Opinion of the Scientific Panel on Animal Health and Welfare on a request from the Commission related to

"The Risk of a Rift Valley Fever Incursion and its Persistence within the Community"

EFSA-Q-2004-050

Adopted by the AHAW Panel on 5th July 2005

Chapter 11 on Human Infection and Relevant Public Health Aspects and the relevant parts of Chapter 18 were adopted by the BIOHAZ Panel on 7 and 8 September 2005

Summary

EFSA was invited by the EU Commission to produce a scientific opinion on "The Risk of a Rift Valley Fever Incursion and its Persistence within the Community".

This scientific opinion was adopted by the Scientific Panel on Animal Health and Welfare (AHAW) on 5th July 2005. The Chapter 11 on Human Infection and Relevant Public Health Aspects was adopted by the Scientific Panel on Biological Hazards (BIOHAZ) on 7th and 8th September 2005.

According to the mandate of EFSA, ethical, socio-economic, cultural and religious aspects are outside the scope of this opinion.

Rift Valley fever (RVF) is a mosquito-borne viral disease which is causing epidemics of in sub-Saharan Africa at irregular intervals mainly affecting sheep, goats and cattle, although many other mammals, including humans are also susceptible to the disease.

Humans can become infected after intensive contact with acutely infected animals (caretaking) and after handling such animals and tissues at slaughter and butchering, presumably via skin abrasions or aerosol droplets. Besides being mosquito-borne, RVF can also be an occupational hazard.

There is no evidence that RVFV infection can be acquired by humans via the consumption of animal products or meat.

The qualitative risk assessment was structured into 3 linked components. A release assessment of the risk of the introduction of RVF into the EU. An exposure assessment of the risk of susceptible livestock within the EU becoming exposed to RVF infection. A consequence assessment analysing the risk of infection of livestock with the EU, the risk of persistence of the virus within the EU, and the risk of human infection within the EU.

The release assessment concludes that most pathways have a negligible risk with the following exceptions. The risk of virus entry through legal importation of cattle and cattle products as well as zoo animals is considered to be low, but increases if exporting countries experience epidemic periods. Illegal importation of animal products is associated with moderate to high risk of introduction of virus during epidemic periods in source countries. The risk of virus entry through infected vectors, humans or contaminated fomites is negligible during inter-epidemic periods. During epidemics in source countries, the risk increases to low to moderate for infected vectors, to very low for infected humans, and to low for contaminated fomites. The risk posed by RVF vaccines remains negligible as long as no live-virus vaccines are imported, as does the risk posed by imports of non-RVF vaccines potentially RVF contaminated, as long as they are sourced from production facilities subject to relevant quality control procedures.

The exposure assessment concludes that the probability of introduction infected live animals and contaminated animal products legally imported is negligible for sheep and goats, at least low for cattle, but likely to increase during epidemic periods in the source country, and is greater than negligible for zoo animals. The risk for infected live animals and contaminated animal products illegally imported is considered to be associated with much uncertainty dependent upon route and economic situation but probably:

negligible to low for live animals and moderate to high for contaminated animal products; and in both cases it will increase during epidemic periods in the source country. The probability of exposure resulting from infected vectors and humans as well as contaminated fomites is negligible during inter-epidemic periods, but increasing to low to moderate during epidemic periods in the source country. Imported RVF vaccines represent a negligible exposure risk as long as no vaccines based on live virus are imported. It increases to low if vaccines based on modified live-attenuated virus are imported. Non-RVF vaccines represent a negligible risk of exposure as long as they are produced subject to the necessary quality control procedures.

The consequence assessment concludes that infection of EU livestock with RVF through exposure to infected and infectious vectors is moderately likely, and that it will increase in case of multiple exposures. The probability of persistence of RVF virus within the EU as a result of entry of infection into the vector population of the EU is considered higher than negligible, possibly much higher depending upon climatic conditions. The likelihood of infection of humans through handling or consumption of products derived from animals infected within the EU as a result of infection in the livestock population is considered higher than negligible for slaughterhouse staff and related occupations, and possibly even high during epidemic activity, whereas it is considered negligible for general consumers.

The recommendations resulting from this risk assessment include the development of early-warning systems based on prediction of RVF epidemics in countries endemic with infection and setting up sentinel herds in EU countries identified to beat the highest risk as a result particularly of wind-borne movement of mosquito vectors. The ecology of potential European mosquito vectors needs to be better understood so that rises of introduction and persistence can be better estimated, and effective vector control can be applied in case of introduction of infection. Contingency plans need to be developed to allow a rapid and effective response during an RVF outbreak in EU countries. This includes setting up the required laboratory capacity to be able to handle RVF diagnostics, as well as development of effective vaccines for livestock and humans. Veterinary staff needs to be trained such that they are able to recognise the disease.

Key words: Rift Valley Fever Virus, infected and infectious vectors, illegal import, prevalence, climatic conditions, sentinel herd.

Table of Contents

List of Figures

List of Tables

Glossary

AGAL-FAO: Livestock Information, Sector Analysis and Policy Branch – Food and Agriculture Organisation

AVHRR: Advanced Very High Resolution Radiometer. AHVRR's detectors, researchers can measure the intensity of light coming off the Earth in visible and near-infrared wavelengths and quantify the photosynthetic capacity of the vegetation in a given pixel (an AVHRR pixel is 1 square km) of land surface

BERMS: Basin Excess Rainfall Monitoring System. New statistics derived from satellite data used to predict periods when floding might occur.

BIP: Border inspection Post

BSL: Bio Safety Level

CDC: Centers for Disease Control and Prevention. Department of Health and Human Services. Biosafety in Microbiological and Biomedical Laboratories (BMBL).

Cryptic infection: unrecognised or hidden infection

El Niño: Is a disruption of the ocean-atmosphere system in the Tropical Pacific having important consequences for weather and climate around the globe.

ELISA: Enzyme-linked Immune Sorbent assay

GMP: good manufacturing practice

Ig: Immunoglobulin

Inter-epidemic period: Time period between epidemics

MIPLD$_{50}$: Mouse Intraperitoneal 50% Lethal Dose.

NDVI: Normalised Difference Vegetation Index. To determine the density of green on a patch of land. It's used for measuring vegetation, together with the Enhanced Vegetation Index (EVI) and improves scientists' ability to measure plant growth on a global scale.

NOAA: National and Oceanic Atmospheric Administration. http://www.elnino.noaa.gov/ is the National Oceanic and Atmospheric Administration, which has primary responsibilities for providing forecasts for the U.S.A.

OIE: World Organisation for Animal Health

PCR: Polymerase Chain Reaction.

PFU/ml: Plaque Forming Unit/millilitre

Pre- epidemic period: Time period immedidately prior to an epidemic

(RT)PCR: Reverse transcriptase-polymerase chain reaction

RSSD: Remote Sensing Satellite Data. These data enable national and regional monitoring of rainfall and climatic patterns and their effects upon the environment.

RVF: Rift Valley Fever

RVFV: Rift Valley Fever Virus

SST: Sea Surface Temperature index

SOI: Southern Oscillation Index

$TCID_{50}$: Tissue Culture Infecting Dose. The level of dilution of a virus at which half of a series of laboratory wells contain active, growing virus

WHO: World Health Organisation

1. Terms of Reference

1.1. Background

Rift Valley fever (RVF) is a mosquito-borne viral disease that is 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. It is listed by the World Organisation for Animal Health in the category of multiple species diseases. The causative agent is the Rift Valley fever virus, member of the *Phlebovirus* genus of the *Bunyaviridae*, a group of enveloped RNA-viruses. The family encompasses a large group of arthropod borne viruses, members of which infect humans, animals and plants. It is a negative stranded spherical enveloped RNA virus that is spread by a large variety of biting insects especially mosquitos.

Epidemics of this disease have occurred in sub-Saharan Africa at irregular intervals mainly affecting sheep, goats and cattle, although many other mammals, including humans are also susceptible to the disease. In 1977 a disease outbreak occurred in Egypt involving a large number of severe human cases. Cases of RVF in Egypt were again reported in 1993 and more recently in 1997 and 2003. The outbreak of RVF that occurred in south-western Saudi Arabia and Yemen in 2000-2001 was the first outside of Africa and Madagascar.

The virus is generally transmitted via mosquito vectors. During the inter-epidemic periods, eggs of floodwater-breeding species of *Aedes* mosquitoes, together with the RVF virus that they may carry due to transovarial virus transmission, may remain viable in the mud of dried-up surface water pools. A low level of virus activity occurs during the inter-epidemic periods. For epidemics to occur, climatic or environmental conditions must be present that encourage a massive build-up in mosquito vector populations in the presence of susceptible hosts. The latter usually happens when there are warm conditions and unusually heavy and persistent rainfall that cause surface flooding and lead to the hatching of infected *Aedes* spp. mosquito eggs and large numbers of vector mosquitoes.

There is no known carrier state in mammals. The disease may cause high mortality rates in young animals and high abortion rates in ruminants.

In humans there may be a benign febrile illness. In severe cases, meningo-encephalitis, haemorrhagic syndrome with jaundice, petechiae and death may occur. Retinopathy and blindness can occur as a sequel to benign or severe infection.

1.2. Mandate

The European Commission requested the European Food Safety Authority (EFSA) to issue a scientific opinion on 'The risk of an RVF incursion and its persistence within the Community'.

1. Concerning the animal health implications, the scientific opinion should include

- a qualitative assessment of the risk of introduction of this virus into the enlarged EU by vectors, animals and animal products,

- a qualitative assessment of the risk of an epidemic or virus persistence being maintained in the EU, considering vectors, climatic situation and livestock production in Member States, in particular following enlargement of the EU, and
- an assessment of the relevance of the current recommendations of World Organisation for Animal Health (OIE) and Food and Agriculture Organisation (FAO) for Member States most at risk, in particular with respect to adequate means of vector control, host protection against vectors and vaccination of hosts.

2. Concerning the public health implications, the scientific opinion should include

- a qualitative assessment of the risk for humans in the Member States, during an outbreak within the EU, by handling or consumption of products derived from infected animals.

The mandate outlined above was accepted by the Panel on Animal Health and Welfare (AHAW) at the Plenary Meeting, on 30th and 31st March 2004. It was decided to establish a Working Group of AHAW experts (WG) chaired by one Panel member. Therefore the Plenary entrusted a scientific risk assessment to a working group under the Chairmanship of Prof. Dirk Pfeiffer. The members of the Working Group are listed at the end of this report.

This risk assessment is considered for the discussion to establish the relevant conclusions and recommendations forming the scientific opinion by the AHAW Panel.

1.3. Approach

This risk assessment (RA) addressing the risk questions specified by the Commission follows the methodology for RA (which can be summarised as: assessing risk release, exposure, consequences and overall risk estimation), as defined by the World Organisation for Animal Health (OIE 2004).

2. Risk Question(s) used in this Risk Assessment

The following risk questions are those used to undertake this risk assessment:

Release assessment (Risk Question 1)

- What is the probability of viable RVF virus entering the EU?

Exposure assessment (Risk Question 2)

- What is the probability of exposure of livestock within the EU to viable RVF virus?

Consequence assessment (Risk Question 3)

- What is the probability of:
  a. infection of livestock within the EU with RVF virus,
  b. persistence of RVF virus within the EU (in vectors, livestock, or other susceptible species excluding humans) and
  c. infection of humans by the handling or consumption of products derived from animals infected within the EU?

3. Risk Pathways

Risk pathways describe the series of events required in order that the hazard under consideration results in the unwanted outcome specified. In this risk assessment, the hazard is defined as the pathogenic organism RVF virus. The unwanted outcomes are defined in the risk questions (see Section 2). To assess the risk, the probability that each stage in the risk pathway will occur needs to be separately assessed and data (information) is necessary to assess this. The following provides an overview of the risk pathways, and data required to assess the risks.

3.1. Release assessment: Risk Question 1

What is the probability of viable RVF virus entering the EU?

In order that viable RVF virus can enter the EU, there need to be one or more geographical sources (countries or regions of the world) in which the RVF virus is present. The virus then needs to have available one or more possible routes of entry into the EU from at least one source. In addition, the virus must remain viable whilst 'in transit'. Finally, the safeguards in place to prevent virus entry (if any) must fail. To assess the overall probability of this series of events (steps in the risk pathway) occurring, the probability of each event is assessed separately.

Implicit within this risk question is the requirement to assess the risk from the whole of the remainder of the world. Therefore for the first step of the risk pathway, the probability of viable RVF being present needs to be assessed for all relevant geographical areas around the world. The *ideal data* would therefore be the prevalence of RVF infection in susceptible species (including vectors) in each geographically and epidemiologically defined source region of the world. Such data allows the assessment of the probability that any animal (including humans) or vector contains viable virus. However, such prevalence data are rarely available for any pathogen, and RVF is no exception. In addition, there is an implicit requirement to categorise the remainder of the world in some RVF-related way. The data or information therefore required by this risk assessment is the best approximation possible to the ideal data, to enable an assessment of the probability of RVF virus being present (and the probable level of its presence) in each RVF-related and defined geographical region of the world.

In order to assess the probability that any virus present remains viable after pre-export processing (i.e. animal products and vaccine), *ideal data* required includes a description of the processing undergone, plus the effect on the virus of each of the conditions it will encounter (e.g. pH, salination, dessication, temperature etc, including the effect of time).

The data described to date in turn allow an estimate of the probability of the presence of virus in all types of 'exported items', prior to the application of safeguards.

The next step in the risk pathway comprises safeguards intended to reduce the probability of any exported item of harbouring viable pathogen. Such pre-export safeguards include, for example, restrictions on geographical sources from which importation is allowed, requirements for imports only from specific herds or flocks, allowing only the import of certain processed products, pre-export testing, quarantine and certification requirements. The effectiveness of these will depend upon their

efficiency, and where this includes pathogen detection stages, their 'sensitivity' in detecting pathogen. This is likely to include the sensitivity of clinical inspection and examination. Thus, data is required both on the pre-export safeguards in place, and on their effectiveness and sensitivity.

For the next step in the risk pathway, the possible routes of entry of viable RVF virus each need to be identified. In this risk assessment the following have been identified: Live animals via both legal and illegal routes, animal products (again, legal and illegal routes), vectors, humans, fomites, and vaccines. In order to assess the probability of entry via each of these routes, *ideal data* needed includes the quantity of importation/movement into the EU for each item via each of these routes, the time in transit, and transit conditions (temperature, humidity etc.). Some relevant data is usually available, but rarely is it complete, especially for illegal imports or vectors.

The final step in the release assessment risk pathway comprises safeguards at the border. These include border inspections, border testing and quarantine, inspection of luggage for illegal imports etc. These will reduce the probability of travel past the border of infected or contaminated items. The effectiveness of such safeguards will again depend upon their efficiency, including their 'sensitivity' in detecting pathogen. Thus data is required both on the safeguards in place, and on their sensitivity and effectiveness.

Figure 1, below, summarises this general risk-release pathway, and Figure 2, following that, summarises the potential routes of entry of RVF virus to the EU and the types of data required to assess the probability of entry. Items in Figure 1 include live animals, animal products, viable virus on fomites, and vectors.

A further source of virus entry is that of deliberate import of the virus by terrorists. However this has not been included either in Figure 2, or the text, as the decision was made by the Working Group not to include it within this risk assessment.

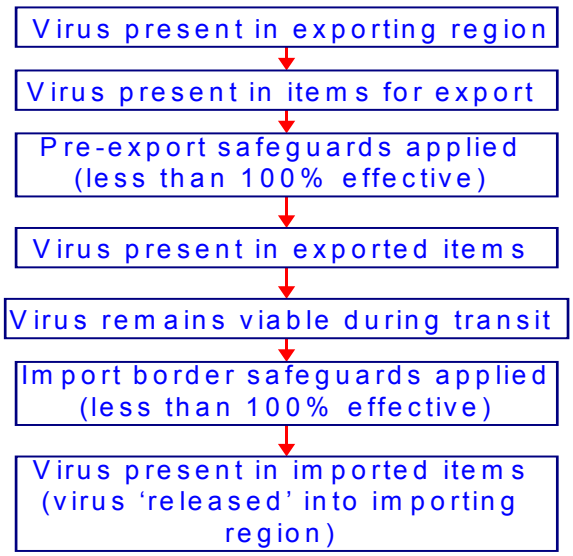

Figure 1: General import risk release pathway for viral pathogen

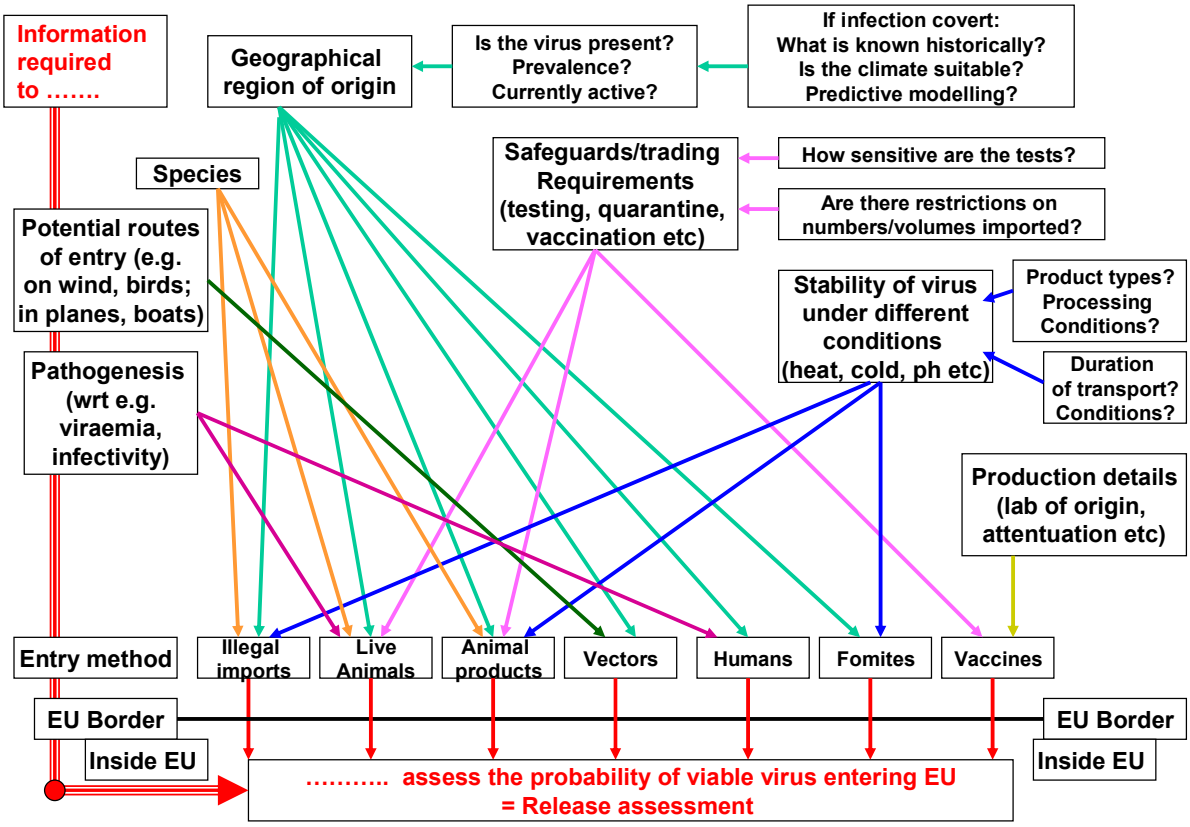

Figure 2: The potential routes of entry of RVF virus to the EU and the types of data required (by route) to assess the probability of entry

3.2. Exposure assessment: Risk Question 2

What is the probability of exposure of susceptible livestock within the EU to viable RVF virus?

The release assessment assesses the probability (risk) that RVF virus will enter the EU. Given the situation that RVF virus has entered the EU, the exposure assessment must identify the possible routes by which EU livestock would be exposed to the virus. It should be noted therefore, that all exposure routes (whether direct, or indirect - in particular, via vectors) are part of the exposure pathways, and thus part of the exposure assessment. Routes identified are:

- viable virus arrives within competent vector, and EU livestock are directly exposed to competent vector,
- viable virus arrives in EU in vaccine (via import or laboratory escape); livestock are directly exposed to imported or escaped vaccine,
- viable virus arrives in the EU by any route other than vector or vaccine (this includes infected livestock, and as a contaminant on animal products, or on fomites); livestock are directly exposed to this viable virus,
- viable virus arrives in competent vector, transmits to other competent vectors of the same species within EU (most likely via transovarial and potentially sexual transmission within the same vector species) and EU livestock are then directly exposed to the other competent infected vectors, and

- viable virus arrives in the EU by any route other than vector or vaccine [this includes infected livestock, and as a contaminant on animal products, or on fomites; competent vectors within the EU are directly exposed to this imported viable virus and become infected; EU livestock are then directly exposed to either these directly infected competent vectors, or to other competent vectors infected (transovarially or potentially sexually) from these initially infected vectors].

Ideally, data is then required to assess the probability of exposure via each of these routes.

It should be noted that the last two of these exposure routes for livestock require the prior infection of EU vectors, and thus an implied establishment of infection within these EU vectors. Thus estimating the probability of establishment within vectors is an integral part of estimating the probability of infection of EU livestock. The probability of contact between livestock and vectors will be affected by the density, activity and longevity of competent vectors, which will in turn be affected by environmental and climatic factors which may change over time. Given infection within the vectors, these changes in vector demographics will also affect the activity of RVF virus, giving periods when activity is intense, alternating with periods when activity is difficult to detect. Human intervention in the environment may also result in change to vector habitat.

It is unlikely that data to estimate the probability of each of these exposure routes directly exists. The data or information therefore required by this risk assessment is the best approximations possible to the ideal data. Data (information) likely to be useful in assessing the probability of exposure include:

- livestock species density and location within the EU and their proximity to points of entry (e.g. border inspection posts or BIPs, probable illegal entry points, probable vector entry routes),
- the probability of the presence of competent vectors within the EU; their species, density and location; their proximity to probable entry points, and to EU livestock,
- the effect of prevailing environmental and climatic factors on the density, activity and longevity of competent vectors, both those native to the EU, and those which enter from outside,
- the effect of environmental and climatic factors on the population persistence and vector capacity of competent vectors native to the EU,
- the probability of infection of EU vectors either by entry of infected vector from outside EU, or the introduction of viable virus as a contaminant on animal products or fomites,
- the probability of establishment of infection within vector communities following the infection of EU vectors,
- the location and biosecurity measures of any research, diagnostic or vaccine production laboratories storing or handling RVF virus within the EU, and data on the use (or potential use) of RVF vaccine from any source in the EU,
- the intended use and destination of imported livestock and the probability of direct contact with EU livestock, and
- the potential exposure pathways to livestock and vectors for viable virus which is contaminating animal products or fomites (e.g. waste dumping in countryside; vehicles travelling to countryside etc) and the probability of virus remaining viable throughout this process.

Part 1 of Figure 3 represents these exposure pathways.

3.3. Consequence assessment: Risk question 3 (all three parts)

What is the probability of

- infection of livestock within the EU with RVF,
- persistence of RVF virus within the EU (in vectors, in livestock or other susceptible species excluding humans), and
- infection of humans through the handling or consumption of products derived from animals infected within the EU?

A summary of the risk pathways for each of these consequences of interest will now be considered.

Infection of livestock within the EU with RVF

Given that exposure of livestock to RVF virus occurs within the EU, the probability of infection will depend upon susceptibility of the exposed livestock, route of exposure (essentially vector, non-vector or vaccine), and dose of viable virus.

Persistence of RVF virus within the EU (in vectors, livestock or other susceptible species excluding humans)

Since RVF is essentially a vector-borne disease, the probability of RVF virus persistence (and thus establishment) within the EU will depend upon establishment within a competent vector species. This will depend upon the presence of competent vectors. This in turn will depend upon the presence of appropriate environmental and climatic conditions for the vectors. Establishment within vectors is an integral part of one exposure pathway for livestock. Thus its probability has been assessed in the exposure assessment.

The subsequent probability of establishment of infection within EU livestock (and other susceptible animal species) will depend upon the probability that they become infected from the vectors in a continuing cycle. This probability will depend upon both exposure (as for risk question 2) and the factors affecting infection (see previous consequence). Intra-community movements of infected livestock, and climatic change, may affect the probability of establishment of infection in new areas of the EU.

Infection of humans by handling or consumption of products derived from animals infected within the EU

Given the presence of animals infected with RVF within the EU, the probability of infection of humans via handling or consumption of products derived from such animals will depend upon the probability of such handling or consumption. In addition, it will depend upon species and individual susceptibility, route of exposure (handling or consumption), and dose of viable virus.

It is unlikely that data to assess the probability of each of these three consequences directly exists. Therefore the data or information required by this risk assessment is the best approximation possible to the *ideal* data. Data (information) likely to be useful in assessing the probability of these consequences includes:

- susceptibility of livestock species by route and dose,
- susceptibility of other vertebrate species (including humans) by route and dose,
- density and distribution of livestock within the EU (as for Section 3.2),

- density and distribution of other susceptible vertebrate species within the EU,
- the probability of establishment of infection within vector populations (as for Section 3.2),
- intra-community movement of livestock (routes and numbers),
- surveillance currently or potentially undertaken to identify vectors and their infection status and infection within susceptible vertebrates, and the effectiveness (i.e. the sensitivity) of such surveillance, including that of diagnostic tests, and
- contingency plans in the event of the detection of RVF virus in vectors or susceptible vertebrates, and their effectiveness.

Part 2 of Figure 3 represents these consequences, and indicates some of the data required to assess their probability.

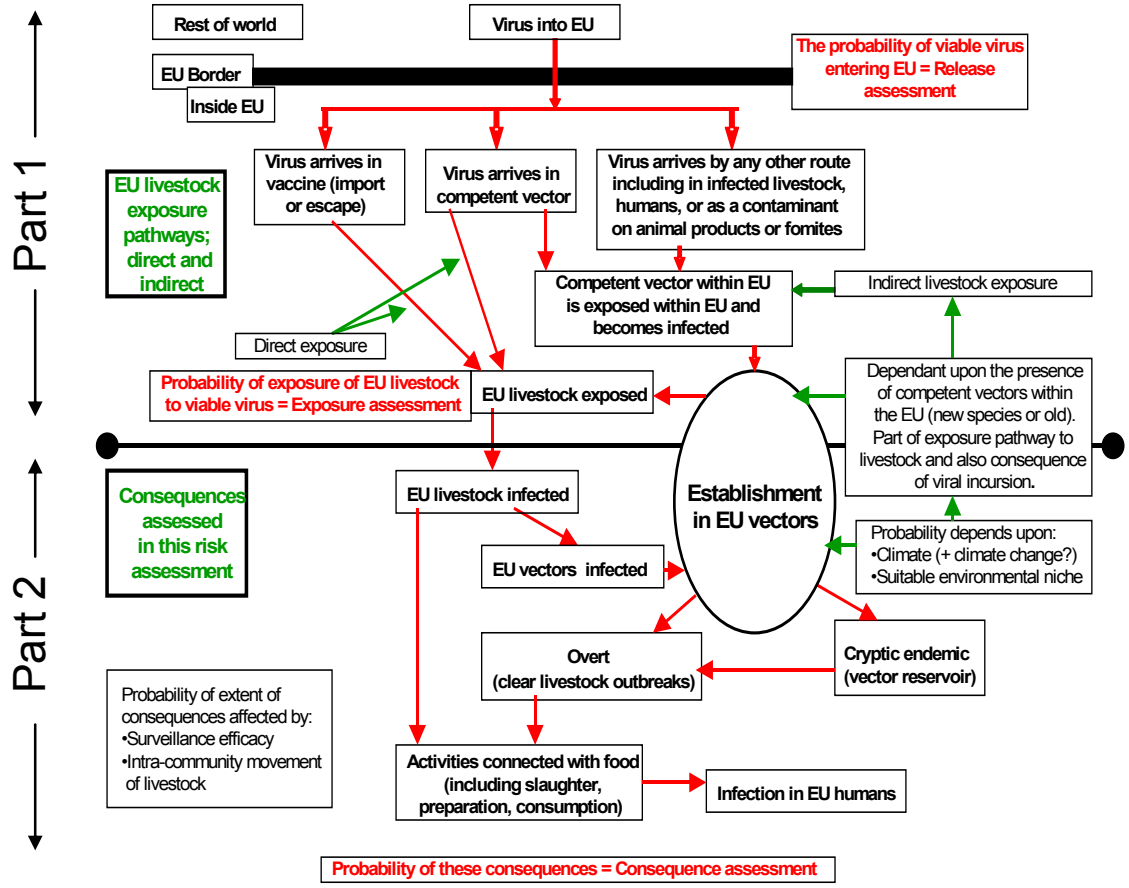

Figure 3: Diagrammatic summary of potential exposure and consequence assessment pathways after an entry of RVF virus to the EU (note: this includes all pathways which were considered in the risk assessment prior to making a decision about their importance)

Although part of the risk assessment, Figure 3 does not specifically show the potential exposure pathways for infection of susceptible species other than livestock. The exposure pathways are identical to those for livestock, and the consequence is that of infection in other susceptible species.

4. Overview of Pathogenesis

This section is adapted from Swanepoel and Coetzer (2004).

Mosquito bites are the most important transmission mechanism to livestock. The initial resorptive viraemia following infection is often too low to be detected. Virus is conveyed from the inoculation site by lymph drainage to regional lymphnodes where replication occurs and subsequent systemic viraemia. There is within- and between species variation in the manifestation of disease. Viraemia becomes demonstrable in lambs less than one week old within 16 hours of infection with small doses of virus, and perists for the duration of infection ending fatally within 36-42 hours. In older ruminants, viraemia becomes demonstrable 1-2 days post infection and persist for up to seven days, usually being most intense on the second to fifth day. Maximum titres recorded have been 10^{10} $MIPLD_{50}$/ml in lambs, 10^{7} in sheep and calves, 10^{8} in kids and 10^{5} in goats, although individual animals may not develop viraemia.

The intensity of viraemia in domestic ruminants and humans is sufficient to infect mosquito vectors of RVFV through ingestion of blood meals, with threshold levels of viraemia required to infect 50% of mosquitoes varying between 10^{5} and 10^{8} $MICLD_{50}$/ml (mouse intracerebral 50% lethal dose) for various species of putative vectors.

The proportion of mosquitoes in which infection, internal dissemination of infection and transmission of virus occurs, and the duration of the incubation period, tend to be characteristic for each vector species and do not appear to be influenced by the RVFV strain, but by increased doses of RVFV. Higher ambient temperatures during incubation produce disseminated infection in a greater proportion of mosquitoes and result in higher transmission rates and shorter incubation periods.

Morbidity and mortality of the host species are influenced by the virulence of the virus strain, virus dose, herd immunity, the host's susceptibility and pre-disposing conditions. In newborn lambs and kids mortality can be as high as 90%. Small ruminants older than two weeks may develop inapparent, peracute but most likely acute disease. Mortality in pregnant sheep/goats varies between 5 and 60%, and abortion in sheep between 40 and 100%. Infection in adult cattle is generally inapparent, but abortion rates can be up to 40%. Mortality in calves can be up to 20% and 10% for older cattle.

5. Vertebrate Species Susceptibility

5.1. Overview of information required

Data on species susceptibility is required both in the release assessment, in order to identify those imported species which might be infected with RVF virus, and in the consequence assessment, in order to identify those species which might be infected within the EU by the virus.

In addition, data on species susceptibility, in conjunction with data on animal demographics can be used to assist in identifying regions where RVF might be present, both outside the EU and, following incursion, within the EU. There are separate Sections in this document on the infection in humans, and in vectors.

5.2. Data

5.2.1. Background

RVF infection and clinical disease has been most frequently described in domestic ruminants and humans. Clinical disease is generally more severe in those animal genotypes which have been imported into the African continent from Europe or elsewhere. There is considerable variation in the susceptibility of the different breeds and genotypes of cattle, sheep and goats within the African continent. Some countries in Africa with large domestic ruminant populations have not reported any clinical disease over the past 75 years, despite the presence of RVF virus throughout the country.

5.2.2. Data on sheep, cattle and goats

Many cited laboratory observations on the pathogenicity of RVF virus for farm animals were made in breeds exotic to Africa and the experiments were conducted outside the continent. Exotic sheep and cattle are less resistant to RVF than indigenous African livestock (Davies 1981), but some indigenous African breeds may also be severely affected by the disease (Eisa 1977; Ksiazek 1989; Matumoto 1950).

Lambs are extremely susceptible and can be infected lethally with as little as 0.1 $MIPLD_{50}$ (mouse intraperitoneal 50 per cent lethal doses) of RVF virus. It is often difficult to produce disease in non-pregnant sheep and cattle of susceptible strains or breeds with high doses of virulent virus (Coackley 1967; Easterday 1962; Harrington 1980; Scott 1981; Swanepoel 1986). There is a clearly increased level of resistance to RVF infection with age.

Goats were considered to be more resistant to the disease than sheep in the Egyptian outbreak of 1977-78 (Daubney 1931; Eisa 1977; Imam 1979; Imam 1981; Ksiazek 1989; Malik 1981; Shimshony 1983). Abortion in goats and mortality in kids were recorded in Kenya in 1930, the Sudan in 1973, South Africa and Namibia in 1974-75, and in West Africa in 1987. In the mixed flocks of nomadic pastoralists in the semi arid zones of Africa in 1997-8, they show similar or slightly lower abortion rates than sheep.

