12	14	16	18	20	24	31	38	44	51
Number of viraemic calves /total	5/6	6/6	6/6	4/6	4/6	3/6	3/6	3/6	2/6	1/6	0/6	1/6	0/6

Gard and Melville (1992) provided information on the duration of viraemia in cattle naturally infected with EHDV-2, -5, -7 and -8 (Table 6). Although the authors reported that the number of viraemic animals could be underestimated because of the isolation techniques used at the beginning of the study it still represents valid field information

**Table 6:** Duration of viraemia in 130 sentinel cattle (Adapted from Gard and Melville 1992)

	Duration of viraemia in weeks (viral isolation)				
	<1	1-2	2-3	3-4	4-5
EHDV-2	46*	24	6	4	1
EHDV-5	31	3	0	1	1
EHDV-7	0	1			
EHDV-8	12				

\*Number of viraemic animals per week

According to Quist et al., (1997), animals infected with EHDV might show high levels of gamma interferon starting from day 4 pi until the appearance of detectable antibodies (10 dpi). Following infection with EHDV-1 neutralising antibodies were detected between days 10-14 (Gibbs and Lawman 1977). The immunological response is not able to completely eliminate the virus from circulation and in infected animals it is possible to find simultaneously neutralising antibodies and EHDV. As for BTV, this is due to the strong association between the virus and the red blood cells

In young deer whose mothers had been infected with EHDV seroneutralizing maternal antibodies were found in serum up to 17-18 weeks of age. The presence of passive immunity could not prevent infection or viraemia on fawns naturally exposed to EHDV challenge but was able to protect against the severe clinical form of the disease (Gaydos et al., 2002b). Infection with a particular EHDV serotype confers protection against the infection by the homologous serotype, as shown by Ibaraki outbreaks in Japan where after the first outbreaks in 1959 many cattle in the epizootic areas had antibody against the virus and both morbidity and mortality decreased during the 1960 outbreaks in same area (Omori et al., 1969). Duration of this protection is unknown but evidence for monitoring of natural infection, and from the use of live attenuated vaccines<sup>4</sup> suggests it may last for life (Stalknecht et al., 1991).

Also there is partial cross-protection against severity of disease between the North American serotypes EHDV-1 and EHDV-2, probably due to some antigenic relationship. However, it does not protect from infection and viraemia (Gaydos et al., 2002b). It is not known if cross-protection between other serotypes does exist. No cross-protection was observed between BTV and EHDV when EHDV previously infected deer were subsequently infected with BTV (Quist et al., 1997).

EHDV is a vector borne disease. Nevertheless, oral and faecal shedding of EHDV-1 from white-tailed deer has been reported (Gaydos et al., 2002a) but the epidemiological role of this transmission route is probably insignificant. Virus has also been recovered from vulvar swabs of a sheep that had recently

<sup>4</sup> Kyoto Biken Laboratories inc.

lambled (Gibbs and Lawman, 1977), the most likely explanation being blood contamination of the samples. Transplacental transmission has been demonstrated by isolation of EHDV-2 (Ibaraki) from internal organs of aborted fetuses (Ohashi 1999). The isolation of BTV from semen has been reported (Howard et al., 1985) although the presence of the virus in semen appears to be a rare event (Melville et al., 1996). No reports of EHDV isolation in semen have been found. The experimental infection of sheep by inoculation with semen from bulls infected with EHDV-2, 5 and 7 did not lead to infection, however in the trial virus was only isolated from blood and not from semen (Gard et al., 1989).

## **2.4. EHD clinical signs and lesions**

EHDV was initially recognised as a disease of white-tailed deer but clinical signs have been observed also in cattle and other wild and domestic ungulates. The disease is characterized by high morbidity and mortality in white-tailed deer while in cattle morbidity varies from 1 to 18% but mortality is usually low.

### **2.4.1. EHD in cattle - Japan**

The first EHDV strain demonstrated to be pathogenic for cattle was the Ibaraki virus strain (EHDV-2). The disease occurring in Japan was characterized by fever, anorexia and difficult in swallowing, which may lead to dehydration and emaciation with aspiration pneumonia constituting the major cause of death in affected animals (Inaba, 1975). 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, foetal malformations and stillbirths have also been reported in the 1997 epidemics in Japan (Ohashi 1999). The swallowing disorder is caused by damage to the striated muscles of the pharynx, larynx, oesophagus and tongue. The lesions are histologically characterized by hyaline degeneration, necrosis and mineralization of striated muscle accompanied by an infiltration of neutrophils, lymphocytes and histocytes.

### **2.4.2. EHD in cattle - North America**

North American isolates of EHDV are able to cause viraemia in cattle and in sheep, usually without inducing clinical disease (Abdy et al., 1999; Thompson et al., 1988). There are occasional anecdotal reports of natural clinical disease in cattle associated with EHDV-2 activity (House et al., 1998, [http://www.cfsph.iastate.edu/Factsheets/pdfs/epizootic\\_hemorrhagic\\_disease.pdf](http://www.cfsph.iastate.edu/Factsheets/pdfs/epizootic_hemorrhagic_disease.pdf)), however, it has not been substantiated that North American strains of EHDV can cause disease in cattle.

### **2.4.3. EHD in cattle - EU neighbour countries**

During the summer of 2006 outbreaks of EHD caused by EHDV-6 were reported from Turkey, Morocco, Tunisia, Algeria and Jordan in dairy and beef cattle. In these outbreaks the animals presented clinical signs similar to the ones described for EHD in Japan, with low case fatality. In Morocco almost half of the premises affected had high morbidity rates (98 out of the 218 herds had rates of over 33%) but many of these farms were small, with the largest number of cases being 8 on a single farm. The overall morbidity rate was 18% (329 cases out of 1814 susceptible animals), while the case-fatality rate was 2.2%. In Israel during the autumn of 2006 an outbreak of EHD resembling Ibaraki disease was reported. The isolated virus was typed as EHDV-7 (Yadin et al., 2008) and was the first identification of serotype 7 causing significant clinical signs in cattle. Within-herd morbidity ranged from 5 to 80 per cent in infected dairy herds, with variable involvement of replacement heifers; the case-fatality was less than 1 per cent. The duration of the disease in individual animals was reported to range between three and 30 days. There were no reports of a distinctive disease syndrome in sheep or goats in these areas. Affected cows showed a 10 to 20 per cent reduction in milk



production and loss of appetite, followed by clinical signs similar to Ibaraki. Some or all of the clinical signs of EHD were reported in a total of 105 cattle herds (80 dairy herds, 21 beef herds and four feedlots). A study conducted in Israel indicated losses in milk production of 140 kg milk per milking cow during the disease period of 3 month and an increase of 1.31% in the mortality rate. Extra use of medicines and losses due to abortion, infertility problems and inferior milk quality were noted as result of the disease (Hagai Yadin personal communication). A summary of information regarding morbidity and mortality is presented in Table 7.

**Table 7:** Morbidity and mortality of EHDV infection in cattle

Serotype	Location	Start	End	Apparent morbidity	Apparent mortality	Morbidity per herd	Case fatality rate	Reference
2 (Iba)	Japan	Sep 59	Nov 59	1.96 %*	0.2%*	0.01-12.6% a)	10.3%	Omori et al., 1969
2 (Iba)	Japan	Sep 60	Nov 60	1.16%*	0.06%*		5.8%	Omori et al., 1969
2	USA	Aug 96	Oct 96			Up to 20%		House et al., 1998
NK	French Reunion	Jan 09		3.23%	0	-	0	Breard et al., 2004
7	Israel	Sep 06	Nov 06			5-80%	Less than 1%	Yadin et al., 2008
6	Turkey	Jul 07	Aug 06					Temizel et al., 2009
6	Morocco	Jul 07	Sep 07	18%	2.2%			OIE-WAHID 2006
6	Algeria	Jul 06		8%	0.5%			OIE-WAHID 2006

\* total of animals in districts involved in the outbreaks

a) depending on district

NK Not known

#### 2.4.4. EHD in wild ungulates - North America

Three clinical forms of EHD have been described in white-tailed deer, peracute, acute and chronic forms. 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. Hemorrhagic 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 tissues 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 may 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 and crawl on their knees or chest. Deer with the chronic form might also develop ulcers, scars, or erosions in the rumen (Iowa State Centre for Food Security and Public Health) extensive damage to the lining of the rumen can cause emaciation even when there is no shortage of food. Histopathologic lesions may include widespread vasculitis with thrombosis, endothelial swelling, haemorrhages, degenerative changes, and necrosis in many organs especially the tongue, salivary glands, walls of forestomachs, aorta and papillary muscle of the left ventricle of the myocardium.

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

The morbidity and mortality due to EHD in wildlife is difficult to determine. The estimated number of affected animals and populations in at risk areas suggests an infection rate of 29% and mortality rate of 20% in white-tailed deer (Gaydos et al., 2004). Similar seroprevalence has been reported in Pronghorn antelope (*Antilocapra americana*) (Drolet et al., 1990; Dubay et al., 2006a). White-tailed deer are the most severely affected by the disease while the survival rate is much higher in mule deer (*Odocoileus hemionus*), black-tailed deer (*Odocoileus hemionus columbianus*) and pronghorn antelope (Hoff and Trainer, 1981; Work et al., 1992).

## 2.5. Welfare impact of EHD

There can be a considerable welfare impact of this infection although the degree will vary according to serotype of virus and species of animal infected. Severe pain and distress are likely to occur. Clinical signs indicative of severe pain include salivation (ulceration and erosion in the mouth, tongue and lips) and lameness (coronary band lesions). Severe distress is likely to be associated with fever, dehydration (resulting from diarrhoea, salivation and nasal discharge), anorexia and increased metabolic rate (leading to emaciation and lethargy). Productivity, another measure of poor welfare, is decreased manifesting as poor growth, lowered milk production, and reduced fertility as a result of abortion, resorption, foetal malformations and stillbirths. In the chronic form animals are ill for several weeks but gradually recover but not always completely. Thus chronic wasting is a welfare issue as the animals do not thrive.

## 3. Vectors

EHDV is transmitted between its ruminant hosts, as is BTV, by species of *Culicoides* biting midges. Such 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, seasonality and control (see also Carpenter et al., 2008), and the effect of climatic variables upon vectors including criteria for the vector-free season is likely to be the same as has already been provided as part of the EFSA opinions on BT (EFSA 2007a, 2008b).

Nevertheless, the levels of competence that individual vector species and populations express for EHDV and hence, their vector capacities, may 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 will also be different than for BTV. Such differences may form the basis of the occasional observed differences in the current regional and global distributions between EHDV and BTV as exemplified, particularly, in Europe and North America.

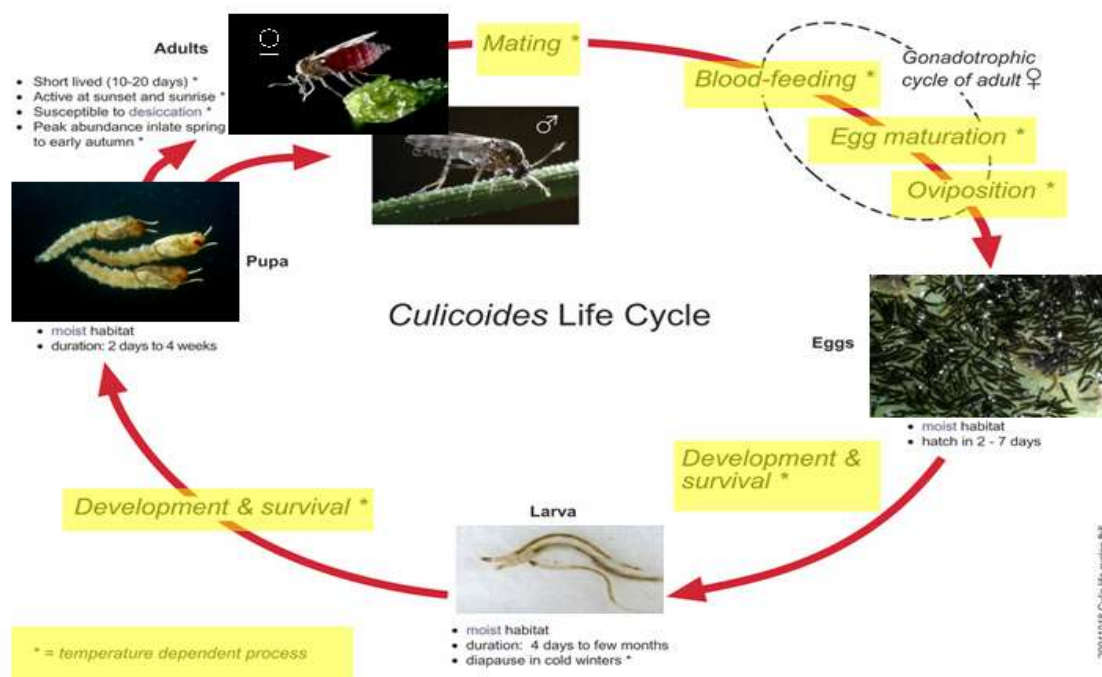
### 3.1. *Culicoides* biology and taxonomy

*Culicoides* are small biting flies belonging to the family Ceratopogonidae. In different parts of the English speaking world they are variously known as gnats, midges, punkies and no-see-ums. The family Ceratopogonidae contains some 125 genera with about 5500 species. Of these genera, four are



known to contain species that suck the blood of vertebrates: *Austroconops*, *Culicoides*, *Forcipomyia* subgenus *Lasiohelea*, and *Leptoconops*. *Culicoides* are easily differentiated from these others by wing characters.

More than 1400 species of *Culicoides* are known worldwide of which about 96% are obligate blood suckers attacking mammals (including humans) and birds. The life cycle of *Culicoides* (fig 1) includes egg, four larval stages, pupa, and imago. Immatures require a certain amount of free water or moisture and are found in an astonishingly wide range of habitats that meet that criterion. Breeding sites include pools, streams, marshes, bogs, beaches, swamps, tree holes, irrigation pipe leaks, saturated soil, animal dung, and rotting fruit and other vegetation (Blanton and Wirth 1979 Wirth and Hubert 1989). Eggs are usually laid in batches adhering to the substrate. They are white when laid but darken rapidly. They are not resistant to drying and usually hatch within two to seven days (Blanton and Wirth 1979, Meiswinkel et al., 1994). Larvae are vermiform and swim with a characteristic serpentine or eel-like motion. The duration of the four larval stages varies with the species and ambient temperature, from as little as four to five days to several weeks). In temperate countries these periods may be considerably extended because most species overwinter as fourth-instar larvae in diapause (Kettle 1984). Pupae may be free floating or loosely attached to debris. The pupal stage is brief, usually lasting for only two to three days but occasionally three to four weeks, depending on species and temperature. Most adult *Culicoides* are crepuscular, and therefore peak activity is around sunset and sunrise and to a lesser extent through the night, though a few species bite during the day. Females undertake flight activity to seek a mate, a blood meal, or an oviposition site. Males do not blood-feed. The flight range of *Culicoides* usually is short and most species disperse only a few hundred meters from their breeding sites (Kettle 1984) or at most 2 to 3 km (Lillie et al., 1981). However, *Culicoides* are capable of being dispersed passively as aerial plankton over much greater distances (Hayashi et al., 1978). In general, adult *Culicoides* are short-lived and most individuals probably survive for fewer than 10 to 20 days but exceptionally they may live for much longer periods (44 to 90 days) and during this time may take multiple blood meals.