5.2.3. Data on buffaloes

High prevalence of antibody was found in domesticated Asian water buffaloes *(Bubalis bubalis*) during the 1977-78 epidemic in Egypt, and abortion and mortality rates of 12.1 and 7.2 percent, respectively, were recorded in buffalo on government farms. Such abortion rates differ little from those usually experienced and it is not clear that the losses were confirmed to be due to RVF infection (Darwish 1981; Ghaffar 1981; Imam 1981).

Antibody to RVF virus was found in a few African buffalo (*Syncerus caffer*) sera collected after the 1997 epidemic in Kenya, and there was a low prevalence of antibody in sera from some species of antelope (Davies 1975; Davies 1981).

Experimental RVF infection of a seven month old African buffalo (*Syncerus caffer*) in Kenya produced fever and malaise (Daubney 1932), and in another experiment 4 of 5 individuals exhibited transient viraemia and one of two pregnant females aborted (Davies 1981). In 1999, RVF infection caused abortion in 6 African buffalo females held in breeding pens in the Kruger National Park in South Africa (R. Swanepoel, unpublished laboratory records). A low prevalence of antibody to RVF virus has been found in African buffaloes, but no evidence of disease was recorded (Swanepoel 1990).

Both, the African buffalo (*Syncerus caffer*) and the Asiatic water buffalo (*Bubalis bubalis*), have been infected during outbreaks, when abortions have been reported, or shown to be susceptible.

5.2.4. Data on antelopes

A low prevalence of antibody to RVF virus has been found in a few species of antelopes in East Africa (Davies 1975) and in Zimbabwe, but no evidence of disease was recorded (Swanepoel pers. comm.).

It was noted on some properties involved in the 1950-51 epidemic in South Africa that abortion occurred in springbok (*Antidorcas marsupialis*) and blesbok (*Damaliscus dorcas phillipsi*) antelope, but this was not confirmed to be due to RVF (Joubert 1951).

5.2.5. Data on camels

Camels have been affected during the epidemic periods in Africa, Arabia and Egypt. Camels show a brief period of viraemia following inoculation with RVF virus, which may be difficult to detect. Neonatal deaths and abortions occurred in camel flocks simultaneously with the sheep and goat abortions during the 1997 RVF epidemic in the semi arid lands of North East Kenya. RVF antibody was detected in camels in a part of Kenya, where abortions occurred during a RVF epidemic in 1962, and again during a survey following the 1978-79 epidemic (Davies 1985; Scott 1963). However, while there was only one isolation of RVF virus from a camel during the 1977-78 Egyptian epidemic, 56 deaths and one abortion were ascribed to the disease (Imam 1981).

5.2.6. Data on horses and donkeys

Horses develop only low grade viraemia following experimental infection (Daubney 1931; Erasmus and Coetzer 1981; Yedloutschnig 1981b). During the Egyptian epidemic, there was one isolation of virus from a horse and four abortions in donkeys were

ascribed to RVF, while a low prevalence of antibody to the virus was detected in the two species (Imam 1981).

5.2.7. Data on pigs

Pigs are resistant to experimental infection, but develop transient viraemia if a high dose of virus is administered (Daubney 1931; Easterday 1962; Scott 1963). No isolations of RVF virus have been made from pigs during epidemic periods and no antibodies were detected in pigs subsequent to the Egyptian epidemic (Imam 1981).

5.2.8. Data on rodents

Many rodents have been shown to be susceptible to RVF virus at the laboratory but several studies have suggested that they play no role in the natural history of RVF in the field (Findlay 1931; Daubney and Hudson 1932; Mims 1958; Weinbren and Mason 1957; Scott and Heisch 1959; Henderson *et al.* 1972; Davies 1977; Swanepoel *et al*. 1978).

In one study hamsters were extremely susceptible to aerosol infection with an LD_{50} estimated to be equivalent to 0.525 mouse intraperitoneal LD_{50} ($MIPLD_{50}$) (Miller *et al.* 1963). Persistence of RVF virus was shown to occur in immune mice for 63 days following challenge with virulent virus (Kasahara 1973).

5.2.9. Data on non-human primates

RVF antibody has been detected in wild primates and some are susceptible to RVF virus in the laboratory, but they appear not to be important in the natural cycles of infection (Findlay 1932; Smithburn *et al*. 1948; Pelissier and Rousselot 1954; Davies *et al.* 1972; Davies and Onyango 1978). While no end-point was determined for aerosol infection of rhesus monkeys in one study, it is thought to be low, since they were infected by an estimated 76 $MIPLD_{50}$ (Miller *et al.* 1963).

5.2.10. Data on other species

Species of rodents, bats, dogs and cats may develop viraemia or antibody to RVFV following experimental or field infections, but no disease develops (Findlay 1931; Yedloutschnig and Walker 1981). Laboratory studies have shown that many species, such as the above with woodmice, voles, dormice, lauchas, gerbils and squirrels may develop viraemia (Findlay 1931; Easterday 1965). However, they do not manifest disease nor have any apparent involvement in RVF virus activity during field outbreaks (Findlay 1931; Bres 1981; Shimshony and Barzaloi 1983).

5.2.11. Summary of data on species susceptibility

Vertebrates are classified in Table 1 with respect to susceptibility to peripheral infection with RVF virus on the basis of laboratory and field observations (Anderson 1988; Daubney 1931; Daubney 1932; Daubney 1933; Davies 1972; Davies 1978; Davies 1986; Easterday 1962; Easterday 1963; Easterday 1965; Easterday *et al*. 1962a; Easterday *et al*. 1962b; Findlay 1932a; Findlay 1932b; Findlay 1936; McIntosh 1961; Meegan 1989; Mitten 1970; Peters 1981; Scott 1963; Shimshony 1983; Swanepoel 1978).

Table 1: Susceptibility of vertebrates to RVF virus infection (information derived from sources cited in the text; note: both mortality and development of disease are considered for defining the susceptibility categories)

Extremely susceptible **(70-100 per cent mortality)**	***Highly susceptible*** **(20-70 per cent mortality)**	***Moderately susceptible*** **(<10 per cent mortality)**	***Resistant*** **(Inapparent infection)**	***Refractory*** **(Not susceptible)**
New-born lambs*	Sheep	Cattle		Birds
New-born-kids	Calves	Goats	Equines	Reptiles
Puppies	Certain rodents*	Camels African buffalo	Pigs	Amphibians
Kittens		Asian buffalo	Dogs	
Mice		South American monkeys	Cats	
Hamsters		Asian monkeys	African monkeys	
Certain other rodents*		Baboons	Rabbits	
		Humans	Guinea pigs	
			Certain other rodents*	

** Note that there is much genotypic variation, for example some breeds of sheep and goats may be classified in the moderately susceptible or resistant groups.*

6. Cattle and Small Ruminant Distribution in Europe, Africa and Asia

Cattle and small ruminant census data from FAOSTAT was used to produce maps of animal densities (head / square kilometres) in Europe, Africa and Asia (see Figure 4). These maps show that there is a high concentration of cattle in Western Europe and some countries of Africa and Asia. The small ruminant population in Europe is more concentrated in the Mediterranean basin, in the UK and in Eastern Europe. This distribution of small ruminants continues towards Southern Asia (Source: FAO Animal Production and Health Division - AGAL Service).

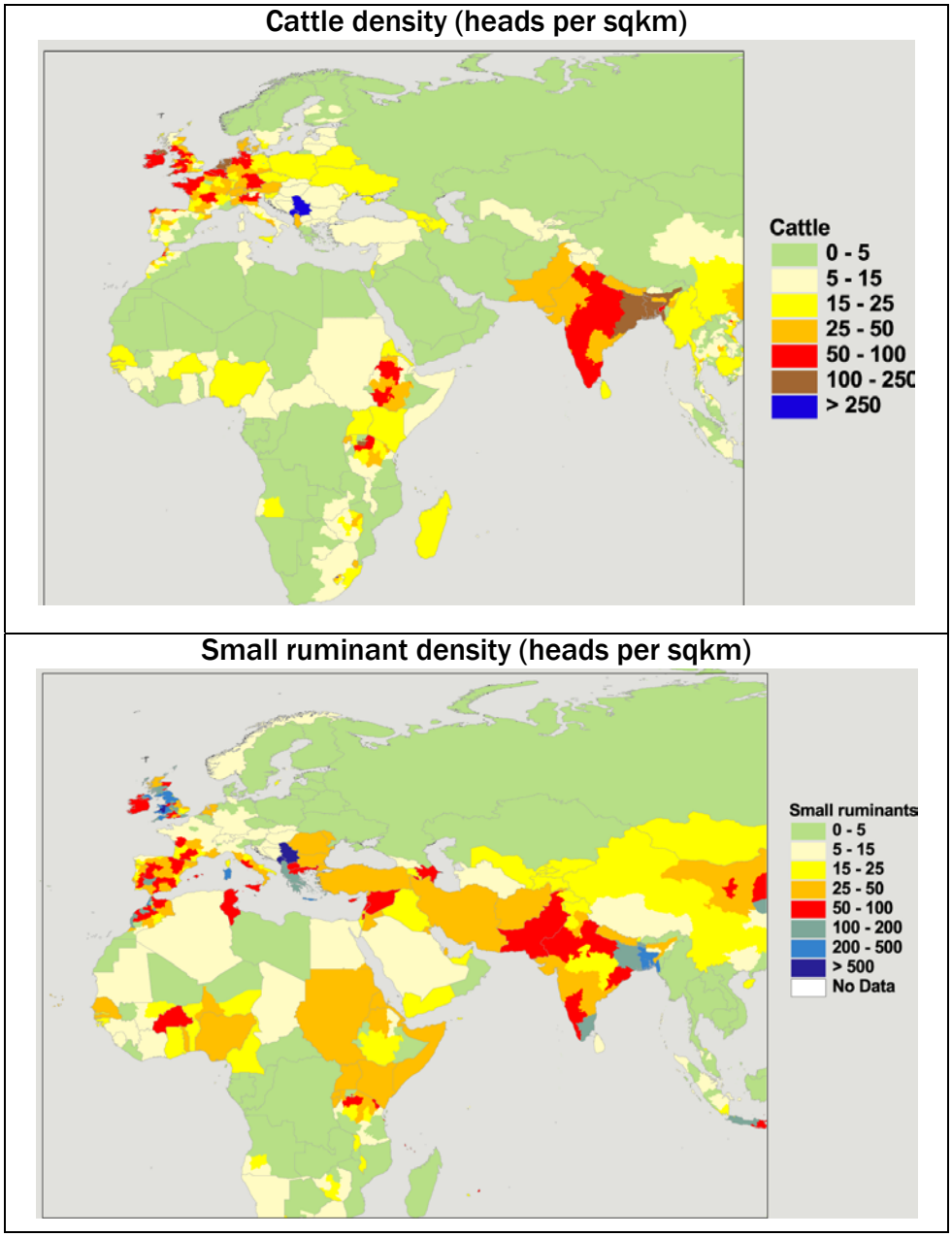

Figure 4: Geographical distribution of cattle and small ruminants (source: FAOSTAT)

7. Vectors

7.1. Overview of information required

Since RVF is essentially a vector transmitted disease, it is necessary to know which species can or may be able to act as vectors. It is also necessary to know where these species are found (in particular their presence or proximity to the EU) and what kind of environmental conditions they require. Information on the natural history of the disease is also required. In order to estimate the probability of infected vector entry to the EU, information on probable travel distances is required.

In the evaluation of vector importance, the terms vector competence and vector capacity are used. They are defined as follows:

- Vector competence represents the ability of a blood-sucking insect to become infected with an arbovirus after ingestion of an infective blood meal, and to transmit the virus subsequently when feeding on a vertebrate host.
- Vector capacity represents the combination of vector competence and all exogenous factors of ecological nature that affect it. Indeed, to be effective, the vector must, not only be competent, but have, in a given environment, a favorable bio-ecology for the transmission, i.e. to be abundant, to have a long longevity, to maintain close contact with the vertebrate reservoir (if any) and its vertebrate hosts, etc. It is only under these conditions that its vector capacity will be high. Variations in population density and age are often at the origin of the seasonal pattern of transmission of many vector-borne infectious diseases.

7.2. Known and potential vector species

A review of the literature was undertaken to produce summaries of the known RVFV vector species, including those from which RVFV has been isolated in the field and those that have been proven to be capable of infection and transmission in the laboratory.

Table 2 and Table 3 provide a nearly complete record of natural RVFV infection and experimental susceptibility/vector competence of mosquito vectors. Also included are some of the other dipteran species that most probably would be involved as mechanical vectors and, theoretically, also as potential vectors in certain instances.

The *culicoides* biting midges and phlebotomine sandflies are competent vectors of other veterinary viruses and have been implicated through virus isolation of RVFV during outbreaks.

The role of other arthropods, namely argasid and ixodid ticks, has not been considered in this report. The literature only provides hints at the role of these potential vectors with their long lifespan and ability to transmit other zoonotic viruses transovarially and non-viraemically (so-called “saliva-activated transmission”).

Table 2: Arthropods infected with Rift Valley fever virus in nature (note: that these reports are less a reflection of vector specificity than of high levels of viraemia in host species combined with the vctors ability to become infected orally and transmit biologically)

Genus (Subgenus)	Species	Locality (year)	Reference
Aedes (Aedimorphus)	*cumminsii*	Kenya (1981-84)	Linthicum *et al.* (1985b)
		Burkina Faso (1983)	Saluzzo *et al.* (1984)
	dalzieli	Senegal (1974, 1983)	Fontenille *et al.* (1998)
	dentatus	Zimbabwe (1969)	McIntosh (1972)
	durbanensis	Kenya (1937)	Mulligan (1937)
	ochraceus	Senegal (1993)	Fontenille *et al.* (1995)
	tarsalis	Uganda (1944)	Smithburn *et al.* (1948)
	vexans arabiensis	Senegal (1993)	Fontenille *et al.* (1995)
		Saudi Arabia (2000)	Jupp et al. (2002)
Aedes (Neomelaniconion)	*circumluteolus*	Uganda (1955)	Weinbren *et al.* (1957)
		South Africa (1955, 1981)	Kokernot *et al.* (1957), Jupp *et al.* (1983)
	mcintoshi	Zimbabwe (1969)	McIntosh (1972)
		South Africa (1974-75)	McIntosh *et al.* (1980a)
		Kenya (1981-84)	Linthicum *et al.* (1985b)
	palpalis	Central African Republic (1969)	Digoutte *et al.* (1974)
Ochlerotatus [1] *(Ochlerotatus)*	*caballus*	South Africa (1953)	Gear *et al.* (1955)
	caspius [2]	Egypt, suspected (1993)	Turell *et al.* (1996)
	juppi	South Africa (1974-75)	McIntosh *et al.* (1980a)
Aedes (Stegomyia)	*africanus*	Uganda (1956)	Weinbren *et al.* (1957)
	demeilloni/dendrophilus	Uganda (1944)	Smithburn *et al.* (1948)
Aedes (Diceromyia)	*furcifer* group [3]	Burkina Faso (1983)	Saluzzo *et al.* (1984)
Anopheles (Anopheles)	*coustani*	Zimbabwe (1969)	McIntosh (1972)
		Madagascar (1979)	Clerc *et al.* (1982)
	fuscicolor	Madagascar (1979)	Clerc *et al.* (1982)
Anopheles (Cellia)	*christyi*	Kenya (1981-84)	Linthicum *et al.* (1985b)
	cinereus	South Africa (1974-75)	McIntosh *et al.* (1980a)
	pauliani	Madagascar (1979)	Clerc *et al.* (1982)
	pharoensis	Kenya (1981-84)	Linthicum *et al.* (1985b)
	squamosus	Madagascar (1979)	Clerc *et al.* (1982)
Culex (Culex)	*spp.*[4]	Madagascar (1979)	Clerc *et al.* (1982)
	antennatus	Nigeria (1967-70)	Lee 1979
		Kenya (1981-84)	Linthicum *et al.* (1985b)
	neavei	South Africa (1981)	McIntosh *et al.* (1983)
	pipiens	Egypt (1977, 1978)	Hoogstraal *et al.* (1979), Meegan *et al.* (1980)
	poicilipes	Senegal (1998)	Diallo *et al.* (2000)
	theileri	South Africa (1970)	McIntosh (1972)
		Zimbabwe (1969)	McIntosh (1972)
	tritaeniorhynchus	Saudi Arabia (2000)	Jupp *et al.* (2002)
	vansomereni		
		Kenya (1981-84)	Linthicum *et al.* (1985b)
	zombaensis	South Africa (1981)	McIntosh *et al.* (1983)
		Kenya (1981-84, 1989)	Linthicum *et al.* (1985b), Logan *et al.* (1991b)
Culex (Eumelanomyia)	*rubinotus*	Kenya (1981-84)	Linthicum *et al.* (1985b)
Eretmapodites	*chrysogaster*	Uganda (1944)	Smithburn *et al.* (1948)
	quinquevittatus	South Africa (1971)	McIntosh (1972)
		Kenya (1981-84)	Linthicum et al. (1985b)
Coquillettidia	*fuscopennata*	Uganda (1959)	Williams *et al.* (1960)

Genus (Subgenus)	*Species*	*Locality (year)*	*Reference*
(Coquillettidia)	*grandidieri*	Madagascar (1979)	Clerc *et al.* (1982)
Mansonia (Mansoniodes)	*africana*	Uganda (1959, 1968)	Williams *et al.* (1960), Henderson *et al.* (1972)
		Central African Republic (1969)	Digoutte *et al.* (1974)
		Kenya (1989)	Logan *et al.* (1991)
	uniformis	Uganda (1959)	Williams *et al.* (1960)
		Madagascar (1979)	Clerc *et al.* (1982)
Other Diptera	*Culicoides* spp.	Nigeria (1967)	Lee (1979)

[1] Former subgenus of *Aedes*, recently elevated to generic rank by Reinert (2000).
[2] Suspected due to being the predominant mosquito species during the outbreak, vector competence confirmed in the laboratory.
[3] Representing a species complex consisting of 3 possible species, *Ae. furcifer, Ae. cordellieri & Ae. taylori.*
[4] *Culex* spp. may include *annulioris, antennatus, simpsoni & vansomereni.*

Table 3: Arthropods species which have demonstrated vectorcompetence in the laboratory

Genus (Subgenus)	*Species*	*Mode of transmission*	*Reference*
Aedes (Aedimorphus)	*fowleri*	biological	Turell *et al.* (1988a)
Aedes (Neomelaniconion)	*circumluteolus*	biological	McIntosh *et al.* (1983)
	macintoshi	biological	McIntosh *et al.* (1980a)
Aedes (Protomacleaya)	*triseriatus*	biological	Gargan *et al.* (1988)
Aedes (Stegomyia)	*aegypti*	biological, mechanical	McIntosh *et al.* (1980a), Hoch *et al.* (1985)
	aegypti formosus	Mechanical	Gillett and Mims (1956)
	albopictus	biological	Turell *et al.* (1988b)
Ochlerotatus (Finlaya)	*notoscriptus*	biological	Turell and Kay (1998)
Ochlerotatus (Ochlerotatus)	*caballus*	biological	Gear *et al.* (1955)
	canadensis	biological	Gargan *et al.* (1988)
	cantator	biological	Gargan *et al.* (1988)
	caspius	biological	Gad *et al.* (1987), Turell *et al.* (1996)
	excrucians	biological	Gargan *et al.* (1988)
	juppi	biological	McIntosh *et al.* (1980a)
	sollicitans	biological	Gargan *et al.* (1988)
	taeniorhynchus	biological, mechanical	Gargan *et al.* (1988), Hoch *et al.* (1985)
	vigilax	biological	Turell and Kay (1998)
Anopheles (Cellia)	*multicolor*	biological	Gad *et al.* (1987)
	pharoensis	biological	Gad *et al.* (1987)
Coquillettidia (Coquillettidia)	*versicolor*	biological	Daubney and Hudson (1933)
Culex (Culex)	*annulirostris*	biological	Turell and Kay (1998)
	antennatus	biological	Gad *et al.* (1987), Turell *et al.* (1996)
	neavei	biological	McIntosh *et al.* (1973b)
	perexiguus	biological	Turell *et al.* (1996)
	pipiens	biological, mechanical	Turell *et al.* (1996), Hoch *et al.* (1985)
	poicilipes	biological	Jupp and Cornel (1988)
	quinquefasciatus	biological	McIntosh *et al.* (1980a)
	salinarius	biological	Gargan *et al.* (1988)
	tarsalis	biological	Gargan *et al.* (1988)
	theileri	biological	McIntosh *et al.* (1973b, 1980a)
	univittatus	biological	McIntosh *et al.* (1980a)
	zombaensis	biological	McIntosh *et al.* (1983)
Culex (Neoculex)	*territans*	biological	Gargan *et al.* (1988)
Eretmapodites	*chrysogaster*	biological	Smithburn *et al.* (1949)
	quinquevittatus	biological	McIntosh *et al.* (1980a)

Genus (Subgenus)	Species	Mode of transmission	Reference
Other Diptera	*Stomoxys calcitrans*	mechanical	Hoch *et al.* (1985)
	Glossina morsitans	mechanical	Hoch *et al.* (1985)
	Lutzomyia longipalpis	mechanical	Hoch *et al.* (1985)
	Phlebotomus dubosqi	mechanical	Dohm *et al.* (2000)
	Culicoides variipennis	mechanical	Hoch *et al.* (1985)

7.3. Potential vectors within the EU

7.3.1. Species in the EU

Table 4, Table 5 and Table 6 list the most probable mosquito vectors in Europe by country, respectively as species present in the EU, species present in the EU candidate and applicant countries and species present in those European countries that are not currently part of the EU. Amongst these, only *Aedes v. vexans* is not a known vector of RVF.

A distribution chart for the European culicid species has been made available by Prof. K. Snow of the University of East London in Great Britain through MOTAX, the working group of European mosquito taxonomists (http://www.sove.org/motax/imatges/chart.xls). This chart was used firstly to identify those RVFV vector species with distributional ranges in the Paelearctic Region, with particular reference to European Union member states, and secondly those species that have taxonomic affinities with known vectors.

One new suspected RVF vector, *Mansonia (Mansonioides) uniformis*, has recently been identified from Bulgaria (Gratz 2004). Its vector status is not certain but specimens collected during an outbreak in Kenya in 1989 had tested positive for the virus (Logan *et al.* 1991). A known RVF vector, *Culex (Culex) tritaeniorhynchus*, which was identified as the amplifying vector in the Arabian Peninsular outbreak (Jupp *et al.* 2002), is present at the periphery of the EU, in Turkey.

Not shown in this list but of potential significance is the proportionately large number of species in the genus *Ochlerotatus* in Europe. Forty-five species, 3 in the subgenus *Finlaya*, 36 in the nominate subgenus, *Ochlerotatus*, and the remainder in the subgenus *Rusticoidus* have been identified from territories in and adjacent to the European Union. Furthermore, Europe has 2 species of *Coquillettidia*, a genus that contains at least one species that has been collected as infected specimens in the field. There are several members of the culicid subfamily *Anophelinae* present, none of which have been implicated in RVFV transmission but should be tested for susceptibility at some stage; RVFV has been isolated from several species of *Anopheles* in the Afrotropical region.

Table 4: Mosquito vectors of Rift Valley fever virus with known distributions in the European Union

Country	Aedes vexans vexans	Ochlerotatus caspius s.l.	Culex theileri	Culex pipiens	Culex perexiguus/ univittatus
Austria	X	X		X	
Belgium	X	X		X	

Country	*Aedes vexans vexans*	*Ochlerotatus caspius s.l.*	*Culex theileri*	*Culex pipiens*	*Culex perexiguus/ univittatus*
British Isles: UK	X	X		X	
British Isles: Ireland		X		X	
Cyprus		X		X	?
Czech Republic	X	X		X	
Denmark	X	X		X	
Estonia	X	X		X	
Finland	X	X		X	
France (mainland)	X	X	X	X	
France: Corsica	X	X	X	X	
Germany	X	X		X	
Greece	X	X	X	X	X
Hungary	X	X	X	X	
Italy (mainland)	X	X	X	X	X
Italy: Sardinia	X	X	X	X	
Italy: Sicily	X	X	X	X	X
Latvia	X	X		X	
Lithuania	X	X		X	
Luxembourg					
Malta		X		X	
Poland	X	X		X	
Portugal	X	X	X	X	X
Slovakia	X	X	X	X	
Slovenia	X	X		X	
Spain (mainland)	X	X	X	X	X
Spain: Balearic Islands	X			X	
Sweden	X	X		X	
The Netherlands	X			X	

Table 5: Mosquito vectors of Rift Valley fever virus with known distributions in European Union candidate (*) and applicant (H) countries

Country	*Aedes vexans vexans*	*Ochlerotatus caspius s.l.*	*Culex theileri*	*Culex pipiens*	*Culex perexiguus/ univittatus*
Bulgaria*	X	X	X	X	X
Croatia*	X	X		X	
Macedonia[H]	X	X	X	X	X
Romania*	X	X	X	X	
Turkey*	X	X	X	X	X

Table 6: Mosquito vectors of Rift Valley fever virus with known distributions in non-EU European countries

Country	*Aedes vexans vexans*	*Ochlerotatus caspius s.l.*	*Culex theileri*	*Culex pipiens*
Albania			X	X
Belarus	X	X	X	X
Bosnia-Herzegovina	X			
European Russia	X	X	X	X
Moldova		X	X	X
Norway	X			X
Switzerland	X		?	X
Ukraine	X	X	X	X
Serbia and Montenegro	X	X		X

7.3.2. Comparison of vectors, with respect to probable competence, in RVF infected areas, and in the EU

An attempt has been made to predict the most likely species that may become involved in the transmission of RVFV in the European Union should the virus be introduced from Africa or Saudi Arabia. Even though certain traditional African vectors of Rift Valley fever virus (RVFV) are present in member states of the European Union, their relative abundances are different and they have adapted physiologically and behaviourally (so-called "post-translational adaptations") to the more temperate climate. Furthermore, because of the Sahara and Mediterranean forming a barrier to gene flow between Afro-tropical (formerly the Ethiopian Region) and Paelearctic populations of these species, they are expected to exhibit different genetic traits, which may include vector competence for RVFV, through the evolutionary process known as genetic drift.

The Egyptian outbreak introduced *Culex (Culex) pipiens* into the list of suspected vectors, yielding 2 isolates from that species and leading to the lab experiments that confirmed that *Cx. pipiens* was susceptible to and could transmit RVFV efficiently (Hoogstraal *et al.* 1979; Meegan *et al.* 1980). This species occurs throughout Eurasia, Africa and North America. It displays great genetic and phenotypic plasticity and has a complicated taxonomic history (Barr 1982; Mattingly *et al.* 1951; Munstermann and Conn 1997; Vinogradova 2000). *Cx. pipiens* in southern Africa is morphologically and behaviourally different from *Cx. pipiens* in North Africa and Europe, which is, in turn, morphologically different from *Cx. pipiens* in America.

The natural history of RVF in Egypt also involves *Ochlerotatus (Ochlerotatus) caspius sensu lato.* Consisting of species that require temporary ground pools along river systems for oviposition and larval/pupal development (therefore pre-adapting them to take advantage of agricultural irrigation systems), the *Oc. caspius* group is believed to constitute the Egyptian maintenance vectors of RVFV (Gad *et al.* 1999).

The evidence on the importance of the *Aedes* subgenus *Aedimorphus* as vectors of RVF virus in West Africa in the 1990s (Traoré-Lamizana *et al.* 2001; Zeller *et al.* 1997) was confirmed in the 2000 epidemic/epidemic in Saudi Arabia, where *Ae. (Aed.) vexans* subspecies *arabiensis* was the maintenance vector (Jupp *et al.* 2002). Both, *Oc. caspius* and *Ae. vexans* occupy the kind of habitat that favours endemic cycles of RVFV transmission and both species occur in Europe. There are subspecific differences between the Afrotropical and Paelearctic populations, which make a simple prediction of vector capacity problematic. For example, *Ae. vexans arabiensis* occurs in sub-Saharan Africa and in the narrow geographical region delineated by the Sarawat mountain range in the Arabian Peninsula, westwards to the Red Sea and southwards to the Indian Ocean. However, the species is represented by another subspecies, *Ae. vexans vexans*, in the Mediterranean region and further north.

Laboratory studies relating to vector competence of RVF virus and the Egyptian strain of *Cx. pipiens* have demonstrated that not all individuals within a species are vector competent (Gargan *et al.*, 1983; Turell *et al.*, 1984). This population variation concerning vector competence is apparently dependent on many extrinsic and intrinsic factors such as genetic factors, age, rearing temperature and virus dose. With the exception of the studies on vector competence of laboratory strains of Egyptian *Cx. pipiens*, detailed laboratory investigations have not been conducted on most of the principal species of sub-Saharan that would allow us to quantify the importance of

different populations within different geographic areas. This may be due mostly to the difficulty of establishing colonies of many of the floodwater *Aedes* species.

In relation to the 5 potential RVF vectors present in Europe (Table 4), it needs to be considered that European populations of an African RVF vector may not have the same competence for the virus and may therefore not transmit it or transmit less efficiently. In addition, other European species not listed in table 4, particularly in the *Ochlerotatus* sub-genus ubiquitous in the Palearctic region, could be competent for RVF virus and play a vector role if showing a bio-ecology in terms of abundance, biting activity, feeding habits and longevity compatible with its transmission.

7.3.3. Conclusion

Many of the vector species involved in RVF to date are present in the EU. Differences may exist, due for example to adaptation physiologically and behaviourally to a more temperate climate, and genetic drift. The density of the potential vectors will vary depending on seasonal climatic conditions. Nevertheless, there is considered to be a high probability that, given the introduction of infection, at least some of these species would be competent vectors.

7.3.4. Recommendations

Laboratory investigations need to be conducted to determine competence and capacity of the European mosquitoes to transmit RVFV between domestic bovine, ovine and caprine livestock species. Such studies should also quantify the rates of transovarial transmission. Other studies should determine if sexual transmission of RVFV from infected males to uninfected females or vice-versa can occur and if it does occur, as well as how important is this to the amplification and maintenance of endemic infection in the species. Another very important question is the survival of RVFV infected mosquito eggs.

7.4. Role of vectors and climate in the natural history of RVF

7.4.1. Endemic/epidemic cycles of RVF

The history of RVF prior to 1977 provides insight into the cycle between so-called "floodwater aedine" mosquitoes as maintenance vectors and *Culex* species as amplifying vectors in the epidemic stages, and their respective habitat and vertebrate host requirements.

The virus is transmitted transovarially by *Aedes (Neomelaniconion) spp.* floodwater breeding mosquitoes. There are long periods, when virus activity does not occur or is limited to short periods with emergence of infected mosquitoes only in wetter ecozones, in forest or forest edge situations, where periodic flooding is more likely to occur. Without flooding, the existence of the virus may be extremely difficult to demonstrate during these inter-epidemic periods. However intensive longitudinal mosquito and field studies have shown that some emergence of these mosquitoes occurs when flooding persists for short periods of time (less than 3 weeks). Adults may be seen and RVF virus isolated, however the amplification of virus by secondary vector species does not occur unless the flooding persists (Davies 1975; Linthicum *et al.* 1985; Linthicum *et al.* 1990).

Conversion from endemic to epidemic virus activity follows persistent and heavy rains, which lead to flooding in the bushed and wooded grassland zones in Africa. *El Niño* phenomena predispose to the above changes. The flooding may occur in the wet highland grasslands, in the drier *Acacia-Commiphora* grasslands or in the semi-arid zones, where there are floodplains. Persistent flooding allows amplification of the virus to occur by secondary transmission cycles in the enormous numbers of *Culex*, *Aedes*, *Ochlerotatus, Anopheles* and *Mansonia* spp. which appear in these conditions. Many of these can transmit RVFV.

In the semi-arid to arid zones of the Horn of Africa, an inter-epidemic period of 35 years was recorded (Scott *et al*. 1963; Davies *et al*. 1985).

There are changes in global weather patterns since 2000, with greater amplitude of fluctuation of the Southern Ocean Sea Surface temperatures (SST and SOI indices). The sea surface temperatures drive the evaporation rates and globally there are now more severe droughts and periods of flooding than in previous decades. These changes may have an influence upon the periodicity of RVF epidemics in the future, and the previous patterns of epidemic activity may be altered (Davies *et al*. 1985; Linthicum *et al*. 1987; Linthicum *et al*. 1990; Linthicum *et al*. 1999).

Figure 5 provides a pictorial summary of the RVF cycle of infection.