**Figure 1:** The *Culicoides* life cycle (Adapted from Purse et al., 2005)

Vectorial capacity represents the number of infections a population of a given vector would cause per case per day at a given place and time assuming conditions of non immunity (Garret-Jones 1964). It depends upon a series of variables including vector competence, biting rate, vector density, vector survival rate and duration of the extrinsic incubation period. However, there is little information on any of these variables that applies specifically to the transmission of EHDV by its vectors.

The duration of the median extrinsic incubation period(EIP), i.e. the number of days required for 50% of competent females to complete the development time of the virus, has estimated at different temperatures for EHDV-1, in *C. sonorensis* (table 8). The theoretical minimum temperature for EHDV-1 replication in a vector to a transmissible level has been estimated to be 15.2oC (Wittmann et al., 2002).

**Table 8:** Estimated duration of median extrinsic period (MEP) for EHDV-1 in *C. sonorensis* at different temperatures

Temperature	MEP (days)
15	-
20	18.2
25	5.7
30	4.8

### 3.2. EHDV vectors and their geographic distribution

Table 9 shows the *Culicoides* species that have been implicated as confirmed or suspect vectors of EHDV, their taxonomic relationships and the geographic locations where the studies were undertaken.

Brown et al., (1992) reported two isolations of EHDV-4 from *Anopheles vagus* mosquitoes collected in Bali, Indonesia. However, it is likely that these reflect chance isolations as there is no evidence in this paper or elsewhere to indicate that any mosquito species can act as a biological vector of EHDV (or BTV). Consequently, it is unlikely that mosquitoes play any significant role in the epidemiology of EHD.

**Table 9:** EHDV confirmed/suspect vector species

Subgenus	Species Complex	Species	Identified EHDV Serotypes	Geographical Location	Vector implication criteria*				References
					1	2	3	4	
Avaritia	Imicola	<i>C. imicola</i>	2, 3, 5, 6, 7, 8	South Africa	X				Paweska et al., 2005.
Avaritia	Imicola	<i>C. bolitinos</i>	1, 2, 3, 4, 5, 6, 7	Experimental infection		X			
Meijerehelea		<i>C. leucostictus</i>	5, 6, 7			X			
Culicoides	Pulicaris	<i>C. magnus</i>	2			X			
Beltrammyia		<i>C. nivosus</i>	5, 7			X			
Avaritia	Gulbenkiani	<i>C. gulbenkiani</i>	2			X			
Hoffmania		<i>C. zuluensis</i>	7			X			
Hoffmania		<i>C. onderstepoortensis</i>	8			X			
Monoculicoides		<i>C. cornutus</i>		South Africa	X				Barnard et al., 1998
Remnia		<i>C. nevilii</i>		South Africa	X				
Avaritia	Imicola	<i>C. imicola</i>		Sudan	X		X		Aradaib et al., 1999
Oeacta/ Remmia	Schultzei	<i>C. king/ C. schultzei</i>	4	Sudan	X		X		Mohammed and Mellor. 1990.

Oecacta/ Remnia	Schultzei	<i>C. schultzei</i>	Ibar 49630	Nigeria	X	X	Lee VH. 1979
		<i>C. mohave</i>		North America Arizona	X		Rosenstock et al., 2003 (Detection by RT- PCR)
Haematomyidium	Paraensis	<i>C. lahillei</i>	2	North America Experimental infection	X	X	Smith et al., 1996b
Monoculicoides	Variipennis	<i>C. sonorensis</i>	1,2	North America	X	X	Foster et al., 1977 Foster et al., 1980 Smith et al., 1996a Jones et al., 1977 Greiner et al., 1984
Haematomyidium	Paraensis	<i>C. debilipalpis</i>		North America	X	X	Mullen et al., 1985 a and b
Haematomyidium	Paraensis	<i>C. paraensis</i>				X	
Haematomyidium	unknown	<i>C. stellifer</i>				X	
Avaritia	Obsoletus	<i>C. obsoletus</i>				X	
Hoffmania	Hoffmania	<i>C. venustus</i>		North America Experimental infection	X		Jones et al., 1983
Avaritia 1955	Fox, Imicola	<i>C. brevitarsis</i>	2, 5, 6, 7, 8	Australia	X	X	St. George et al., 1983 Parsonson and Snowdown 1984
Oecacta/ Remnia	Schultzei	<i>C. oxystoma</i>		Japan	X	X	Yanase et al., 2005
Hoffmania	Peregrinus	<i>C. lungchiensis/ peregrinus</i>			X	X	
Culicoides	Pulicaris	<i>C. punctatus</i>			X	X	

\*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 (Adapted from WHO 1967)

Table 10 shows those *Culicoides* species that have been implicated as confirmed or suspect vectors of BTV and/or EHDV. Those species that occur within Europe are shown in bold.

**Table 10:** *Culicoides* (Latreille, 1809), regarded as vectors or potential vectors of BTV and or EHDV.

Subgenus	Species Complex	Species	BTV	EHDV
Avaritia Fox, 1955	Imicola	<i>C. imicola</i>	<i>x</i>	<i>x</i>
		<i>C. brevitarsis</i>	<i>x</i>	<i>x</i>
		<i>C. bolitinos</i>	<i>x</i>	<i>x</i>
	Obsoletus	<i>C. obsoletus</i>	<i>x</i>	<i>x</i>
		<i>C. scoticus</i>	<i>x</i>	<i>NK</i>
	Dewulfi	<i>C. dewulfi</i>	<i>x</i>	<i>NK</i>
	Orientalis	<i>C. fulvus</i>	<i>x</i>	<i>NK</i>
<i>C. dumdumi</i>		<i>x</i>	<i>NK</i>	

		<i>C. orientalis</i>	x	NK
	Grahamii	<i>C. actoni</i>	x	NK
	Pusillus	<i>C. pusillus</i>	x	NK
	Suzukii	<i>C. wadai</i>	x	NK
	Gulbenkiani	<i>C. brevipalpis</i>	x	NK
		<i>C. gulbenkiani</i>	x	x
		<i>C. tororoensis</i>	x	NK
<i>Culicoides</i> Latreille, 1809	Pulicaris	<b><i>C. pulicaris</i></b>	x	NK
		<i>C. magnus</i>	x	x
		<b><i>C. punctatus</i></b>	x	x
<i>Silvicola</i> Mirzaeva and Isaev, 1990	Cockerellii	Species unknown	x	NK
<i>Monoculicoides</i> Khalaf, 1954	Variipennis	<i>C. sonorensis</i>	x	x
	Nubeculosus	<b><i>C. nubeculosus</i></b>	x	NK
		<b><i>C. puncticollis</i></b>	x	NK
<i>Remmia</i> Glukhova, 1977	Schultzei	<i>C. oxystoma</i>	x	NK
		<i>C. nevilli</i>	x	x
		<i>C. kingi</i>	NK	x
		<b><i>C. schultzei</i></b>	x	x
Oecacta/Remmia	Schultzei	<i>C. oxystoma</i>	x	x
<i>Hoffmania</i> Fox, 1948	Guttatus	<i>C. insignis</i>	x	NK
		<i>C. filarifer</i>	x	NK
	Peregrinus	<i>C. peregrinus</i>	x	x
	Milnei	<i>C. milnei</i>	x	NK
		<i>C. venustus</i>	NK	x
<i>Haematomyidium</i> Goeldi, 1905	Complex unknown	<i>C. stellifer</i>	x	x
	Paraensis	<i>C. lahillei</i>	NK	x
	Paraensis	<i>C. debilipalpis</i>	x	x
	Paraensis	<i>C. paraensis</i>	x	x
<i>Oecacta</i> Poey, 1853	Furens	<i>C. furens</i>	x	NK
<i>Meijerehelea</i> Wirth and Hubert, 1961	Complex unknown	<i>C. pycnostictus</i>	x	NK
Subgenus unknown	Complex unknown	<i>C. trilineatus</i>	x	NK
Subgenus unknown	Complex unknown	<i>C. mohave</i>	NK	x

NK: Not Know

*Culicoides imicola* is an Afro-Asiatic species but has established itself across southern Europe and is now widespread in Portugal, Spain, the Balearics, southern mainland France, Corsica, mainland Italy, Sardinia, Sicily, mainland Greece and several Greek islands adjacent to Anatolian Turkey (Mellor et al., 2008). *Culicoides obsoletus* and *C. punctatus* are northern Palaearctic species belonging to the *Obsoletus* and *Pulicaris* complexes. These species are widespread across most of northern and central Europe (Mellor et al., 2008, Purse et al., 2005). *Culicoides schultzei* is widespread in Africa and the Middle East but in Europe has only been recorded from south Attica, in southern mainland Greece (Mellor et al., 1984).

### 3.3. Vector seasonality

It is well documented that BT is a disease of the late summer and autumn, and peak numbers of cases occur at this time of the year (Mellor et al., 2000, Purse et al., 2005, Yonguc et al., 1982, Mellor and Boorman 1995, Mellor 1994). In Africa and southern Europe where *C. imicola* is the major vector this is not surprising as the population abundance of this species also peaks in late summer and autumn, and is virtually coincident with the peak of outbreaks in animals. However, in northern Europe, where *C. imicola* is absent, the main BTV vectors are *C. obsoletus* (group), *C. dewulfi*, *C. chiopterus* and possibly *C. pulicaris* (group) species, whose populations' peak in late spring and early summer with much smaller peaks in late summer/autumn (Birley and Boorman 1982, Takken et al., 2008, Fassotte et al., 2008). Yet the pattern of disease cases in livestock remains the same as in southern Europe - i.e. the vast majority are in late summer and autumn.

The patterns of virus and vector activity in northern Europe suggest that the occurrence of the first and by far the largest vector peak of the year is not reflected by an increase in virus activity but the much smaller second peak is. Currently, the reasons for this anomaly are uncertain. It is likely that, due to the low winter temperatures in northern Europe virus survival from one vector season to the next in these regions will be at a very low level - and this is evidenced by the absence of cases in livestock during this period in Europe (Wilson et al., 2007). Furthermore, it has been established that the transmission of BTV and EHDV is enhanced by high temperatures (Mullens et al., 1995; Wittmann et al., 2002).

In North America the distribution of EHDV, like BTV, generally reflects the distribution of *C. sonorensis*. Also, most cases of BT and EHD occur in the late summer and autumn (Stallknecht and Howerth, 2004) at the same time that *C. sonorensis* abundance peaks (Gerry and Mullens, 2000, Gerry et al., 2001). However, there are a few reports of disease as far north as New Jersey in the USA and western Canada that beyond the normal range for this vector species (Stallknecht and Howerth, 2004, Chalmers et al., 1964, Shapiro et al., 1991). Canada is usually free of EHDV except for sporadic outbreaks that have occurred in the Okanagan valley of British Columbia (Pasick et al., 2001). It is likely that in such locations different *Culicoides* species will have different vectorial capacities for EHDV strains, as has been seen with the introduction of BTV-2 in Florida where the major vector is *C. insignis* not *C. sonorensis* (Mecham and Nunamaker, 1994; Tanya et al., 1992).

## 4. Epidemiology of EHD

EHD is a vector-borne viral disease its distribution is consequently limited to the distribution of competent *Culicoides* vectors. According to reported cases the range of EHDV lies approximately between latitudes 35°S and 49°N. Within this range EHD has been observed in North America, Australia, Asia and Africa. Recently, it has also been recorded in countries surrounding the Mediterranean Basin including: Morocco, Algeria, Tunisia, Israel, and Turkey (Figure 3). So far there has been no report of EHDV being present in Europe. Conversely in Europe there have been at least 12 incursions of 8 serotypes of BTV (1, 2, 4, 6, 11, 8, 9, 16) since 1998 and many of the viruses have spread widely, involving countries from Greece to Portugal and reaching as far north as southern Norway (60°N) (Mellor et al., 2008, ProMed Archive no. 20090221.0729, 21st Feb 2009)). The reasons for these different distributions of BTV and EHDV that have a similar range of host species and are transmitted by similar species of *Culicoides* have yet to be elucidated.

### 4.1. EHD geographical distribution

The global distribution of EHD is summarized in Table 11 and represented in Figure 2.

**Table 11:** EHD global geographical distribution

Geographical Location	Date of occurrence	Infected Host	Status*	Identified Serotypes	References
British Columbia (Canada)	1987-1988	Cattle	2	2 (Serol)	Dulac et al., 1989
British Columbia (Canada)	1999	White-tailed deer and California bighorn sheep	2	2 (Serol /RT_PCR study on sentinel cattle)	Pasick et al., 1999.
West Virginia (USA)	1993	White-tailed deer ( <i>Odocoileus virginianus</i> )	3	2	Gaydos et al., 2004
Indiana (USA)	2000-2002	Cattle	NK	NK	Boyer et al., 2008
Indiana and Illinois (USA)	2006	White-tailed deer	3	6 (VI)	Allison et al., 2009.
Kansas (USA)	1980-2002	White-tailed deer and mule deer	3	1, 2	Flacke et al., 2004
Kansas (USA)	2008	White-tailed deer	3	6 (VI)	Allison et al., 2009.
Texas (USA)	1985	Beef cattle		1, 2 (Serol)	Fulton et al., 1989
Texas (USA)	2008	White-tailed deer	3	6 (VI)	Allison et al., 2009
Colorado (USA)		Dairy cattle		1, 2 (VI)	Foster et al., 1980
Missouri (USA)	1996	White-tailed deer	3	2 (VI)	Beringer et al., 2000
Missouri (USA)	1988	White-tailed deer	3	2 (VI)	
Missouri (USA)	2007-2009	White-tailed deer	3	6 (VI)	Allison et al., 2009
Arizona (USA)	2002	Mule deer	2	1, 2 (Serol)	Dubay et al., 2006b
Arizona (USA)	2001-2002	Pronghorn	2	1, 2 (Serol)	Dubay et al., 2006a.
Florida (USA)	1998	Black bear	2	NK (Serol)	Dunbar et al., 1998
California(USA)	1987-1991	black-tailed and mule deer	2	NK (Serol)	Chomel et al., 1994
Midwestern states (USA)	1996	Cattle	3	2 (Serol)	House et al. 1998
Mexico	1994	White-tailed deer	2	NK (serol)	Martinez et al., 1999
Sudan	1982	Cattle (sentinel cattle)	2	4, 6 (VI) (EHDV 318)	Mohammed and Mellor. 1990.
Nigeria	1967-1970	<i>Culicoides</i> spp.	2	3, 4 (VI)	Lee,1979.
South Africa	1995-1997	Cattle	3	NK (VI)	Barnard et al., 1998
Japan	1959-1960	Cattle	3	2 (Ibaraki strain) (VI)	Omori et al., 1969
Japan	1997	Cattle	3	2 (Ibaraki strain) (VI)	Ohashi et al., 1999
Australia	1992	Cattle	2	1, 2, 5, 7, 8 (VI)	Weir et al., 1997. Gard and Melville. 1992
Indonesia	1991	Cattle, Buffaloes, sheep	2	5 (Serol)	Sendow et al., 1991
La Reunion	12/01/2009	Cattle	3	NK	OIE WAHID Disease