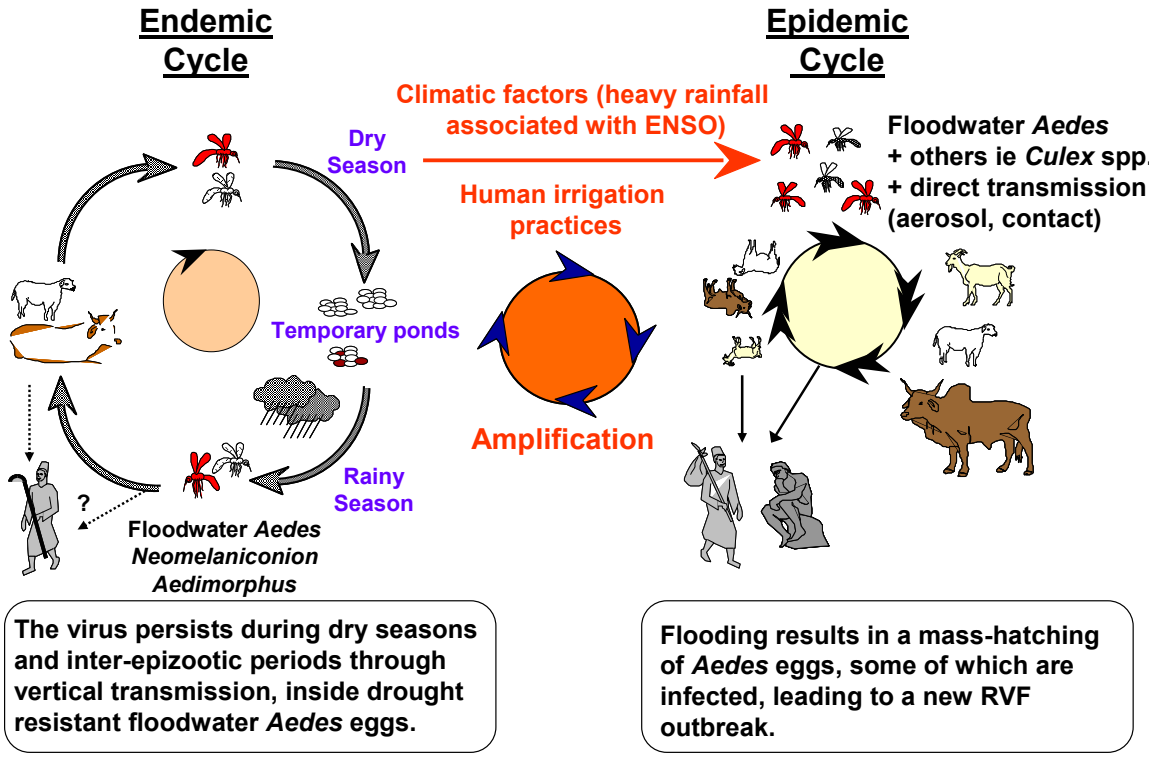

Figure 5: Cycle of RVF infection

To illustrate these cycles, the long-term pattern of antibody prevalence to RVF is shown in Figure 6 for national surveillance data from Senegal (Thonnon *et al*. 1999; Thiongane, unpublished data). Following the 1987 RVF epidemic the sero-prevalence was 70%, and it reduced to 30% in the next year, and continued to decrease until 1993. This reduction in RVF activity was associated with periods of low rainfalls. During the periods of heavy

rainfalls in 1994, 1999, 2002 and 2003, RVF activity re-emerged as epidemics in the Senegal River Basin and some adjacent areas such as the Ferlo region.

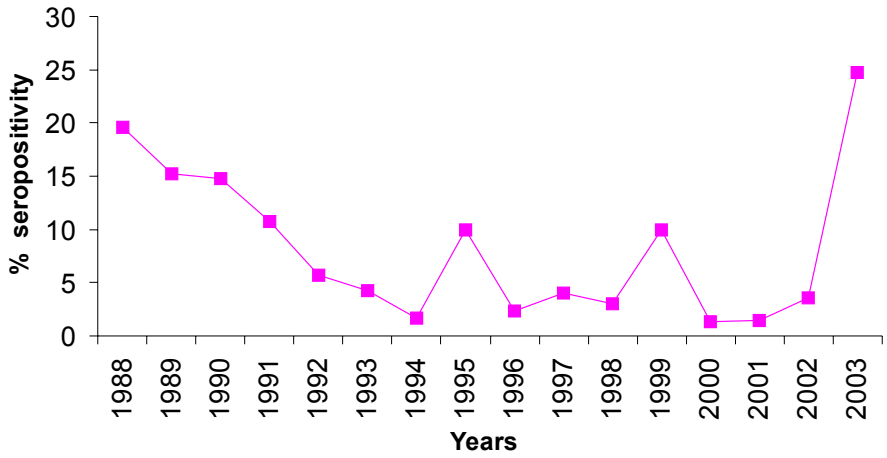

Figure 6: RVF sero-prevalence in domestic ruminants in Senegal, 1988 to 2003 (Thiongane, unpublished data)

Improving water storage and utilisation systems in semi-arid zones increases the areas of suitable habitat for floodwater breeding and other mosquito species. The backing up of water in riverine feeder systems following the construction of dams is an excellent example of this. All irrigation systems create and extend the areas which are suitable habitat for mosquitoes. Many epidemics such as those in the Sudan, Mauretania/Senegal and Egypt have been associated with such habitat. The characteristics of five types of ecological systems found commonly in Africa and Arabia where RVF may occur are presented in Table 7.

Table 7: RVF related characteristics of different ecological systems potentially suitable for RVF occurrence

Variable	***Ecological and epidemiological characteristics of different systems***				
Vegetation types	Forest and derived grasslands	Moist bushed and wooded grasslands, *Combretum* and *Hyparrhenia*	Drier, Acacia and *Commiphora* type grasslands	Semi-arid lands, dry scrub and low Acacia.	Desert
Virus activity in inter- epidemic periods	Likely most years	Possible every 3-5 years	Unlikely, usually in alluvial fans and flood zones	Usually not, alluvial fans and riverine flood zones	Alluvial fans and raadha
% sero-conversions in ruminants and man/ year	3-15%	Less than 5%	Less than 1%	Less than 1%	No
Epidemic Virus Activity	Yes,	Yes,	Less,	Usually not	Rare
% sero-conversions in ruminants and man	30-90%	25-70%	10-70%	5-70%	

Variable	*Ecological and epidemiological characteristics of different systems*				
Duration of epidemics	Peak at 3-6 months, but cases for 1-3 years	Peak 3-6 months, some cases for 1 year	3-6 months	3-4 months	3-4 months
Periodicity of epidemics	Usually 5-15 year cycles	Usually 5-15 years	5-25 years	25-35 years	25-35 years ?
Exotic animals present	Many	Often many	Few, some crossbreds	No	No
Clinical disease	Yes, main zone for exotic cattle and sheep	Yes, exotic cattle and sheep	Few pure bred, many cross breeds and indigenous types	Camels, indigenous cattle sheep and goats	Camels, some sheep and goats
Risks of flooding	Highest	Moderate	Low	Low	Exceptional
Risk of RVF virus activity during IEP periods	Occurs	Occurs	Low, flood associated	Very low, flood associated	Very low, flood associated

7.4.2. Influence of flooding

Flooding, whether local and limited or extensive, in any of the above zones determines the occurrence and extent of RVF virus activity. The persistence of flooding for 10-15 days is necessary to allow the emergence of RVFV infected *Aedes* floodwater breeding spp. (Logan *et al*. 1991). The persistence of the floodwaters for a further 4-6 weeks and their colonisation by secondary mosquito vectors, can allow amplification of the virus to epidemic proportions (Linthicum *et al*. 1983). This will only occur if many amplifying hosts (cattle, sheep and goats) are present.

Shorter periods of flooding may allow some infected *Aedes* to emerge, and evidence for this may be obtained by sero-conversions in sentinel ruminants. The disappearance of the floods after 2-4 weeks does not usually allow amplification of RVFV to occur to a level where it becomes clinically apparent in susceptible disease hosts (Linthicum *et al*. 1990; Logan *et al*. 1991a).

Inter-epidemic periods may be from 3-15 years in the moist highland or coastal grasslands and 15-35 years in the semi arid zones.

7.4.3. Predicting RVF epidemics

The environmental conditions which increase the likelihood of a change from an endemic to an epidemic situation have been defined, and therefore can be used for intensified surveillance. Predictive tools indicating a pre-epidemic phase have been successfully used in Kenya (Davies unpublished data; Davies *et al*. 1985a; Linthicum *et al*. 1990).

Historical information has shown that the greatest periods of RVF virus activity in Africa have occurred during periods of widespread, frequent, heavy and persistent rainfall. These periods are now recognised as *El Niño*. They occur on a wide regional basis. For example, RVF epidemics have involved East Africa and Egypt during the same periods. An analysis of the rainfall patterns allowed a prediction to be made for a RVF epidemic in Kenya in 1977. The lead-time was 6 weeks (Davies unpublished data).

Subsequently, a composite statistic based upon measurements of the rainfall characteristics, gave a clear positive value during the pre- and epidemic periods (Davies *et al*. 1985b). The measurement of rainfall was correlated with the normalised difference vegetation index (NDVI) derived from an advanced very high resolution radiometer (AVHRR) sensor on a satellite operated by the National Oceanic and Atmospheric Administration (NOAA) of the USA. This was made at sites in the highlands of Kenya known to be involved in RVF episodes. These studies showed the potential of Remote Sensing Satellite Imagery (RSSD) in monitoring and prediction of periods of RVF virus activity and epidemics (Linthicum *et al.* 1987; Linthicum *et al.* 1990; Logan *et al.* 1991a). The predictive models were greatly improved by the addition of the Pacific and Indian Oceans' Sea Surface Temperature data, together with the rainfall and NDVI data. An accuracy of 95-100% was claimed for the prediction of Kenyan epidemics of RVF. A lead time of 2-5 months was possible using these systems (Linthicum *et al*. 1999).

The gradual increase in NDVI values at known RVF virus emergent sites for the vector mosquito spp. in East Africa indicates a rising water table, and changes from brown to green in the vegetation, to the point where flooding occurs. These changes can be seen over a period of at least 2-3 months and allow predictions to be made of the likelihood of flooding and subsequent RVF virus emergence, usually when the NDVI value is greater than 0.43.

These predictive tools cannot be used, where the flooding occurs in river flood plains in the semi-arid zones. The rainfall producing such flooding has occurred often 1000's kilometres distant in the mountain watersheds for the river systems involved. Examples are the Nile river systems passing through Sudan and Egypt, the rivers running from the Kenya and Ethiopian highlands into the arid NE of the Horn of Africa, the rivers in the tihama of Arabia, the Senegal River in West Africa and the Kafue in Zambia (Davies *et al.* 1992). Flooding may suddenly occur as the water reaches these floodplains, without the preceding gradual build up of the water table, which drives the NDVI changes. The changes in NDVI follow the flooding and the extent of this is markedly different from the non RVF epidemic years. The RSSD derived Basin Excess Rainfall Monitoring Systems (BERMS) do allow predictions to be made of the expected river flow however, for they measure rainfall in the mountain watersheds. This data is now being examined to evaluate its use in a predictive model (Davies, pers. comm.).

The density of actual and potential vectors will therefore depend on seasonal as well as specific climatic events occurring at longer time intervals, which influence the availability of surface water at environmental temperatures favourable for mosquito breeding.

7.5. Transmission of RVF virus by vectors

The role of anopheline and culicine mosquito species (Diptera: Culicidae) in RVFV epidemiology may be a complicated one, consisting of biological, mechanical and transovarial/vertical transmission.

7.5.1. Transmission between vectors and vertebrates

RVF virus replicates to very high titers in mosquito vectors (Jupp and Cornel 1988).

Biological transmission is defined as requiring a persistent systemic infection in the vector that is transmissible through salivation during blood-feeding, mainly involving

mosquitoes but also potentially ticks (Acari: *Ixodidae*) and other haematophagous flies, e.g., phlebotomine sand flies (Diptera: *Psychodidae* subfamily *Phlebotominae*) and *Culicoides* midges (Diptera: *Ceratopogonidae*).

Mechanical transmission occurs when infectious viral particles are attached to the mouthparts after an interrupted feed on a viraemic host and are re-inoculated when the mosquito resumes feeding on a second host. This mode of transmission is especially efficient with the larger haematophagous flies, e.g., horse and deer flies (Diptera: *Tabanidae*) and tsetse flies (Diptera: *Glossinidae*), because of the greater surface area of their mouthparts compared to that of a mosquito. Due to the higher viremia in RVF infected animals, any larger haematophagous arthropods that feed readily on domestic animals and herbivores would be able to act as mechanical vectors.

7.5.2. Transmission within vector populations

Transovarial transmission consists of a systemic infection that disseminates to the ovaries of the vector, infecting the eggs prior to oviposition. RVFV was isolated from adult male and female mosquitoes hatched from field collections of larvae of *Aedes mcintoshi* in Kenya (Linthicum *et al.* 1985). The collections were made at a known RVF endemic site, which strongly suggested that the virus is transmitted trans-ovarially in this mosquito *spp*. It is likely that trans-ovarial transmission is effective in most African *Aedes* floodwater belonging to the sub-genera *Neomelaniconion* and *Aedimorphus* which are competent for RVF virus.

Laboratory experiments have not been successful because of the difficulty in colonizing the African maintenance vectors. However, transovarial transmission is an essential part of the ecology of RVF virus, explaining the long-term survival of the virus in the absence of suitable vertebrate hosts and/or environmental conditions for extensive mosquito population growth and associated virus transmission.

7.6. Movement of vectors in air currents

The migration of large numbers of mosquitoes for considerable distances is possible in air currents.

In the week immediately preceding the first outbreaks of the Egyptian epizootic in 1977, prevailing winds were from Sudan in the south, where infections had been recorded in the past. The distance traveled would have been at least 500 kilometers. Only circumstantial evidence is available to support this hypothesis. It was considered more probably that RVF introduction to Egypt was due to importation of infected ruminants (Baldet, pers comm.).

The active flight capacity of most potential RVF vectors is about 1km distance. In a RVF endemic area of Kenya, Linthicum and Bailey (1985) carried out a study on the migration of *Ae. mcintoshi* from a known located breeding habitat during the dry season. The mean dispersal of both males and females was limited to 0.25 km or below during a 45 day period following the first emergence of adult mosquitoes. At least, in this environment, there seems to be no need for the vectors to migrate between the larval habitats. Numerous females feed on cattle as they came to drink water and graze around the flooded habitat. This easy access to blood meals by potential vectors lessens the requirement of these mosquitoes to leave the breeding area in search of a blood

source of oviposition substrate. Attempts to study the vertical movements of these mosquitoes provided only negative results (Baldet, pers comm.).

In contrast, long vector dispersal has been described in association with the dissemination of Bluetongue disease, a non-zoonotic arthropod-borne disease transmitted by *Culicoides* biting midges, in Portugal (Sellers *et al.*, 1978), in Cyprus (Sellers *et al.*, 1979), in Western Turkey (Sellers and Pedgley, 1985), in Israel (Braverman, 1992 ; Braverman and Chechick, 1996), in Corsica by *Culicoides imicola* (Delecolle and de La Rocque, 2002) ; also in British Columbia from Washington State, USA (Sellers and Maarouf, 1991) and in northern Australia from Indonesia (Dyce, 1982) by others *Culicoides* species.

Aedes spp have been known to travel up to 175 km or more in wind currents at probably 1-2 km altitude in favourable temperatures (15-35°C; Sellers *et al.* 1977).

It has been suggested that a distance of 300 km could be covered in 6 hours on a moderate wind of 50 km hour, on the basis of experience with *Anopheles pharoensis* in the Eastern Mediterranean (Garret Jones 1962).

Dispersal distances are influenced by the local topography and the environment as well as intrinsic factors linked to the species. Some species will disperse further than others, because they occupy wind-exposed areas, and because their flight behaviour brings them into contact with the prevailing high winds. For example, when species such as *Ae. taeniorhynchus* fly up at a steep angle to a height of 12 m shortly after emergence in the evening before levelling out (Haeger, 1960), this takes them out of the protection of the boundary layer and they are more likely to be swept along by winds, sometimes for long distances (Haeger, 1960; 1985; Nayar, 1985). Such behaviour seems to be a response to high larval density, because in the laboratory temporary larval crowding produces a rising flight in adults during twilight (Nayar and Sauerman, 1969). According to Service (1997), it can be ecologically advantageous for a population to disperse out of an area when densities increase to levels where there is hard competition for resources, such as food in either the larval or adult stage. *Ae. taeniorhynchus* is the mosquito referred to most often with respect to long-distance dispersal. Whether or not, some mosquito species are inherently dispersive and others are not is not known.

8. Diagnostic Methods for RVF

Both, the release assessment and the consequence assessment, require data on the sensitivity of diagnostic tests used. Diagnostic assays are the primary tools for determining presence or absence of infection in animals and humans, as well as for the detection of contaminated animal products and fomites.

8.1. Definitions

8.1.1. Case definition of Rift Valley fever

"Rift Valley fever" is caused by an infection with Rift Valley fever virus resulting in a systematic infection of susceptible vertebrates which is characterized by a rapid humoral immune response and a high level of replicating virus in various tissues and blood.

8.1.2. Test performance characteristics

8.1.2.1. Definition of terms

- Diagnostic sensitivity

The proportion of known infected reference individuals that test positive in the assay; infected individuals that test negative are considered to have false-negative results.

- Diagnostic specificity

The proportion of known uninfected reference individuals that test negative in the assay; uninfected reference individuals that test positive are considered to have false-positive results.

- Analytical sensitivity

An integral part of the diagnostic sensitivity is the analytical sensitivity - the smallest detectable amount of the analyte (antibodies, antigens, nucleic acid or live organisms) in question.

8.1.2.2. Interpretation of diagnostic sensitivity / specificity

If the sensitivity of a diagnostic test is low, then the true number of infected individuals will be higher than those identified in the testing regime. This will mean that unless a correction is made, the assessed risk will be lower than the true risk. Therefore, it is very important in a risk assessment involving diagnostic assays, to take into account their accuracy for the targeted populations of animals and humans.

Conversely, with respect to the specificity, false-positive results will overestimate the risk. An animal identified as positive (whether correctly or not) is unlikely to be used for importation or moved within the EU. Therefore, problems associated with a low level of an assay's diagnostic specificity are likely to be much less significant in assessing the risk. It should be noted however that false-positive results may be significant in economic and cost-benefit terms.

8.2. Data on diagnostic methods for RVF

8.2.1. General considerations

There are health and safety issues to be addressed when considering the laboratory diagnosis of RVF. The virus presents a high bio-hazard risk for laboratory personnel. Biosafety level (BSL) 3 or 4 facilities are considered necessary by international organisations such as WHO and OIE, for handling the virus and others in the haemorrhagic fever group. The awareness of the serious hazard presented by members of the haemorrhagic group of viruses such as Marburg and Ebola, which occur in similar habitat to RVFV has created a necessity for suspicious material to be handled at BSL 3/4 levels of security (CDC 1999). There is a scarcity of such facilities throughout Africa, which is the endemic area for RFVF. There is no vaccine available to protect laboratory staff. Therefore, suspicious material may not always be subject to diagnostic tests and examination, thus reducing the probability of diagnosis. The OIE Terrestrial Manual describes various diagnostic techniques, which are required by the International Animal Health Code for testing animals before they are moved internationally (OIE 2004). However, this manual does not provide any data or references, which could be used for risk assessment of RVF and also there are a number of drawbacks with respect to serological assays recommended by OIE. For example estimates for diagnostic sensitivity and specificity of traditional serological assays (virus neutralisation, inhibition haemagglutination) are not given, neither are references evidencing their validation and/or diagnostic accuracy are provided. Also, references for the recommended cut-offs for these tests are not provided and the reference to the recommended indirect ELISA for the detection of IgG antibodies refers to a study, which did not follow the current OIE's guidelines for assay validation.

The South African outbreaks of 1950-1951 and the Egyptian outbreaks of 1977 - 1978 were not recognized as RVF until several months had elapsed with the deaths of thousand of animals, and in the Egyptian outbreaks, of many deaths in humans. In both these instances, delays occurred because the disease was previously unknown in those geographical areas and the possibility of RVF was not at first a consideration (Portefield and Della Porta 1981).

However, clinical, pathological, histological and laboratory diagnosis of RVF outbreaks can be relatively simple when veterinary, medical, and laboratory personnel who are in the best position to observe and report disease activity receive regular and continuous training and when suitably equipped laboratory facilities are available.

RVF epidemics have a number of concurrent and interrelated features, which allow for making fairly accurate tentative diagnosis. They include

- unusually heavy and persistent rainfall resulting in flooding over a wide area and subsequent massive build up in vector competent populations,
- sudden simultaneous and multifocal onset of abortions among sheep, goats, cattle or camels and high mortality rate particularly in newborn lambs, kids and calves over a wide area,
- other severe, often haemorrhagic clinical signs and gross and histological lesions, especially in livers of young animals or aborted foetuses, and
- usually benign febrile illness among people involved in handling the blood, tissues, secretions or excretions of infected animals, especially after abortion, or involved in slaughtering and autopsying of infected animals.

During RVF epidemics isolation of the virus from vertebrates and mosquito vectors should not be difficult because of high-level viraemia and high virus concentrations in various tissues, high-level of virus amplification in competent vector populations, virus stability and high susceptibility of different *in vitro* and *in vivo* isolation systems.

Infection with RVF virus induces rapid production of class-specific immunoglobulins. They are easily detectable at the early stage of infection that allows rapid diagnosis on a single serum sample.

During the long inter-epidemic periods the virus is maintained silently within the cryptic cycle and only sporadic, small and local epidemics may occur. Therefore, unless very intensive and well-designed surveillance activities are in place virus activity usually remains undetected.

Guidelines for collection and transport of specimens for laboratory confirmation of RVF and diagnostic techniques have been recently reviewed (FAO 2002; FAO 2003; OIE 2004).

For comprehensiveness of this document, diagnostic approaches to RVF are briefly discussed. References are provided for more detailed description of clinical, gross and histological lesions as well as for the differential diagnosis, and laboratory techniques. Special emphasis is made on the applicability of the current diagnostic assays in RVF non-endemic areas.

8.2.2. Clinical diagnosis

8.2.2.1. Clinical diagnosis in domestic animals

There are marked differences in the patterns of disease, which are observed in the field. Infection with RVF virus may present in domestic animals with moderate or serious clinical disease. RVF most commonly presents with abortions in 15-30% of the pregnant animals (sheep, goats or cattle). Neonatal deaths may be a feature, or in older animals depression with fever, inappetence, vomiting with haemorrhagic diarrhoea, and some mortality. However, considerable RVF virus activity may occur in ruminants, cattle sheep or goats, without any obvious clinical signs. Brief periods of viraemia with or without a febrile response would not be detected. Diagnostic activities are problem driven, and cryptic virus activity may occur unnoticed.

Ninety percent or more of RVF infection in Africa is sub-clinical and in-apparent in most of the indigenous ruminants. Those breeds or strains, which are exotic to the continent, are usually highly susceptible to RVFV and show clinical signs. A viraemia does occur in these indigenous breeds and in camels however. This is asymptomatic and of comparatively short duration, but the virus is foetopathic and some animals may subsequently abort.

RVF has a short incubation period of 12–36 hours. A fever of up to 41- 42°C may develop, and it remains high until shortly before death. Affected animals are listless, depressed, loose their appetite and are disinclined to move. They may show enlarged superficial lymph nodes and abdominal pain. Lambs under the age of two weeks rarely survive longer than 36 to 42 hours after the onset of signs of illness with mortality rate of 95% or higher. Animals older than 2 weeks may die peracutely, acutely or may develop a subclinical infection. Some animals may show melaena or bloody, foul-

smelling diarrhoea and bloodstained mucopurulent nasal discharge. Icterus may sometimes be observed, particularly in cattle. In addition to these signs, adult cattle may show lachrymation, salivation and dysgalactia.

In pregnant sheep, the mortality and abortion rates vary from 5% to almost 100% in different outbreaks and between different flocks. Very high abortion and mortality rates in neonates have been encountered amongst highly susceptible sheep breeds (Daubney *et al.* 1931; Findlay 1931; Davies 1975). Situations have been encountered, where exotic sheep flocks suffer 80-90% abortion rates with very high mortality in lambs, whilst indigenous animals in the same area show no clinical signs, with a 5-10% abortion rate, which may be difficult to distinguish from expected levels (Davies 1975; Davies 1981).

In goats, clinical signs are less common than in sheep (Easterday 1962a; Easterday 1962b; Easterday *et al*. 1962; FAO 2002; Botha *et al*. 1996; FAO 2003; OIE 2004; Swanepoel and Coetzer 2004).

Clinical signs in calves may be obvious and serious in susceptible breeds or mild with 10 to 15% mortalities, but in adult cattle abortion (10 to 40%) is sometimes the only clinical sign.

Diseases to be taking into consideration in the differential diagnosis of RVF include (FAO 2002; FAO 2003; Swanepoel and Coetzer 2004):

- Wesselsbron disease, bluetongue, ephemeral fever, Nairobi sheep disease, Rinderpest, peste des petits ruminants, Thogoto virus infection, Q fever, toxoplasmosis, brucellosis, salmonellosis, pasteurellosis, leptospirosis, chlamydiosis, anthrax, poisonous plants (causing hepatic lesions, haemorrhages, and/or icterus)
- In instances where teratology is involved, infections with the following agents should be considered: Simbu serogroup bunyaviruses, Palyam and Bluetongue serogroup orbiviruses, Wesselsbron and other flaviviruses such as West Nile and Banzi.

Suspicion of RVF based on clinical sign and symptoms requires laboratory confirmation.

8.2.2.2. Clinical diagnosis in humans

The disease in humans is characterized by an acute, undifferentiated febrile affection that is manifested in 4 possible clinical syndromes (Daubney *et al*. 1931; Findlay 1931; Swanepoel and Coetzer 2004; Gerdes 2004):

- An uncomplicated, febrile, influenza-like illness and hepatitis occurs in virtually every case, with vomiting and diarrhoea.
- A haemorrhagic fever with liver involvement, thrombocytopenia, icterus and bleeding tendencies.
- Encephalitis following a febrile episode with confusion and coma. Death is infrequent but there may be some residual damage
- Ocular involvement with reported blurred vision and loss of visual acuity due to retinal haemorrhage and macular oedema.

Fatality rates in humans were considered to be usually < 1% (Gubler 2002), but seems to have increased in outbreaks which have occurred since the seventies. Mortality figures are quite variable from one outbreak to the other. An exceptionally high case

fatality rate of 14% was encountered in the September 2000 outbreak in Saudi Arabia and Yemen (Balkhy and Memish 2003) which may have been related to coexisting hyper-endemic malaria (Davies, pers. comm.).

In hospitalised patients, case-fatality rate associated with severe infection can be as high as 33% as a result of hepatorenal failure, shock, and severe anaemia (Al-Hazmi *et al.* 2003). Usually, individuals who become affected are involved in the livestock industry, farm labourers who salvage carcasses for human consumption, veterinarians, and abattoir workers, thus aiding differential diagnosis. RVF virus has caused serious human infection in laboratory workers (Kitchen 1934; Smithburn *et al*. 1949; Schwentker and Rivers 1934).

In the early stages, many differential diagnoses are possible, including influenza. Clinical diagnosis can thus be problematic in the early stages.

8.2.2.3. Sensitivity of clinical diagnosis

8.2.3. Pathological diagnosis

8.2.3.1. Gross lesions

The hepatic gross lesions of RVF are very similar in all species. The most severe lesion occurring in aborted foetuses and newborn lambs is a moderately to greatly enlarged, soft, friable liver with a yellowish-brown to dark reddish-brown colour with irregular congested patches. Numerous greyish-white necrotic foci are invariably present in the parenchyma, but may not be clearly discernible. In adult sheep, the lesions are less severe and pinpoint reddish to greyish-white necrotic foci are distributed throughout the parenchyma. Haemorrhage and oedema of the wall of the gallbladder are common. Hepatic lesions in lambs are almost invariably accompanied by numerous small haemorrhages in the mucosa of the abomasum. The contents of the small intestine and abomasum are dark chocolate-brown as a result of the presence of partially digested blood. In all animals, the spleen and peripheral lymph nodes are enlarged, oedematous and may have petechiae (Daubney *et al.* 1931; Findlay 1931; Findlay 1932; Findlay 1933; Easterday *et al*. 1962; Coetzer 1977; Coetzer 1982; Coetzer and Ishak 1982; FAO 2002; FAO 2003; Schultz 1951; Swanepoel and Coetzer 2004).

8.2.3.2. Histological lesions

Microscopically, hepatic necrosis is the most obvious lesion of RVF in both animals and humans. In foetuses and neonates of cattle and sheep, foci of necrosis consist of dense aggregates of cellular and nuclear debris, some fibrin and a few inflammatory cells. There is a severe lytic necrosis of most hepatocytes and the normal architecture of the liver is lost. In about 50% of affected livers, intranuclear inclusion bodies that are eosinophilic and oval or rod-shaped are found. Mineralisation of necrotic hepatocytes is also seen. In adult animals, hepatic necrosis is less diffuse and in sheep, icterus is more common than in lambs (Daubney 1931; Coetzer 1977; Coetzer 1982; Coetzer and Ishak 1982; FAO 2002; FAO 2003; Rippy *et al.* 1992; Schultz 1951; Swanepoel and Coetzer 2004).

8.2.4. Laboratory diagnosis

8.2.4.1. Facilities necessary for laboratory diagnosis

The awareness of the serious human health hazard presented by members of the haemorrhagic group of viruses which also include RVF has created a necessity for suspicious material to be handled at biosafety security levels (BSL) 3 to 4. There is a scarcity of such facilities throughout Africa, which is the endemic area for RVF. There is no properly certified vaccine available to protect laboratory staff (an experimental inactivated RVFV vaccine has however been widely used in many laboratories). Therefore, suspicious material may not always be subject to diagnostic tests and examination, thus reducing the probability of diagnosis.

Handling and processing of specimens from suspected viral haemorrhagic cases, and virus isolation and amplification procedures are not recommended for use outside endemic areas unless high-level bio-containment facilities are available and laboratory workers are adequately protected.

Historically, primary virus isolation was made in the disease hosts and later in hamsters or mice. Subsequently, the development of tissue cultures replaced experimental animal inoculation for primary virus isolation and identification. The OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals provides guidelines for the various diagnostic and serological tests used with RVFV.

Table 8 lists the laboratories which perform diagnostic work for RVFV in Africa and Europe.

Table 8: Laboratories conducting diagnostic work for RVF in Africa and Europe

Continent	*Country*	*Laboratory*	*Activities*
Africa	Ethiopia	Sebata Veterinary Laboratory, Addis Ababa	serological diagnosis
	Guinea	Laboratoire de Diagnostic Vétérinaire, Conakry	serological diagnosis
	Kenya	Kabete Veterinary Laboratory, Kabete	serological diagnosis
	Mali	Laboratoire Central Vétérinaire, Bamako	serological diagnosis
	Mauritania	Centre National d'Elevage et de Recherches Vétérinaires (CNERV), Nouakchott	serological diagnosis
	Senegal	Institut Pasteur (ISRA), Dakar	P3 facilities, serology and virus isolation, RT-PCR diagnosis
		Laboratoire national d'Elevage et de Recherches vétérinaires, Dakar-Hann	serology and virus isolation diagnosis
	South Africa	National Institute for Virology, Pretoria	serology and virus isolation, RT-PCR diagnosis
		Onderstepoort Veterinary Institute, Onderstepoort	serology and virus isolation, RT-PCR diagnosis
	The Gambia	International Trypanotolerance Center, Banjul	serological diagnosis
Europe	United Kingdom	Special Pathogens Reference Unit, Health Protection Agency, Porton Down, Salisbury	

Council Directive Dir 2000/54/EC (EC, 2000) is the relevant EU legislation dealing with human protection at the lab for RVF.

There is no specific EU legislation for laboratories dealing with diagnosis for OIE List Diseases that specifies/put down requirments for the BSL in the laboratories, as this is mostly for the human protection (hence referrring to the 2000/54/EC).

8.2.4.2. Isolation

RVF virus can be isolated from serum and whole blood collected during the febrile stage of the disease, from liver, spleen and brain of animals that have died, or from tissues of aborted foetuses (OIE 2004). Primary isolation can be performed in a number of cell lines such as Vero, Baby Hamster Kidney and mosquito cell lines (Anderson *et al*. 1989; El Karamany *et al*. 1979; Ellis *et al*. 1988; Johnson and Orlando 1968; Johnson and Orlando 1969; Lecatsas and Weiss 1968; Ohder *et al*. 1970; Orlando *et al*. 1967), as well as in in-vivo systems (Findlay 1931; Pini *et al*. 1970; Anderson *et al*. 1989; Easterday and Murphy 1963; Matumoto *et al.* 1959; Peters and Anderson 1981). Isolation and titration of RVF virus in sucking mice has been considered the traditional and the most sensitive method, but it has been suggested that laboratory cell lines might be a more practical and efficient alternative (Anderson *et al*. 1989). Also, it is easier and safer to work with cell cultures than animals, especially in high containment laboratories.

Estimates of sensitivity for various isolation systems are not available. But virus isolation is generally considered to be highly sensitive, and is therefore often used as a gold standard for diagnosis.

8.2.4.3. Detection and identification

8.2.4.3.1. Agar gel immunodiffusion

A rapid diagnosis can be made by demonstrating viral antigen in tissues or in serum of febrile animals by agar gel immunodiffusion test (Hahon 1969; Levitt and Polson 1964). This assay might be useful in laboratories without tissue-culture facilities.

8.2.4.3.2. Immunofluorescent staining

Specific identification of RVF virus antigen may be made rapidly by immunofluorescent staining of cover-slip preparations or 8-chamber tissue culture slides. The 8-chamber slides provide a means of preliminary identification and titration requiring minimal resource. To each a diagnosis before appearance of cytopathic effect in cell cultures, they can be screened daily, or even within a few hours post inoculation, as RVF virus can be detected and identified within hours (El Mekki and Van der Groen 1981). Thus, a tissue culture system combined with the fluorescent antibody technique can provide a rapid means of diagnosis for RVF (Easterday and Jaeger 1963; El Mekki and Van der Groen 1981; Ellis *et al*. 1988; Hahon 1969; Ohder *et al*. 1970). The virus may also be detected by immunofluorescence carried out on impression smears or tissue section of liver, spleen and brain (Easterday and Jaeger 1963; Easterday and Murphy 1963; Pini *et al.* 1970).

8.2.4.3.3. Antigen immunocapture ELISA

RVF virus replicates to a very high titers in many species (e.g. viraemia of 10^4 to 10^9 PFU/ml for several days) and mosquito vectors. Hence, a variety of specimens contain sufficient antigen for diagnostic purposes (Anderson *et al*. 1989; Easterday and Murphy

1963; Easterday *et al*. 1962a; Easterday *et al*. 1962b; Niklasson and Gargan 1985; Niklasson *et al*. 1983; Turell and Rossi 1991).

Detection of RVF virus by antigen ELISA may be the method of choice for diagnosis and surveillance, especially in non-endemic areas, since the assay can be performed entirely with inactivated reagents.

The plaque assay and the ELISA had similar sensitivity (100% vs. 93%, respectively) and specificity (94% vs. 94%, respectively) in detecting mosquitoes capable of transmitting RVF virus to susceptible hamsters (Niklasson and Gargan 1985). An ELISA could detect RVF in 29.3% of 82 viraemic human sera collected during the 1987 RVF epidemic in the Senegal River basin, and was shown to 96.7% specific in 61 sera negative for virus isolation (Meegan *et al.* 1987).

A sandwich ELISA utilizing hyperimmune mouse serum as capture antibody was reported to detect $10^{3.5}$ PFU/ml of virus in viraemic rhesus monkeys (Niklasson *et al*. 1983), and was shown to be useful in detection of RVF antigen in sera from experimentally infected lambs (Peters *et al*. 1989). A sandwich ELISA utilizing sheep serum as a capture antibody was shown to have a detection limit ranging form $10^{2.5}$ $TCID_{50}$/ml of sheep viraemic serum to $10^{2.8}$ $TCID_{50}$/ml of mosquito pool homogenate. It also detected RVF virus in fluids from infected BHK-21 cells at least 12 h after inoculation (Paweska *et al*. 2005b).

8.2.4.3.4. Nucleic acid techniques

Delay in diagnosis associated with traditional virus isolation and identification techniques may represent a significant problem for regulatory healthcare authorities faced with an epidemic of RVF, especially outside its traditional geographical confines. Hence, considerable efforts have been recently made to develop nucleic acid techniques for rapid detection of RVF virus genome in mosquitoes (Ibrahim *et al*. 1997), human and animal sera (Sall *et al*. 2001).

The real-time reverse transcription (RT) - PCR could detect less than 10 $TCID_{50}$/ml of RVF-infective cell culture supernatants (Garcia *et al.* 2001) or 9-16 RNA copies per assay (Drosten *et al*. 2002). When using real-time RT- PCR, results can be available within few hours after arrival of the samples in the laboratory. However, it has been suggested that definitive diagnosis or exclusion of viral haemorrhagic fevers should not be based on a single PCR result. Rather, nucleic acid based techniques should be embedded in a diagnostic procedure involving additional test, e.g. virus isolation in cell culture, antigen capture ELISA, or detection of virus-specific immunoglobulins (Drosten *et al*. 2002).

8.2.4.3.5. Serological tests

Due to a very short viraemia in RVF-infected animals, serological assays may sometimes represent the only tool for testing live animals. The classical methods for the detection of antibodies to RVFV include haemagglutination-inhibition, complement fixation, indirect immunofluorescence, and virus neutralisation tests (Clarke and Casals 1958; Matumoto *et al*. 1950; Swanepoel *et al*. 1986). Although classical serological techniques have proved useful, they suffer from a number of drawbacks. In general terms, these drawbacks relate to a combination of inadequate diagnostic performance, lack of standardization and/or poor cost efficiency (Wright *et al*. 1993). Disadvantages of these techniques also include health risk to laboratory personnel (Smithburn *et al.* 1949), and restrictions for their use outside RVF endemic areas. ELISA has the potential

to solve all these problems and compared to classical methods it was shown to be more sensitive in detection of the earliest immunological responses to infection or vaccination with RVF virus (Paweska *et al.* 2003).

The fact that RVF virus consists of a single serotype makes the serological diagnosis and preparation of diagnostic reagents relatively easy. Antigenic cross-reactivity studies in animals failed to provide any evidence that other African phleboviruses could hamper the diagnosis of RVF (Davies 1975; Swanepoel 1976; Swanepoel 1981; Swanepoel *et al.* 1986). However, results of other studies suggest that heterologous reactions should be considered between RVF virus and other viruses in the phlebotomus fever virus group when serological test are used, including the virus neutralization tests (Meegan *et al.* 1987; Shope *et al.* 1980; Shope *et al.* 1981). Especially sera showing low titres could represent cross-reacting or non-specific antibodies that may confound interpretation of RVF sero-diagnosis and sero-surveys results.

8.2.4.3.6. Virus neutralisation

Virus neutralisation (VN) tests including micro-neutralization, plaque reduction neutralisation and neutralisation in mice have been used to detect antibodies against RVF virus in the serum of all animal species.

The VN test is highly specific with little or no cross-neutralisation with other phleboviruses (Tesh *et al.* 1982). Its sensitivity has been traditionally assumed to be 100%, although there is only very limited data available to support this assumption. Although regarded as gold standard, the VN test is extremely laborious, expensive, and requires several days for completion. It can be performed only when standardized stock of live virus and tissue cultures or mice are available. Therefore, it is rarely used, and not recommended for use outside endemic areas or in laboratories without appropriate biosecurity facilities and vaccinated personnel.

8.2.4.3.7. Inhibition haemagglutination

It has been suggested that the inhibition haemagglutination (IH) test can be employed with great confidence in non-endemic areas, as it has a high level of sensitivity and specificity. However, sera from individuals that have had previous infections with phleboviruses other than RVF virus may cross-react with RVF virus antigen (Kitchen 1934; Easterday *et al.* 1962a; Botha *et al.*1996).

8.2.4.3.8. Enzyme-linked immunosorbent assay

Different formats of enzyme-linked immunosorbent assay (ELISA) for rapid detection of specific anti-RVF virus IgM and IgG antibodies and viral antigen have been reported (Meegan *et al.* 1987; Niklasson *et al.* 1984; Paweska *et al.* 1995; Swanepoel *et al.* 1986). However, the use of ELISA in the diagnosis of RVF in humans and animals was until recently limited to a few reference laboratories worldwide. This was partly due to very limited validation data in field-collected sera and also because methods used for the expression of the ELISA absorbance readings and the selection of the cut-off values is now obsolete. In response to increasing international demand for highly accurate tests, standardized and safe reagents for the serological diagnosis of RVF and for the use of validated diagnostic methods, the OIE Reference Laboratory for RVF at the Onderstepoort Veterinary Institute, Onderstepoort, South Africa and the WHO Collaborating Centre for Arboviruses and Viral Haemorrhagic Fevers at the National Institute for Communicable Diseases, Sandringham, South Africa, undertook development and intensive validation of various ELISAs for accurate and safe detection

of RVF-specific antibodies in humans, domestic and wild ruminants. Results in field-collected, experimental and post-vaccination sera demonstrated that these assays could be useful for epidemiological surveillance and control programmes, import/export veterinary certification, early diagnosis of infection, and for monitoring of immune response after vaccination. As highly accurate robust and safe tests, they have the potential to replace traditional diagnostic methods, which pose biohazard risks limiting their use outside of endemic areas to high containment facilities (Paweska *et al.* 2003a; Paweska *et al.* 2003b; Paweska *et al.* 2005a; Paweska *et al.* 2005b).

8.3. Summary of diagnostic tests

Table 9, Table 10 and Table 11 summarise the diagnostic test information required by this risk assessment.

Table 9: Diagnostic sensitivity of different ELISA tests in field-collected sera (virus neutralisation test (VNT) used as gold standard; n.t. = not tested)

	Sensitivity (%)						
ELISA Test	***Human***	***Cattle***	***Goat***	***Sheep***	***African Buffalo***	***Camel***	***Reference***
Indirect ELISA	n.t.	84.3	99.2	98.9	92.6	n.t.	Paweska *et al.* 2003b
IgG-sandwich ELISA	100	96.3	100	99.1	n.t.	n.t.	Paweska *et al.* 2003a; Paweska *et al.* 2005a;
IgM-capture ELISA	96.5	n.t.	n.t.	see table 9	n.t.	n.t.	Paweska *et al.* 2003a; Paweska *et al.* 2005a
Inhibition ELISA	99.5	100	99.6	100	100	100	Paweska et al, 2005b

Table 10: Diagnostic sensitivity of IgM-capture ELISA in sheep infected with wild type AR 20368 strain and in sheep vaccinated with live-attenuated Smithburn strain of RVF virus (Paweska et al. 2003a)

	Sensitivity (%)	
Days post infection	***Infected***	***Vaccinated***
1-3	0	0
4	75	10
5-42	100	50
49	75	80
58	37.5	90
65	12.5	100
72	12.5	

Table 11: Diagnostic sensitivity of haemagglutination-inhibition (HI), virus neutralisation (VN) and IgG-sandwich ELISA in sheep infected with wild type AR 20368 strain, and in (sheep vaccinated with live-attenuated Smithburn strain of RVF virus (Paweska et al. 2003a)

	Days post infection	***Sensitivity (%)***		
		HI	***VN***	***ELISA***
Infected	1-3	0	0	0
	4	0	25	50
	5	25	75	87.5
	6	62.5	100	100
	7-72	100	100	100
Vaccinated	1-3	0	0	0
	4	0	10	30
	5	0	40	50
	6	20	60	70
	7	50	80	90
	8	80	100	100
	9	90	100	100
	10-34	100	100	100

8.4. Conclusions on diagnostic tests

For the reasons already given in Section 8.1, the most important feature of diagnostic tests in import risk assessments are their diagnostic sensitivities under field conditions.

Data presented in Table 10 and Table 11 show that ELISA sensitivity varies with the number of days post-infection and the number of days post-vaccination.

From Table 9 it can be seen that under field conditions the sensitivity of the four different types of ELISAs varied in different host species, although not all combinations were investigated. In addition, it is unlikely that each test would always produce exactly the same sensitivity value under different sets of field conditions, a range being the usual finding, but only one value is given for each of the above. For humans, the sensitivity values given are (rounded, percentages) 96, 99, and 100; for cattle, they are 84, 96, 100; for goats, 99, 100, 100, for sheep, 99, 99, 100, and for other species from 92 to 100. The majority of sensitivity values are therefore in the high nineties, although the value of 84 for cattle in one of the studies is markedly lower.

In conclusion, diagnosis of RVF with a high level of sensitivity can be achieved when serological tests are used in combination with clinical observation and epidemiological history and/or when sero-conversion to the virus is demonstrated. Antibody detection techniques are also widely used to demonstrate freedom from a disease, and also in epidemiological investigations.

The degree to which the reference animals represent all of the host and environmental variables in the population targeted by the assay has a major impact on the accuracy of test result interpretation. Although the currently available validation data for ELISAs showed their high diagnostic accuracy and potential for large-scale diagnostic use, they were derived from testing sera collected in Africa. An assay validated by using sera from Africa may not give valid results for populations of humans, domestic and wild ruminants in Europe.

Validation data for traditional serological tests, virological and molecular detection and identification techniques are not available.

Current laboratory diagnosis of RVF is mostly based on techniques or production of diagnostic reagents, which require the highest level of bio-containment.

8.5. Recommendations on diagnostic testing

Regular training about clinical, pathological, histological and laboratory diagnosis of RVF of veterinary, medical, and laboratory personnel would increase the probability of early detection of infection.

When handling live RVFV and clinical specimens suspected to contain the virus, this work should be carried out in at least biosafety level 2 laboratory facilities, and the personnel needs to have received the training essential to the required level of biosafety.

At least one reference laboratory at BSL 3 within the EU, where work with haemorrhagic fever group viruses can be routinely performed, should be identified to handle any blood or tissues collected from suspected RVF affected animals or man. EC, 2000. It is recommended that they have the capacity to isolate RVF virus from tissues and to carry out the serum neutralisation and PCR testing for the specific identification of any suspected RVF isolates.

8.6. Future Research

It is recommended that currently available diagnostic assays, especially for the detection of antibody to RVFV, are validated under European settings.

Research aiming at development and validation of a new generation safe diagnostic immuno-reagents and assays, e.g. ELISAs based on RVFV recombinant antigens is strongly recommended. Cloning and expressing RVFV antigen would avoid any risk of residual virus in the test reagents, making it safer for routine use in RVF non-infected areas.

9. Viral Tissue Tropism

9.1. Overview of information required to assess the probability of contamination of products of animal origin with viable RVF virus

The probability that products of animal origin are contaminated with virus depends on

- the probability that the animal from which it is produced is infected,
- the part of the animal from which the product is produced,
- the processing undergone to produce the product, and
- the probability of the virus remaining viable through the processing procedures.

This section considers the second of those points. In order that the probability of viable RVF virus in animal products can be estimated, data on the probability of virus in specific tissues is required. Also required is any information on the level of virus likely to be found those tissues in at the point of slaughter (for meat products) or tissue harvest (for milk, germplasm etc.).

9.2. Data on tissue tropism and levels of virus by tissue type

9.2.1. Level and duration of viraemia in the live animal

RVF virus replicates to very high titers in many species (e.g. viraemia of 10^4 to 10^9 PFU/ml for several days). However, RVF-infected animals have a very short period of viraemia (Daubney *et al.* 1931; Findlay 1831; Easterday *et al.* 1962a; Easterday *et al.* 1962b; Coakley *et al.* 1967).

9.2.2. Virus in blood

RVF virus in blood presents a real hazard to humans who may come into contact with it, for the virus titres may approach 10^9 mouse lethal doses per ml in susceptible lambs or calves, at the peak of viraemia (Daubney *et al.* 1931; Findlay 1831; Easterday *et al.* 1962a; Easterday *et al.* 1962b; Coakley *et al.* 1967).

Animals may be viraemic for up to 3-days in sheep and 1-7 days in cattle (McIntosh *et al.* 1973a). Contact is most likely to occur, when viraemic animals are presented for slaughter. In particular, the slaughter of livestock by ritual methods, have been associated with infection and disease in man (Davies, pers. comm.). Infection has frequently been described amongst staff at large-scale slaughtering facilities. Those involved in the killing, bleeding, skinning, eviscerating and handling of offals and hides have been mostly affected. Those staff who handle the carcases after they have set for 2-8 h do not become infected (Swanepoel *et al.* 1981).

Blood is thought to infect directly via skin cuts and abrasions, or by droplet or aerosol infection via mucous membranes of the mouth and nares (Francis and McGill 1935; Kitchen 1950; Weiss 1957).

Dried or processed blood products prepared from infective animals may also present some risk, for the blood protein would serve to protect RVF virus. Those involved in the preparation of such products would appear to be at risk, but there is no data available to confirm this possibility. Similarly, the processed offals (liver, spleen and kidney or other tissue), which are frequently used in animal feeds, would present a similar risk to those

involved in their drying and preparation. Their presence in feedstuffs is unlikely to present a hazard if illegally fed to animals in the EU even without any heat treatment and to those handling processed feeds (van Velden *et al*. 1977; Joubert *et al.* 1951). There is no data suggesting that outbreaks might have been caused by contaminated feed.

9.2.3. Virus in muscle meat

RVF virus may be present at a high titre in muscle tissue at and immediately after slaughter, particularly if the animal is viraemic. At such times, it presents a serious hazard to those handling the carcase meat for immediate cutting and preparation as food. Within hours, the fall in pH to below 6.8 associated with the process of maturation and setting of the carcases is deleterious to the virus. The fall in pH results in the elimination of the virus after 4-8 h (Mims 1956). If febrile animals are killed however, rigor mortis and lactic acid formation does not occur and the pH changes may be minimal (Yedloutschnig *et al.* 1981c). In these situations, it is possible that infected tissues may find their way into animal or even human feedstuffs, where they may present a hazard. Historically, there is no evidence to suggest that human or animal infections have been produced in this manner.

9.2.4. Virus in internal organs

During and after the viraemic phase of a RVF virus infection in livestock, the virus is found in the liver, kidney, spleen, intestine, endocrine organs and carcase lymph nodes as well as the brain. The liver is considered to be the primary site of multiplication of virus (Findlay 1931; Mims 1956; Easterday 1965). The uterus, placenta, the foetus and foetal fluids are also highly infectious. Such animals may not necessarily show any clinical signs of disease. The fall in pH, which occurs in muscle meat after slaughter, when it sets, does not occur in these organs and tissues. Infectious blood is likely to be present in all these tissues for up to 7 days after infection, and remain a hazard for considerably longer. In one study (Yedloutschnig *et al.* 1981c), the virus persisted for 20-21 days in the brain, liver and spleen, and was found in the spleen after 30 days.

9.2.5. Virus in milk

Virus has been found in milk (Haig *et al.* 1953; Jouan *et al*. 1989), but historically there has not been any direct evidence that milk plays any role as a source of infection for humans nor animals during epidemic periods (Easterday 1965; Keefer 1972). Lambs suckling infected ewes do not become infected (Daubney *et al.* 1931). Two studies have suggested that there may be an association of RVF infection with milk based on epidemiological analysis of data from pastoral population groups handling animals (Jouan *et al*. 1989; Woods *et al.* 2002). There is a possibility that these included the family members, who were milking the cattle, camels or goats as well as handling the milk. Those animals, which have aborted, would have highly infectious discharges at the perineum, which could contaminate the udder and infect those milking them, as well as contaminate the milk. Field observations during many epidemic periods show that RVF infections occur amongst those directly involved with the infected animals, which includes those milking them by hand (sheep, goats, camels and cattle). Those living on farms, not associated with actual livestock, but handling milk for commercial, marketing or domestic use are not infected. Drinking milk is not identifiable as a source of infection. Milk products likewise would not appear to be a likely source of infection

(Daubney *et al.* 1931; Findlay 1932; Keefer 1972; Henning 1952; Easterday *et al.* 1962a; Davies, pers. comm.; Swanepoel, pers. comm.).

9.2.6. Virus in semen

There is no evidence for this. But it was concluded by McDiarmid and Thompson (1997) in their qualitative risk assessment that it is possible for virus to be present in semen.

9.2.7. Virus in embryos, foetus and placenta

The uterine contents, the uterine caruncles and placentomes, the foetal membranes and fluids and the foetus itself are known to contain high concentrations of virus (Yedloutshnig *et al.* 1981a; Imam *et al.* 1981; Davies, pers. comm.).

9.2.8. Virus in cadavers

Veterinarians are routinely infected whilst carrying out investigative post-mortem examinations of animals, which have died in the field (Daubney *et al.* 1931; Smithburn *et al.* 1949; Mundel and Gear 1951). RVF virus may be present at a high titre in all tissues and organs (especially blood) after a death in the acute or sub-acute stage of an infection, whether clinical or sub-clinical in character.

9.3. Conclusions on presence and level of virus in animal tissues at slaughter or harvest

Viable virus may be present in fresh blood, meat, internal organs, foetus, and milk of infected animals. The probable quantity of virus, and thus the infectivity of the fresh product, will depend upon the time at slaughter following infection. The virus has been found to persist in the parenchymatous organs such as liver and kidney. Thus high levels of virus are likely to persist in internal organs for longer than in muscle meat. The process of carcase setting is accompanied by a fall in pH to levels where the virus is inactivated (Chambers and Swanepoel 1980; Swanepoel 1981).

Blood, organs, fresh meat, foetal fluids and tissues, and hides all present a serious hazard to humans at and immediately after slaughter. This hazard persists in organs such as the liver, spleen and kidneys but rapidly disappears from meat as the pH changes, which is associated with setting of the carcase. Handling, processing and preparing fresh meat and organs for food creates a real danger of infection. Handling and processing offals and hides in the immediate post slaughter period is dangerous. Humans are at serious risk of infection from the foetal tissues and from the fluids, which heavily contaminate the perineal area and udder after abortions have occurred in livestock. Laboratory staff handling blood and tissues from suspected RVF carcases have historically experienced most accidental infection by RVF. Post-mortem procedures are a real source of danger to veterinarians from RVF infection. Milk is not considered to be a hazard. The importance of blood and bone or offal meal products as a vehicle for RVF virus has not been evaluated. These dangers only exist at such times as there is epidemic RVF virus activity in the country involved.

Use of carcases from febrile animals in the human or animal food chain would be dangerous, but this should not happen if appropriate inspections prior to slaughter are conducted. The danger would be likely to remain, if slaughtering was for family or private use at home or at a small slaughtering facility for immediate use.

The process of evisceration during and after slaughter exposes those humans involved to the greatest risk of RVF infection. Handling, trimming and cutting any of the above organs or tissues present the greatest hazard. In particular the foetal fluids are infectious and contact or droplet infections are likely to occur.

Internal organs can find their way into meat and bone meals (bone marrow would be heavily infected) and be a hazard to those in the production process if fresh, but digestive pH changes would be likely to inactivate any residual virus in the meal product.

Shepherds, farmers, milkers and slaughtermen are all exposed to RVF infection from these sources. The majority of infections of humans in the field are thought to occur in this way. The infection is likely to be via skin abrasions and contact with the highly infectious foetal fluids or by aerosol or droplet infection of mucous membranes.

RVF virus may be present at a high titre in all tissues and organs (especially blood) after a death in the acute or sub-acute stage of an infection, whether clinical or sub-clinical in character. The precautions routinely taken in most professional institutions should minimise such sources of infection, however veterinarians in the field are always at risk.

10. Stability of RVF Virus

10.1. Overview of information required

As indicated in Section 3.1 (release assessment outline), there are many possible mechanisms for transport of RVF virus from one region to another. The ability of the virus to survive in an infectious state in each of these transport mechanisms will impact greatly on the resultant risk at importation.

Data is therefore required on the ability of the virus to survive in animal tissues after slaughter or harvest; through preservation and processing of animal products; and in the environment as a contaminant, including in aerosols. Preservation and processing methods commonly include heating (especially cooking), cooling (chilling and freezing), and changes in pH (including post mortem in fresh meat), salinity, and water content (in air drying, salt curing etc). Environmental conditions which the virus may experience as a fomite contaminant include similar factors, plus the application of disinfectants and bleaches.

10.2. Data on the stability of RVF virus

10.2.1. Effect of heat

Experiments by Findlay (1931) showed that the virus was stable at 27°C in buffered solutions within the pH range 6.9-7.3 for at least 24h. The virus is destroyed at pH 8 within 20h at room temperature and 37°C. The virus may be isolated from carcase tissues, such as the spleen and liver between 36-72h after death. Virus remains viable in small aliquots of tissue in buffered saline for several weeks, when stored at 4°C. It is stable in serum at 4°C for several months, but only 3h at 56°C. Pasteurisation temperatures would be expected to result in rapid inactivation of the virus (Smithburn *et al*. 1949; Klein *et al.* 1969; Mims and Mason 1956; Craig *et al*. 1967; Findlay 1932; Davies, pers. comm.).

10.2.2. Effect of pH

RVFV is rapidly inactivated by acid pH below 6.8 (Findlay 1931; Kaschula 1953; Peters and Meegan 1981).

10.2.3. Stability in blood, blood derivatives, liver and bile

Infected sheep plasma retained RVFV infectivity after 8 years of storage and shipment under a variety of conditions of refrigeration (Easterday 1961).

RVFV infectivity was maintained in 'ordinary citrate' for at least a week at room temperature. In citrate or oxalate-carbol-glycerin preservative at 5°C the virus remained 'fully as virulent as [in] fresh blood' for up to 54 days. Reduced infectivity remained demonstrable for up to 147 days (Daubney *et al.* 1931).

Viraemic blood collected in an oxalate-carbol-glycerin preservative retained infectivity after 8 months at 4°C (Findlay 1932).

Defibrinated viraemic blood with 0.5% carbolic acid added retained infectivity for 6 months at 4°C (Findlay 1932).

Viraemic blood in phosphate buffer, pH 7.2, retained infectivity after 20min at 56°C, but not after 40 minutes (Findlay 1932).

RVFV in infected mouse blood was viable after being held for 20h at pH 6.6-7.3 and 'room temperature', but not at pH 8.0. At 37°C infectivity was lost at both pH 6.6 and 8.0, but not at pH 6.9-7.3 (Findlay 1932).

Infective titres of RVFV in serum from viraemic mice declined slightly (by 0.3-2.1 log10 mouse intracerebral LD50) after 1h at 56ºC, and titres of 5.0 log10 were still recorded after 3h at 56ºC (Mims and Mason 1956).

RVFV infectivity in both liver suspension and blood of infected mice was destroyed by mixing with a 1:50,000 solution of methylene blue [a photodynamic dye] and exposing the mixture to a 100 candle power light for 30 minutes (MacKenzie 1935).

RVFV infectivity in liver suspension or blood of infected mice was destroyed by exposure to a 0.25% concentration of formalin at 4°C for 3 days (MacKenzie 1935).

A suspension RVFV-infected mouse liver held in 20% ether at 4°C for 18-24h was doubtfully [sic] infective for mice at a dilution of 1:100, whereas control suspension was infective at a dilution of 1:10,000 (Andrewes and Horstmann 1949). Along with other lipid-enveloped viruses, RVFV is regarded as 'ether-sensitive'.

In confirmation of the above observation, 25% ether reduced the mouse intraperitoneal LD50 titre of RVFV in mouse blood and brain by 3.0-5.0 $\log_{10}$. RVFV suspended in a 1:1,000 concentration of sodium desoxycholate decreased in titre by 2.0 $\log_{10}$ (Theiler 1957). The temperature and duration of the holding period was not stipulated, and the method of titrating infectivity was not stated. Along with other lipid-enveloped viruses, RVFV is regarded as 'sensitive to bile salts'.

10.2.4. Stability in cell cultures

RVFV grown in cell cultures and stored in culture fluid at 4ºC retained infectivity for 30d without loss of titre (Craig *et al.* 1967).

Aliquots of RVFV were grown in cell cultures and stored variously at pH values of 6.2, 7.0, and 7.8, with 10, 20 and 40% serum added to the medium as a preservative and at temperatures of -20, -65 and -175ºC. All retained infectivity with a slight decline in titre when tested after 250d, but pH values of 7.0-7.8 and temperatures of -65ºC and -175ºC were optimal for preservation of virus infectivity (Klein *et al.* 1969).

RVFV in infected cell culture monolayers was inactivated by fixation in acetone at -70°C for 18h, but not in 15min (Easterday and Jaeger 1963).

RVFV-infected cell culture monolayers fixed and stored in acetone at -60ºC retained a 'small fraction' of infectivity after 48h, reported as 0.01%, but this was destroyed by 5-10min irradiation with two 15W ultraviolet lamps at a distance of 10cm (Hahon and Zimmerman 1969; Findlay and Howard 1951).

10.2.5. Stability in aerosols

Aerosols produced in cloud chambers at 23ºC (75ºF) in incandescent light (i.e. not ultra-violet light) and at 50 or 85% relative humidity from RVFV present in plasma of viraemic lambs, or in cell culture fluid, and tested by collection of samples in liquid impingers, retained 25% of the initial infectivity after 1 hour in the aerosol form (Miller *et al.* 1963). Loss of infectivity in the aerosols was estimated to range from 1 to 5% per minute. The authors remarked that the ease with which aerosol infection can be produced in animals accords well with the fact that RVFV is well known for causing infections in laboratory workers and those engaged in the livestock industry.

10.2.6. Stability in the environment

A laboratory assistant who had not worked with RVFV was diagnosed with infection 4mths after the virus had last been handled in the laboratory (Francis and Magill 1935). Fifteen days before his onset of illness he helped scrape and paint the walls and floor of a room where infected mice had been held more than 3mths previously. The case is remarkable not only for the implication that virus may have survived in the animal room for more than 3mths, but also for the putative length of the incubation period, which is generally <6 days.

10.3. Conclusions for persistence of infectivity in processed animal products and the environment

There appear to be no recent publications dealing specifically with the stability of RVFV, and some of the older findings are clearly contradictory. However, RVFV is a remarkably stable virus and the following conclusions can be drawn.

Infectivity is maintained in protein-rich medium (eg plasma or serum) for up to 20h at 'room temperature' (conventionally 22°C), 8mths at 4-5°C, and 8 years under a variety of (unspecified) conditions of refrigeration. Infectivity survives heating to 56°C for up to 3h, is most stable at pH 7.0-7.8, labile at pH < 6.8 or > 8.0, sensitive to ether and bile salts, destroyed by low concentrations of formalin, or by methylene blue in the presence of light.

Infectivity is stable in aerosols at 23°C and 50-80% relative humidity, with 25% of the initial infectivity being retained at 1h. Miller *et al.* (1963) remarked that the ease with which aerosol infection can be produced in animals, accords well with the fact that RVFV is well known for causing infections in laboratory workers and those engaged in the livestock industry.

No specific studies have been conducted to assess the survival of viable virus in animal products. But it can be inferred from the above data that it would be unlikely to survive in meat whether fresh, chilled or frozen due to the reduction in pH. If present in animal products, it could present a hazard to those handling these but virus would be immediately inactivated through heating, such as cooking or pasteurisation of milk.

The probability of the virus remaining viable when being carried as a contaminant on, - for example, shoes, tyres has not been investigated, but is not negligible as evidence of survival in blood and a recorded case of infection in a laboratory assistant 4mths after virus handling in the laboratory suggests.

11. Human Infection and Relevant Public Health Aspects

11.1. Human infections in countries with RVF

In countries where RVFV is endemic and where epidemics in animals (sheep, goats, camel, cattle) occur, human infections are frequent and may even reach epidemic proportions with many deaths.

11.1.1. Epidemics in humans

Major epidemics of RVF in humans occurred in Egypt in 1977 and 1993. During the 1977 epidemic, 200.000 human infections and 60 deaths were recorded. In 1987, an epidemic also occurred in Mauritania leading to 200 reported deaths. Further outbreaks occurred in 1991 both in Madagascar and Eastern Africa. In the latter epidemic, 89.000 cases of infection and more than 500 deaths were reported. A more recent epidemic in humans was observed in 1997-1998 in Kenya, Tanzania and Somalia. In 2003, an outbreak of RVF in humans occurred among farmers in a rural area of Egypt, while no cases in animals were reported. There were 45 human cases of clinical disease and 17 fatalities (WHO 2003).

In 2000, the first confirmed epidemic of RVF outside Africa in any animal species or humans was reported in Jemen and Saudi Arabia. In Saudi Arabia, 453 humans with suspected haemorrhagic fever required hospitalization from August to October (WHO 2000c); the case-fatality was 19 percent with a median age of 47 years and an age range varying from 1 to 95 years. In Jemen, the number of similarly suspected patients amounted to 1087 and 121 died. Here, the mean age of fatalities was 32,2 years and the age ranged between 1 month and 95 years (CDC 2000 a, 2000 b, 2000 c).

11.1.2. Clinical disease in humans

The forms of the clinical disease in humans are described in Section 8.2.2.2 of this monograph. In summary, RVF in humans in characterized by an acute febrile affection which is manifested as 4 possible clinical syndromes (Daubney et al. 1931; Findlay 1931; Swaenepoel and Coetzer 2004; Gerdes 2004), viz.

- An uncomplicated influenza-like illness, with headache, muscular pain, malaise, anorexia and prostration, usually leading to recovery
- Heamorrhagic fever with liver involvement, thrombocytopenia, icterus and bleeding tendencies, often with a fatal course
- Ocular involvement with retinitis and impaired vision
- Meningo–encephalitis characterised by confusion and coma following a febrile episode.

Fatality rates in infected humans are usually <1 % (Gubler 2002) but vary markedly from one outbreak to another. Mortality rates appear to have increased in recent years and were exceptionally high in the 2000 Saudi outbreak (14%) and in Jemen (Balkhy and Memesh 2003). In hospitalized patients, case fatality rates can be as high as 33 % (Al-hazmi et al. 2003). Data on the diagnosis of RVF in different animal species including humans are discussed in Section 8.2.

11.1.3. Modes of human infection

This part of the monograph brings together current knowledge on the mode(s) and risks of RVFV infection in humans as experienced and studied in the course of the epidemics mentioned previously. There is some overlapping with Section 9, where virus tissue tropisms and virus prevalence in tissues, secretions and excretions of acutely infected animals (sheep, goats, camels, cattle) have already been discussed. In Section 9, there are a number of references to hazards and infections for humans. The present section is intended to provide a schematic overview of the modes by which such infections in humans take place. The modes by which RVFV infections can occur in humans are as follows:

11.1.3.1. Infection via insect vectors

RVF in humans is, in principle, a vector-propagated and vector-based viral infection and occurs during an concurrent epidemic in animals, particularly in sheep, goats, camels and cattle. Viraemic animals are a source of virus for mosquitoes and for vector-based transmission. This route of transmission is established via anthropophilic mosquitoes and is the main mode of infection during epidemics in countries in which RVF occurs in animals and humans. Vectors can also be responsible for transmitting RVF among humans during the viraemic stage of infection.This mode of infecftion is also called the "urban peridomestic transmission cycle" (Gerdes 2004).