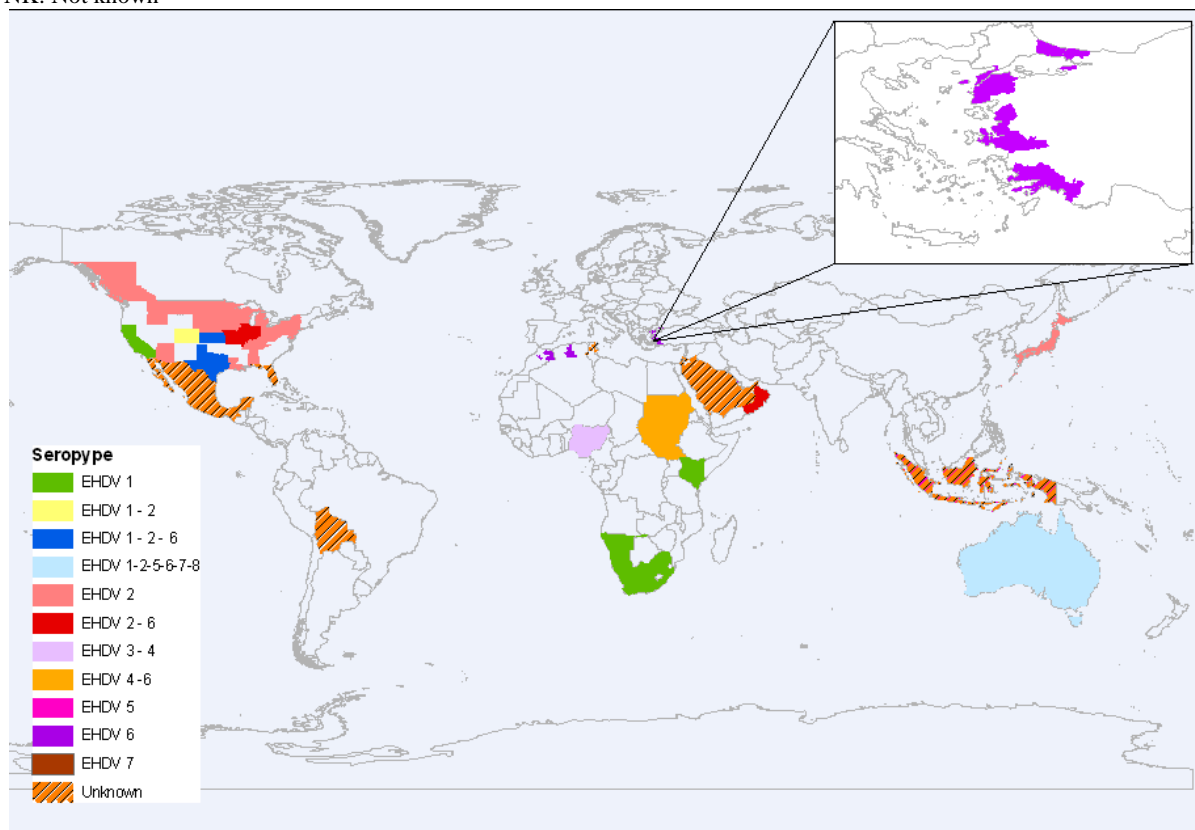


La Reunion	start date 2003	Cattle	3	RT-PCR NK RT-PCR	Information 2009; 22(9). Bréard et al., 2004
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\*Status : (1) Disease suspected but not confirmed (2) Confirmed infection but no clinical disease (3) Confirmed clinical infection

Detection: (Serol) Serological detection or (VI) viral isolation

NK: Not known



**Figure 2:** Map with the localization of the EHDV infection worldwide.

Where no geographical details as district/province etc, are not reported, the whole country has been indicated as infected.

#### 4.2. EHD in the Mediterranean countries

The geographical distribution of EHDV in EU neighbouring countries is summarized in Table 12 and illustrated in Figure 3. Before 2000 there were only scanty reports on the presence of EHDV in the Mediterranean Basin. To meet the first report we have to go back to 1951 when in Israel a disease similar to BT was described in cattle and sheep (Komarov and Goldsmith, 1951). At that time no typing was made. Other more recent evidence of EHDV presence was described in Turkey where EHDV antibodies were detected in cattle and sheep (Burgu, et al., 1991). As occurred for BTV at the end of the 90es, the EHDV Mediterranean situation changed after 2000. In 2001 clinical cases were described in cattle in Israel. EHDV was identified as the cause of the outbreak but no information on the serotype was available. In 2004, an outbreak caused by EHDV-6 (318) was reported in Morocco. The same serotype was responsible for another outbreak in the same country two years later. In the same year, EHDV-6 caused outbreaks in Algeria and Tunisia and one year later in Turkey (Temizel et al., 2009). Also in 2006, clinical cases were described in Israeli cattle. The first cases were observed in the region bordering Jordan. In contrast with the other Mediterranean outbreaks, EHDV-7 was identified as the responsible serotype. In the same year suspect clinical cases of EHDV were described in Jordan. In summary, between 2006 and 2007, outbreaks caused by EHDV infection have been reported in Algeria, Tunisia, Morocco, Israel, Jordan and Turkey. Two different serotypes were

involved, serotype 6 (EHDV 318) and serotype 7. Both isolates were pathogenic and capable of causing disease in cattle. There was no information available about the possible origin of the two strains. In the late 80's EHDV-6 was identified in Sudan, Bahrain and Oman, and it is likely that this serotype has remained in the region until the recent outbreaks. Conversely the only place where the presence of EHDV-7 has been evidenced other than Israel is Australia.

Even though the origin of the EHDV serotypes which affected the African and Asian Mediterranean countries remains unclear, it is important to notice that there is an alarming similarity between the recent EHDV scenario and that of BTV which took place at the end of the 90es. During that period some BTV serotypes initially circulated in Algeria, Tunisia, Turkey and Israel, were able to cross the Mediterranean Sea and invade Southern Europe through western, eastern and southern corridors.

**Table 12:** EHD geographical distribution in Europe neighbour regions

Geographical Location	Date of occurrence	Infected Host	Status of infection	Identified Serotypes	References
Turkey	07/2007	Cattle	3	6*	Temizel et al., 2009
Turkey	Approx 1986-87	Cattle and sheep	2 (AGID)	NK	Burgu, et al.,1991, 31/568 cattle sera +ve 16/230 sheep sera +ve
Israel	28/08/2006	Cattle	3	7	OIE Disease Information, 28 Sep 2006, Vol 19 - No. 39 <a href="http://www.oie.int/eng/info/hebdo/AIS_77.HTM#Sec8">http://www.oie.int/eng/info/hebdo/AIS_77.HTM#Sec8</a>
Israel	2001	Cattle	3		Yadin et al. 2008 Promed, archive number 20060925.2739
Israel	1950	Cattle	1	NK	Komarov and Goldsmith, 1951.
Jordan	09/2006	Cattle	1	NK	Promed, archive number 20061215.3525
Morocco	28/07/2006	Cattle	3 (RT-PCR and ELISA)	6*	OIE Disease Information, 2 Nov 2006, Vol 19 - No. 44 <a href="http://www.oie.int/eng/info/hebdo/AIS_78.HTM#Sec1">http://www.oie.int/eng/info/hebdo/AIS_78.HTM#Sec1</a>
Morocco	2004	Cattle	3	6*	Promed, archive number 20061214.3513
Tunisia	03/09/2006	Cattle	3 (AgELISA)	6	OIE Disease Information, 28 Dec 2006, Vol 19 - No. 52 <a href="http://www.oie.int/eng/info/hebdo/A_CURRENT.HTM#Sec4">http://www.oie.int/eng/info/hebdo/A_CURRENT.HTM#Sec4</a>
Algeria	19/07/2006	Cattle	3 (RT-PCR)	6*	OIE Disease Information Vol. 19 No. 51 <a href="http://www.oie.int/eng/info/hebdo/AIS_65.HTM#Sec11">http://www.oie.int/eng/info/hebdo/AIS_65.HTM#Sec11</a>
Algeria	Aug-Sep/2008	Cattle	2 (ELISA)	?	Madani personal communication Medreonet serological survey 66/849 cattle – 7.8%, CI 95%:6.2%-9.9% 35/152 (23%) herds with at least 1 positive animal
Bahrein	approx 1985	cattle	3	6*	Mellor personal communication 1992 (cited in Rabenau et al 1993)
Oman	1987-1988	Cattle and goats	2 (AGID , SN)	2 6*	Al-Busaidy and Mellor, 1991



Saudi Arabia	1991	Deer	NK	NK	Abuelzein, et al., 1992
Saudi Arabia and the United Arab Emirate	1999-2001	Arabian oryx	2 (AgELISA)	NK	Frölich et al., 2005

\*Status of infection : (1) Disease suspected but not confirmed (2) Confirmed infection but no clinical disease (3) Confirmed clinical infection

NK: Not known

\*Previously identified as EHDV-318



**Figure 3:** Location of the most recent EHD outbreaks (2006–2007) in countries bordering the Mediterranean Basin.

Where no geographical details as district/province etc, are not reported, the whole country has been indicated as affected.

### 4.3. EHD in North America

The epidemiological situation of EHD in North America has been the subject of considerable research. In wild ungulates, EHD is seasonal, occurring from mid-summer through to late autumn, and usually peaks in September reflecting the 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 (Couvillon et al.1981). In epidemics areas, the disease occurs in a long 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.

Deer surviving infection with EHDV develop long lived neutralizing antibodies (Stallknecht et al., 1991) and the herd immunity conferred by neutralizing antibodies may be one epidemiologic factor responsible for the spatial and temporal distribution in the Southeastern USA (Davidson and Doster 1997). Natural infection with multiple serotypes of BTV and EHDV has been demonstrated in White-tailed deer survey in Texas by serum neutralization tests without evidence of haemorrhagic disease. The absence of clinical signs in relation to antibody prevalence (overall 84%) could not be explained by poor detection and several factors may contribute to the observed enzootic stability: innate host

resistance, maternal antibody transfer, vector species composition and seasonality (Stalknecht et al. 1996). Similar findings of serologic detection of EHDV-1, 2 and BTV-10 and 11 were reported in Mule deer and Proghorn antelope in Arizona (Dubay et al., 2006a, 2006b) and in sentinel cattle in Texas (Fulton et al., 1989). A field sample that was real-time RT-PCR positive for both BTV and EHDV has also been reported (Wilson et al., 2009) but there are no reports of re-assortment between BTV and EHDV.

A phylogenetic analysis of the EHDV-2 strains based on samples collected throughout the eastern United States over three decades revealed that closely related genotypes were widely distributed in space and time (Murphy et al., 2005). Additional analysis of these data indicated that in outbreaks within the same year, genetic and spatial distances are positively correlated and that the virus is evolving at a rate similar to that seen in other vector-borne RNA viruses (Biek 2007). The fact that evidence for a demographic expansion of the virus was not apparent from the genetic data suggests that EHDV-2 dynamics are limited by factors other than deer host density. The abundance of competent vectors, for example, may have a much more pronounced effect on virus population sizes (Biek 2007). In addition, phylogenetic analysis of the outer capsid serotype-specific protein from US field strains of EHDV-2 indicated regionalized genetic types, suggesting limited movement of virus populations across the U.S.

Historically only EHDV-1 and -2 have been reported in North America. In 2006, a non- EHDV-1 or -2 virus was recovered from moribund or dead white-tailed deer (*Odocoileus virginianus*) in Indiana and Illinois. This virus was later typed as EHDV-6 by serological and genetic testing (Allison et al., 2009). EHDV-6 was originally described from Australia and is an emerging pathogen of cattle in Morocco, Algeria and Turkey. Subsequently, additional isolations of EHDV-6 from white-tailed deer in Missouri in 2007; Kansas and Texas in 2008 (Allison et al., 2009), and Missouri and Michigan in 2009 (Stallknecht - personal communication), suggesting that the virus has overwintered and may be endemic in a geographically widespread region of the United States. How this newly identified virus type has spread in the USA is difficult to determine. It is possible that the virus has been present for some time since genetic characterization indicated that this virus is a reassortant. The serotype specific outer capsid proteins genes (VP2 and VP5) are derived from an exotic EHDV-6 strain, while the remaining genes were genetically similar to indigenous EHDV-2 (Alberta strain) (Allison et al., 2009).

## 5. Monitoring and Control Measures

For EHD as for other viral infections countries might have to face two possible scenarios: they are free of infection and don't want to introduce it in their territory or they have the infection but they don't want it to spread and/or they want to protect animals if the infection is able to cause disease. Once established, EHD, as a viral disease transmitted by vectors, may be difficult to control or eradicate. Unpredicted and uncontrollable variables such as climatic and geographic factors as well as abundance of suitable EHDV insect vectors are all important for the outcome and persistence (re-appearance) of EHDV in an area.

### 5.1. Diagnosis

Early detection of EHDV infection is crucial for the implementation of any monitoring or control programme. EHD is an OIE listed disease since May 2008, and currently it is included into the Manual of Diagnostic Test and Vaccines (2008 edition) together with BT, but no details are included on specific diagnostic techniques for EHD.

Clinical diagnosis of EHD is inconclusive, as clinical signs in wild ruminants and cattle are indistinguishable from those of BT, and they may be similar to signs found in other cattle diseases like bovine viral diarrhoea/mucosal disease, infectious bovine rhinotracheitis, vesicular stomatitis,

malignant catarrhal fever and bovine ephemeral fever. Definitive diagnostic of EHD or EHDV infection requires the use of specific laboratory tests.

EHDV can be recovered from blood or tissues including spleen and lung of infected animals by inoculation into cell culture (Vero, BHK, or insect cell lines e.g. C6/36), embryonated hens eggs or in susceptible hosts (Aradaib et al., 1994b). The serotype-specific identification of the virus is usually based on serum neutralization and plaque inhibition test using known reference antisera (Pearson et al., 1992a, Stott et al., 1978). Virus isolation procedures, although time consuming, still remain important for recovery of infectious virus and for understanding the biology, transmission, ecology, genetic diversity and molecular epidemiology of the disease.

In recent years efforts have been directed towards development of molecular techniques like the reverse transcriptase polymerase chain reaction (RT-PCR) (Wilson et al., 1992; Aradaib et al., 1994a; Wilson et al., 2009a) or nucleic acid hybridization (Nel and Huisman, 1990; Wilson et al., 1992, Wilson et al., 1990). Use of RT-PCR has proliferated because of its simplicity, rapidity, reliability, reproducibility, sensitivity and specificity. However, as for other viruses, there are major limitations when attempting to relate RT-PCR positivity and infectious status, if virus isolation is not attempted. The RT-PCR technique detects viral RNA with a very high level of sensitivity but it is not possible to determine with the test whether the sample contains infectious virus or not. The duration of EHDV positivity by RT-PCR in blood is largely unknown but there is evidence that it lasts longer than the period over which infectious virus can be isolated (Aradaib et al., 1994a). Information about published techniques is included in Tables 13 and 14. A real time PCR kit for EHDV detection and identification of all its serotypes has recently been made commercially available<sup>5</sup>.