11.1.3.2. Infection via contact with live RVFV- infected animals

No horizontal transmission from infected animals to man occurs through casual or indirect contact. However, when such contact is intensive and direct, and when there is exposure to high virus quantities, virus transmission to humans may occur. Direct contact and the risk of infection mainly occur during animal handling and caretaking, when assistance is offered during parturition, when aborted tissues and fetuses are manipulated or when other close handling procedures are carried out (Zeki et al 1995; Wilson et al. 1994). In these circumstances transmission will mainly occur from animals during the viraemic stage, since they may carry virus titers as high as 109 mouse lethal doses per ml blood. Secretions and excretions may also contain high quantities of virus at this stage of the infection.

During the 1997-1998 outbreak in Northeastern Kenya, it was shown that, when contact with sheep body fluids and amniotic fluids had occurred and in cases where livestock were sheltered in one's home, there was a significant association with human infections (Wood et al. 2002). It has also been suggested that discharges from aborted animals may contaminate the perineum and can give rise to infecion in humans during the act of hand milking (Davies, personal communication). RVF in humans is thus an occupational hazard involving farmers (and their family members), shepherds, animal caretakers, veterinarians and other persons working in direct contact with animals in livestock industry. This occupational route of infection is also called the "sylvatic transmission cycle" (Gerdes 2004).

11.1.3.3. Infection at slaughter and butchering

High titers of RVFV are present not only in the blood of viraemic animals but also in the parenchymatous organs (see Section 9). Slaughter of such animals has been frequently associated with human infection, particularly in persons working in slaughtering

facilities and those involved in the slaughter, bleeding, skinning, eviscerating of animals and those handling tissues or organs (Wilson et al. 1194). Also, human infections occur during handling and processing of animal offals and hides (Van Velden et al. 1977; Joubert et al. 1951). This mode of infection truly represents an occupational risk and also belongs to the so called “sylvatic cycle” of transmission.

Low virus quantities have been recovered from the spleen and liver of experimentally infected sheep up to 20 days after inoculation (Yedloutschnig et al. 1981). Slaughtering animals at this late stage of infection, when virus quantities are low, represents a slight but real risk of occupational exposure during organ/offal processing. It has been suggested that human infection resulting from butchering tissues from sheep with a persistent infection may have played a role in the introduction of RVFV in Egypt in 1997 (Yedloutschnig et al., 1981).

RVFV is present in muscle meat when viraemic animals are slaughtered. However, if rigor mortis is allowed to occur, the pH in the meat falls below 6.8 within 2 to 8 hours after slaughter and all infectious virus present is inactivated at this acid pH (Mims, 1956; Swaenepoel et al., 1981; Swaenepoel and Coetzer, 2004). There is a risk of human infection when meat is handled, cut or otherwise prepared shortly after slaughter. Likewise, meat from viraemic animals that has been frozen immediately after slaughter contains infectious virus and therefore also presents a risk during later processing. Also, as rigor mortis does not occur, or only occur to a limited extent, e.g. in animals that are slaughtered when febrile (Yedloutschnig et al. 1981c), meat derived from such animals may present a further risk of infection for handlers. Moreover, since acid pH formation does not occur in parenchymetous organs, secretions or excretions,pH-induced virus inactivation does take place. Consequently these organs remain infectious for an extended period.

Persons who are not in direct contact with animals and who are not engaged in the milking of animals, but who may handle milk and dairy products derived from RVFV-infected animals in a location remote from such animals (e.g. in the course of marketing and distribution), have not been shown to become infected (Swaenepoel pers.comm.; Davies pers. comm.)

11.1.3.4. Infection at autopsy and laboratory processing

Handling of tissues (especially blood) or organs during autopsy of animals that have died in the acute or subacute stage of the disease has often resulted in infections in veterinarians (Daubney et al. 1931; Mundel and Gear 1951). Such risk not only exists when post-mortem examination is carried out on sick animals but also when subclinically infected animals are autopsied. Laboratory staff who process blood, tissues and tissue preparations from suspected RVF cases have experienced accidental infections; again such exposure represents a high level of risk (Mindel and Gear 1951; Van Velden et al. 1957).

11.1.3.5. Infection by milk or meat consumption

There is no evidence that the consumption of meat or raw mil kfrom infected animals can lead to RVFV infection in humans. Experimental feeding of high titers of virulent RVFV to lambs, puppies and kittens failed to induce infection (Easterday 1962; Keefer 1972), indicating that the oral route of infection does not occur, possibly as a result of lability of the virus to the acid pH in the stomach.

It is possible that, on occasion, infected animal organs or tissues have been incorporated in food intended for human consumption, and in animal feedstuffs; however, there is no indication that human infection has arisen in this manner.

11.1.4. Routes of human infection

While it has not been directly proven, there is much evidence to suggest that in humans, RVFV infection can occur via skin cuts or abrasions. It is also considered that inhalation of virus-containing droplets and aerosols created during the handling of infected animal material can result in human infections, particularly if virus titers are sufficiently high (Francis and McGell 1935; Kitchen 1950; Weiss 1957). The presence of such high virus quantities appears to be a necessity to facilitate virus entry into the human body. A primary site of replication at the natural portal of entry of the body is not known to exist, either at the level of the intact skin or at mucosal surfaces.

11.2. Risk of human infection during presumptive outbreak in EU-countries

In the event of a presumptive RVF outbreak in animals in the EU, the risk of transmission of RVFV to humans during handling and caretaking of acutely affected animals is high for persons engaged in the occupations at risk mentioned above for RVF countries (viz. farmers, animal caretakers, veterinarians, laboratory staff, etc.), since the animal handling procedures would be similar. The risk of infection in slaughterhouse staff is present but may be considered as being lower than that in RVF-endemic countries, as more precautions are likely taken in the larger slaughterhouses under direct regulatory supervision and inspection. However, the risk of exposure remains if slaughtering is carried out in a smaller, unregulated slaughtering facility or for family or private use at home and in particular, in cases where immediate processing of the carcases and offals of freshly slaughtered animals is carried out. In any event, the risk of infection is negligible for consumers of animal products.

11.3. Conclusions on the risk of human infection with RVFV in countries in which RVF is endemic and in the EU during a presumptive outbreak

In countries where RVFV is endemic and where epidemics are a regular occurrence in animals, human infections do occur. Most infections are mosquito-borne. However, infection in humans also results from direct and close contact with viraemic animals, and through direct contact with their secretions and excretions during caretaking, and with their carcases and organs including offals during, autopsy, slaughtering and butchering. Generally, for the infection to become established in humans, exposure to high virus titres generally needs to occur. RVF infection in humans, therefore, is clearly an occupational hazard. For this reason, males are relatively more likely to be exposed to infection. Virus entry presumably occurs via skin abrasions or via inhaled droplets. There is no evidence that either indirect contact with infected animals, ingestion of virus or the consumption of animal products containing the RVFV, leads to human infection.

In the event of an outbreak of RVF in animals within the EU the risk of human infection from close contact and handling of viraemic animals would be similar to that in countries in which RVF is endemic, since the persons thus exposed would be performing similar procedures. Slaughter of RVFV-infected animals in large well-supervised and

inspected slaughterhouses, in which more precautions would be applied, may decrease the risk. However, when slaughter takes place for personal or private use at home or in a small facility, the risk of human infection would be similar as in RVF countries. The risk for the consumer is low to non-existent.

11.4. Recommendations

Recommendations are made for consideration by risk management as follows:

- Public education during epidemics should be done with the objective of reducing human illness and death.
- The introduction of inspection and preventive measures and procedures at licensed slaughterhouse to minimize the risk of human RVFV infection during an epidemic.
- Prohibition of slaughter of food animals for personal use during an epidemic and for a period thereafter.
- Use of plastic gloves and face masks when assisting animals, suspected as RVF cases, at the time of parturition or abortion, and when handling aborted foetal material or organs from RVF-infected animals.
- Avoidance of exposure to, or contact with, infected droplets and aerosols during the slaughter of infected animals or in the course of manipulation of virus-containing material.
- Use of a P-3 level of biosecurity by laboratory workers when handling blood and other tissues from clinical or suspected RVF cases.

11.5. Future research

- Vaccine and immuno-modulating preventive measures need to be developed for rapid protection of persons with occupational hazard during an epidemic. Effort should be made to ensure that such vaccines become available for widespread use.
- Research requires to be conducted on the precise route(s) of entry of the RVF virus in humans, using animal models (skin, conjunctiva, aerogenic, oral, ...) so as to identify more effective preventive measures that can be prescribed during an epidemic.
- Proof is needed that ingestion or consumption of virus containing material does not lead to human infection.

12. EU Import Legislation for Live Animals and their Products

12.1. Legislative background

A comprehensive set of EU legislation exists detailing the requirements to be met in order to import live animals and their products into the EU. Specific requirements differ between live animals and their products. The most relevant legislative texts can be found in the EU website: http://europa.eu.int/comm/food/animal/index_en.htm. A summary providing 'General guidance for third country authorities on procedures to be followed when importing live animals and animal products into the EU' can be found on the same website.

12.2. Pre-export control mechanisms for live animals and their products

12.2.1. Data

12.2.1.1. Domesticated live animals and their products

Imports into the EU of live animals and their products are only allowed from approved countries, or approved regions within countries. A description of the approved region of the country is also given where appropriate. When a third country or part thereof has been listed in the appropriate Council or Commission Decision (the specific decision number varies with species) then it is approved in principle to export that species to the EU. However, further steps are needed before exports of live animals can take place.

An assessment of the specific disease situation is carried out. Special conditions may be required to minimise potential disease risks. These conditions will be defined in specific legislative texts and are reflected in the requirements laid down in the veterinary animal health certificate which must accompany all animals entering the EU. Animal health certificates for import must conform to EU requirements.

As of the date of this report, no countries from within the RVF region indicated on the map in Figure 7 are approved for the import of live animals of susceptible species.

Council Decision 79/542/EEC (EC, 1979a) lists third countries approved for imports of live animals and fresh meat of bovine, caprine, ovine and porcine species into the EU, animal health requirements for such imports and lays down the model animal health certificates to be used. Animal health requirements laid down in these certificates must be complied with and certified by the official veterinarian signing the certificates. For the listed domestic species no pre-export quarantine is foreseen.

Animal health requirements of the EU for RVF are:

- The exporting country (or region) must be free of RVF for 12 months (no testing or surveillance requirements for RVF).
- In the exporting country no vaccination against RVF has been carried out during the last 12 months and imports of domestic cloven-hoofed animals vaccinated against this disease are not permitted.
- Animals have remained on the territory since birth, or for at least the last 6 months (for breeding and productive animals, and 3 months for slaughter animals) before dispatch to the EU and without contact with imported cloven hoofed animals for the last 30 days.

However, pre-export quarantine provisions have been adopted since 2004 for certain ungulate species where no harmonised rules have yet been established (such as camelids) or for animals which were not for at least 6 months in an approved country of origin. For this purpose a Community quarantine station has been approved on the island of St. Pierre and Miquelon. Animals must stay for a period of at least 60 days in quarantine during which testing is carried out, which can then lead to a necessary extension of this time period.

During quarantine in St.Pierre and Miquelon animals must be subjected to the following tests for RVF:

1. ELISA, virus neutralisation test, or other recognised test in accordance with the protocols described in relevant sections of the OIE manual.
2. (Timing: the animals have to be tested twice: the first within two days from their arrival in the quarantine station and the second after at least 42 days from the first test.
3. Options for action following testing: If any animal displays evidence of exposure to Rift Valley Fever agent, then all animals present in the quarantine station are not considered eligible for entry into the EC.

The laboratory tests must be carried out in an EU approved laboratory and all laboratory tests and their results, vaccinations and treatments must be enclosed with the health certificate. In order to keep animal interventions to a minimum, sampling, tests and any vaccinations must be grouped as far as is possible whilst respecting the minimum time intervals required by the testing protocols.

According to information from the European Commission (H.Batho, pers. comm.), no importation has yet taken place via St. Pierre and Miquelon since approval of the quarantine station.

Fresh meat imports from currently 4 African countries (Botswana, Namibia, Swaziland, South Africa) and from none of the Asian countries relevant to this risk assessment (Saudi Arabia and Yemen) are approved.

No animal health requirements specific to RVF have been defined for imports of fresh meat or other products.

12.2.1.2. Circus and zoo animals

Council Decision 79/542/EEC does not cover imports of non-domesticated animals for shows or exhibitions where such animals are not regularly kept or bred, and those non-domesticated animals forming part of circuses, or intended for scientific including conservation or experimental purposes in a body, institute or centre that has been approved in accordance with Annex C of Directive 92/65/EEC (the approval requirements are defined in this Directive. According to information from the European Commission, they are in the process of harmonising import certificates for zoos and circuses (as currently being done for the intra-Community-movements of circuses). Import regulations in relation to zoo and circus animal importation are therefore the responsibility of the Member countries. Again, according to information from the European Commission so far no official body, institute or zoo has been approved in third countries for these purposes.

12.2.2. Conclusion

Import regulations for the relevant species are based on the FMD status of the exporting countries. Currently this overlaps with RVF occurrence and therefore is considered to provide some protection against importation of RVF infected animals and their products. This means that currently cattle, sheep, goats and pigs can only be imported from very few countries, none of which belong to the African continent or the parts of Asia (Saudi Arabia or Yemen) which is considered to have endemic RVF infection. This is different for zoo and circus animals which are the subject to import regulations that are the responsibility of member countries.

Current EU import legislation does not require any testing or surveillance specifically for RVF in the country of origin. Only "country freedom" is needed, which relies on the willingness and ability of a country to report RVF disease outbreaks. Therefore, if the FMD status of a country were to change and imports from such a country into the EU would be allowed, the risk of introducing RVF infected animals or their products would increase.

12.3. Safeguards (import control mechanisms) applied at the EU border

All live animals and products of animal origin imported into the EU from third countries must undergo veterinary checks at an approved Border Inspection Post (BIP) to verify their compliance with EU legislation. Therefore for legal importation, imports must arrive at officially approved BIPs. BIPs must be approved to carry out the required checks. Approval is given separately for live animals, products of animal origin, and germplasm. BIPs are currently (as at date of this report) listed in Commission Decision 2001/881/EC (EC 2001a).

Table 12 gives details of current (as of date of this report) EU BIPs.

Table 12: Type and Number of Border Inspection Posts in EU Member States as of 1 May 2005 (EC 2001a; EC 2004) (LA: live animals, P: products)

Country name	Port		Airport		Rail		Road		Total	
	LA	P	LA	P	LA	P	LA	P	LA	P
Austria	-	-	(2)	2	-	1	(2)	2	(4)	5
Belgium	-	4	(3)	4	-	-	-	-	(3)	8
Cyprus	-	1	1	1	-	-	-	-	1	1
Czech Rep.	-	-	(1)	1	-	-	-	-	(1)	1
Germany	(4)	9	(10)	10	-	1	(2)	2	(16)	22
Denmark	-	11	(2)	2	-	-	-	-	(2)	13
Estonia	1	3	-	-	1	-	(1)	2	(2)	5
Greece	(1)	2	(2)	2	3	(1)	(5)	6	2 (9)	11
Spain	(4)	22	(11)	19	-	-	-	-	(15)	41
Finland	(1)	2	(1)	1	-	-	(1)	2	(3)	5
France	(2)	14	1 (9)	15	-	-	(1)	2	1 (12)	31
Hungary	-	-	(1)	1	-	3	(4)	4	(5)	8
Ireland	-	1	2	(1)	-	-	-	-	1 (1)	2
Italy	(3)	17	(7)	14	2	(1)	(1)	2	1 (12)	35
Latvia	-	3	-	-	(1)	2	(2)	3	(3)	8
Lithuania	-	3	(1)	1	-	3	(1)	5	(2)	12
Luxembourg	-	-	(1)	1	-	-	-	-	(1)	1
Malta	-	1	(1)	1	-	-	-	-	(1)	2
Netherlands	-	6	(2)	2	-	-	-	-	(2)	8
Poland	(1)	3	(1)	1	-	-	(4)	4	(6)	8

Country name	Port		Airport		Rail		Road		Total	
	LA	P	LA	P	LA	P	LA	P	LA	P
Portugal	(1)	10	(4)	5	-	-	-	-	(5)	15
Sweden	(2)	5	3	(2)	-	-	-	-	(5)	8
Slovenia	-	1	(1)	1	(1)	1	(2)	2	(4)	5
Slovakia	-	-	-	-	-	1	(1/-)	1	(1)	2
United Kingdom	-	21	(6)	9	-	-	-	-	(6)	30
TOTAL									6 (122)	287

12.3.1. Import checks on live animals

Council Directive 91/496/EEC (EC, 1996) defines the principles governing the organisation of veterinary checks on animals entering the Community from third countries. Upon presentation at the BIP, Member States' official veterinarians examine the animal health certificates and all accompanying documentation to ensure they fulfil all the requirements provided for in European legislation. The checks always include a check of documentation, an identity check to ensure that the animals are as described in the documentation, and may include a physical check to ensure the animals do not show any signs of poor health.

Animals intended for immediate slaughter shall be conveyed without delay to the slaughterhouse of destination where they shall be slaughtered within five working days;

Animals intended for breeding, production or fattening purposes, and animals intended for zoos, amusement parks and hunting or wildlife reserves, shall be conveyed without delay to the holding of destination where they shall remain for a minimum period of 30 days before further movement outside the holding, except in the case of direct dispatch to a slaughterhouse. However, separation from animals already present on the holding is not required.

Clinical checks of imported animals at the holding of destination are carried out by the competent authority on a random basis and not systematically on all consignments in a non discriminatory way. There are no testing requirements for RVF or for any other disease.

12.3.2. Import checks on animal products

For products of animal origin from third countries Council Directive 97/78/EEC (EC 1997b) is relevant, and the following is a summary of veterinary checks at BIPs based on this directive. There are three main types of veterinary check; documentary, identity, and physical. The documentary check is carried out on all consignments to ensure all documentation is present and fully completed. The identity of all consignments is also verified by checking that the description on the consignment matches that on the documentation. In particular, health marks identifying the country of origin and establishment of origin must be present. For sealed containers, either an official seal has to be present, or the container is opened and checked as above. However, Community legislation foresees a reduction of the frequency of physical checks for products of animal origin, when the country of origin is an approved third country (see list in Council Decision 79/542/EEC), has a list of approved establishments and a model certificate for animal health and public health (EC, 1994a). The following frequencies are required 20% of consignments of fresh meat including offal and products of bovine, caprine, porcine and equine species, casings; 50% of consignments of milk and milk products for human consumption, processed animal protein; and

between 1% and 10% of consignments of semen, embryos, products, hides and skins, horns and horn products. Although physical checks are required on all consignments, for the majority of products only a percentage are normally checked, and this varies with the product.

12.3.3. Checking the efficacy of BIPs

Checking of imports at BIPs is one of the main safeguards for ensuring that imports, both of live animals and their products, pass the required legal standards. In order to ensure that BIPs are functioning efficiently, checks of BIPs are routinely implemented (Commission decision 2001/881/EC). Details of such checks are given in EU reports. Full details of the aims, legal basis, methods, findings, conclusions and recommendations can be found in these documents. The first aim of the inspections is to ensure the BIPs are acting in accordance with EU requirements for checking of imports.

12.3.4. Conclusions

The conclusions reached in the BIP inspection reports are that these safeguards are not 100% effective in ensuring that everything which enters the EU 'legally' conforms to EU animal disease and veterinary public health requirements. For example, 'consignments posing a potential risk cannot be fully excluded' [DG(SANCO)/8735/2002 - MR Final], and referring to potential risks for public and animal health - 'these risks cannot be completely excluded' [DG(SANCO)/9001/2002-GR].

13. Quantity and Sources of Legal Imports into EU and Movement Patterns within the Expanded EU

13.1. Overview of information required

The source country or region from which imports occur will affect their probability of infection or contamination with viable RVF virus. Countries with RVF present will be the most likely to carry the virus. However, given the possibility of unrecognised spread of infection to further countries, it is also of use to consider those where it is believed this spread is most likely to occur, particularly where they are on a recognised trading route to the EU. Given the specific unit risk for any item and route, the total quantity will affect the total risk of entry of viable virus to the EU. Therefore data on sources and quantities of legal imports of susceptible animals and their products into the EU is necessary.

Movement of animals and their products within the EU will affect the probability of establishment and spread of the RVF virus, given incursion and local infection. Therefore information on this movement is required.

13.2. Data on EU legal importations and movements within the expanded EU

13.2.1. Import of live animals

Table 13 indicates that small ruminants were not imported into the EU during the period 1995 to 2003 from any of the RVF endemic countries or those in their neighbourhood. Limited numbers of cattle importations were recorded during 1995-2002 from Turkey, Egypt, Algeria and Morocco, but none in 2003. This information was sourced from FAOSTAT and crosschecked with EuroStat, and it is to be noted that the EU did not approve any imports of live animals of susceptible species from RVF endemic countries (see 12.2.1.1).

Table 13: Cumulative number of cattle and small ruminant imports into EU from countries located in the RVF endemic regions or in its neighbourhood (source: FAOSTAT)

	1995-2002			*2003*		
Country of origin	***Cattle***	***Goat***	***Sheep***	***Cattle***	***Goat***	***Sheep***
Egypt	183	0	0	0	0	0
Morocco	96	0	0	0	0	0
Lebanon	40	0	0	0	0	0
Algeria	21	0	0	0	0	0
Turkey	9	0	0	0	0	0
Kuwait	6	0	0	0	0	0

13.2.2. Import of animal products

Trade of animal products (ovine and bovine meat) follows the same pattern as for live animals with most ovine meat traded within the EU. African countries such as Namibia, Botswana and Zimbabwe have significant bovine meat exports to the European Union (see Table 14).

*Table 14: Bovine and ovine meat imported into European countries from countries located in the RVF endemic regions or in its neighbourhood (tons carcase weight; * countries where RVF is likely to be present and endemic; source: FAOSTAT)*

	1995-2002		2003	
Country of origin	**Bovine**	**Ovine**	**Bovine**	**Ovine**
Algeria	65		3	
Angola	9			
Bahrain	36			
Benin *	98			
Botswana *	124,737		10,850	
Burkina Faso	11			
Burundi	14			
Chad *	31			
Congo *	103			
Egypt *	505	8		
Eritrea *	195	2		
Gabon *	105			
Gaza Strip	107			
Ghana *	73			
Iran	154			
Israel	333	35		
Ivory Coast *	306			
Jordan	224			
Kenya	0	49		
Kuwait	147			
Lebanon	85	56		
Liberia	8			
Libya	50			
Madagascar *	7814	43		
Mauritania *	21			
Morocco	213	8	1	
Mozambique *	0	38		93
Namibia *	92,792	34	11,724	13
Niger	3	8		
Nigeria	3			
Oman	36			
Qatar	21			
Saudi Arabia	265			
Serbia	83			
Sierra Leone	10			
South Africa *	3,224	10		81
Swaziland	4,153		1014	
Syria	140		13	4
Tanzania *	3			
Tunisia	19			
Turkey	360	17	101	
Uganda *	500			
United Arab Emirates	101			
Zaire	38			
Zambia *	22			
Zimbabwe *	82,922		360	

13.2.3. Origin of legal imports from countries with RVF

The legal requirements for export to the EU are detailed in Section 11.1. In summary, the animals and derived products coming from the trading countries identified in

Sections 13.2.1 and 13.2.2 which also have RVF usually originate from areas considered by National Veterinary Services to be disease-free.

14. Illegal Imports of Animals and their Products

14.1. Definition of illegal imports

Illegal importation of animals and their products includes all attempted third country importations at places other than BIPs, and importations at BIPs which attempt, by whatever means, to evade the normal import requirements. In the UK, responsibility for detection now rests with HM Customs and Excise, and each Member country will have its own enforcement agency.

14.2. Illegal importation at recognised international entry points

For imports declared at BIPs, including cargo in ships and aircraft, details of the checks undertaken are given in Section 12.3. Illegal importation may be attempted via incorrect and misleading documentation. Some illegal imports may comprise items concealed within cages, containers etc., which will only be discovered if the contents are physically examined. In the UK, not all consignments declared of animal origin are physically examined. The proportion of consignments declared to be of non-animal origin which are physically examined is even lower than that for those declared to be of animal origin (UK Defra International Animal Trade Division, pers. comm.). The situation regarding physical examination in other EU countries is believed to be similar.

Illegal importation may also be attempted simply by concealment without any attempt to make a declaration, and this may occur either at a BIP or other recognised international border entry point, including those for land, sea and airport entry. Detection then relies upon the vigilance of the enforcement authorities.

Incorrect disposal of aircraft and ship waste may also constitute illegal entry.

14.3. Other entry routes

Illegal importation may be attempted via routes not recognized as border entry points. Such routes include small boats or aircraft entering at places other than ports or international (customs) airports, and land border crossings not at border posts. Again, detection then relies upon the vigilance of the enforcement authorities. Although a potential route, no information is currently available, on probability or frequency of smuggling by this route.

14.4. Eastern borders

The eastern side of the EU is characterized by a long land border, lending itself to opportunities to effect illegal importation. It might be assumed that a land border is more easily crossed than sea or air. Although a potential route, no information is currently available, on probability or frequency of smuggling by this route.

14.5. Illegal imports: GB as a case study

In April 2001, UK Defra set up the Illegal Animal Products Seizures (ILAPS) Database, to allow all those concerned with detecting illegal imports into GB to record seizures following attempted illegal importation of animal products. Data has been collected on meat (including poultry), fish, dairy products, and other goods, for example honey. The

database has a number of limitations; in particular the initial recording of data was poor. Places from which seizure was made comprise: cargo, personal baggage, shop/warehouse or transit shed. Product descriptions of items seized include fresh and frozen meat products, and a variety of processed products. For some seizures the species from which the product was derived has been identified and stated, but for some this was not possible, and some contain mixed products. Routes of entry include ports and airports (M Wooldridge, *pers comm*.). A full description of the database, its contents and its limitations is given in Adkin *et al.* (2004), an illegal import risk assessment for Great Britain for products of ruminant and porcine origin. In this risk assessment, it was estimated that only a small fraction of attempted illegal imports of meat products was detected (between approximately 0.01% and 1%, and the total quantity illegally imported per year was estimated to be between 4,398 and 28,626 tonnes.

15. Vaccines against RVF and Contamination Issues

15.1. Overview of information required

One of the identified routes of RVF virus incursion into the EU was via vaccine. The vaccine itself could be imported, the vaccine could be used in animals in countries with RVF, and the animals imported whilst infectious due to live vaccine virus, or the vaccine could be made in the EU and viable virus escape from the manufacturing laboratory. It may also be a vaccine intended to protect against a different organism, but contaminated with RVFV.

In order to assess the probability of entry via RVF vaccine, relevant information on the properties of RVF vaccine are needed e.g. is the vaccine live attenuated or killed, what is the evidence for infectiousness in vaccinated animals, are vaccinated animals allowed into the EU legally. Relevant information on vaccine imported into the EU and made in the EU is also needed, including quantities imported, quantities made in the EU, biosecurity at EU laboratories etc.

The likelihood of contamination of non-RVF vaccines with RVFV is dependent on the prevalence of infection in the country of origin, and whether GMP (good manufacturing practice) in used during the vaccine production process.

15.2. Production of RVF vaccine

Various killed vaccines have been made, but not used extensively (Randall *et al.* 1964; Roth and Spickler 2003).

The only vaccine against RVF which has been extensively used, is the Modified Live Virus Vaccine (MLVV) developed by Smithburn (1949). It is cheap to produce. Another modified live virus vaccine strain has been developed, the MP 12 strain, but this has similar foetopathic effects, has no other benefits and has not been used in the field.

MLVV Smithburn vaccines are/were being made in Cairo, S Africa, Kenya, and maybe Botswana (Davies, pers. comm.).

Table 15: Rift Valley Fever vaccines (after Roth and Spickler 2003)

Country	*Manufacturer*	*Strain of Organism*	*Type of Vaccine*	*Adjuvant*
Egypt	Veterinary Serum and Vaccine Research Institute	Smithburn strain	Live	None
Egypt	Veterinary Serum and Vaccine Research Institute	ZH-501 strain	Killed	Aluminum hydroxide
South Africa	Onderstepoort Biological Products	Smithburn strain	Live	None
South Africa	Onderstepoort Biological Products	South African isolate	Killed	Aluminum hydroxide

There is no Community Pharmaceutical legislation that prohibits vaccine companies to produce such vaccine on EU territory and there is no obligation to notify this to the European Commission. However, there is currently no evidence suggesting that such production takes place nor that any Member country has given marketing authorisation for such a vaccine.

According to Council Directive 2001/82/EC (EC 2001b), in the case of a serious outbreak of an epidemic disease, a Member country may provisionally allow the use of immunological veterinary medicinal products without a marketing authorisation, in the absence of a suitable medicinal product and after informing the Commission of the detailed conditions of use.

15.3. Legal aspects of RVF virus vaccine import and use within the EU

The EU follows a non-vaccination policy for exotic diseases (diseases not present in the Community) such as RVF. The general control measures to be taken in case of an outbreak of RVF within the EU are described in Council Directive 92/119/EEC (EC 1992) and are based on a stamping-out policy. Vaccination against RVF may not be carried out except as a supplement to control measures used in case of an outbreak (emergency measures). When a Member country intends to introduce vaccination against RVF, it must submit the details of the vaccination campaign (area, duration, vaccine used…) to the Commission and this must be approved with the agreement of the other Member States. Vaccination of animals on suspected farms as well as the use of hyper-immune serum injection is prohibited. Vaccinated animals are subject to certain movement restrictions.

Under exceptional circumstances, e.g. in a situation of extreme urgency, the decision to vaccinate can be taken by the Member countries concerned, but must be revised by the Commission in co-operation with Member countries. As RVF has not yet occurred in the EU, vaccination against RVF has never been used.

15.4. Biological properties of RVF vaccine

15.4.1. Use in humans

The vaccine developed by Randall *et al*. (1964) has been used to immunise humans, and 3 separate inoculations were found to give a neutralising antibody response. This vaccine has been used to protect laboratory workers in America and Africa with apparent success. This vaccine is not available commercially nor has it been approved for use in humans by any drug licensing board.

15.4.2. Use in farmed livestock

Killed vaccines have been generally poor immunogens, requiring 2-3 doses to develop protection against abortion in livestock (Randall *et al*. 1964).

Many millions of doses of the Smithburn MLVV vaccine have been used in Africa. This strain may be foetopathic and abortigenic in a proportion of susceptible small ruminants but does not produce any clinical effects in cattle. Up to thirty percent or more pregnant sheep and goats may abort in highly susceptible breeds following vaccination (depending upon their susceptibility) and there may be foetal abnormalities following its use (Yedloutschnig *et al.* 1979*;* Davies pers comm.). However in Egypt and many African countries, no complications follow its use in the indigenous breeds of sheep and goats (Davies pers comm.). Routine use of the vaccine in non-pregnant animals has been widely and successfully practised in high-risk areas, where valuable exotic breeds are kept. The vaccine provides life long immunity (Davies pers comm.).

The use of vaccine in an outbreak situation is hazardous. Virulent virus activity occurring at a low level can be greatly amplified by needle propagation by vaccination teams in the field.

15.5. Conclusions

The probability of Smithburn modified live virus vaccine containing pathogenic RVFV is negligible, but iatrogenic transmission may occur through use of the same needle in multiple animals in an outbreak situation.

The probability of RVFV contamination of non-RVF vaccines sourced from infected countries is not negligible.

15.6. Recommendations

The quality control and safety testing procedures required for any vaccines imported into the EU should ensure that both laboratory and animal inoculation tests have been carried out with the imported batch. Detailed and strict protocols should be prepared and followed by the producer should any RVFV vaccine be imported into the EU. This is to eliminate the possibility that the vaccine could be contaminated by other vaccine seeds or contaminants which are prepared or are endemic in the producing country.

Controls should be implemented to allow detection of possible RVFV contamination in imported live vaccines.

16. Risk Assessment for Risk Question 1: Release Assessment

What is the probability of viable RVF virus entering the EU?

16.1. Overview of information required

The information required to assess the probability of viable virus entering the EU is indicated in Section 3.1. To summarise, the *ideal* data requirements are:

- The countries/regions where RVF is present
- Species susceptibility to RVF
- The prevalence of RVF (by species) in those countries/regions where RVF is present (including humans and insect vectors)
- The probability of detection, given infection
- Pre-export safeguards (including diagnostic testing, restriction of sources etc)
- EU Border safeguards
- All potential mechanisms for transport of virus into the EU
- Numbers/quantities of exports (legal and illegal) of each susceptible species (including humans) and its products into the EU; routes and duration of journey
- Types of pre-export processing for animal products (freeze, cook, dry-cure etc)
- Viral tropism, levels of virus, and stability of virus under all conditions during pre-export processing and transport to EU
- Natural insect vector incursion into EU; routes and numbers

Where the ideal data is not available, the best available information (including, where necessary, expert knowledge) is used to allow estimates of the probability of entry via each of the potential mechanisms identified.

16.2. Information on countries and ecological zones affected by RVF

All countries in Sub-saharan Africa are infected with RVF virus (see Figure 7). The Arabian Peninsula and the Egypt are also infected, but to date there is no evidence that any of the N African (Mahgreb) countries from Libya to Morocco have been infected with the virus. Many individual countries in Africa currently do not or will not provide any reliable information on RVFV activity in their countries. There are many reasons for this; technical inability, awareness of the negative impact upon livestock trade, many are in denial that they have RVFV at all, despite published work showing its presence. Thus the assessment of the extent of infection includes both published data and expert opinion or the authors of the risk assessment.

Currently there is no evidence to suggest that RVF virus is or ever has been active in Eastern Asia, India or South America. The recent emergence of RVF virus as an epidemic in Arabia should drive active surveillance for RVFV in neighbouring countries, particularly Turkey, Iraq and Iran.

Within the affected countries, the presence and prevalence of RVF will vary with the ecology. A brief description of the ecological zones in Africa, where RVF occurs is given below:

- Sahelian Acacia savannah
- West and East Sudanian savannah

- Flooded savannah zones (flood plains, inland delta's, dams, irrigation schemes etc.)
- *Acacia-Commiphora* bushed savannah and alluvial fans
- Grassland and shrubland systems (moist to dry)
- Forest savannah mosaic systems

The periodicity of RVFV activity at epidemic level is much less frequent in the drier zones of Africa, than in the wetter highland and coastal forest and bushed and wooded grassland zones.

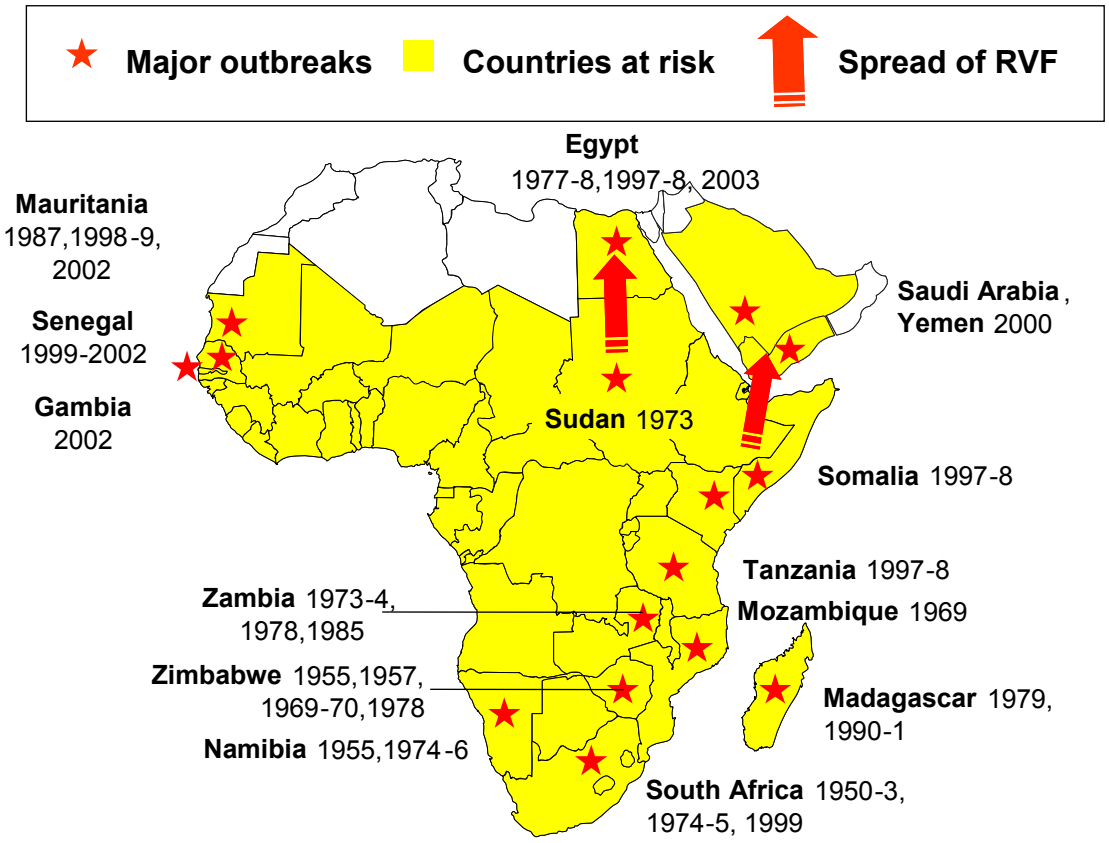

Figure 7: RVF distribution, outbreaks and spread

16.3. Factors influencing prevalence of RVF infection in source countries

16.3.1. Presence of susceptible species

A list of susceptible species is given in Section 5, with an indication of susceptibility. Additional information on routes of infection for humans is given in Section **Error! Reference source not found.**.

Information on the distribution of cattle and small ruminants within Africa and Asia is given in Section 6. Vectors are, by definition, present in each country and region in which RVF occurs. Humans are also present.

16.3.2. Stage in the endemic/epidemic cycles of RVF

See Section 7.4.1 for details on the endemic/epidemic cycle, including the effect of the climate and flooding on vector activity and the probable levels of virus activity, given infection in the vectors.

16.3.3. Conclusions on the probability that a randomly selected animal, human or insect vector in a country in which RVF is present is infected with RVF

The prevalence levels of RVF in infected regions change from season to season with the prevailing climatic conditions. However, no specific data to indicate the likely point prevalence of infectiousness in any species is available either for endemic or epidemic stages in the cycle.

The prevalence levels amongst susceptible species are likely to be negligible during inter-epidemic periods, when there is little or no RVFV activity in Africa, Egypt and Arabia. This is the case for domestic livestock, humans and vectors.

During an epidemic period, the prevalence in susceptible species (including humans and vectors) in the affected regions is likely to be much higher, as suggested by the serological data from Senegal (Section 7.4.1, Figure 6). During a human epidemic, the prevalence is likely to be highest amongst those who work with animals and their products; farm workers, veterinarians, abattoir workers etc. Models are being developed which, given the appropriate climatic and other data, are able to predict the probable onset of an epidemic period.

During an epidemic period in a given region, the conservative assumption is that probability of infection in any susceptible species within that region is at least moderate and may be high, and in humans particularly so if they work with animals and their products.

16.3.4. Recommendations

If pre-epidemic conditions have been recognised, disease risk can be substantially reduced if all animal-related trade with high risk countries is temporarily discontinued. Predictions produced by RVF models incorporating climatic and other relevant data need to be used to inform these decisions.

Predictive models based on remote sensing satellite data will become available that will allow informing RVF specific surveillance. Sentinel herd data is available from several countries in the region, but this is retrospective, and the information is only available after the period of highest risk has passed. Agreement might be reached to allow the EU to manage its own sentinel herds within countries considered to pose greatest risk.

Sentinel herds should be established in those countries considered to be at highest risk from RVF. These should be monitored for RVF antibody, particularly during any period, when epidemic RVF is occurring in Egypt, the Horn or East Africa. RVF could enter and be maintained without manifestation of obvious clinical disease. There are several other insect borne virus diseases of African livestock, which present disease risks to the EU (ie African horse sickness, bluetongue, ephemeral fever, Akabane virus, Lumpy skin disease). A common surveillance project should be instituted to screen sentinel animals for all of these. They could all enter the EU with vectors in the air-currents of the Eastern Mediterranean Convergence Zone.

The disease-free status of a country, zone or compartment should be based on results from specific RVF serological sample surveys in regions and their neighbours, not on passive surveillance,

16.4. Probability of detection given infection

The probability of detection depends upon a number of factors. It will vary depending upon whether the infection is endemic or epidemic. It will vary with the quality of the surveillance system in the country or region. It will vary with the number of animals suffering disease, and whether or not humans are infected and clinically affected. It may well also vary with the political will of the region to discover the infection. These factors will also themselves vary between countries and regions, and some will also be affected by recent weather patterns, and longer-term climate changes. Thus to assess the probability of detection is both very difficult, and changing.

16.5. Methods by which viable RFV virus could travel from source country/region to the EU border

These have been identified in Section 3, Figure 2. Summarising, the potential routes are:

- infected live animals legally imported,
- contaminated animal products legally imported,
- infected live animals illegally imported,
- contaminated animal products illegally imported,
- infected vectors,
- infected humans,
- fomites (on shoes, tyres, etc) and
- vaccines (via imports or laboratory escapes).

Given infection present in the source country/region, for each of these potential routes, the probability of successful movement of virus into the EU needs to be considered.

Livestock, other susceptible vertebrates and meat products may be transported from the region of origin both legally and illegally. To assess the effect of legislation, it is necessary to know what legal safeguards are in place.

With all methods of virus entry, given the probability with which any single item is infected or contaminated, the total quantity of importation will affect the overall probability of importation of viable virus.

16.6. Probability of introduction of viable RVF virus into the EU via infected live animals legally imported

16.6.1. Factors influencing import of infection via legally imported live animals

The probability that live animals of species susceptible to RVF are infected at the point of export depends upon the probability of infection within the region of concern. This, in turn, depends upon the prevalence of the infection, whether or not any testing or selection is required or undertaken before export, and the sensitivity of any tests used. If infected at export, the probability that they are infectious (i.e. harbour viable virus) at the point of import depends and the probability that infection will be recognised in transit or at the EU border. This in turn depends upon the duration of the journey and the incubation period of the disease. Animals may travel from RVF infected areas to arrive at their destination within the incubation period of the disease.

Given the probability of infection per imported animal, the total probability of introduction depends upon the total numbers imported.

The best available information on the regions in which RVF is present and the prevalence of infection in those regions is given in Sections 16.2 and 16.3, along with an outline of the environmental and climatic factors affecting prevalence.

Legislation on the importation of live animals into the EU, including source, testing and selection requirements, is detailed in Section 11.1. This section also details the checks required at BIPs and gives an indication of the efficacy of BIPs.

Diagnostic methods for RVF, including clinical examination, and an estimate of their sensitivity are presented in Section 0. The incubation period is given in Section 8.2.2.1.

Import numbers and countries of origin for live animal imports for cattle sheep and goats are given in Section 13.2.1. No specific information is given on probable journey duration.

Zoo animals can be imported subject to EU member countries regulations, and no information on numbers of animals or possible infection prevalence is available.

16.6.2. Conclusions on probability of legally imported animals carrying viable RVF virus into the EU

The presence of FMD precludes countries with endemic RVFV from exportation of livestock and livestock-related products to the EU.

The Eurasian Ruminant Street could be a source of introduction of transboundary animal diseases into Europe. However, a comparison of the regions in which RVF is currently known or believed to be present, and those from which the major legal EU importation of live animals susceptible to RVF takes place shows that for sheep and goats, these regions do not overlap. This leads to the conclusion that the current probability of importing RVF via these species is negligible. If this trade pattern continues and the FMD infection status of RVF endemic countries does not change, and if the geographical distribution of RVF endemic regions does not change, the probability of importing RVF in legally imported sheep and goats would remain negligible.

The following conclusions are based on the assumption that the EUROSTAT data used to draw conclusions about risk as a result from animal imports. A comparison of the regions from which cattle are imported and RVF areas shows Egypt in both categories, RVF being described as likely to be endemic in Egypt. In addition, Morocco and Algeria, both countries from which cattle have been imported, neighbour countries which have had RVF outbreaks. Risk reduction for legal imports from these countries therefore relies on the import regulations applied and the probability of detection if RVF is present, either in cattle for export or at the EU border. However, the regulations require that animals originate from disease-free zones monitored by the National Veterinary Services and trans-shipment through infected zones is not allowed (see Section 11.1). From section 16.4, it can be seen that under appropriate surveillance circumstances the probability of detection is likely to be high. Assuming that imports will only be allowed from countries with such surveillance systems, the probability of importing RVF with legal cattle importation is currently assessed as low, although not negligible. However, it

should be noted that the above countries are not on the list of approved countries for importation of live animals (79/542/EEC).

If livestock are imported from geographical zones close to those in which RVF is endemic, the probability of infection in those livestock will increase during epidemic or pre-epidemic periods.

The probability of importation of RVF infected zoo animals is likely to be higher than negligible, if not higher specifically if it occurs during RVF outbreaks in the source country.

16.7. Probability of introduction of viable RVF virus into the EU via contaminated animal products legally imported

16.7.1. Factors influencing import of infection via legally imported animal products

Animal products include meat (fresh and processed), milk (fresh, pasteurised and processed), hides, wool, germplasm and any other animal product.

The probability that animal products of species susceptible to RVF are contaminated depends first upon the probability that the animal from which they are derived was infected, which is the probability of infection within the region of concern (as per Sections 16.2 and 0). The probability of contamination at the point of export then also depends any legal safeguards prior to export (that is, upon whether or not any testing or selection of the live animals or their products are required or undertaken (Section 11.1). Also, it will depend upon the sensitivity of any tests used (Sections 0 and 16.4).

It then further depends upon the pathogenesis of the organism, in particular tissue tropism and probable level of tissue contamination, and then upon the specific processing undertaken. If contaminated at export, the probability that the products harbour viable virus at the point of import depends upon the level of pathogen at the point of export, the duration and conditions of the journey. Given the probability of contamination per imported item (which will vary with type), the total probability of introduction depends upon the total numbers imported.

Information on tissue tropism and probable levels of virus per tissue at slaughter is given in Section 9.

Information on the effect of environmental conditions, e.g. temperature, humidity, pH, etc, on RVF virus in animal tissues is given in Section 10.

Import quantities and countries of origin for imports for bovine and ovine meat are given in Section 13.2.2. No specific information is given on the product type (i.e. fresh, frozen, cooked, cured, other processing), nor for any other species. No specific information is given on probable journey duration.

16.7.2. Conclusion on total probability of introduction of viable RVF virus into the EU via contaminated animal products legally imported

As for live animals, a comparison of the regions in which RVF is currently known or believed to be present, and those from which the major legal EU importation of animal

products from species susceptible to RVF takes place shows that for ovine meat, these regions do not overlap. This leads to the conclusion that the current probability of importing RVF via ovine meat is negligible. If this trade pattern continues, and if the extent of RVF does not increase, the probability of importing RVF in legally imported ovine meat would remain as negligible. No legal importation of goat meat is recorded, and it is believed that none currently occurs.

Bovine meat once again has an historical import pattern which includes countries with RVF present; Namibia, Botswana and Zimbabwe, although importation from Zimbabwe is no longer allowed. Risk reduction for legal imports from these countries therefore again relies on the import regulations applied, in turn based on the probability of detection of RVF in the area sourcing the product. As for live animals, the regulations require that the products originate from disease free zones monitored by the National Veterinary Services. Although, as described in Section 16.4 active surveillance has a high probability of detecting RVF, expert opinion indicates that the probability of effective active surveillance beign conducted is actually low. Given this, the probability of products for legal export being sourced from cattle infected with RVF is currently assessed as low, or possibly even greater than low.

If any bovine product from such sources does contain viable virus at the point of slaughter, conclusions presented in Section 9.3 indicate that contamination of fresh meat with viable virus can fall rapidly with time as the pH falls in meat after slaughtering and inactivates the virus. Chilled carcases and hides are unlikely to contain viable virus 4-6 hours after slaughter. However, virus may persist in meat and blood if the animal was fevered when slaughtered, as well as in internal organs, as virus-destructive pH changes are then unlikely to occur. Chilled viscera (liver/spleen) may remain a source of viable virus. Section 11.1 indicates that importation of offal is not allowed, and meat must be deboned. Removal of offal from legal importation is a safeguard which will reduce the probability of viable RVF virus being present in legally imported animal product. However, Section 10.3 indicates that RVF is a remarkably stable virus, and although susceptible to heating, it can survive at 'room temperature', and in particular under refrigeration, for extended periods.

In the absence of any data to the contrary, if the conservative assumption is made that the majority of legally imported animal product is frozen for export soon after slaughter the conclusion would be that the majority of any viable virus present would survive until thawing post-import. Thus the probability of import of the virus cannot be assumed to decrease due to processing and the probability of viable virus being imported from such bovine product therefore remains low, or possibly even greater than low, rather than decreasing to negligible.

Milk has not been incriminated as a source of RVF infection thus it is concluded that imported milk is unlikely to contain viable virus whether legally or illegally imported.

16.8. Probability of introduction of viable RVF virus into the EU via infected live animals illegally imported

16.8.1. Factors influencing import of infection via illegally imported live animals

The probability that live animals of species susceptible to RVF are infected at the point of export depends upon the prevalence of the infection at source. Since the movement

is illegal, the conservative assumption is that no testing or selection is undertaken prior to export, thus there is no change in probability of infection. If infected at export, the probability that they are infectious (i.e. harbour viable virus) at the point of import, depends upon the duration of the journey, the incubation period of the disease, and the probability that the attempted illegal importation will be detected at the border and stopped. Given the probability of infection per imported animal, the total probability of introduction depends upon the total numbers imported.

With the conservative assumption being made that no testing or selection is undertaken for illegal export, the probability of infection at the point of export is the same as that of the probability of infection within the region of concern.

No information is available on the illegal importation of live animals by number or route. However Section 14 indicates some of the factors which need to be considered. Illegal import may be attempted at BIPs by the use of forged documentation as to the animal's source. Illegal import may also be attempted at non-BIP entry points, via the long land border of eastern EU, or from small boats or aeroplanes.

Economic factors are generally considered to play a large part in illegal activities, and no information on this is available.

16.8.2. Conclusions on probability of illegally imported live animals carrying viable RVF virus into the EU

Concealment of live animals of species susceptible to RVF through BIPs is assessed as being unlikely due to the high probability of detection. Illegal import through BIPs by the use of incorrectly completed or forged documentation is assessed as being more likely, and the probability of infection then depends upon the animal's origin. However such animals would be included in data on legal importation, since this is how they would appear. Thus for sheep and goats to present a significant risk, they would first have to have travelled from a country infected with RVF to one of those from which legal imports are recorded. The probable costs of doing this suggest this is unlikely. For cattle the situation is slightly more difficult to assess. Imports of cattle from Egypt with forged documents may in some circumstances make economic sense.

Illegal importation of live animals along the lengthy eastern EU border, for any species from a country with RVF again means they must previously have travelled considerable distances. However, movement across from Egypt, for example, in small boats or aeroplanes with arrival at an isolated or concealed EU destination may again on occasion make economic sense.

The overall conclusion therefore is that from reasonably proximate countries, if favourable economic conditions exist, the probability of illegal importation of live animals may be higher than negligible. And, given no legal safeguards, if RVF is present in those countries, the probability of infection and introduction is also higher than negligible. However, as the costs and practicalities of moving live animals are considerable, compared with animal products, the numbers may be restricted and the latter the more likely route.

16.9. Probability of introduction of viable RVF virus into the EU via contaminated animal products illegally imported

16.9.1. Factors influencing import of infection via illegally imported animal products

The probability that animal products of species susceptible to RVF are contaminated depends first upon the probability that the animal from which they are derived was infected, which is the probability of infection at source. Since the intended importation is illegal, the conservative assumption is that no testing or selection is undertaken prior to use of the animals, thus there is no reduction in probability of infection. The other factors relevant to contamination at the point of export are then as in Section 16.7.1 i.e. as for legally imported goods, as are those relevant to contamination at the point of import.

As for live animals, illegal import may be attempted at BIPs by the use of forged documentation as to the product's source and illegal import may also be attempted at non-BIP entry points, via the long land border of eastern EU, or from small boats or aeroplanes. As with live animals, given the probability of contamination per imported item (which will vary with type), the total probability of introduction depends upon the total quantities imported.

Unlike for live animals, some data is available for illegal imports, at least into GB (Section 14). In the absence of any information to the contrary, the assumption can be made that the illegal importation of animal products into other parts of the EU is broadly similar in quantity, product type, routes and sources, and estimated success rates. Then there is the addition of potential illegal importation via the long eastern EU land border.

16.9.2. Conclusions on probability of illegally imported animal products carrying viable RVF virus into the EU

Based on the GB illegal imports of animal products risk assessment, it is reasonable to conclude that a substantial amount of animal product is successfully illegally imported into the EU via each international port and airport on a regular basis, in both personal and commercial luggage, and from every region of the world including those where RVF is present. Given lack of export safeguards, it can therefore be concluded that the probability of exporting to the EU concealed animal products contaminated with RVF virus is higher than negligible, and under conditions of high viral activity may even be moderate to high.

A wide variety of conditions of transport and product types were identified in the GB risk assessment. Given the stability of RVF virus under various tissue and environmental conditions, and assuming an equal diversity, it is concluded that if contamination is present at the point of export, the probability of at least some virus retaining viability at the point of import into the EU is high.

16.10. Probability of introduction of viable RVF virus into the EU via infected vectors

16.10.1. Factors influencing import of infection via infected vectors

The probability that vectors within a source country are infected with RVF virus depends upon the presence and prevalence of RVF virus within the vectors in the source country. Given presence (see Section 16.2), the prevalence will depend upon the prevailing stage in the endemic viral cycle (see Sections 7.4 and 0), which will in turn depend upon the climatic conditions currently or recently prevailing.

Vectors may enter the EU naturally, on wind currents, and the distance from source country and prevailing air currents will affect the probability of their successful arrival. Data on distances vectors are believed to travel on the wind is given in Section 7.6. Vectors may also enter within, for example, aircraft, cars, and boats. No specific information is currently available regarding this probability. Under these conditions, the distance from the source country is of less significance. Vectors arriving naturally will be subject to no legal safeguards. However, it is possible to include safeguards (e.g. insecticidal spraying before disembarkation) for vectors which arrive by other means.

16.10.2. Conclusions on probability of infected vectors carrying viable RVF virus into the EU

It can logically be assumed that windborne vectors from countries closest to the EU are more likely to arrive successfully and in greater numbers than from more distant countries. From Section 16.2 it can be seen that the regions closest to the EU currently infected with RVF are Egypt and the Arabian countries. During intense viral activity - i.e. an epidemic period - the prevalence of RVF infection in vectors has been assessed as high (see Section 16.3.3), but negligible during an inter-epidemic period.

It is therefore concluded that during a period of intense RVF virus activity in Egypt or Arabia, infected vectors could be carried in low level wind currents into Turkey, Cyprus and possibly other countries in SE Europe with a probability greater than negligible, and possibly as high as moderate. This incursion has happened in the past, with African horse sickness and bluetongue. The characteristics of the Red Sea Convergence Zone are a determining factor for such north-west wind currents from Egypt and the Middle East.

Although no specific information is available it is reasonable to conclude that, compared with numbers of vectors likely to be borne on wind under favourable conditions, the numbers entering within aircraft, cars etc is likely to be very small. A large number of mosquitoes under these circumstances would most likely be unpleasant, noticed and dealt with. However, whatever the baseline risk, it is also reasonable to conclude that the probability of infected vectors entering within aircraft, cars etc. is increased during periods of high viral activity in the country of origin unless specific safeguards are in place.

16.11. Probability of introduction of viable RVF virus into the EU via infected humans

16.11.1. Factors influencing import of infection via infected humans

The probability that humans within a source country are infected with RVF virus depends upon the presence and prevalence of RVF virus within other susceptible species in the source country. Given presence (see Section 16.2), the prevalence will depend upon the prevailing stage in the endemic viral cycle (see Sections 7.4 and 0), which will in turn depend upon the climatic conditions currently or recently prevailing. The probability of human infection is also dependent upon the exposure of humans to the RVF virus, and this depends in large part on their occupation and living conditions (see Section **Error! Reference source not found.**).

The major safeguard against infected humans travelling to the EU is the likelihood that clinical disease (possibly preventing travel) and/or diagnosis of infection occur prior to transport. This will also depend upon the sensitivity of clinical or other diagnostic testing. Information on clinical symptoms and likelihood of diagnosis is given in Section **Error! Reference source not found.**. Section **Error! Reference source not found.** gives data on two recorded cases of infection identified in international travellers distant from countries with RVF.

16.11.2. Conclusions on probability of infected humans carrying viable RVF virus into the EU

Whilst travelling to the EU when infected with RVF has been demonstrated as a possibility, the number of records of humans identified as infected after international travel is only two in total (Davies, pers comm.). This is more likely to happen with humans experiencing incubating or a mild inapparent infection. Clinical illness would tend to reduce the desire to travel, and in general the agricultural occupations associated with infection are probably not associated with the level of salary making long-distance international travel frequent.

Even allowing for under-reporting, therefore, the probability of this method of introduction of viable virus is assessed as negligible to very low, depending upon the level of viral activity in the source country.

16.12. Probability of introduction of viable RVF virus into the EU via contaminated fomites (e.g. shoes, tyres etc)

16.12.1. Factors influencing import of infection via contaminated fomites

The probability that fomites within a source country are contaminated with viable RVF virus depends upon the presence and prevalence of RVF virus within the source country. Given presence (see Section 16.2), the prevalence will depend upon the prevailing stage in the endemic viral cycle (see Sections 7.4 and 16.3), which will in turn depend upon the climatic conditions currently or recently prevailing. The probability of fomite contamination will also depend upon the probability of exposure of the fomite to the virus. No specific information exists on this probability, but in general exposure of fomites (e.g. soles of shoes; car and lorry tyres etc) will be associated with human movement into an infected area, and thus to an extent subject to similar travelling and occupational factors.

Given contamination of fomites, the stability of the virus in the environment (see Section 10) and thus the duration and conditions of the journey, will affect the probability of viable virus being present at the EU border.

16.12.2. Suspected or possible transmission by fomites

Many authors have described the failure of RVF virus to spread from animal to animal by contact (Daubney *et al*. 1931; Davies, unpublished data). However, low levels of transmission of RVFV have been described from infected to uninfected sheep under experimental conditions where these have been held in very close contact. One in four animals exposed became infected in one series of experiments (Yedloutschnig *et al.* 1981c). The precise route and mechanism of transmission is not known, but fomites may play a part. Other workers have found this contact transmission difficult to reproduce. In the field, new cases cease within 3-5 days, when livestock are moved from an environment where the vectors are present to an area where they are not (Daubney *et al.* 1931).

16.12.3. Conclusions on probability of contaminated fomites carrying viable RVF virus into the EU

As for infected humans, in general the agricultural occupations associated with proximity to infection of relevant fomites (e.g. shoes, car tyres) are probably not associated with the level of salary making long-distance international travel frequent. One type of fomite more likely to travel long distances is a lorry or other container collecting meat from slaughterhouses. If the container is emptied of infected meat prior to travel to the EU, not cleaned effectively, and then travels on to the EU, there is a possibility that drops of contaminated debris remain.

Section 10.3 concludes that RVF virus is remarkably stable, and can survive in, for example, plasma or serum for up to 20 hours at room temperature. Therefore, if it is present on fomites within such a medium (for example within drops of fluid from an infected animal), it must be concluded that it would be possible under some conditions to survive the duration of a rapid international journey.

In summary, whilst fomites may be a vehicle for transmission of RVFV, the probability is considered low.

16.13. Probability of introduction of viable RVF virus into the EU via vaccines (importation of vaccine, vaccinated animals, or laboratory escape)

16.13.1. Factors influencing import of infection via vaccines

RVF vaccine entry into the EU will depend upon the requirements surrounding its import, the import of vaccinated animals or, if produced within the EU, laboratory biosecurity (see Sections 11.1 and 13). Whether the vaccine contains viable virus or not will depend upon its method of manufacture, and its type (i.e. killed, live attenuated etc) (see section 15.4).

Entry of non-RVF vaccine contaminated with RVFV will depend on the prevalence in the source control and the efficacy of laboratory quality control methods during production.

16.13.2. Conclusions on probability of vaccines carrying viable RVF virus into the EU

Reversion of RVF vaccines remains a theoretical possibility, however the use of tens of millions of doses during inter-epidemic periods has not been associated with any untoward incidents. Such intensive use of vaccine has happened in South Africa, Zimbabwe, Kenya and Egypt.

Introduction of RVF through importation of RVF contaminated non-RVF vaccines is a theoretical possibility, as it has never been reported elsewhere. The risk may increase if as a result, for example. of a bluetongue outbreak in the EU increased quantities of vaccine are imported, and are sourced from production facilities in RVF endemic countries not applying good manufacturing practice.

16.14. Tabular summary of probability of introduction of viable RVF virus into the EU by method

A summary of the assessed probability of importation of viable RVF virus into the EU by method of introduction considering current trade patterns is presented in Table 16.

Table 16: Summary of probability of RVF introduction into EU

Method of entry of viable virus into the EU	***Assessed probability of entry***	***Section***
Infected live animals legally imported	For sheep and goats: negligible For cattle: low, not negligible: increases during epidemic periods in source country For zoo and circus animals: greater than negligible	16.6
Contaminated animal products legally imported	For sheep and goat products: negligible For cattle products: low, or possibly even greater than low: increases during epidemic periods in source country	16.7
Infected live animals illegally imported	Much uncertainty: dependent upon route and economic situation: negligible to low? Increases during epidemic periods in source country	16.8
Contaminated animal products illegally imported	Moderate to high: increases during epidemic periods in source country	16.9
Infected vectors	Negligible during inter-epidemic periods; low to moderate during epidemic periods in source country	16.10
Infected humans	Negligible during inter-epidemic periods; very low during epidemic periods in source country	16.11
Contaminated fomites (e.g. shoes, tyres)	Negligible during inter-epidemic periods; low during epidemic periods in source country	16.12
Vaccines (imported or laboratory escape; contamination)	RVF vaccines: Negligible as long as no vaccines based on live virus are imported Low, not negligible if vaccines based on modified live-attenuated virus are imported Non-RVF vaccines: Negligible as long as GMP compliant production	16.13

16.15. Recommendations to reduce the probability of viable RVF virus entering the EU

Early-warning surveillance systems for EU countries need to be developed which incorporate surveillance data information from RVF endemic countries as well as from predictive models.

The movement and export of livestock should only take place when there is no likelihood of any RVFV activity occurring at the point of origin. Disease risk can be minimised if importations of live animals during pre-epidemic periods, and periods of epidemic virus activity in Africa are discontinued. Such a ban may be imposed regionally, to include Egypt and the Horn of Africa, Central and South Africa and the West according to the prevailing climatic influences, which operate at these regional levels.

The OIE Recommendations for RVF provide good guidelines for trade with one reservation. The recommendation that a country, which has been infected with RVF virus, may be declared free from infection after a period of 4 years, is however hazardous. A country, which has been infected, and experienced ongoing RVFV transmission cycles, may have established a cryptic infection of floodwater breeding mosquitoes, which can transmit the virus transovarially. The conditions for the emergence of these floodwater breeding species may not occur within a four year period, but require exceptional conditions, to allow emergence to a level where the test systems would be sensitive enough to detect virus activity.

The role of quarantine measures in preventing the introduction of viable RVF virus into European countries is difficult to evaluate, thus recommendations on this are problematic. If infected animals enter a quarantine station before export, and there are mosquitoes or other biting flies present, further transmission and amplification of the virus could occur. Insect proofed quarantine facilities are only possible where relatively small numbers of animals are involved. Further 'pre-release' quarantine at a BIP in Europe would again suffer the same risk, if viraemic animals were present.

Import regulations for zoo and circus animals need to be standardised for all EU countries, and take possible RVF infection into account.

16.16. Future Research

Identification of the climatic conditions allowing long-distance aerial movement of vectors is necessary.

Existing predictive models need to be developed further to provide reliable forecasts of high risk periods in RVF-endemic countries.

17. Risk Assessment for Risk Question 2: Exposure Assessment

What is the probability of exposure of susceptible livestock within the EU to viable RVF virus?

17.1. Overview of exposure pathways

17.1.1. Summary of identified livestock exposure pathways

The following is a summary of the potential livestock exposure pathways identified in Section 3.2.

Direct livestock exposure routes:

- virus arrives in vector; livestock directly exposed
- virus arrives in vaccine (via import or EU laboratory escape); livestock directly exposed
- virus arrives by any other route (includes infected livestock, contaminant on animal products or fomites); livestock are directly exposed

Indirect livestock exposure routes:

- virus arrives in vector, transmits to vectors of the same species (most likely via transovarial and possibly by sexual transmission); livestock exposed to EU infected vectors
- virus arrives by any route other than vector or vaccine (includes infected livestock, contaminant on animal products or fomites); vectors are directly exposed and become infected; livestock exposed to EU infected vectors

The two indirect exposure routes both involve the infection of competent vectors present within the EU. The types of information required are also summarised in Section 7.3.

17.1.2. Components involved in assessment of exposure to viable virus

Exposure of EU livestock to the viable virus of RVF relies first on exposure of livestock to the transporter of the virus i.e. the potentially infectious animal or vector, or the potentially contaminated animal product or fomite, and the level of exposure to this transporter mechanism. Part of the exposure assessment is the assessment of the probability of this exposure.

Exposure to viable virus then depends upon the probability of this transporter mechanism being infected and infectious (prevalence of infection), or contaminated with viable virus at the point of import (for an import risk assessment).