A sandwich ELISA has been developed for detection of EHDV in tissue culture and infected *Culicoides* (Thevasagayam et al., 1996) using polyclonal antibodies raised in two different species. This technique showed a detection threshold of 102.6TCID<sub>50</sub>/ml. No cross-reaction was observed with BTV or other orbiviruses like Palyam virus, AHSV, or Tilligery virus. Also, a capture ELISA has been developed for detection of VP7 of EHDV in cell culture supernatant, with the aid of a rabbit polyclonal and a mouse monoclonal antibody (Mecham and Wilson, 2004). None of these techniques are commercially available.

**Table 13:** Summary of the group-specific gel-based and real time RT-PCR assays developed for EHDV detection

Genome target	Real-Time/Gel based	Analytical Specificity	Limit of detection	References	Annotations
Segment 10	Real time	No cross reaction with 24 BTV serotype reference strains	-----	Wilson et al., 2009	TaqMan. 7 primer sets and 3 probes
Segment 6	Nested; gel based	No cross reaction 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	No cross reaction with North American BTV		Harding et al., 1996	
Segment 3	Gel based	-----	-----	Ohashi et al., 1999	Tested with EHDV-1, 2, 7, 8, 9, 10

<sup>5</sup> EHDV - Kit TaqVet™ Epizootic Hemorrhagic Disease Virus, for specific detection of EHDV by real-Time PCR, www.lsvet.com.

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	No cross reaction with BTV and AHSV strains	-----	Wilson, 1994a	Used also on semen
Segment 6 of BTV and EHDV Multiplex PCR	Conventional gel based			Aradaib et al., 2003	Nested PCR Tested with EHDV-1 and 2 and BTV (2, 10,11,13,17)
Segment 6 and 10 of EHDV Multiplex RT-PCR	Real Time of based	No cross reaction observed with 24 reference strains of BTV		Wilson et al., 2009a	TaqMan Tested with EHDV 8 serotypes Differentiate BTV and EHDV infection

**Table 14:** Summary of the serotype-specific RT-PCR assays developed for EHDV detection

Genome target	Real-Time/Gel based	No cross reaction observed	Limit of detection	References	Annotations
Segment 2 of EHDV-1	Gel based	Tested for EHDV-2 and all North American BTV serotypes (2,10,11,13,17)	0.1fg	Aradaib et al., 1995a	With chemiluminescence hybridization
Segment 2 of EHDV-2	Gel based	Tested for EHDV-1 and all North American BTV serotypes (2,10,11,13,17)	0.1fg	Aradaib et al., 1995b	With chemiluminescence hybridization

The serological techniques applied for diagnosis of EHDV infection include the agar-gel immunodiffusion (AGID) test (Aradaib et al., 1994b), the complement fixation test (CFT) (Pearson et al., 1992b), and the enzyme-linked immunosorbent assay (ELISA). This latter technique has been found to be more sensitive than earlier serological tests (Mohammed and Mellor, 1990). Competitive ELISA (cELISA) techniques making use of monoclonal antibodies against EHDV VP7 have been used for the detection of EHDV serogroup specific antibodies (Luo and Sabara, 2005, Mecham and Wilson, 2004, Mecham and Jochim, 2000), and currently are the preferred technique. Reports on the validation of these tests are not available. c-ELISA can also be used to detect specific antibodies to EHDV-1 and 2 serotypes. Serum neutralisation however is still the test of choice for typing and quantifying EHDV antibodies. More detailed information on published ELISA techniques is shown in Table 15. None of these ELISA techniques are currently available commercially.

**Table 15:** Summary of the ELISA developed for EHDV serological diagnosis.

ELISA	Antigen	Monoclonal antibody	Notes	Specificity (Sp)	Sensitivity (Se)	References
Competitive	VP7 recombinant	VP7 MAb 18B2	Tested with EHDV-1 and BTV (serotype	-----	-----	Luo and Sabara, 2005

				unknown)				
Capture	VP7 recombinant	VP7 MAb 4F4.H1	Tested with: EHDV-1, EHDV-2. Does not cross react with BTV antibodies	with: -----	-----	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	with: -----	-----	Mecham and Jochim, 2000		
Competitive	Purified EHDV-1	VP7 MAb C.31	Tested with: EHDV-1, EHDV-2, and field samples	with: 95.1%*	90.0%*	Afshar et al., 1997		
Competitive	Purified EHDV-1	-----	Tested with: EHDV, AHS and BTV	with: -----	-----	Thevasagayam et al., 1995		
Competitive	Purified EHDV	Ig G1 bovine MAb	Tested with: EHDV-1-4 and field sera	with: 99.3%*	91.5%*	Afshar et al., 1992		

\* Sp and Se are relative to the AGID test. It should be noted that AGID is not a highly specific test and so the relative sensitivity and specificity of the other tests might be biased.

## 5.2. Monitoring and control measures in endemic zones

In most endemic zones in the world there is no official surveillance or control programme for EHDV. This is explained by the fact that the infection rarely has a significant economic impact on the cattle industry (beef or dairy), and no clinical signs of EHDV have ever been reported in sheep or goats. However, in some countries the disease can apparently have a great impact on captive (farmed) deer as well as wild deer or cattle. In USA, EHDV surveillance in wild species has been supported since 1980 through cooperative agreements with the South-eastern 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 (<http://www.usaha.org/committees/btbr/btbr.shtml>) through submitted samples for testing. Surveillance programmes are directed to monitor the circulation of the different EHDV serotypes and disease activity. A combination of surveillance methods may be applied depending on the purpose and the relevance of the infection on the different areas. Passive surveillance on clinical cases of EHD in susceptible wild ruminants is a useful tool for detecting disease, based on lesions of haemorrhagic disease combined with viral detection techniques (virus isolation and RT-PCR). The main disadvantages of EHD passive surveillance are that it does not allow detecting subclinical infection, and that in general, disease in wildlife is often under-reported (Beringer et al., 2000). Cross-sectional serological studies allow identifying infection, which may be important in species in which infections result in mild clinical signs or are subclinical. An EHDV-specific serological technique needs to be used, especially in areas where other ruminant orbiviruses (like BTV) are present (Stallneck and Howerth, 2004).

In countries in the Mediterranean Basin like Morocco, Algeria, Tunisia, Turkey and Israel, the control measures undertaken to tackle the recent EHDV incursions have included control of wildlife reservoirs, quarantine, vector control of infected premises/establishment(s), and awareness campaign for veterinarians and farmers. Reinforced surveillance for detection of clinical signs of the disease is in place. To date, there are no detailed studies on the effect of control measures applied in these countries, where the disease has mainly affected cattle.



### 5.3. Vaccination

As an effort to control the disease, vaccines have been developed in USA through support of the National Deer Farmers Association for captive wildlife deer farmers and in Japan for use in cattle. Apart from these two limited settings, there was only a minor interest from laboratories and vaccine companies elsewhere to develop vaccines in order to control the disease and/or the virus circulation.

Autogenous inactivated vaccines have been prepared in North America from EHDV isolates originated from ill or dead animals in affected premises. Its use is limited to the original and adjacent affected herds. Prior to release, these vaccines have to be tested for purity and safety but they don't need to be tested for efficacy. Application has to be approved by the USDA. Most of the applications are by deer farmers. The Missouri White-tail Breeders and Hunting Ranch Association (MWBHRA) sponsors the use of the EHD/BT autogenous vaccine. The most common product<sup>6</sup> contains killed viruses of EHDV types 1 and 2 and BTV type 17. Its use is allowed in the Missouri deer farms. The vaccine can be administered subcutaneously or intramuscularly any time by dart or syringe. A booster dose 2-4 weeks following the first injection and a yearly shot are required.

In Japan, both<sup>7</sup> live modified and inactivated vaccines have been developed to control Ibaraki disease. The live attenuated vaccine derives from the Ibaraki-2 strain and has been used following the outbreaks in 1980s. The vaccine has to be administered once subcutaneously during the low vector season. A National surveillance and intensive monitoring of yearlings as sentinel cattle in place for a number of years showed no evidence of Ibaraki disease nor seroconversion until 1997, when new cases of the disease were observed (Ohashi et al., 1999). It is worthwhile to point out that the 1997 outbreak was characterized by abortion and stillbirths, clinical signs never observed in the previous outbreaks. The inactivated vaccine includes bovine ephemeral fever and Ibaraki viruses grown in cell cultures and inactivated by formalin, as an aluminium-gel adjuvant vaccine. Both vaccines are used on a voluntary basis according to the epidemiological situation.

### 5.4. Vector control

The methods used to control *Culicoides* worldwide have been fully reviewed as part of previous EFSA reports on bluetongue (EFSA 2007a, 2008b). In addition, Carpenter et al. (2008) review the control techniques for *Culicoides* biting midges in use in Europe and elsewhere and cover:

- i) Application of insecticides and pathogens to vector breeding sites.
- ii) Environmental interventions to remove vector breeding sites.
- iii) Application of insecticides to adult vector resting sites including to host animals.
- iv) Screening of animal housing to prevent entry of adult vectors.
- v) Use of repellents to repel adult vectors from host animals.
- vi) Use of kairomones to lure adult vectors away from host animals and kill them.

However, for the major vectors of BTV and EHDV in Europe (*C. obsoletus* group, *C. dewulfi*, *C. chiopterus*, *C. pulicaris* group) there is apparently scant data on breeding habitats, resting behaviour and host-orientated responses. However, data extrapolated from vector control campaigns elsewhere indicate that the treatment of livestock and animal housing with synthetic pyrethroids, the use of midge-proofed animal housing and the promotion of good farm hygiene to partially or wholly remove vector breeding sites are the best options currently available.

### 5.5. EU import regulation

Directive 2004/68/EC, lays down the animal health requirements for the importation into and transit through the community of live ungulates. The third countries allowed importing into the Community

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<sup>6</sup> Manufactured by the Newport Laboratories, Worthington, MN, USA

<sup>7</sup> Both types of vaccine have been produced by the Kyoto Biken Laboratories Inc.

certain live ungulate animals and their fresh meat are according to the Council Decision 79/542/EEC, Annex I: Canada (Whole country, except the Okanagan valley of British Columbia), Switzerland, Chile, Greenland, Croatia, Iceland, New Zealand, St. Pierre et Miquelon. Also, Montenegro, FYR Macedonia and Serbia are listed but only for transit of live ungulates from a EU Member State to another.

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 (Okanagan valley of British Columbia), additional guarantees regarding BT and EHD diagnostic tests are required in the veterinary certificate.

The imported animals are accompanied by veterinary certification issued by the official veterinarians of the exporting third country and the animals are inspected at an approved EU Border Inspection Post (BIP). Concerning EHD, the relevant information can be found in Council Decision 79/542/EEC. The veterinary certificate includes the following:

- The country of origin is free from EHD for the last 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 6 months for animals for breeding and 3 months for animals for slaughter 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
- In and around the territory where the animals remaining before dispatch 40 days, there has been no case/ outbreak of bluetongue and EHD, during the previous 60 days
- Animals are not to be killed under a national programme for the eradication of diseases, nor have they been vaccinated against the disease
- Animals have reacted negatively to a serological test for detection of antibody to BTV and EHDV, 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 (only for Okanagan valley , British Columbia, Canada).
- The animals have to be examined within 24 hours of loading by the official veterinarian and showed no clinical sign of disease at the time of examination.

In addition, special quarantine conditions have been laid down for the importation of camelids from St Pierre et Miquelon.

## 5.6. Monitoring and control measures applicable for EHD

EHD of deer is a compulsory notifiable disease according to Annex I of Council Directive 92/119/EEC. This Directive applies to the listed diseases with the exception of those for which, subsequently, specific provision has been made at Community level, as for example, BT . Its aim is to lay down general measures to prevent the spread of certain animal diseases of major economic importance, and in particular to control the movements of animals and products liable to spread the infection. General control measures, applicable to all the listed diseases in Annex I, are also included.

No EHDV outbreaks in EU have been ever reported with the exception of French Reunion outbreaks in 2003 and 2009. Only one limited serological survey was carried out in Germany in wild species (Frolich et al., 2005) with negative results. Whether or not EHDV is present in Europe causing subclinical infection is unknown.

In case of an outbreak, the use of a reliable diagnostic test (e.g. RT-PCR) should allow to identify viraemic animals in the herd, and these should be slaughtered or culled. Immediate insecticide treatment to destroy possible *Culicoides* vectors which could have fed on the infected host should be performed. Animals in the affected farm should again be retested after 7-10 days, to detect other possible infected animals which were non-viraemic at the first test carried out in the premises (the incubation period of the disease has been reported to be 4days, Abdy et al., 1997). Movement restrictions of susceptible species (with a radius adapted to the geographical meteorological and epidemiological situation) should be established until an epidemiological investigation has been performed.

An immediate epidemiological investigation should be carried out for understanding of the situation, including:

The extent of EHD infection and disease (in space, and potentially also in time), based on reliable diagnostic test(s)

- The likely source or method of initial introduction.
- The stage of the epidemic (whether this is the index case or not),
- The presence of susceptible species (domestic, wild animals) in the locality
- The vector situation (which species, potential/known competence).

The epidemiological investigation in the surrounding farms (with a radius adapted to the geographical meteorological and epidemiological situation) will allow to determine the stage of the incident (whether this is the index case or not), and if there was extension of the infections to other farms. Positive RT-PCR and negative serological results are indicative of recent infection. Presence of potential vectors in the area should be investigated by trapping of *Culicoides* in the farms. If more infected herds are identified the approach will depend on the epidemiological situation, the use of culling slaughter or not will depend on the number of infected herds.

Trace-forward and trace-back activities (in cases where animal movement has played a role in spread of infection), to determine the origin of infected animals, and whether there is an explanation, other than vectors, for introduction of infection should be done; as well as a qualitative risk assessment of the risk of future spread (based on animal movements, vector activity etc). If the risk of new introductions does exist, a long-term surveillance should be in place including monitoring ruminant hosts and insect vectors.



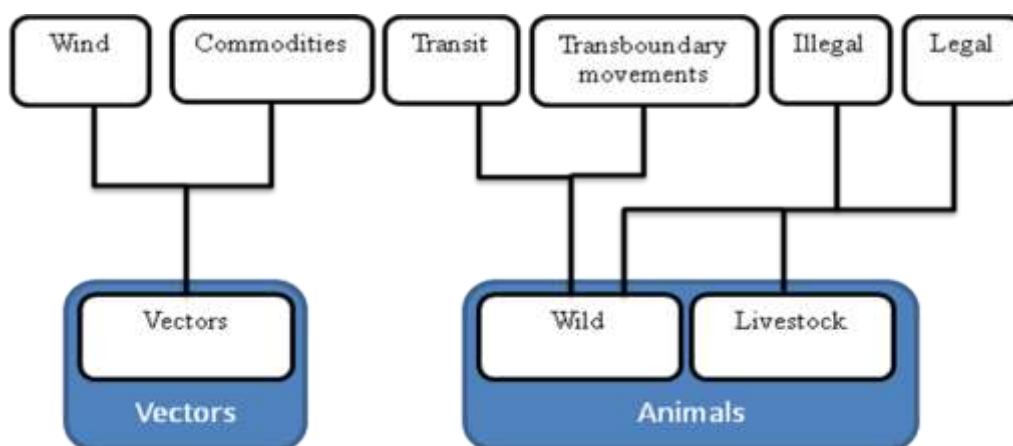
## 6. Risk assessment

### 6.1. Scope of the RA

The scope of the risk assessment was to estimate, “The possibility of EHD to spread to and persist within the EU considering epidemiology, vectors, livestock and climatic conditions.”