The final aspect of exposure is the probable quantity of viable virus to which the livestock is exposed, since the consequences will depend upon this quantity. With respect to infected animals and vectors, this probable quantity will depend upon the stage of pathogenesis at which exposure occurs; available quantities of viable virus can both increase and decrease. However with viable virus as a contaminant, whether on animals products or fomites, the total quantity once separated from live hosts can only decrease due to the effects of its environment and the duration in that environment. Part of the exposure assessment is the assessment of the probable level of exposure.

Thus these aspects all need to be taken into account.

17.2. Direct exposure of livestock to viable virus in vectors arriving wind-borne from outside the EU

17.2.1. Factors influencing the probability of exposure of livestock to viable virus in vectors arriving wind-borne from outside the EU

The probability of exposure to arriving vectors will depend upon the proximity of livestock to these vectors, and the total numbers of both arriving vectors and native livestock in this close proximity to each other. The probability of exposure to virus depends upon the probability that these vectors are infected and infectious.

Section 7 indicates the probability of vector arrival and most likely incursion areas. Section 6 indicates the distribution of livestock by species within the EU. Section 16.3.3 gives the probability that vectors are infected. An assumption is made that if vectors come from an infected area, and are themselves infected, they will pass through an infectious period.

17.2.2. Conclusions on probability of exposure of livestock to viable virus in vectors arriving wind-borne from outside the EU

There are significant populations of susceptible livestock species present in those areas where vector incursion is assessed as being likely. It was also concluded that, during certain wind patterns, many vectors could arrive en masse. The probability of exposure to vectors under these conditions is therefore assessed as high.

The probability of exposure to infected vectors depends upon the stage of viral activity within the countries with RVF present. During inter-epidemic periods it is assessed as negligible, but during periods of high viral activity in Egypt and the Middle East may be up to moderate.

17.3. Direct exposure of livestock to viable virus in vaccine (via import or EU laboratory escape)

17.3.1. Factors influencing the probability of exposure of livestock to viable virus in vaccines

For import of vaccines, the most obvious route of exposure is use of the vaccine. Currently RVF vaccine is not used for livestock in the EU, as RVF is not considered to be present. If the use of vaccines becomes likely in the future, then assessments of the number of animals vaccinated would be necessary to estimate probability of exposure. Section 16.13 assesses the probability of infectious virus being present in the vaccine.

For laboratory escape, the probability of exposure will depend upon the probability of viral escape, and proximity of the production or storage laboratory to livestock populations. The Smithburn modified live virus vaccine strain is innocuous for man (it has been used as a human vaccine on occasion). No laboratory escape has been reported or documented.

The probability of infection from use of contaminated imported non-RVF vaccines depends on GMP compliance at manufacturing level and quality control procedures on imported batches.

17.3.2. Conclusions on probability of exposure of livestock to viable virus in vaccines

The probability of direct exposure of livestock to RVF vaccines within the EU (from any source) is currently assessed as negligible. However this could change in the future if RVF vaccines are produced or used within the EU.

The probability of exposure of EU livestock to non-RVF vaccines could increase during outbreaks of diseases such as bluetongue, but the probability of RVF infection as a result of vaccine contamination should be negligible as long as good manufacturing practices are adhered to during production and quality control procedures are used on imported batches.

17.4. Direct exposure of livestock to viable virus arriving in the EU by any other route

17.4.1. Factors influencing the probability of exposure of livestock to viable virus from any other route

Section 7 indicates that RVF is a vector-borne infection and therefore, in general, livestock will only become infected from a vector. Sections 16.6 to 16.9, 16.11 and 16.12 assess the probability of entry of viable virus into the EU by methods other than vector or vaccine.

The probability of exposure of EU livestock to imported animals depends upon the probable use to which the animals are being put. Thus the exposure to any imported animals which are infected (whether infected by natural means, or infectious due to the shedding of vaccine virus) depends upon the same factors. For imported animals going straight to slaughter, the probability of exposure of native livestock to these animals or any virus they carry is very low. For breeding animals, or animals going via a market, the probability of native exposure will be much higher, but exposure to infectious virus will also depend upon whether imported animals are 'quarantined' on farm or in any other way post-import but prior to mixing. Section 16.8 assesses the probability that imported animals are infected. Given the probability of infection in any individual imported animal, the total probability of exposure of native livestock to viable virus will depend upon the total numbers of animals imported, by intended use. Although Section 13.2.1 gives data on import numbers, there is currently no information on intended use.

Most animal products imported are likely to be primarily for human use, in particular for consumption. Meat and edible products in particular are likely to have a lengthy and complex exposure pathway to livestock via, for example, retail, home, cooking, then depositing of scraps in the countryside. Due to the primary use of the product, both the quantity of meat product, and the quantity of any viable virus (of any type) available for livestock exposure tend to decrease by a large proportion at each step in the exposure pathway. The effect on the quantity of viable virus is due not only to the reduction in quantity of meat product at each step, but also the combined effect of duration and environmental conditions on a virus, cooking often being a major factor. Details of the exposure pathway for illegally imported meat products can be found, for GB, in the risk assessment for illegal imports of meats undertaken for Defra (Adkin *et al.* 2004). The livestock exposure pathways for legally imported meat are likely to be similar to illegal, once they reach retail, and it is reasonable to assume that those for other countries within the EU will be broadly similar to those in GB. The GB risk assessment concluded

that the probability of exposure of livestock to viable virus on contaminated meat products, for a number of viruses (though not specifically RVF virus), was very low.

For viable virus arriving as a contaminant on fomites, although no specific data is available, it is reasonable to assume that exposure pathways are once again likely to be complex, unless the fomite is directly intended for farm or livestock use. As for animal products, the viability of the virus through the complex exposure pathway will depend upon the duration, the environmental conditions, and the matrix in which the virus resides.

17.4.2. Conclusions on probability of exposure of livestock to viable virus from any other route

Based on the known biology of RVF virus, the assumption is made that for direct transmission (i.e. without vector) a high titre is necessary, thus the assessment concentrates on the exposure of EU livestock *to a high titre* of viable virus from other routes.

The probability of exposure to imported livestock will vary with their intended use. Section 16.6 assesses the probability that such animals will be infected, which varies with species, legality of importation, and in particular viral activity in source country. With respect to infectious animals, since a group of animals in the epidemic phase generating a high quantity of virus is likely to be clinically obvious (see Section 8.2.2.1), the probability of export, deliberate mixing and thus exposure to a high virus titre is also assessed as likely to be negligible to very low at worst (i.e. in epidemic periods).

The probability of exposure to a *high virus titre* from contamination on either animal products or fomites is assessed as likely to be negligible due to the complexity of the exposure pathways and the effect of these pathways (e.g. dilution, environment, cooking etc.) on the concentration and viability of virus.

17.5. Indirect exposure of livestock via infection of an EU vector from an arriving vector of the same species

17.5.1. Factors influencing the probability of exposure of livestock to a vector infected within the EU from another infected vector of the same species arriving in the EU

Species similarity is necessary in order that, given infection, sexual and transovarial transmission directly occurs. This would also represent an early stage in the establishment of infection in local populations. The first essential requirement for this is contact between arriving and native vectors of the same species, so arriving vectors must enter in areas with EU vectors of the same species in close proximity. Data on the species found in the EU is given in Section 7.3.1, and Section 7.3.2 indicates that there are a considerable number of species of vectors within the EU which are known as competent transmitters of RVF outside the EU. No specific information is available on their EU distribution, but favourable climatic conditions for their habitat are outlined in Section 7.3.

Section 16.10 assesses the probability of infected vectors entering the EU and the probability of these arriving vectors being infected was assessed as varying from negligible to moderate, depending upon viral activity in the source country. The

probability of infection of local vectors will depend upon the total numbers of infected vectors arriving.

Given that local vector infection occurs, the next factor is the proximity of susceptible livestock species. Section 6 indicates the presence of susceptible livestock species throughout the majority of the EU.

17.5.2. Conclusions on probability of exposure of livestock to a vector infected within the EU from another infected vector of the same species arriving in the EU

It was concluded (Section 16.10.2) that, during certain wind patterns, many vectors could arrive en masse. It is reasonable to argue that climatic conditions in the EU most favourable to those vector species also found in RVF endemic areas are those in relatively close proximity to the Southern and South-Eastern EU borders. If this argument is correct, the probability of exposure of local vectors to arriving vectors of the same species is high. Exposure to infected vectors leading to infection of local vectors is therefore assessed as varying from negligible (during inter-epidemic periods) to moderate (during epidemic periods). Over time, the presence of periods of moderate likelihood in the cycle is likely to lead to at least some local infection.

There are significant populations of susceptible livestock species present throughout the majority of the EU, and the probability of livestock exposure to local vectors in any area where vectors are present is assessed as high. If these local vectors have become infected, then the total probability of livestock exposure to infected vectors will depend upon the prevalence of infection of local vectors, which is likely to vary (as in already endemic RVF areas) and experts in Working Group vary in their assessment as considering it between negligible and moderate.

17.6. Indirect exposure of livestock via infection of an EU vector which was infected by viable virus arriving via any route other than a vector

17.6.1. Factors influencing the probability of exposure of livestock to a vector infected within the EU from any route other than vector

The exposure routes other than an arriving infected vector by which an EU vector species (or potential vector species) might be exposed include imported infected livestock, viable virus as a contaminant on animal products or fomites, and infected humans. Sections 16.6 to 16.9, 16.11 and 16.12 assess the probability of entry of viable virus into the EU by methods other than vector or vaccine.

One major factor here is that the potential vector exposed to the above may belong to a species which is potentially competent, but has not previously been associated with transmission in endemic RVF countries - perhaps because it is not present in those countries. Thus exposure of all potentially competent vector species must be considered. Information on such vectors and potential vectors in the EU is given in Section 7, where it was concluded that there is a high probability that, given the introduction of infection, at least some EU species would be competent vectors.

Unless specific protective measures are in place, exposure of EU vectors to all other virus transport mechanisms (animals, animal products, fomites, humans) could occur as

early as the BIP or other place of EU entry, although animal products are likely to be packaged in some way, reducing immediate exposure. If infection or contamination is present, exposure to the highest viral titres is likely to be from infected animals or humans. The arrival of infected animals in Europe, to an area where there are many mosquitoes and biting flies, could allow amplification of the virus in the vector populations. As infection of the vector from these sources represents the expected method of transmission if a competent vector is exposed to, and feeds from, an infected animal or human at the appropriate stage of the disease, transfer of infection would be expected. The specific primary vector species only become relevant, if the virus is to become established in a transovarial transmission cycle.

Given exposure, infection of vectors from contamination on animal products or fomites will depend upon the dose and route of contact. However, the probability of exposure of vectors to a high titre on such products or fomites is assessed as negligible to low.

Given local vector infection, the second factor is the proximity of susceptible livestock species. Section 6 indicates the presence of susceptible livestock species throughout the majority of the EU.

17.6.2. Conclusions on probability of exposure of livestock to a vector infected within the EU from any route other than a vector

The probability of exposure of potentially competent EU vectors to imported livestock and humans travelling to the EU is assessed as high. If those imported livestock, or humans, are infected with RVF, then the probability of exposure to a high virus titre is assessed as high. However, the probability of any individual animal or human entering the EU being infected with RVF was assessed as negligible to low (see Section 16.14), the worst case being at times of high viral activity in source countries. Overall therefore, the probability of exposure of EU vectors to RVF virus from imported livestock or travelling humans is assessed as from negligible to low.

The probability of exposure of potentially competent EU vectors to imported animal products or fomites is assessed as moderate to high. However, even given contamination, the probability of exposure to a high titre of viable virus under any of these conditions is assessed as being negligible to low.

Overall, it is concluded that the probability of potentially competent EU vectors being exposed to high quantities of viable virus from routes other than infected hosts or vectors varies from negligible to low. Infection will depend upon the route, dose present and the dose required for infecting such vectors.

However, if local vector species do become infected, from whatever source, then exposure of susceptible livestock species to RVF virus is assessed as from negligible upwards, for the same reasons as given as in Section 17.5.2.

17.7. Tabular summary of probability of exposure of EU livestock to viable RVF virus by exposure route

A summary of the assessed probability of exposure of EU livestock to viable RVF virus by exposure route is presented in Table 17.

Table 17: Summary of probability of RVF exposure of EU livestock

Exposure route of livestock within the EU	***Assessed probability of exposure***	***Section***
Direct exposure from viable virus in vectors arriving wind-borne into the EU	Exposure to vectors: high Exposure to infected vectors: negligible in inter-epidemic periods, may go to moderate during epidemic periods in Egypt and the Middle East	17.2
Direct exposure from viable virus in vaccines (from any source) arriving in the EU	RFV vaccines: Currently negligible, but could change if vaccine produced or used within EU Non-RVF vaccines: Negligible, as long as vaccines are produced based on GMP	17.3
Direct exposure of livestock to viable virus arriving in the EU by any other route including livestock, contaminated animal products, fomites. (Note: As RVF is a vector-borne infection, it is considered that only exposure to a *very high titre* of virus by any route other than vectors of vaccine is significant)	Exposure to imported livestock: depends on intended use of imports. Exposure to *a high virus titre* from imported livestock: negligible to very low at worst (i.e in epidemic periods). Exposure to *a high virus titre* from animal products or fomites: negligible.	17.4
Indirect exposure of livestock via infection of an EU vector from an arriving vector of the same species	Exposure of local vectors to arriving vectors: high Exposure of local vectors to infected arriving vectors: varying from negligible to moderate Infection in local vectors due to infected arriving vectors of same species: negligible to moderate at any specific time, thus likely over time to lead to at least some local infected vectors. Exposure of livestock to local vectors: high. Exposure of livestock to local infected vectors: from negligible upwards; probability increases with time and varies with weather pattern and climate.	17.5
Indirect exposure of livestock via infection of an EU vector of any competent species from any source other than an arriving vector, including infected livestock and humans, contaminated animal products and fomites.	Exposure of local vectors to incoming livestock, humans, animal products and fomites: high Exposure of local vectors to infected animals or humans: negligible to low Exposure of local vectors to a high virus titre from contaminated animal products and fomites: negligible to low Exposure of livestock to local vectors: high. Exposure of livestock to local infected vectors: from negligible upwards; probability increases with time and varies with weather pattern and climate.	17.6

18. Risk Assessment for Risk Question 3: Consequence Assessment

What is the probability of

- infection of livestock within the EU with RVF,
- persistence of RVF virus within the EU (in vectors, in livestock or other susceptible species excluding humans), and
- infection of humans by the handling or consumption of products derived from animals infected within the EU?

18.1. Probability of infection of livestock within the EU with RVF

18.1.1. Factors influencing probability of infection of livestock within the EU with RVF

Exposure to the virus is necessary before infection can occur, and Sections 17.2 to 17.6 assess the probability of exposure of EU livestock to RVF virus from different sources; vectors, vaccines, and other sources (contaminated animal products and fomites).

RVFV would be likely to be pathogenic for most breeds of ruminant species in European countries. The outcome of the animal/virus contact and the infection/disease syndrome produced will vary with the susceptibility of the exposed populations, the dose of virus to which the animals are exposed, and the route of exposure. The outcome will differ with genotype, age, and, for females, stage in reproductive cycle (see Section 5.2).

18.1.2. Conclusions on probability of infection of livestock within the EU with RVF

Section 17 assessed that exposure to a high virus titre would be very unlikely from any source other than infected vectors. However, this is the normal transmission method, and the exposure of susceptible species to vectors in an infectious state is assessed as moderately likely to result in infection. This probability would increase with numbers so exposed.

18.2. Probability of persistence of RVF virus within the EU (in vectors, in livestock or other susceptible species excluding humans)

18.2.1. Factors influencing probability of persistence of RVF virus within the EU

18.2.1.1. Presence of competent vectors

The major factor underpinning the persistence of RVF in the EU is the presence of competent vectors. As RVF is essentially a vector-borne disease, without these, the probability of establishment in any other species is negligible. Section 7.3 gives information on this.

Given the presence of these vectors, an appropriate climate and habitat is necessary to allow the vectors, and thus the virus, to flourish. Emergence sites for the primary *Aedes* vectors are in poorly draining soils susceptible to flooding, with high clay content. Alluvial flood plains, sub surface water drainage areas along the network of river drainage systems, where local flooding occurs (gleysols, fluvisols, vertisols, planisols). In highland grassland areas, these can be recognised by their plant communities, typically

with *Cyperus*, *Setaria* and *Digitaria* spp. They are often described as dambos in central and South Africa. Alluvial fan systems emerging from the sides of the Rift Valley both in Africa and Arabia are foci of RVFV activity. The areas may be of limited or widespread extent. Similar zones, which share these geophysical characteristics, undoubtedly exist in the eastern Mediterranean, Turkey and Southern European countries

Secondary propagation of the virus can occur, wherever there are large mosquito populations associated with lakes, dams and river systems. Barrages on the river systems especially produce a back up of water flow in the system to allow more extensive flood zones to develop. Local or extensive irrigation schemes provide ample habitat for secondary vectors. These occur along the Nile in Sudan and Egypt for example.

The identification of areas suitable for persistence of RVF in local mosquito vector populations requires a detailed study of the mosquito species present seasonally and particularly after flooding, and their potential as vectors of RVFV. The areas described below are selected on the basis of knowledge of the natural history of RVF in Africa and Arabia. At greatest risk from introduction of RVFV by aerial transport of infected vectors from Africa, are the islands of Sicily, Cyprus, Crete and Turkey. This would likely be by air currents from Egypt driven by the Eastern Mediterranean Convergence Zone system. Table 18 presents the ecological systems which may be able to support vector persistence.

Table 18: High risk vector habitat areas in selected EU (including candidate) countries

Country	*High risk areas*
Cyprus	Coastal zones associated with river systems. Sub surface and surface water drainage systems in plateau and valleys.
Turkey	Riverine systems in low plateau, valleys and flood plain areas associated with rivers exiting from the mountain zones, in fan areas or flood plains. There are many river systems from the Ceyhan in the east to Mut, Antalya and many in the western coast from Marmaris, Izmir to Istanbul. The mountain grasslands will also have suitable habitat in the network of sub and surface drainage systems. These would be found in Western Turkey along the Bulgarian border in Thrace, along the Evros river system.
Greece	The many riverine systems emerging from the Bulgarian mountains and entering the sea East and West of Thessalonica. There are likely to be flood plains associated with these systems. Coastal zones and river systems to the South and on the islands, including Crete, and up the West coast to Albania.
Italy	Floodplains are associated with major rivers in Sicily near Catania and in Sardinia. All river systems, which emerge from the Apennines, Alps and Dolomites to the West, East and North. In particular, the area Vicenzo-Bologna-Turin-Milan, which has many river systems.
Spain	Alluvial plains and river systems exiting the mountains in the plains from North to South on the Mediterranean coast.
Portugal	Atlantic coast has river systems and probably flood plain zones.
France	Coastal strip from Cannes to Perpignan, the Rhone valley and the Garonne and Dordogne river systems to the West.

Although the only species where a trans-ovarial transmission has been demonstrated to occur is not currently seen in Europe, another *Aedes* spp. for which circumstantial evidence strongly suggests that transovarial transmission of RVF probably occurs, is widely distributed in the EU countries. Currently it is not known whether the European race of the species is capable of transmitting RVF virus.

18.2.1.2. Exposure and infection of susceptible livestock and other susceptible species

Sections 17.2, 17.5 and 17.6 have already provided information on the presence of susceptible livestock, and the assessment of their probability of exposure to, and infection with, RVF virus given infection in vectors. No specific information is given on other species, but if any are present, they would merely add to the probability of infection and establishment. The movement of animals from infected areas could allow RVFV to become seasonally established in Cyprus, Turkey or other Southern European countries.

18.2.1.3. Identification of presence of established RVF in susceptible species

The arrival of RVFV in Europe, at a situation where there was little potential for amplification of the virus in vector populations, other than at a low level, may not be recognised. Low levels of abortion, a few sporadic deaths in neonates, might be unremarkable. Adult non pregnant or male animals of beef breeds may show only an inapparent infection. RVF might not be recognised for several years, unless specific investigations are made. The arrival of RVFV in a European country and its amplification to a high level in vector populations at a time when most breeding animals were pregnant would be likely to be associated with massive abortion storms in cattle sheep and goats. There would be a high mortality in neonates of all species, with some severe disease, morbidity and mortality in older age groups. Deaths would occur in the acute febrile stages of the disease and also from hepatitis and jaundice in succeeding weeks. In dairy cattle, milk yields would fall dramatically and there may be severe gastro intestinal signs with haemorrhages on mucosal membranes.

18.2.1.4. Intra-community movements

In the early stages of establishment of infection, disease would be seen in domestic animals, but may not be recognised. Therefore the movement of animals within the community may allow viraemic animals to travel from a cryptic focus of RVF virus activity. The local and sub regional movement of mosquitoes in wind and low level air currents would be a more likely vehicle for the movement of RVF virus within the community. Humans are not considered to present a hazard for the movement of RVF within a country or region.

18.2.2. Conclusions on probability of persistence of RVF virus within the EU

Suitable habitats for flood water breeding mosquitoes exist widely in the EU. Many of the species associated with RVF in other countries exist in the EU, and it is considered possible (expert opinion of the authors) that some other EU species could also become competent vectors, given initial infection.

In addition, the potential effects of climate change (see Section 7.4.1) and global warming may alter the range of mosquito species which transovarially transmit the virus and of those which are known to be involved in the secondary transmission cycles associated with epidemics of the disease. The probability of this within countries in the Eastern Mediterranean, and Cyprus, Turkey, Italy and Greece will be that much greater. There is thus an increasing probability of the northern extension of both the mosquito borne virus diseases such as RVF and of the *Culicoides* transmitted viruses into Europe.

Susceptible livestock species are widespread in the EU, early signs of infection and disease might be missed, and intra-community movement routinely occurs.

Thus, given infection within vectors in the EU, the probability of establishment within vector and livestock populations is assessed as at least higher than negligible and possibly much higher if the climate changes significantly.

18.3. Probability of infection of humans by the handling or consumption of products derived from animals infected within the EU

18.3.1. Factors influencing probability of infection of humans by handling or consumption of products derived from animals infected within the EU

In humans, RVF is in principle a vector-propagated and vector-based virus disease during an epidemic or in endemic regions. However, other routes of infection may also occur (see Section **Error! Reference source not found.**).

18.3.2. Conclusions on probability of infection of humans by handling or consumption of products derived from animals infected within the EU

Viraemic animals during the acute phase (up to 1 week after infection) present a risk for humans during:

- intensive contact with and by handling of live animals at caretaking, help at parturition, contact with foetal tissues, hand milking, etc.,
- handling animals and tissues at autopsy, and
- slaughtering, bleeding, eviscerating, processing tissues, hides and offal.

Infection presumably occurs via skin abrasions or aerosol droplets. High virus titers are needed to start the infection in humans.

RVFV infection in humans, therefore, can be classified as an occupational risk for farmers, shepherds, animal caretakers, veterinarians, laboratory staff, slaughterhouse staff, butchers or animal offal processors.

If carcases are allowed to undergo rigor mortis, then RVFV is inactivated in meat when the pH drops below 6.8. However, such drop in pH does not occur in meat from animals slaughtered during fever, in meat which is frozen immediately after slaughter or in parenchymatous organs. The virus is destroyed by cooking processes. No evidence exist that consumption of meat or milk, even when derived from acutely infected animals, leads to human infection.

18.4. Tabular summary of probability of each consequence of interest

A summary of the assessed probability of the consequences of interest is presented in Table 19.

Table 19: Summary of probability of each consequence of RVF exposure within EU

Consequence	*Assessed probability of consequence*	*Section*
Infection of EU livestock with RVF	Exposure to infected and infectious vector: moderately likely. For multiple exposures, probability increases.	18.1
The persistence of RVF virus within the EU	If infection enters the vector population of the EU: higher than negligible, and possibly much higher depending	18.2

	upon climate.	
Infection of humans by the handling or consumption of products derived from animals infected within the EU	If infection in the livestock population: slaughterhouse staff and related occupations: higher than negligible; possibly high during epidemic activity. General consumer: negligible.	18.3

18.5. Overall conclusion

Aerial movement of RVFV infected vectors presents the greatest hazard to the EU countries.

The greatest danger is that a cryptic focus of RVF virus activity could develop within the EU. If only adult animals, either sheep goats or cattle, should be available at the point of introduction of the virus, then overt clinical signs may not occur or may not be observed. This might be the case when the exposed animals were not pregnant, were all males and when the highly susceptible neonatal age groups were not present. Such a scenario would not be exceptional and could allow amplification of virus to occur and endemic foci to develop, if mosquitoes with the potential for transovarial transmission became infected.

Many of the following recommendations are based on these two factors.

18.6. Recommendations regarding consequences in the EU

Early-warning surveillance systems for EU countries need to be developed which incorporate surveillance data information from RVF endemic countries as well as from predictive models.

The implementation of surveillance systems appropriate for early detection of RVF in livestock populations in endemic countries needs to be supported.

Sentinel herd systems should be established in defined, high risk areas within the EU closest to the endemic areas of Africa and Arabia. These should be monitored for RVFV and also for other African virus diseases, which present a hazard to the EU countries (Akabane virus, Lumpy Skin disease, Ephemeral fever etc).

In the EU, a surveillance system should be implemented in order to detect an abnormal increase of abortions and mortalities in young and in very young animals of different species (by order of priority: sheep > goats > bovine) and particularly in southern European countries and during periods favourable for the development of vectors. In case of abnormal increase of abortions, any dead animal, or aborted female should be submitted for a viral investigation or for a research of specific antibodies (using the general recommendations for protecting laboratory staff and veterinarians).

Laboratory capacity needs to be set up in the EU to be able to deal with diagnostic requirements during an outbreak situation.

An emergency plan should be developed for susceptible countries with a list of measures to be taken in case of an alert or introduction of RVF. Conditions of an emergency plan of vaccination (availability of vaccines, conditions of vaccination) should be defined in order to limit the extension of the disease in susceptible animals and to prevent human infections.

Veterinarians and other professions who are most likely to recognise RVF infected livestock need to be trained so that an outbreak can be controlled as soon as possible. Therefore, the AHW Panel refers to the recommendations published in the EFSA web site (http://efsa.eu.int) on the adopted statement concerning the "Core competencies required by the private veterinary practicioner, the official veterinarian and the state veterinarian".

18.7. Future Research

18.7.1. Vectors

Scientific research programmes should make assessments of the seasonal mosquito populations in receptive areas in Europe and their potential for transmission of RVF and establishing trans-ovarial transmission cycles.

Some assessment and definition should be made of the potentially receptive areas for RVFV in Cyprus, Turkey and other south-east European countries. Mapping should be performed of those geo-physical areas identified as highly receptive for RVF in Africa and elsewhere. Ground truth data should be gathered on the mosquito populations in the potentially receptive zones during the summer season, and possible vector species should be identified and their population biology studied.

Detailed laboratory investigations of the European mosquitoes to determine susceptibility and ability to transmit RVFV between domestic bovine, ovine and caprine livestock species.

18.7.2. Detection

Epidemiological models need to be developed for European countries that will allow definition of high risk areas for increased surveillance during high risk periods, and to develop optimised control strategies during outbreak situations.

Rapid diagnostic tools compatible with the standard systems currently in use for diseases with similar clinical presentation need to be developed.

18.7.3. Control of an outbreak

Tailored disease control strategies need to deal with potential outbreaks in the European Union to prevent establishing of infection in vector populations. These need to be informed by results from modelling exercises.

Vaccine and immuno-modulating preventive measures need to be developed for rapid protection of persons with occupational hazard during an epidemic.

Safety instructions need to be developed to minimise risk of infection from occupation exposure for persons involved in the control operation, as well as anybody handling animals during this time.

Vaccines for animals need to be developed that are marked, safe in all stages of cattle production, prevent colonization of target tissues, and significantly reduce viremia. Effective methods for widespread vaccination of wildlife need to be developed.

Research needs to be conducted to improve our knowledge about the vectors present in susceptible countries (geographic distribution, dynamics of development).

The immune response in susceptible European cattle breeds needs to be characterised.

Research needs to be conducted for the molecular characterization of virulence factors, vector competency, host range specificity, and tissue tropism.

19. References

Abu-Elyazeed R, El-Sharkawy S, Olson J, Botros B, Soliman A, Salib A, Cummings C, Arthur R, 1996. Prevalence of anti-Rift Valley fever IgM antibody in abattoir workers in the Nile Delta during the 1993 outbreak in Egypt. Bulletin of the World Health Organisation, 74:155-158.

Adkin A., Coburn H, England T, Hall S, Hartnett E, Marooney C, Wooldridge M, Watson E, Cooper J, Cox T, Seaman M, 2004. Risk assessment for the illegal import of contaminated meat and meat products into Great Britain and the subsequent exposure of GB livestock. Defra Publication, London, UK. PB9527, 316pp.

Alexander RA, 1951. Rift Valley fever in the Union. Journal of South Africa Veterinary Medical Association, 22:105-109.

Al-Hazmi M, Ayoola EA, Abdurahman M, Banzal S, Ashraf J, El-Bush Hazmi A, Abdullah, M, Abbo H, Elamin A, Al-Sammani el-T, Gadour M, Menon C, Hamza M, Rahim I, Hafez M, Jambavalikar M, Arishi H, 2003. Epidemic Rift Valley fever in Saudi Arabia: A clinical study of severe illness in humans. Clinical Infectious Diseases, 36:245-251.

Ali AM, Kamel S, 1978. Epidemiology of RVF in domestic animals in Egypt. Journal of the Egyptian Public Health Association, 53:255-263.

Anderson GW, Peters CJ, 1988. Viral determinants of virulence for Rift Valley fever (RVF) in rats. Microbial Pathogenesis, 5: 241-250.

Anderson GW, Saluzzo J-F, Ksiazek TG, Smith JF, Ennis W, Thureen D, Peters CJ, Digoutte JP, 1989. Comparison of in vitro and in vivo systems for the propagation of Rift Valley fever virus from clinical specimens. Research in Virology, 140:125-138.

Anderson GW, Slone TW, Peters CJ, 1987. Pathogenesis of Rift Valley fever virus in in-bred rats. Microbial Pathogenesis, 2:283-293.

Andrewes CH, Horstmann DM, 1949. The susceptibility of viruses to ethyl ether. Journal of General Virology, 3:290-297.

Arborio M, Hall WC, 1989. Diagnosis of a human case of Rift Valley fever by immunoperoxidase demonstration of antigen in fixed liver tissue. Research in Virology, 140(2):165-168.

Balkhy HH, Memish ZA, 2003. Rift Valley fever: an uninvited zoonosis in the Arabian peninsula. International Journal of Antimicrobial Agents, 21:153-157.

Barnard BJH, 1979. Rift Valley fever vaccine antibody and immune response in cattle to a live and an inactivated vaccine. Journal of the South African Veterinary Association, 50:155-157.

Barnard BJH, Botha MJ, 1977. An inactivated Rift Valley fever vaccine. Journal of the South African Veterinary Association, 48:45-48.

Barr AR, 1982. The Culex pipiens complex. In WWM Steiner, WJ,Tabachnick, KS Rai, and S. Narang (eds). Recent Developments in the Genetics of Insect Vectors of Disease. Stipes, Urbana, Illinois. 551-572.

Baskerville A, Hubbard KA, Stephenson JR, 1992. Comparison of the pathogenicity for pregnant sheep of Rift Valley fever virus and a live attenuated vaccine. Research in Veterinary Science, 52:307-311.

Battles JK, Dalrymple JM, 1988. Genetic variation among geographic isolates of Rift Valley fever virus. American Journal of Tropical Hygiene, 39:617-633.

Botha WJS, Jaeger HG, Aitchison H, Stoltsz C, 1996. Vaccines against viral diseases. Onderstepoort Biological Products, National Department of Agriculture, South Africa, 1996. pp. 28-42.

Brubaker JF, Turell MJ, 1998. Effect of environmental temperature on the susceptibility of Culex pipiens (Diptera: Culicidae) to Rift Valley fever virus. Journal of Medical Entomology, 35(6):918-921.

Capstick PB, Gosden D, 1962. Neutralising antibody response in sheep to pantropic and neurotropic Rift Valley fever virus. Nature, 195: 583-584.

Cash P, Robeson G, Erlich BJ, Bishop DHL, 1981. Biochemical characterisation of Rift Valley fever and other Phlebotomus fever group viruses. In: TA Swartz, MA Klingberg, N Godblum (eds). Rift Valley fever. Contributions to Epidemiology and Statistics. Vol 3, Basel, Switzerland: S. Karger, 1-20.

CDC, 1999. Centres for Disease Control and Prevention. Department of Health and Human Services. Biosafety in Microbiological and Biomedical Laboratories. 4th Edition, US Government Printing Office, Washington. http://www.cdc.gov.

CDC, 2000a. Outbreak of Rift Valley fever - Saudi Arabia, August - October, 2000. MMWR, 49:905-908.

CDC, 2000b. Centres for Disease Control and Prevention. Department of Health and Human Services Update: outbreak of Rift Valley fever - Saudi Arabia, August - November, 2000. MMWR, 49:982-985.

CDC, 2000c. Centres for Disease Control and Prevention. Department of Health and Human Services Outbreak of Rift Valley Fever — Yemen, August - October 2000. MMWR, 49:1065-1066.