The assessment followed the methodology proposed by the OIE for import risk analysis (OIE 2004).

The Hazard is: Epizootic hemorrhagic disease virus (EHDV), the causal agent of EHD. Family Reoviridae, genus Orbivirus, EHDV serotypes 1 to 7.



**Figure 4:** Pathways of introduction of EHDV

The risk assessment addressed separately the two main possible pathways of EHDV entrance to EU (Figure 4):

- via live animals (legal, illegal and trans-boundary movement) and
- via vectors by wind or carried by commodities (animals , plants, etc.).

Other possible pathways considered were the entrance via germplasm and contaminated vaccines

The risk questions addressed by the risk assessment were:

For each of the two main pathways what is the likelihood of EHDV entrance to EU?

- Probability of importing or entrance of infectious animal.
- Probability of entrance of infectious vector

After the entrance of EHDV in EU what is the possibility of its spread within the considered EU area for each of the two main pathways.

The risk assessment methodology developed allows also for the evaluation of the impact on disease spread of possible control measures such as, removal of infectious animals, control of movements /importations and reduction of vector numbers.

## 6.2. Risk of EHDV introduction via infectious animals

EHDV could only be introduced into EU by animals that are in the incubation period or viraemic at the time of introduction. This event will occur only if all the following conditions take place:

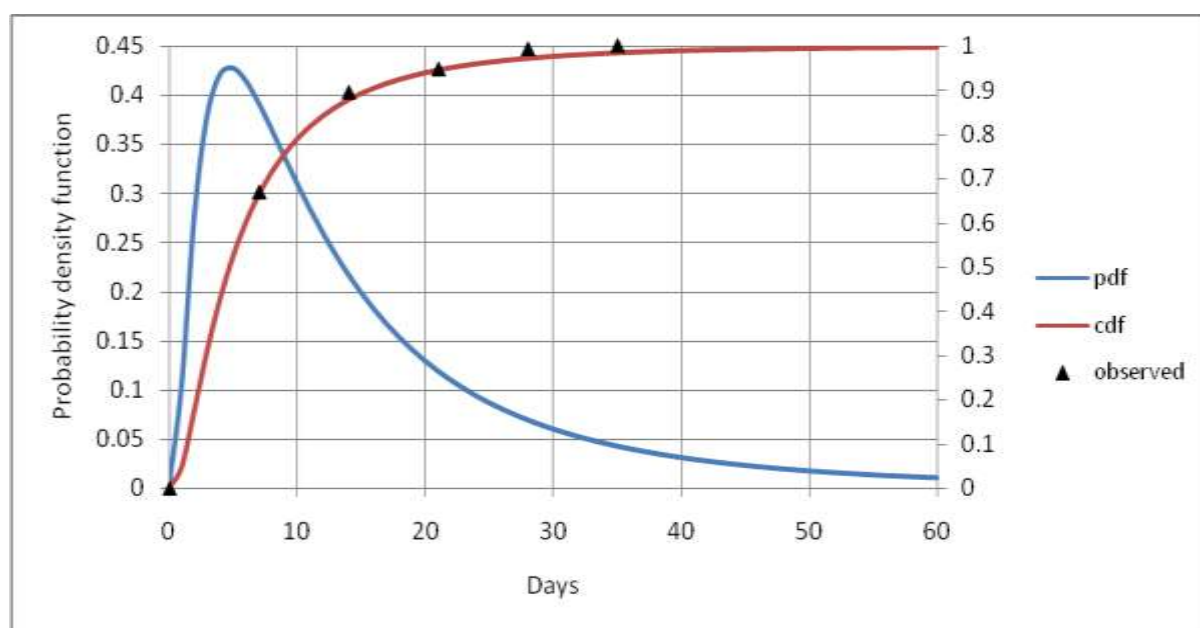
- 1- the imported animal is coming from an infected area where *Culicoides* species are active and
- 2- the imported animal is infected before its movement and
- 3- the imported animal is still in incubation period or infectious at time of its movement

The first condition depends on probability of freedom ( $P_F$ ), hence, on the credibility of the surveillance and reporting system of the exporting country and the evidence given to demonstrate the freedom from EHDV.

The subsequent conditions depend on the number of days post infection at time of movement and the duration of the viraemia for the concerned animal. For an infected animal, to be in incubation or infectious at time of movement it is necessary that the number of days post infection at the time of movement are less than the days of duration of viraemia. Since both of these parameters are variable, the probability distributions of the number of days post infection at time of movement and viraemia duration were estimated .

The probability of introduction when quarantine is applied will depend on the duration of quarantine and whether the imported animal is still in incubation period or infectious at time of its arrival to an EU. If a test is conducted before the animal entrance to an EU MS the sensitivity of the test will also affect the probability of entrance.

The distribution of viraemia duration was estimated using data from Tables 5 and 6 (cattle experimental and natural infection): logNormal distribution (with mean of 1.556 and standard deviation of 0.931) fit well the observed data (Figure 5), using maximum likelihood criteria. Approximately 80% of the animals have viraemia duration of less than 10 days. Data from white-tailed deer showed a slightly longer viraemia duration.



**Figure 5:** Probability distribution of viraemia duration

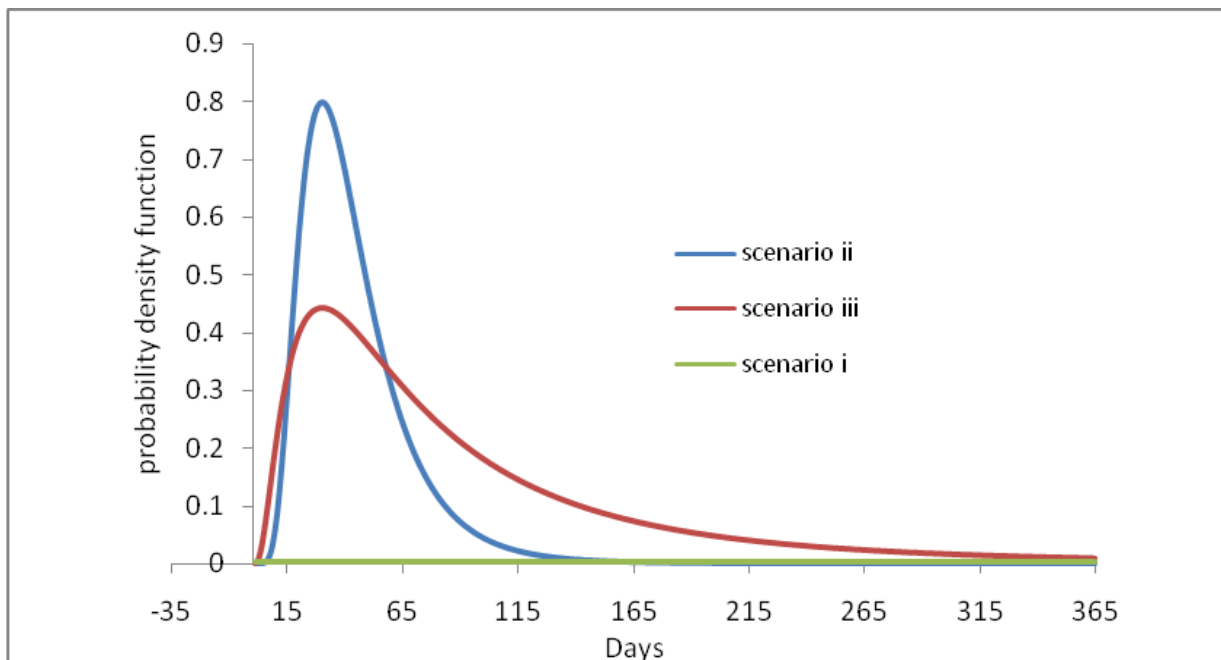
(pdf: probability density function, cdf: cumulative distribution function)

Infections were assumed not to occur between two epidemic seasons hence maximum age of considered infections will be 365 days.

The probability of being infectious at the time of movement was calculated using the model developed for BTV (EFSA 2008). Day of movement and day of infection are random and depend on the specific region of origin, the epidemiological situation, etc., to handle the variability different scenarios were considered regarding the seasonal infection pattern and the distribution of animal movement during time.

For the seasonal/temporal infection pattern, three scenarios were considered (Figure 6):

- **Scenario i:** uniform distribution of infection occurrence from day 1 to day 365,
- **Scenario ii:** logNormal distribution considering seasonal peak after 30 days of the beginning of *Culicoides* seasonal activity and a spread corresponding to standard deviation equal to 0.5 and
- **Scenario iii:** logNormal distribution considering seasonal peak after 30 days of the beginning of *Culicoides* seasonal activity and a spread corresponding to standard deviation equal to 0.9.



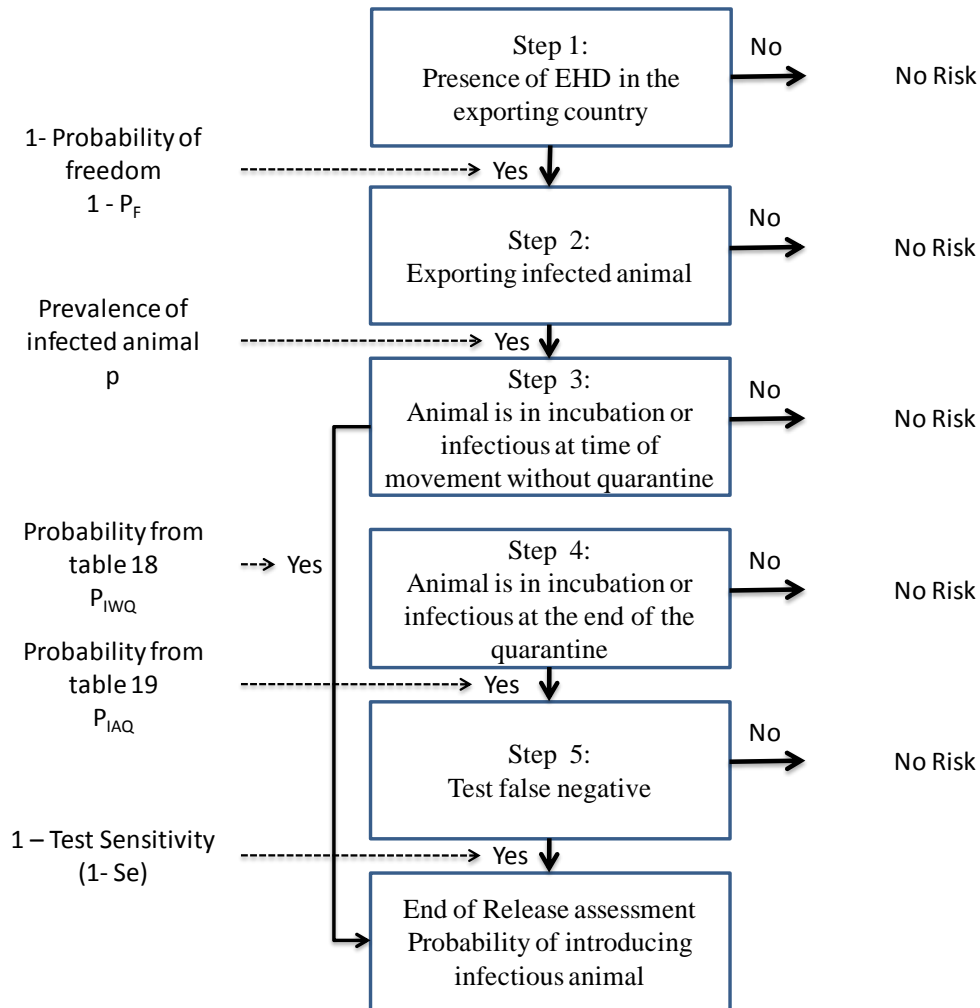
**Figure 6:** Scenarios used for EHDV infection pattern

Day 0 correspond to the beginning of EHDV transmission season.  
(pdf: probability density function)

For the distribution of animal movement three scenarios were considered:

- **Neutral scenario** uniform from 1 to 365 day ,
- **Worst case scenario** triangular surrounding the peak of infection (min=15, mode=30, max=45)
- **Optimistic scenario** where animals are moved after the peak of infection (triangular with min=90, mode=120 and max=150 )

When data on the reliability of freedom and prevalence is available, the likelihood of introducing a viraemic animal from an endemic region into EU MS could be assessed by integrating the 5 following steps:



**Figure 7:** Release assessment

$P_F$ : Probability of Freedom  
 $p$ : Annual prevalence in the country of origin  
 $P_{IWQ}$ : Probability of being infectious without quarantine  
 $P_{IAQ}$ : Probability of being infectious with quarantine

### 6.2.1. Release/entry assessment of infectious animals

#### 6.2.1.1. Illegal import of live animals

A possible route of introduction of EHDV into previously free areas is the illegal import of animals. Estimating the importance of illegal transport (smuggling) is a very difficult task due to lack of reliable observations. The probability of an infected animal being in incubation or viraemic at time of movement was estimated for 9 different scenarios (Table 16) according to

$$(1 - P_F) \times p \times P_{IWQ}$$

Probability of freedom is unknown and so we assumed the worst case scenario  $P_F=0$ , prevalence was assumed to be 1% as a practical example ( $p=1$ ), higher prevalence assumption will worst the risk

estimation (data not shown). If illegal movement of wild animal is considered the estimated risk may be higher due to longer viraemia duration.

**Table 16:** Probability of an infected animal being in incubation or viraemic at time of movement come from an area that satisfies PF=0 and p=1%

EHD infection	Animal Movement		
	Uniform(1,365) (Neutral)	Triangular (15,30,45) (Worst )	Triangular (90,120,150) (Optimistic)
Uniform (1,365) (i)	3.9%	3.9%	3.9%
LogNormal(3.4,0.5)(ii)	2.3%	33.7%	0.3%
LogNormal(3.4,0.9)(iii)	2.3%	24.2%	1.2%

Day 0 correspond to the beginning of EHDV transmission season.

#### 6.2.1.2. Legal import of live animals

The rules for import of live animals are established by Community legislation. Import of live animals from third countries is only allowed from the countries listed in Annex I of the Dir 79/542. None of the countries included in Annex I reported any case of EHDV infection.

The number of ovine and bovines imported into EU MS is detailed in Appendix B tables. No wild cervids have been imported.

If a quarantine of 40 days (CD/79/542) and exclusion diagnostic is applied, the probability of an infected animal being in incubation or viraemic at time of arrival to an EU MS can be calculated as follows:

$$(1-P_F) \times p \times P_{IAQ}$$

Probability of freedom depends on the country of origin and it was assumed to be  $P_F=0$ , prevalence was assumed to be 1% (higher prevalence would probably be reported and importation would be interrupted). The probability of introduction decreases in comparison with illegal pathway (Table 17).

**Table 17:** Probability of an infected animal being in incubation or viraemic at time of arrival to an EU after a quarantine of 40 days come from an area that satisfies PF=0 and p=1%

EHD infection	Animal Movement		
	Uniform(1,365) (Neutral)	Triangular (15,30,45) (Worst )	Triangular (90,120,150) (Optimistic)
Uniform (1,365) (i)	0.124%	0.124%	0.124%
LogNormal(3.4,0.5)(ii)	0.073%	0.608%	0.032%
LogNormal(3.4,0.9)(iii)	0.057%	0.456%	0.049%

Day 0 correspond to the beginning of EHDV transmission season.

When testing is conducted before the animal entrance to an EU MS, it was assumed that the probability of an infectious animal to be positive at the testing is 99%. Similarly to BTV such high sensitivity could be reached by a serological test applied to paired serum samples with an interval of 28 days.

$$(1-P_F) \times p \times P_{IAQ} \times (1-Se)$$

**Table 18:** Expected number of an infected animal being in incubation or viraemic at time of arrival to an EU MS after quarantine of 40 days and testing.

EHD infection	Animal Movement		
	Uniform(1,365) (Neutral)	Triangular (15,30,45) (Worst )	Triangular (90,120,150) (Optimistic)
Uniform (1,365) (i)	0.12*	0.12	0.12
LogNormal(3.4,0.5)(ii)	0.07	0.61	0.03
LogNormal(3.4,0.9)(iii)	0.06	0.46	0.05

Day 0 correspond to the beginning of EHDV transmission season.

\*Number of animals per million of animals imported

From all countries allowed to export live animals into Europe, EHD has been reported only in Canada, and imports into EU are not allowed from the area within Canada where outbreaks were observed (British Columbia).

Taking in consideration the results presented in Table 18 and the low number of cattle imported into EU MS (Appendix B), the risk can be considered negligible.

#### 6.2.1.3. Transit of captive wild animals

Registration of movements of captive wild ruminants in TRACES is compulsory but not for those animals in transit or exchanged without payment and therefore the information found in TRACES does not allow to trace back all captive wild animals. Estimating the importance of this pathway of introduction is a difficult task.

It is possible that these animals have origin in areas of high prevalence of EHDV. The approach used for illegal movement could give an indication on the likelihood of introduction (Table 16).

#### 6.2.1.4. Trans-boundary movement of wild animals

The model previously described was not used for this pathway. As at the moment data on ecology of wild ruminants and EHDV prevalence in this animals in affected EU neighbour countries are not available the assessment of the risk was not made. Furthermore it is likely that in this case the most likely route of introduction would be through infectious vectors.

### 6.2.2. Exposure assessment

This step estimates the probability that a susceptible host is exposed to EHDV once the virus has been introduced within Europe. Biological factors such as the presence of vectors and its activity, the availability of hosts and the viraemia duration should be considered.

Considering that competent vectors, susceptible host species and suitable climatic conditions are available, if entrance of infectious animals occurs, exposure is likely to occur.

### 6.2.3. Consequence assessment

The scope is to assess the possibility of EHDV to spread within EU. The assessment was conducted by calculating the expected number of newly infectious animals per imported animal. The model used

was adapted from the one described in Gubbins et al., 2007 and used in Blue Tongue EFSA assessment (EFSA, 2008a). In this assessment only cattle was considered as potential host.

When an infectious animal is introduced into one geographic area, the risk of EHDV transmission to the secondary animals depends on:

**viraemia duration (d)**

**the number of vector per animal (m)**

**the number of bites per animal and per day (a)** (function of T)

**the probability that infection is transmitted from an infectious animal to a susceptible vector per bite ( $\beta_{hv}$ )**

**the extrinsic incubation period (v)** (function of T)

**the survival of vector individuals ( $1/\mu = \sigma$ )** (function of T)

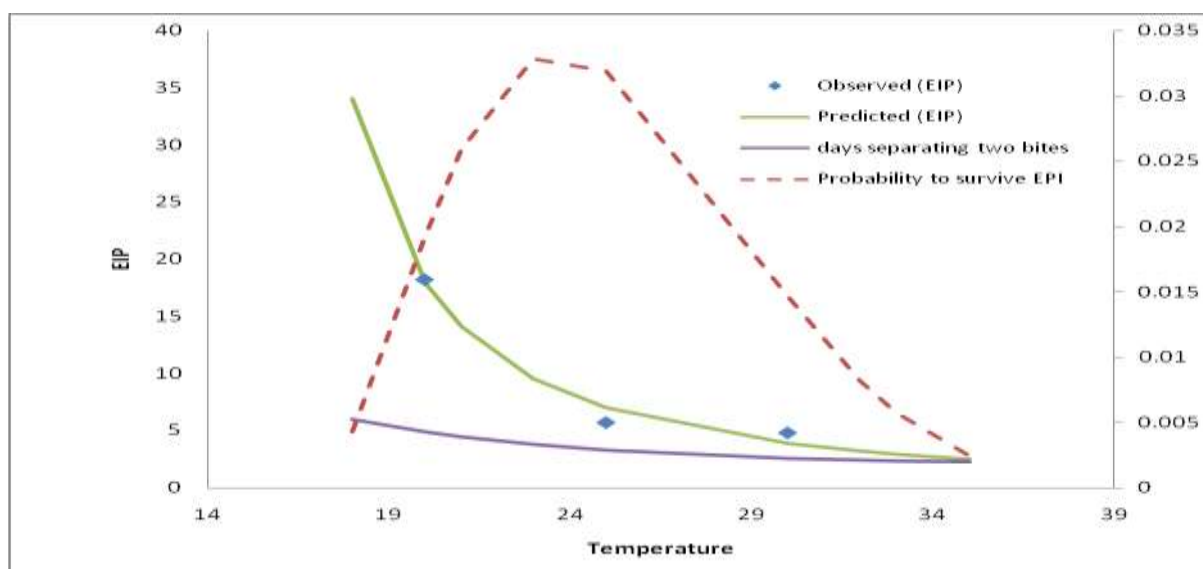
**the probability that infection is transmitted from an infectious vector to a susceptible animal per bite ( $\beta_{vh}$ )**

The expected number of secondary infected animals after introduction of one infectious animal is estimated by the equation below. Please note that the modelled number of infections refers to secondary infected animals of one infected host animal (e.g. the introduced animal).

$$E(i_a) = d \times a \times m \times \beta_{hv} \times e^{-\mu v} \times \frac{a}{\mu} \times \beta_{vh}$$

The formula can be understood as follows: An infected animal remains infectious for (d) days after its entry to an EU MS, during which time it is bitten by susceptible vectors on average a\*m times per day, a proportion  $\beta_{hv}$ , of which results in an infected vector. Out of this infected vectors, on average  $\exp(-\mu v)$  (assuming a constant death rate) will likely survive for the extrinsic incubation period (v) to become an infectious vector. The infectious vector will bite on average a \* 1/ $\mu$  susceptible hosts before dying, a proportion  $\beta_{vh}$ , of which will result a infected host/animal.

The biting rate (a); the vector mortality rate ( $\mu$ ) and the extrinsic incubation period (v) were modelled temperature dependent. Figure 8 shows how the three main inputs vary according to the temperature.



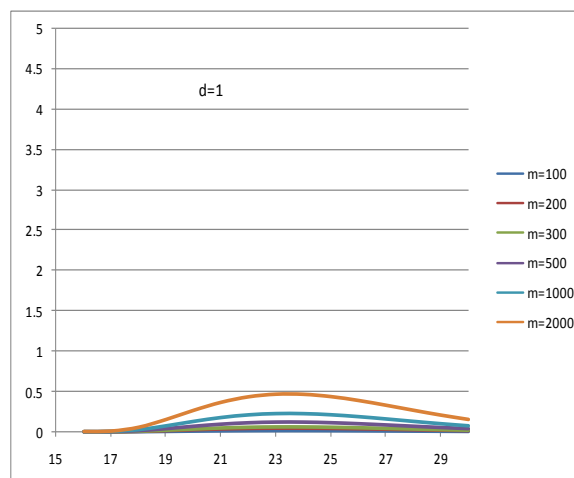
**Figure 8:** Temperature dependency of extrinsic incubation period (EIP: v), biting rate (a), vector mortality rate ( $\mu$ ), the latter is expressed here as the probability to survive the external incubation period v:  $\exp(-\mu*v)$ .

Estimates for each of the parameters influencing the transmission model were obtained from published literature (Table 19). Vector specific parameters with the exception of EIP were taken from BTV studies. The mortality excess due to EHDV infection of vectors was not considered

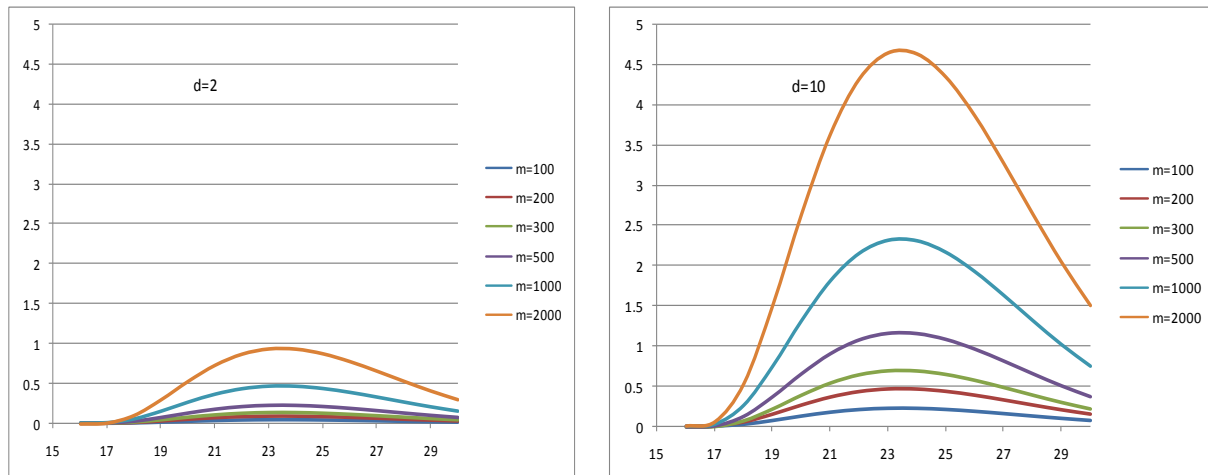
**Table 19:** Parameters used to assess the expected number of newly infected animals from one infectious introduced animal (adapted from Gubbins et al., 2007).

Parameters	Values range or formula	Reference
Probability of transmission from 1 vector to susceptible animal per bite ( $\beta_{vh}$ )	1	O'Connell, 2002
Probability of transmission from animal to vector per bite ( $\beta_{hv}$ )	0.01	Gerry et al., 2001
Biting rate, number of bite per vector per day (a)	depends on temperature $0.0002 * T * (T - 3.7) * (41.9 - T)^{(1/2.7)}$ (Mullens et al., 2004)	Birley and Boorman, 1982; Mullens et al., 1995; Braverman et al., 1985 and Mullens et al., 2004
Vector density, number of vectors per animal (m)	100 to 2000	Mullens et al., (2004)
Viraemia duration in days (d)	<i>Distribution from the release output model</i> (Figure 6)	Gibbs et Lawman (1977) Gard et Melville (1992)Abdy et al., 1997
Extrinsic incubation period in days ( $\nu$ )	depends on temperature $1/\nu = 0.00058 * T * (T - 15.2)$	Wittmann et al., 2002.
Vector survival time $\sigma$ daily mortality rate ( $\mu = 1/\sigma$ )	depends on temperature: $m(T) = 0.009 \exp(0.16T)$ (Gerry and Mullens 2000)	Birley and Boorman, 1982; Braverman et al., 1985; Gerry and Mullens, 2000 and Wittmann et al., 2002
Temperature in °C (T)	15 to 30 °C	Plausible range for vector activity

In order to evaluate the effect of temperature, density of vectors and viraemia duration a uncertainty analysis was performed. Figure 9 shows how the expected numbers of newly infected animals (secondary cases) from the introduced infectious animal vary depending with the temperature, the vector density (m) and the viraemia duration after arrival (d).







**Figure 9:** Expected number of secondary cases (y axis) as a function of the temperature (x axis), vector density (m) and the viraemia duration (d).

The probability of having at least one secondary case was calculated from:

$$P = 1 - e^{-(\text{Expected number of primary cases})}$$

The results are presented graphically in Appendix C.

#### 6.2.4. Risk estimate

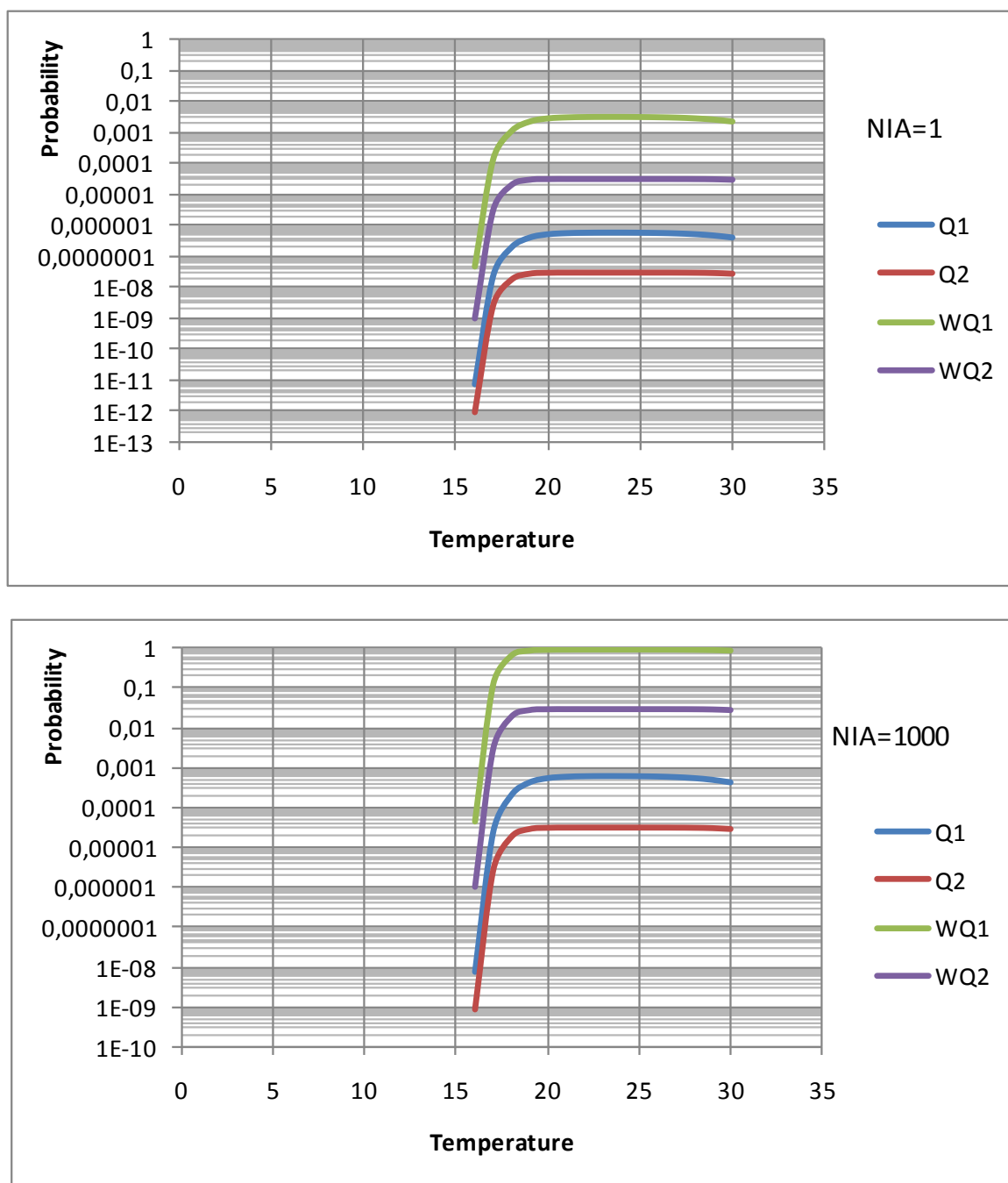
The final risk of introduction and spread per imported animal (R1) is assessed by multiplying the probabilities in Table 16 (without quarantine) or 17 (with quarantine) by the probability of having at least one primary case.

The overall risk associated to the import of animals (IA) is calculated by:

$$RIA = 1 - (1 - R1)^{NIA} \text{ (number of imported animals)}$$

The risk associated with the import of 1 or 1000 animals when  $P=1\%$  and  $PF=0$  if the scenarios ii) of EHD infection (LogNormal (3.4,0.5)) is combined with two different movement patterns (worst scenario: Triangular (15,30,45) and optimistic: Triangular (90,120,150) when vector density is  $m=2000$  was calculated. The results are graphically presented in figure 10.

When no quarantine/testing is applied the risk is below 1% if only one animal is imported, but close to 100% when 1000 animals are moved at the worst movement /infection scenario.



**Figure 10:** Risk estimate of introduction and spread of EHDV

**Q1:** EHD infection pattern LogNormal(3.4,0.5) combined with movement patterns Triangular (15,30,45) and quarantine with testing

**Q2:** EHD infection pattern LogNormal(3.4,0.5) combined with movement patterns Triangular (90,120,150) and quarantine with testing

**WQ1:** EHD infection pattern LogNormal(3.4,0.5) combined with movement patterns Triangular (15,30,45) without quarantine nor testing

**WQ2:** EHD infection pattern LogNormal(3.4,0.5) combined with movement patterns Triangular (90,120,150) and without quarantine nor testing

**NIA:** number of imported animals

### 6.3. Risk of EHDV introduction via infectious vectors

#### 6.3.1. Release/entry assessment of infectious vectors

##### 6.3.1.1. Wind

It is generally accepted that windborne spread of BTV-infected vectors occurred across the Mediterranean Sea from North Africa into Europe. The introduction of BTV-4 and BTV-1 by infectious vectors transported by wind was assessed as highly probable in Spain (MAPA, 2004). The short distance which separates Southern Spain from North Africa together with the absence of geographic barriers, and the presence of the Abrego wind, makes the entrance of wind-borne infected vectors via this route highly likely and unpreventable. The introduction of BTV-2 into Sardinia from North Africa (Algeria and Tunisia) and on Sardinia is also considered to have occurred via wind-borne infected vector *Culicoides*. EHDV is also transmitted by several species of *Culicoides*. The geographical distribution of outbreaks in EU neighbour countries (fig 3) renders this possibility highly likely. Transport of infected vectors by suitable wind conditions was also considered as a likely route of introduction of EHDV in British Columbia (Sellers et al., 1991)

For long-distance wind dispersal process to be of epidemiological significance, the vector must be infected at the source, survive the transport period and still be infectious at arrival. A direct observation of vector movements in the field is very difficult but this pathway has been implicated in known incursions of bluetongue into new territories in the absence of known animal movements (EFSA 2007b) however it is not possible at the moment to estimate the numbers of introduced vectors by wind.

##### 6.3.1.2. Vectors carried by commodities

Other possible routes of entry of EHDV in the EU could also be taken into consideration, as was done for the BTV-8 epizootic in Europe in 2006 (EFSA 2007a). The introduction of EHDV-infected vectors along with horses or other non-ruminant animals entering the EU, or the introduction of infected vectors associated to exotic plants dispatched to the EU from areas where EDHV is endemic could both pose a risk for introduction of the infection into the EU. Although being possible routes, its role in the introduction of ruminant orbiviruses in a free area has never been demonstrated; and it is difficult to estimate.

#### 6.3.2. Exposure assessment

To infect a susceptible host the vector must be infected at the source and survive long enough after landing to replicate the virus. In addition, it needs to find and bite a susceptible host, the probability of which is influenced by the local habitat, the weather conditions, and the number of hosts at each destination.

#### 6.3.3. Consequence assessment

The transmission potential of an infected vector to a susceptible animal is given by:

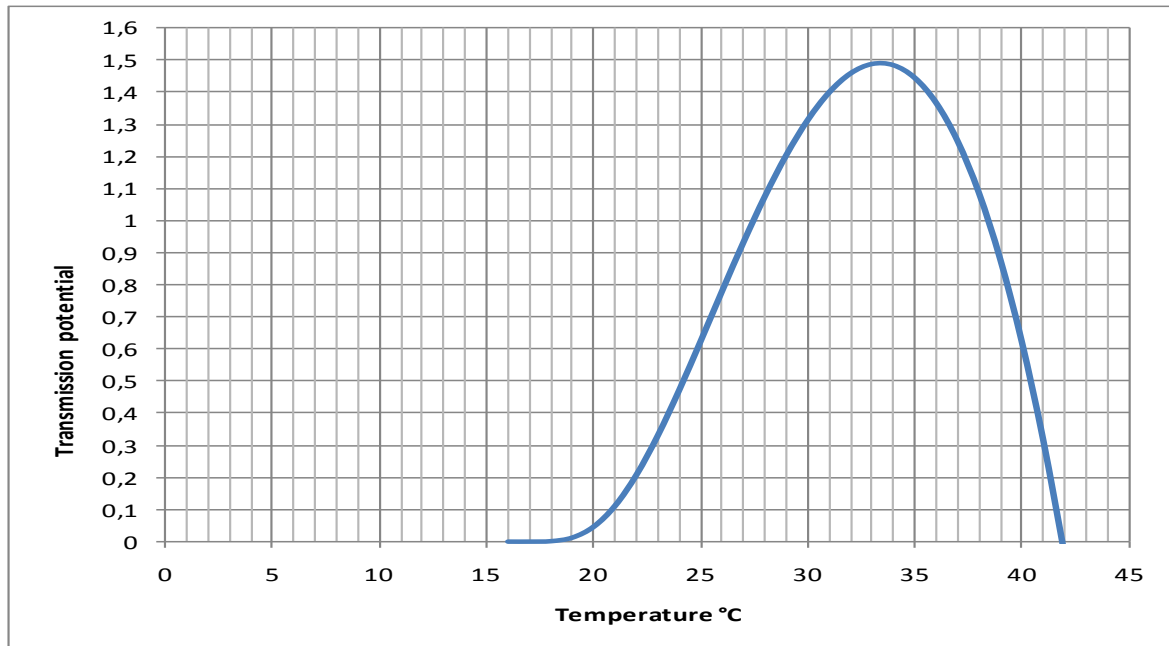
$$\lambda_{vector \rightarrow host} = e^{-\mu v} \frac{a}{\mu} \times b \quad (3)$$

v = the extrinsic incubation period

μ = vector mortality rate

$b$  = probability of transmission from vector to host

Using the parameters in Table 19, the transmission potential of an infected vector to susceptible animal could be presented as a function of temperature (Figure 11).



**Figure 11:** Transmission from vector to host

The entrance of a vector at temperature conditions between 27 and 38°C is associated with a transmission potential higher than one. At temperature lower than 27 the probability of spread per infected vector is low and the spread of EHDV will depend on the repetition of arrival of infected vectors.

Once the single vector infects one susceptible host the risk of spread of infection could be obtained from the spread model presented in sections 6.2.3.

## 6.4. Other possible routes of EHDV introduction

### 6.4.1. Via germplasm

There is only one experimental study trying to assess the transmission of EHDV in semen. Sheep inoculated with semen of EHDV-2, 5 and 7 viraemic bulls did not become infected (Gard et al 1989) however it should be noted that sheep are not the most susceptible species for EHDV. The isolation of bluetongue virus from semen has been reported (Howard et al 1985) although the presence of the virus in semen appears to be a rare event (Mellville et al 1996). Similarly to BTV it is possible that EHDV is transmitted in semen during the viraemic phase but scientific evidence is lacking. No information is available for the possible role of germplasm.

#### **6.4.2. Via vaccines**

A case of EHDV-318 contamination of a cell line has also been reported (Rabenau et al., 1993) however application of good manufacturing practices by current producers and appropriate controls established by the European pharmacopeia should avoid this type of risk.

## CONCLUSIONS AND RECOMMENDATIONS

TOR 1: The significance of the presence, origin and occurrence of EHD in susceptible species (specially livestock animals) in the EU neighbour countries for a better understanding of the possible evolution of the disease in the context of the EU epidemiological situation.

The knowledge on EHDV and its taxonomy was reviewed and the following conclusions were made:

- Evidence indicates that there are seven EHDV serotypes.
- The classification on serotypes only reflects the neutralization characteristics and does not necessarily reflect virulence. Differences in virulence have been observed for different isolates of the same serotype. The basis of differences in EHDV virulence is not understood.
- BTV is the prototype virus of the Orbivirus genus, and in general, limited specific information is available on the physicochemical properties of EHDV, being assumed to be very similar to BTV.

It was recommended:

- Uniform, internationally accepted criteria for EHDV classification and a reviewed list of reference strains and antisera should be established

The available information about host susceptibility to EHDV allowed the following conclusions:

- There is a considerable lack of information about the susceptibility to EHDV and the epidemiological role of many domestic and wild ruminant species in this infection.
- EHDV causes disease in wild cervids, particularly white-tailed deer (*Odocoileus virginianus*). Serotypes associated with disease in white tailed deer are EHDV-1, EHDV-2 and recently EHDV-6.
- EHDV is also a disease of cattle (originally described as Ibaraki disease); serotypes associated to disease are EHDV-2, EHDV-6 and 7.
- Sheep have been shown to be susceptible to EHDV both in natural and experimental infection without showing clinical signs.
- The role of goats as a host for EHDV is uncertain.

It was recommended:

- Detailed and conclusive studies need to be carried out on the susceptibility to EHDV of small domestic ruminant species (sheep, goat) and wild hosts in Europe in particular to serotypes isolated in EU neighbour countries.

The following was concluded regarding immunity to EHDV and cross-protection to serotypes and serogroups

- Duration of viraemia in cattle and white -tailed deer may exceed 50 days.
- Like with BTV infection with a particular EHDV serotype confers protection against the infection by homologous serotype. Duration of this protection is unknown but indirect field evidences suggest it may last for life.



- A partial cross-protection has been demonstrated between the North American serotypes EHDV-1 and EHDV-2. It is not known if this phenomenon also occurs between other serotypes.
- No cross-protection exists between EHDV and BTV.

Regarding the significance of the disease, pathogenesis and transmission routes the following was concluded:

- EHDV was initially recognised as a disease of white-tailed deer but clinical signs have been observed also in cattle and other wild and domestic ungulates
- In recent years serotypes of EHDV normally considered as non pathogenic for cattle have been able to cause disease.
- The disease is characterized by high morbidity and mortality in white-tailed deer while in cattle populations, morbidity varies from 1 to 18% but mortality is usually low.
- Production losses associated with disease in cattle may be significant, especially in dairy farms, in the form of lowered milk production.
- Oral and faecal shedding of EHDV-1 has been reported but the epidemiological role of this transmission route is probably insignificant.
- Transplacental transmission has been demonstrated for the EHDV-2 Ibaraki strain, but it has not been observed with other EHDV strains.
- Unlike BTV no reports of EHDV isolation in semen have been found.
- There can be a considerable adverse welfare impact of EHDV on clinically affected animals.

Epidemiological relevant data were condensed in the following conclusions:

- Epizootic haemorrhagic disease is a vector-borne viral disease, its distribution is consequently limited to the presence distribution of competent *Culicoides* vectors.
- There is a striking epidemiological similarity between the recent EHDV outbreaks occurring in North Africa and West Asia, and the BTV outbreaks in that area between the end of the nineties and early 2000s. During that period some BTV serotypes initially circulated in Algeria, Tunisia, Turkey and Israel, and were able to cross the Mediterranean Sea and invade Southern Europe through western, eastern and southern corridors.
- Despite the similarities, there is a difference between the distributions of BT and EHD, the reasons for the different distributions of these two viruses that have a similar host range and are transmitted by similar species of *Culicoides* have yet to be elucidated.
- In North American endemic areas for EHDV-2, clinical disease appears to occur in white-tailed deer in a two- to three-year cycle.
- Differences in pathogenicity have been observed for different isolates of the same serotype. Whether the reason is a variation in virulence, the susceptibility of the hosts and/or changes in vectors capacities is unknown.
- The factors leading to emergence of EHDV-6 in North America (previously exotic to that part of the world), and of EHDV-7 in Israel (previously reported only in Australia) are unknown.

The knowledge on the circulation of the different EHDV serotypes is scant in many parts of the world, probably due to lack of active surveillance

- Re-assortment of EHDV strains has been observed in the field

TOR 2: The possibility of EHD to spread to and within the EU and to persist considering epidemiology, vectors, livestock and climatic conditions.

- When quarantine and testing for EHDV are in place the probability of importing an infectious animal into a EU MS was considered negligible.
- The probability of introducing an infectious animal through illegal movement was considered not negligible and could be high depending on the animal origin and season of movement.
- The probability of introducing EHDV by an infectious captive wild animal in transit was considered not negligible and could be high depending on the animal origin and season of movement.
- Following EHDV introduction by an infectious animal the risk of exposing susceptible animals during periods of vector activity in EU MS was considered high.
- The risk of introduction and spread could be considered negligible when quarantine /testing are applied but without quarantine and testing it is moderate to high depending on the temperature.
- Based on recent experience with BTV the risk of introduction of EHDV into the EU from neighbouring countries by wind dispersal of vectors was rated as high
- There is no information available regarding EHDV introduction via germplasm to allow for risk estimation or to infer any valid conclusion.
- The risk posed by contaminated vaccines for EHDV introduction was considered negligible for EU authorized medicinal products.
- Vector abundance and climatic conditions would be favourable to sustain EHDV circulation; therefore, presence of EHDV in neighbouring countries poses a significant risk for introduction and establishment of EHDV in EU.

TOR 3: The role played by different vectors in the spread of EHD and the means to control them.

- EHDV, as BTV, is transmitted between its ruminant hosts by species of *Culicoides* biting midges. The data for EHDV and its vectors are much scantier than for BTV. Currently inferences regarding vector capacities based on the knowledge from BTV can only be tentative.
- There is no scientific evidence to suggest that mosquitoes have a role in the transmission of EHDV.
- The major vector of EHDV transmission in the African continent is probably *C. imicola*, in North America *C. sonorensis* and in Australia *C. brevitarsis*. In the Mediterranean Basin *C. imicola* is likely to be the main vector.
- In Europe several possible EHDV vectors are present including potential vectors such as *C. imicola* and suspected vectors within the *Pulicaris* and *Obsoletus* complexes. Other species

may also act as vectors for EHDV but their competence and capacity has not been investigated.

- Where investigations have been conducted the main vectors of BTV have also been shown to be vectors of EHDV.
- The complete “winter” cessation of vector activity depends on the different vector species and the climatic conditions of each country. In certain areas of Europe, the complete cessation of *C. imicola* and *Obsoletus* complex species for more than 2 months has been documented, whereas very low level, activity of adults from the *Obsoletus* and *Pulicaris* complexes species has been detected in many other parts of Europe throughout the winter. These BTV vectors are also possible vectors of EHDV, therefore a SVFP definition may be difficult to achieve instead a definition of vector low abundance season could be developed.

It was recommended:

- There is a need for experimental and other data on the precise spectrum of potential EHDV vectors in Europe and the temperature requirements for virus transmission by them.

TOR 4: The possible measures to control and eradicate the disease including surveillance, control of vectors, availability of suitable vaccines and other elements.

The Availability of different diagnostic techniques was reviewed, with the following conclusions:

- Clinical diagnosis of EHD is inconclusive, as clinical signs in wild ruminants and cattle are indistinguishable from those of BT
- RT-PCR and c-ELISA techniques have been developed which are able to distinguish between infection with EHDV and other orbiviruses.
- RT-PCR detects viral RNA with a very high level of analytical sensitivity and specificity, and it is the technique of choice for detection of infection. However virus isolation may be needed to assess if the sample contains infectious virus or not and for further characterization studies.
- The duration of EHDV positivity by RT-PCR in blood is largely unknown but there is evidence that it lasts longer than the period over which infectious virus can be isolated.
- RT-PCR techniques developed for EHDV diagnose are diverse in the gene segment detected and the primers used.
- Competitive ELISA (cELISA) techniques making use of monoclonal antibodies against EHDV VP7 are currently the preferred technique for detection of EHDV serogroup specific antibodies. At the moment none of these techniques are commercially available.
- AGID test lacks specificity and is not able to discriminate between BTV and EHDV infections.

It was recommended:

- EHD should be considered in the differential diagnosis for cattle suffering from bluetongue-like disease
- Standardised reference strains and sera are needed, for being used in the development and harmonization of diagnostic techniques.

- There is a need to agree which of the RT-PCR techniques available should be preferred at the EU for EHDV detection.
- There is an urgent need to standardise sensitive and specific techniques (as c-ELISA with recombinant antigens and MoAbs) and to make them widely available.

Regarding available measures of surveillance and control the following was concluded:

- EHD of deer is a notifiable disease in EU-MS according to Council directive 92/119/EEC. Active surveillance programmes for EHDV infection are not in place in the EU and are limited in EU neighbour countries.
- Passive surveillance is complicated by the similarity of clinical signs with BT. Monitoring of disease prevalence (active surveillance schemes) is difficult at present due to the non availability of commercial serological diagnostic methods.
- Vaccination programmes with inactivated, autogenous vaccines are claimed to be safe and efficacious to control EHD, but scientific proof of that is not available.
- Modified-life vaccines have been used in Japan against Ibaraki disease, but scientific proof of that is not available.
- As shown for BTV, vaccines may play a primary role for the control of EHDV should the infection enter into the EU. However, at this time no inactivated EHD vaccines are commercially available.

It was recommended:

- A surveillance programme (active and passive) in high risk areas using sensitive diagnostic tests should be established for early detection of disease introduction.
- In case of an outbreak of EHD in the EU, key actions should include detection of infected animals, epidemiological investigation, restriction of movements, and eventually, long term surveillance. The surveillance should be done by serological testing if such tests are available.
- If viraemic animals should be detected in a previously free area culling or slaughter of these animals could be used as a control measure. In addition, mitigation measures on the *Culicoides* vector, as used for BTV control (e.g. insecticides and repellents, good farm hygiene to remove vector breeding sites) could be also used for EHDV.

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## APPENDICES

### A. APPENDIX – LITERATURE REVIEW

Two electronic databases (EDB) were searched for abstracts: Web of knowledge (CABI, Wos, FSTA and Current Contents) and Pubmed.

The search was limited to words in the title or the abstract and no language restriction was imposed. The search was conducted in 29 June 2009

The search terms used were:

((cattle or cow\$ or deer or ungulates or ruminants or dairy or beef or veal or calf or calves or herd\$ or sheep or goat\* or lamb\*) AND (EHD or Epizootic hsem\* disease\$ or Ibaraki disease))

Results :

Web of knowledge (CABI, Wos, FSTA and Current Contents) 770 records (449 from Wos and 321 from CABI).

Pubmed : 130 records (with one duplicate).

All citations were uploaded in End note. Duplicate abstracts were removed using the internal software followed by manual de-duplication.

Total number of de-duplicated records was 865 (software and hand removal)

Abstracts were screened for relevance to the ToRs. The literature review was completed by the WG members personal reference list. All references reviewed are included in the reference list. Total number of records :157.

**B. APPENDIX – ANIMAL IMPORTS INTO EU**

Table 1: Number of bovines introduced in EU - MS from 2005 to 2008 (source: Eurostat, DS-016890-EU27 Import Since 1995 By CN8. Extracted 30/07/2009 16.27.46).

	2005		2006			2007		2008	
	USA	SW	CA	CR	SW	CA	SW	CA	SW
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 2: Number of ovine 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).

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

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).

C. APPENDIX – PROBABILITY OF HAVING AT LEAST ONE SECONDARY CASE

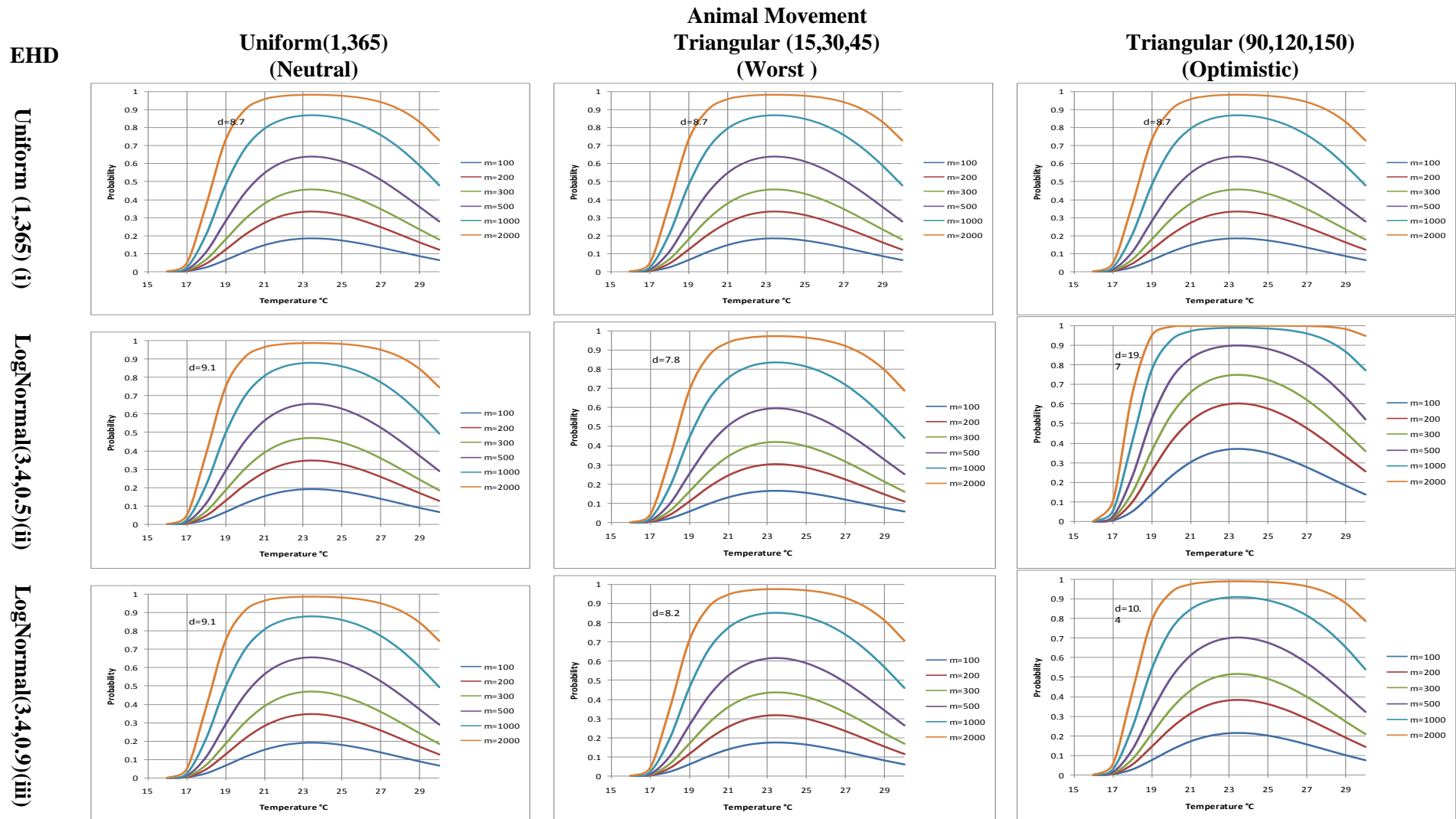
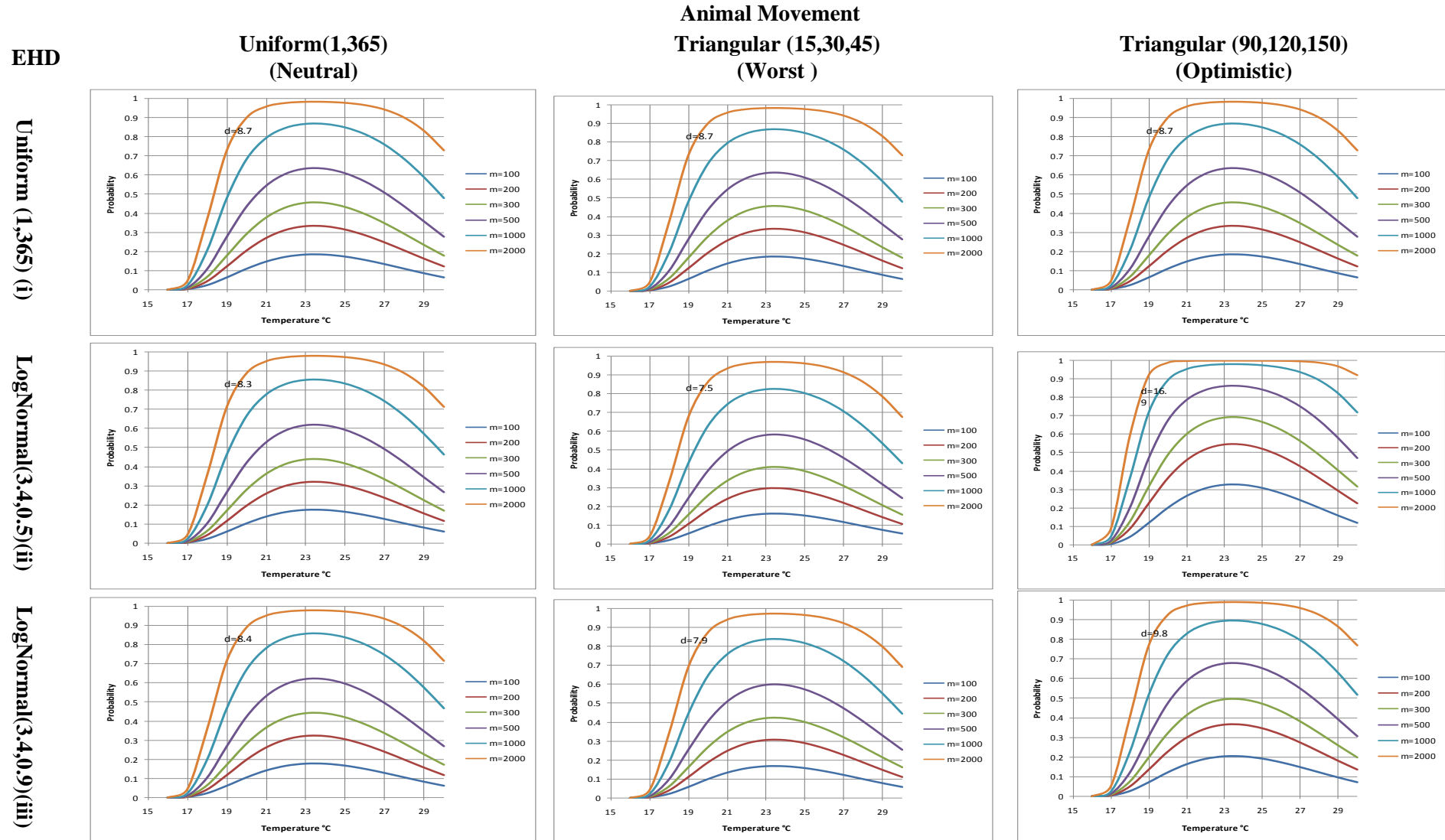


Figure 1: Probability of having at least one secondary case when quarantine and testing are not applied





## GLOSSARY

**Infection**: The establishment/replication of the virus within the mammalian host, which may or may not lead to disease.

**Infectious**: The state of an animal or vector from which the EHDV can spread.

**Clinical case**: An animal that has developed clinical signs characteristic of EHD and confirmed by virological tests or seroconversion.

**Serological case**: An animal that reacts positively for one or more serological tests specific for EHDV.

**Case definition (for evidence of virus circulation)**: Evidence of virus circulation is when in an autoctomous animal:

1. EHDV has been isolated and identified, or
2. A viral antigen or viral RNA specific to one or more of the serotypes of EHDV has been identified, or
3. Seroconversion to EHDV specific serological tests is demonstrated.

**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.

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

**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 phylogenetically 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 EHDV among members of a vertebrate population. It is defined as follows:  $C = ma^2Vp^n/(-\log_2 p)$  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.

## ABBREVIATIONS

AHSV	African Horse Sickness Virus
AGID	agar-gel immunodiffusion
BT	Bluetongue
BTV	Bluetongue virus
cELISA	Competitive ELISA
CFT	complement fixation test
dsRNA	Double stranded RNA
EC	European Commission
EDB	Electronic database
EEV	Equine Encephalosis Virus
EHD	Epizootic Haemorrhagic Disease
EHDV	Epizootic Haemorrhagic Disease Virus
ELISA	enzyme-linked immunosorbent assay
EU	European Union
MS	Member State
MWBHRA	Missouri White-tail Breeders and Hunting Ranch Association
OIE	World Organisation for Animal Health
RT-PCR	Reverse transcription polymerase chain reaction
SCWDS	South-eastern Cooperative Wildlife Disease Study
SN	Sero neutralization
UK	United Kingdom
USA	United States of America
USDA	United States Department of Agriculture
WHO	World Health Organisation