Chartier C, Chartier F, 1988. Enquete sero-epidemiologique sur les avortements infectieux des petitis ruminants en Mauretanie. Revue d'Elevage et de Médecine Vétérinaire des Pays Tropicaux, 41:23-24.

Chomczynski P, Sacchi N, 1987. Single-step method of RNA isolation by acid guanidium thiocyanate-phenol-chloroform extraction. Analytical Biochemistry, 162:156-159.

Cianchi R, Urbanelli S, Sabatini A, Coluzzi M, Tordi MP, Bullini L, 1980. Due entita riproduttivamente isolate sotto il nome di *Aedes caspius* (Diptera, Culicidae). Atti XII Congresso Nazionale Italiane Entomologica, Roma, 2:269-272.

Clarke DH, Casals J, 1958. Techniques for hemagglutination and hemagglutination-inhibition with arthropod borne-viruses. American Journal of Tropical Medicine and Hygiene, 7:561–573.

Clerc Y, Rodhain F, Digoutte JP, Albignac R, Coulanges P, 1982. Le programme exploratoire Arbovirus de l'Institut Pasteur de Madagascar: bilan 1976-1980. Archives de l'Institut Pasteur de Madagascar, 49(1):65-78.

Coakley W, 1963. The effect of recently isolated strains of Rift Valley fever virus on lamb testis cell cultures. Journal of Pathology and Bacteriology, 86:530-532.

Coakley W, 1965. Alteration in virulence of Rift Valley fever virus during serial passage in lamb testis cells. Journal of Pathology and Bacteriology, 89:123-131.

Coakley W, Pini A, Gosden D, 1967a. Experimental infection of cattle with Pantropic Rift Valley fever virus. Research in Veterinary Science, 8:399-405.

Coakley W, Pini A, Gosden D, 1967b. The immunity induced in cattle and sheep by inoculation of neurotropic or pantropic Rift Valley fever viruses. Research in Veterinary Science, 8:406-414.

Coetzer JAW, 1977. The pathology of Rift Valley fever. I. Lesions occurring in natural cases in new-born lambs. Onderstepoort Journal of Veterinary Research, 44:205-212.

Coetzer JAW, 1982. The pathology of Rift Valley fever. I. Lesions occurring in field cases in adult cattle, calves and aborted foetuses. Onderstepoort Journal of Veterinary Research, 49:11–17.

Coetzer JAW, Barnard BJH, 1977. *Hydrops amnii* in sheep associated with hydranencephaly and arthrogryposis, with Wesselbron and Rift Valley fever viruses as aetiological agents. Onderstepoort Journal of Veterinary Research, 44:119-126.

Coetzer JAW, Ishak KG, 1982. Sequential development of the liver lesions in new-borne lambs infected with Rift Valley fever virus. I. Macroscopic and microscopic pathology. Onderstepoort Journal of Veterinary Research, 49:103-108.

Collett MS, Purchio AF, Keegan K, Frazier S, Hays W, Anderson DK, Parker MD, Schmaljohn C, Schmidt J, Dalrymple JM, 1985. Complete nucleotide sequence of the M RNA segment of Rift Valley fever virus. Virology, 144:228-245.

Craig DE, Thomas WJ, DeSanctis AN, 1967. Stability of Rift Valley fever virus at 4C. Applied Environmental Microbiology, 15:446-447.

Darwish MA, Imam IZE, Omar F, 1978. A sero-epidemiological study for Rift Valley fever virus in humans and domestic animals in Egypt. Journal of the Egyptian Public Health Association, 53:153-162.

Daubney R, Hudson JR, Garnham PC, 1931. Enzootic hepatitis or Rift Valley fever. An undescribed virus disease of sheep, cattle and man from East Africa. Journal of Pathology and Bacteriology, 34:545-579.

Daubney R, Hudson JR, 1932. Rift Valley fever. Lancet, 1:611-612.

Daubney R, Hudson JR, 1933. Rift Valley fever. East African Medical Journal, 10:2-19.

Davies FG 1981. Rift Valley fever in Kenya. Proceedings of the 49th General Session of the Office International des Epizooties, Paris, 25-30 May.

Davies FG, 1975. Observations on the epidemiology of Rift Valley fever in Kenya. Journal of Hygiene, 75:219-230.

Davies FG, 1990. Rift Valley fever in the Sudan. Transactions of the Royal Society of Tropical Medicine and Hygiene, 84(1):141.

Davies FG, 1994. Consultation Report in Senegal. TCP FAO/SEN/2252 (E), 20pp.

Davies FG, Adday PAK, 1979. Rift Valley fever: a survey for antibody to the virus in bird species commonly found in situations considered to be enzootic. Transactions of the Royal Society of Tropical Medicine and Hygiene, 72:213-214.

Davies FG, Clausen B, Lund LJ, 1972. The pathogenicity of Rift Valley fever virus for the baboon. Transactions of the Royal Society of Tropical Medicine and Hygiene, 66:363-365.

Davies FG and Onyango E., 1978. Rift Valley fever: the role of the vervet monkey as a reservoir or maintenance host for this virus. Transactions of the Royal Society of Tropical Medicine and Hygiene, 72:213-214.

Davies FG, Highton RB, 1980. Possible vectors of Rift Valley fever in Kenya. Transactions of the Royal Society of Tropical Medicine and Hygiene, 74:815-816.

Davies FG, Karstad L, 1981. Experimental infection of the African buffalo (*Syncerus caffer*) with the virus of Rift Valley fever. Tropical Animal Health Production, 13:185-188.

Davies FG, Kilelu E, Linthicum KJ, Pegrum RG, 1992. Patterns of Rift Valley fever activity in Zambia. Epidemiology and Infection, 108:185-191.

Davies FG, Koros J, Mbugwa H, 1985a. Rift Valley fever in Kenya: the presence of antibody to the virus in camels (Camelus dromedarius). Journal of Hygiene 94:241-244.

Davies FG, Linthicum KJ, 1986. The Sudan dioch (*Quelea quelea aethiopica*) and Rift Valley fever. Transactions of the Royal Society of Tropical Medicine and Hygiene, 80:171-171.

Davies FG, Linthicum KJ, James AD, 1985b. Rainfall and epizootic Rift Valley fever. Bulletin of the World Health Organization, 63:941-943.

Davies FG, Logan TM, Binepal Y, Jessen P, 1992. Rift Valley fever virus activity in East Africa in 1989. Veterinary Record, 130(12):247-248.

Diallo M, Lochouarn L, Ba K, Sall AA, Mondo M, Girault L, Mathiot C, 2000. First isolation of the Rift Valley fever virus from *Culex poicilipes* (Diptera: Culicidae) in nature. American Journal of Tropical Medicine and Hygiene, 62(6):702-704.

Digoutte JP, Calvo-Wilson MA, Mondo M, Traoré-Lamizana M, Adam F, 1992. Continuous cell lines and immune ascitic fluid pools in arbovirus detection. Research in Virology, 143: 417-422.

Digoutte JP, Cordellier R, Robin Y, Pajot FX, Geoffroy B, 1974. Le virus Zinga (Ar B 1276), nouveau prototype d'arbovirus isolé en République Centrafricaine. Ann. Inst. Pasteur, 125B:107-118.

Digoutte JP, Jouan A, Le Guenno B, Riou O, Phillipe B, Meegan J, Ksiazek TG, Peters CJ, 1989. Isolation of Rift Valley fever virus by inoculation to Aedes pseudoscutellaris cells - comparison with other diagnostic methods. Research in Virology, 140:31-41.

Dohm DJ, Rowton ED, Lawyer PG, O'Guinn M, Turell MJ, 2000. Laboratory transmission of Rift Valley fever by *Phlebotomus duboscqi, Phlebotomus papatasi, Phlebotomus sergenti*, and *Sergentomyia schwetzi* (Diptera: Psychodidae). Journal of Medical Entomology, 37(3):435-438.

Drosten C, Götting S, Schilling S, Asper M, Panning M, Schmitz H, Günther S, 2002. Rapid detection and quantification of RNA of Ebola and Marburg viruses, Lassa virus, Crimean-Congo hemorrhagic fever virus, Rift Valley fever virus, dengue virus, and yellow fever virus by real-time reverse transcription-PCR. Journal of Clinical Microbiology, 40:2323-2330.

Easterday BC, 1961. Experimental Rift Valley fever. PhD thesis, University of Wisconsin, USA, cited by Easterday, B.C. (1965) Rift Valley fever. Advances in Veterinary Science, 10:65-127.

Easterday BC, 1965. Rift Valley fever. Advances in Veterinary Science, 10:65-127.

Easterday BC, Jaeger RF, 1963. The detection of Rift Valley fever virus by a tissue culture-fluorescein labeled antibody method. Journal of Infectious Diseases, 112:1-6.

Easterday BC, Murphy LC, 1963. Studies on Rift Valley fever in laboratory animals. Cornell Veterinarian, 53:423-433.

Easterday BC, Murphy LC, Bennet DG, 1962a. Experimental Rift Valley fever in calves, goats, and pigs. American Journal of Veterinary Research, 23:1224-1230.

Easterday BC, Murphy LC, Bennet DG, 1962b. Experimental Rift Valley fever in lambs and sheep. American Journal of Veterinary Research, 23:1231- 1240.

Easterday BC, McGavran MH, Rooney JR, Murphy LC, 1962c. The pathogenesis of Rift Valley fever in lambs. American Journal of Veterinary Research, 23:470-479.

Easterday, B C, Murphy L C, Bennett D G; 1962d. The pathogenesis of Rift Valley fever in lambs. American Journal Veterinary Research, 23, 1231-1240

EC, 1992. Council Directive 92/119/EEC of 17 December 1992 introducing general Community measures for the control of certain animal disease and specific measures relating to swine vesicular disease (OJ L 62, 15.03.93, p. 69) amended by Accession Treaty of 1994, amended by 2002/60/EC (OJ L 192, 20.07.2002, p. 27) amended by (EC) No 806/2003 (OJ L 122, 16.05.2003, p. 1), amended by ACT of 2004.

EC, 1996. Council Directive 91/496/EEC of 15 July 1991 defining the principles governing the organization of veterinary checks on animals entering the Community from third countries and amending Directives 89/662/EEC, 90/425/EEC and 90/675/EEC. Offical Journal, L 268, 24.9.1991, 56.

EC, 1997a. Council Decision 79/542/EEC of 21 December 1976 defining a list of third countries or parts of third countries, and prescribing animal and public health and veterinary certification conditions, for importation into the Community of certain live animals and their fresh meat. Offical Journal, L 146, 14.6.1979, 15.

EC, 1997b. Council Directive 97/78/EC of 18 December 1997 defining the principles governing the organisation of veterinary checks on products entering the Community from third countries. Offical Journal L 24, 30.01.98, 9.

EC, 2000. Directive 2000/54/EC of the European Parliament and of the Council of 18 September 2000 on the protection of workers from risks related to exposure to biological agents at work (seventh individual directive within the meaning of Article 16(1) of Directive 89/391/EEC). Official Journal, L 262, 17.10.2000, p. 21 – 45.

EC, 2001a. Commission Decision 2001/881/EC of 7 December 2001 specifying a list of border inspection posts agreed for veterinary checks on animals and animal products from third countries and updating the detailed rules concerning the checks to be carried out by the experts of the Commission. Official Journal, L 326, 11.12.2001, 44.

EC, 2001b. Directive of the European Parliament and of the Council 2001/82/EC of 6 November 2001 on the Community code relating to veterinary medicinal products (OJ L 311, 28.11.2001, p. 1), amended by 2004/28/EC (Official Journal, L 136, 30.04.2004, p. 58).

Eisa M, Kheir El Sid ED, Shomei AM, Meegan JM, 1980. An outbreak of Rift Valley fever in the Sudan - 1976. Transactions of the Royal Society of Tropical Medicine and Hygiene, 745:417-418.

Eisa M, Obeid HMS, El Saei ASA, 1977. Rift Valley fever in the Sudan. I. Results of field investigations of the first epizootic in Kosti District, 1973. Bulletin of Animal Health and Production in Africa, 24:343-347.

El Akkad AM, 1978. Rift Valley fever outbreak in Egypt, October-December 1977. Journal of the Egyptian Public Health Association, 53:123-128.

El Karamany RM, Imam IZE, Farid AH, Saber MS, 1979. Susceptibility of CER cell line to Rift Valley fever virus. Journal of the Egyptian Public Health Association, 54:105-114.

El Mekki AA, Van der Groen G, 1981. A comparison of indirect immunofluorescence and electron microscopy for the diagnosis of some haemorrhagic viruses in cell culture. Journal of Virological Methods, 3:61-69.

El Rahim IHAA, El Hakim UA, Hussein M, 1999. An epizootic of Rift Valley fever in Egypt in 1997. Revue Scientifique et Technique Office International des Epizooties, 18(3):741-748.

Ellis DS, Shirodaria PV, Fleming E, Simpson DIH, 1988. Morphology and development of Rift Valley fevr virus in Vero cell cultures. Journal of Medical Virology, 24:161-174.

Fagbami AH, Tomori O, Fabiyi A, Isoun TT, 1975. Experimental Rift Valley fever in West African dwarf sheep. Research in Veterinary Science, 18:334-335.

FAO, 2002. Preparation of Rift Valley fever contingency plans. FAO Animal Health Manual 15, ISSN 10205187.

FAO, 2003. Recognizing of Rift Valley fever contingency plans. FAO Animal Health Manual 17, ISSN 10205187.

Findlay GM, 1931. Rift Valley fever or enzootic hepatitis. British Medical Journal, 2:1182-1183.

Findlay GM, 1932. Rift Valley fever or enzootic hepatitis. Transactions of the Royal Society of Tropical Medicine and Hygiene, 25:229-265.

Findlay GM, 1932. The infectivity of Rift Valley fever for monkeys. Transactions of the Royal Society of Tropical Medicine and Hygiene, 26:161-168.

Findlay GM, Howard EM, 1951. Notes on Rift Valley fever. Archiv für die gesamte Virusforschung, 4:411-423.

Findlay GM, Mackenzie RD, Stern RO, 1936. Studies on neurotropic Rift Valley fever virus: the susceptibility of sheep and monkeys. British Journal for Experimental Pathology, 17:431-441.

Fontenille D, Traore Lamizana M, Diallo M, Thonnon J, Digoutte JP, Zeller HG, 1998. New vectors of Rift Valley fever in West Africa. Emerging Infectious Diseases, 4(2):289-293.

Fontenille D, Traore Lamizana M, Zeller H, Mondo M, Diallo M, Digoutte JP, 1995. Short report: Rift Valley fever in western Africa: isolations from *Aedes* mosquitoes during an interepizootic period. American Journal of Tropical Medicine and Hygiene, 52(5):403-404.

Fontenille D, Traoré-Lamizana M, Trouillet J, Leclerc A, Mondo M, Digoutte JP and Zeller G, 1994. First isolations of arboviruses from phlebotomine sandflies in West Africa. American Journal of Tropical Medicine and Hygiene, 50:570-574.

Francis T, McGill TP, 1935. Rift Valley fever. A report of three cases of laboratory infection and the experimental transmission of the disease to ferrets. Journal of Experimental Medicine, 62:433-448.

Gad AM, Farid HA, Ramzy RRM, Riad MB, Presley SM, Cope SE, Hassan MM, Hassan AN, 1999. Host feeding of mosquitoes (Diptera: Culicidae) associated with the recurrence of Rift Valley fever in Egypt. Journal of Medical Entomology, 36(6):709-714.

Gad AM, Hassan AN, Merdan AI, 1989. Transmission of Rift Valley fever virus by different geographic strains of *Culex pipiens* in Egypt. Journal of the Egyptian Public Health Association, 64(5-6):363-379.

Gad AM, Hassan MM, El Said S, Moussa MI, Wood OL, 1987. Rift Valley fever virus transmission by different Egyptian mosquito species. Transactions of the Royal Society of Tropical Medicine and Hygiene, 81:694-698.

Gad AM, Hassan MM, El-Sherif LS, Farid HA, 1992. Electrophoretic separation of two *Aedes caspius* forms from Egypt. Journal of the Egyptian Society of Parasitology, 22:217-230.

Garcia S, Crance JM, Billecocq A, Peinnequin A, Jouan A, Bouloy M, Garin D, 2001. Quantitative real-time PCR detection of Rift Valley fever virus and its application to evaluation of antiviral compounds. Journal of Clinical Microbiology, 39:4456-4461.

Gargan TP, Bailey CL, Higbee GA, Gad A and El Said S, 1983. The effect of laboratory colonization on the vector-pathogen interactions of Egyptian *Culex pipiens* and Rift Valley fever virus. American Journal of Tropical Medicine and Hygiene, 32(5):1154-1163.

Gargan TP, Clark GG, Dohm DJ, Turell MJ and Bailey CL, 1988. Vector potential of selected North American mosquito species for Rift Valley fever virus. American Journal of Tropical Medicine and Hygiene, 38(2):440-446.

Garret Jones C, 1962, The possibility of active long distance migration by *Anopheles pharoensis* Theobald, Bulletin of the World Health Organisation, 27:199-332.

Gear J, De Meillon B, Le Roux AF, Kofsky R, Innes RR, Steyn JJ, Oliff WD and Schulz KH, 1955. Rift valley fever in South Africa; a study of the 1953 outbreak in the Orange Free State, with special reference to the vectors and possible reservoir hosts. South African Medical Journal, 29(22):514-518.

Gerdes G. 2004, Rift Valley Fever. Revue Scientifique et Technique, Office International des Epizooties, 23(2):613-621.

Ghaffar SA, 1981. The epidemiological situation of RVF in Egypt up to December 1980. Journal of the Egyptian Public Health Association, 56:463-467.

Gillet JD, Mims CA, 1956. Relation between RVF virus and its mosquito host. East African Virus Research Institute Report No. 6, pp. 19-20.

Giorgi C, Accardi L, Nicoletti L, Gro MC, Takehara K, Hilditch C, Morikawa S, Bishop DH, 1991. Sequences and coding strategies of the S RNAs of Toscana and Rift Valley fever viruses compared to those of Punta Toro, Sicilian Sandfly fever, and Uukuniemi viruses. Virology, 180:738-753.

Gonzalez JP, Guenno B le, Some MJR, Akakpo JA, Le Guenno B, 1992. Serological evidence in sheep suggesting phlebovirus circulation in a Rift Valley fever enzootic area in Burkina Faso. Transactions of the Royal Society of Tropical Medicine and Hygiene, 86(6):680-682.

Gora D, Yaya T, Jocelyn T, Didier F, Maoulouth D, Amadou S, Ruel TD, Gonzalez JP, 2000. The potential role of rodents in the enzootic cycle of Rift Valley fever virus in Senegal. Microbes in Infection, 2:343-346.

Gratz NG, 2004. The Vector-borne Human Infections of Europe: Their Distribution and Burden on Public Health. WHO Report No. E82481.

Gubler DJ, 2002. The Global Emergence/Resurgence of Arboviral Diseases As Public Health Problems. Archives of Medical Research, 33:330-342.

Hahon N 1969. Immunofluorescent cell-counting assay of Rift Valley fever virus. Am. J. Vet. Ass., 30:1007-1014.

Hahon N, Zimmerman WD, 1969. Intracellular survival of viral and rickettsial agents in acetone at –60C. Applied Microbiology, 17:775-776.

Haig DA, Kaschula VR and Alexander RA (1953). Studies on Rift valley fever; Some properties of the pantropic virus and a report made of its distribution in South Africa. Unpublished data cited in Kaschula V.R. 1953: The propagation of RVF viruses in embryonated eggs and their use as immunising agents for domestic ruminants. Thesis, University of Pretoria.

Harrington DG, Lupton HW, Crabbs CL, Peters CJ, Reynolds JA, Slone TWJ, 1980. Evaluation of a formalin-inactivated Rift Valley fever vaccine in sheep. American Journal of Veterinary Research, 41:1559-1564.

Hawkes R, Marshall ID, 1967. Studies of arboviruses by agar gel diffusion. American Journal of Epidemiology, 86:28-44.

Henderson BE, McCrae AWR, Kirya BG, Ssenkubuge Y, Sempala SDK, 1972. Arbovirus epizootics involving man, mosquitoes and vertebrates at Lunyo, Uganda 1968. Annals of Tropical Medicine and Parasitology, 66(3):343-355.

Hoch AL, Gargan TP 2nd, Bailey CL, 1985. Mechanical transmission of Rift Valley fever virus by hematophagous Diptera. American Journal of Tropical Medicine and Hygiene, 34(1):188-193.

Hoch AL, Turell MJ, Bailey CL, 1984. Replication of Rift Valley fever virus in the sand fly *Lutzomyia longipalpis*. American Journal of Tropical Medicine and Hygiene, 33(2):295-299.

Hoogstraal H, Meegan JM, Khalil GM, Adham FK, 1979. The Rift Valley fever epizootic in Egypt 1977-1978. 2. Ecological and entomological studies. Transactions of the Royal Society of Tropical Medicine and Hygiene, 73(6):624-629.

Huang YM, 1985. A new African species of *Aedes* (Diptera: Culicidae). Mosquito Systematics, 17(2):108-120.

Ibrahim MS, Turell MJ, Knauert FK, Lofts RS, 1997. Detection of Rift Valley fever virus in mosquitos by RT-PCR. Molecular and Cellular Probes, 11:49-53.

Imam IZE, El Karamany R, Darwish MA, 1979. An epidemic of Rift valley fever in Egypt. II. Isolation of the virus from animals. Bulletin of the World Health Organization, 57:441-443.

Imam IZE, El Karmany R, Omar FM, El Kafrawy O, 1981. Rift Valley fever in Egypt. Journal of the Egyptian Public Health Association, 56:356-383.

Jennings M, Platt GS and Bowen ET, 1982. The susceptibility of *Culicoides variipennis* Coq. (Diptera: Ceratopogonidae) to laboratory infection with Rift Valley fever virus. Transactions of the Royal Society of Tropical Medicine and Hygiene, 76(5):587-589.

Johnson RW, Orlando MD, 1968. Growth of Rift Valley fever virus in tissue culture. American Journal of Veterinary Research, 29:463-471.

Joshi MV, Umarani UB, Pinto BD, Joshi GD, Paranjape SP, Banerjee K, 1995. Prevalence of antibodies to Rift Valley fever (or closely related) virus in sheep and goats from Rajasthan, India: a preliminary report. Indian Journal of Virology, 11(1):59-60.

Jouan A, Coulibaly I, Adam F, Philippe B, Riou O, Leguenno B, Christie R, Ould MN, Ksiazek T, Digoutte JP, 1989. Analytical study of a Rift Valley fever epidemic. Research in Virology, 140:175-186.

Joubert JDS, Ferguson AL, Gear JHS, 1951. Rift Valley fever in South Africa. II. The occurrence of human cases in the Orange Free State the North-western Cape Province, the Western and Southern Transvaal. A. Epidemiological and clinical findings. South African Medical Journal, 25:890-891.

Jupp PG, 1974. Laboratory studies on the transmission of West Nile virus by *Culex (Culex) univittatus* Theobald; factors influencing the transmission rate. Journal of Medical Entomology, 11(4):455-458.

Jupp PG, Cornel AJ, 1988. Vector competence tests with Rift Valley fever virus and five South African species of mosquito. Journal of the American Mosquito Control Association, 4(1):4-8.

Jupp PG, Kemp A, Grobbelaar AA, Leman PA, Burt FJ, Alahmed AM, Al Mujalli D, Al Khamees M, Swanepoel R, 2002. The 2000 epidemic of Rift Valley fever in Saudi Arabia: mosquito vector studies. Medical and Veterinary Entomology, 16(3):245-252.

Jupp PG, McIntish BM, Thompson DL, 1983. Isolation of Rift Valley fever virus from *Aedes (Neomelaniconion) circumluteolus* and/or *luteolateralis* collected during an outbreak in cattle in the coastal region of Natal, South Africa. South African Journal of Science, 79(9):377.

Kaschula VR, 1953. The propagation and modification of strains of RVF viruses in embryonated eggs and their use as immunising agents for domestic ruminants. Unpublished Thesis. University of Pretoria, Pretoria, South Africa.

Kitchen SF, 1934. Laboratory infections with the virus of Rift Valley fever. American Journal of Tropical Medicine and Hygiene, 14:547-564.

Kitchen SF, 1950. The development of neurotropism in Rift Valley fever virus. Annals of Tropical Medicine and Parasitology, 44:132-145.

Klein F, Walker JS, Mahlandt BG, Carter RC, Orlando MD, Weirether FJ, Lincoln RE, 1969. Interacting factors that influence long-term storage of live *Pasteurella tularensis* vaccine and Rift Valley fever virus. Applied Microbiology, 17(3):427-434.

Kokernot, R.H., Heymann, C.S., Muspratt J. and Wolstenholme B. (1957). Studies on arthropod-borne viruses of Tongaland. V. Isolation of Bunyamwera and Rift Valley fever viruses from mosquitoes. South African Journal of Medical Sciences, 22(2-3):71-80.

Ksiazek TG, Jouan A, Meegan JM, Le GB, Wilson ML, Peters CJ, Digoutte JP, Guillaud M, Merzoug NO, Touray EM, 1989. Rift Valley fever among domestic animals in the recent West African outbreak. Research in Virology, 140:67-77.

Lecatsas G, Weiss KW, 1968. Electron microscopic studies on BHK-21 cells infected with Rift Valley fever virus. Archiv für die gesamte Virusforschung, 25:58-64.

Lee VH, 1977. Isolation of viruses from field population of *Culicoides* (Diptera: Ceratopogonidae) in Nigeria. Journal of Medical Entomology, 16:76-79.

Levitt J, Polson A 1964. Physical and serological investigation of Rift Valley fever antigens. Journal of Hygiene (Cambridge), 62:239-256.

Linthicum KJ, Anyamba A, Tucker CJ, Kelley PW, Myers MF, Peters CJ, 1999. Climate and satellite indicators to forecast Rift Valley fever epidemics in Kenya. Science, 285:397-400.

Linthicum KJ, Bailey CL, Davies FG, Tucker CJ, 1987. Detection of Rift valley fever viral activity in Kenya by satellite remote sensing imagery. Science, 235:1656-1659.

Linthicum KJ, Bailey CL, Tucker C J, Mitchell K D, Logan T M, Davies F G, Kamau C W, Thande P C, Wagateh J N, 1990. The application of polar orbiting meteorological satellite data, to detect flooding of Rift Valley virus-vector mosquito habitat in Kenya. Medical Veterinary Entomology, 4:433-438.

Linthicum KJ, Bailey CL, Tucker CJ, Angleberger DR, Cannon T, Logan TM, Gibbs PH, Nickeson J, 1991. Towards real time prediction of Rift valley fever epidemics in Africa. Preventive Veterinary Medicine, 11(3-4):325-334.

Linthicum KJ, Davies FG, Bailey CL, Kairo A, 1983. Mosquito species succession in a dambo in an East African forest. Mosquito News, 43:464-470.

Linthicum KJ, Davies FG, Kairo A, 1985a. Rift Valley fever virus (Family Bunyaviridae, genus Phlebovirus). Isolations from Diptera collected during an inter-epizootic period in Kenya. Journal of Hygiene, 95(1):197-209.

Linthicum KJ, Davies FG, Kairo A, Bailey CL, 1985b. Rift Valley fever virus. Isolations from Diptera collected during an inter-epidemic period in Kenya. Journal of Hygiene, 95:197-209;

Linthicum KJ, Kaburia HFA, Davies FG, Lundquist KJ, 1985c. A blood meal analysis of engorged mosquitoes found in Rift Valley fever epizootics areas in Kenya. Journal of the American Mosquito Control Association, 1:93-95.

Linthicum KJ, Logan TM, Bailey CL, Dohm DJ, Moulton JR, 1989. Trans-stadial and horizontal transmission of Rift Valley fever virus in *Hyalomma truncatum*. American Journal of Tropical Medicine and Hygiene, 41(4):491-496.

Logan TM, Linthicum KJ, Wagateh JN, Thande PC, Kamau CW, Roberts CR, 1990. Pretreatment of floodwater *Aedes* habitats (dambos) in Kenya with a sustained release formulation of methoprene. Journal of the American Mosquito Control Association, 6:736-738.

Logan TM, Linthicum KJ, Thande PC, Wagateh JN, Roberts CR 1991a. Egg hatching of *Aedes* mosquitoes during successive floodings in a Rift Valley fever endemic area in Kenya. Journal of the American Mosquito Control Association, 7:109-112.

Logan TM, Linthicum KJ, Davies FG, Binepal YS, Roberts CR, 1991b. Isolation of Rift Valley Fever virus from mosquitoes (Diptera: *Culicidae*) collected during an outbreak in domestic animals in Kenya. Journal of Medical Entomology, 28(2):293-295.

Lupi O, Tyring SK, 2003. Tropical dermatology: Viral tropical diseases. Journal of the American Academy of Dermatology, 49:979-1000.

MacGavran MH, Easterday BC, 1963. Rift Valley fever hepatitis. Light and electron microscopic studies in the mouse. American Journal of Pathology, 42:587-607.

Mackenzie RD, 1935. Immunisation of mice against Rift Valley fever. Journal of Pathology and Bacteriology, 40:65-73.

Mackenzie RD, Findlay GM, 1936. The production of neurotropic variants of Rift Valley fever virus. Lancet, 1:140-144.

Malik SKA, 1981. Epidemiology of Rift Valley fever in domestic animals in Egypt. Proceedings of the 49th General Session of the Office International des Epizooties, Paris, 25-30 May.

Manohar BM, Sundararaj A, Sheela PRR, Elankumaran S, Albert A, Venugopalan AT, 1995. A report on an outbreak of a Rift Valley fever like disease in sheep in Tamil Nadu. Indian Veterinary Journal, 72(6):662-664.

Mariner JC, Morrill J, Ksiazek TG, 1995. Antibodies to hemorrhagic fever viruses in domestic livestock in Niger: Rift Valley fever and Crimean-Congo hemorrhagic fever. American Journal of Tropical Medicine and Hygiene, 53(3):217-221.

Mattingly PF, Rozeboom LE, Knight KL, Laven H, Drummond FH, Christophers SR, Shute PG, 1951. The *Culex pipiens* complex. Transactions of the Royal Entomological Society of London, 102(7):331-382.

Matumoto M, Iwasa S, Endo M, 1950. Complement fixation reaction of Rift Valley fever virus. Japanese Journal of Experimental Medicine, 20:501-508.

Matumoto M, Saburi Y, Nishi I, 1959. Rift Vally fever in the one-day-old chick embryo. Journal of Immunology, 82:219-225.

Maurice Y, 1967. Premieres constatations serologiques sur l'incidence de la maladie de Wesselbron et de la fievre de la Vallee du Rift chez les ovins et les ruminants sauvages du Tchad et du Cameroun. Revue d'Elevage et de Médecine Vétérinaire des Pays Tropicaux, 20:359-405.

MacDiarmid SC, Thompson EJ, 1997. The potential risks to animal health from imported sheep and goat meat. Rev sci tech Off int Epiz, 16(1):45-56.

McIntosh BM, 1961. Susceptibility of some African wild rodents to infection with various arthropod-borne viruses. Transactions of the Royal Society of Tropical Medicine and Hygiene, 55:63-68.

McIntosh BM, 1972. Rift Valley fever. Vector studies in the field. Journal of the South African Veterinary Association, 43:391-395.

McIntosh BM, Jupp PG, de Sousa J, 1972. Further isolations of arboviruses from mosquitoes collected in Tongaland, South Africa 1960-1968. Journal of Medical Entomology, 9:155-159.

McIntosh BM, Dickinson DB, Dos Santos I, 1973a. Rift Valley fever. 3. Viraemia in cattle and sheep. 4. The susceptibility of mice and hamsters in relation to transmission of virus by mosquitos. Journal of the South African Veterinary Association, 44:167-169.

McIntosh BM, Jupp PG, Anderson D, Dickenson DB, 1973b. Rift Valley fever: attempts to transmit the virus with seven species of mosquito. Journal of the South African Veterinary Association, 44:57-60.

McIntosh BM, Jupp PG, Dos Santos I, Barnard JH, 1980a. Vector studies on Rift Valley fever virus in South Africa. South African Medical Journal, 58:127-132.

McIntosh BM, Russel D, Dos Santos I, Gear JHS, 1980b. Rift Valley fever in humans in South Africa. South African Medical Journal, 58:803–806.

McIntosh BM, Jupp PG, Dos Santos I, Rowe AC, 1983. Field and laboratory evidence implicating *Culex zombaensis* and *Aedes circumluteolus* as vector of Rift Valley fever in coastal South Africa. South African Journal of Science, 79:61-64.

Meegan J, Le Guenno B, Ksiazek T, Jouan A, Knauert F, Digoutte JP, Peters CJ, 1989. Rapid diagnosis of Rift Valley fever: A comparison of methods for the direct detection of viral antigen in human sera. Research in Virology, 140:59-65.

Meegan JM, Bailey CL, 1989. Rift Valley fever. In: TP Monath (ed). The Arboviruses: Epidemiology and Ecology. Vol. IV. CRC Press, Boca Raton, Florida. 51-76.

Meegan JM, Digoutte JP, Peters CJ, Shope RE, 1983. Monoclonal antibodies to identify Zinga virus as Rift Valley fever virus. Lancet, 1(8325):641.

Meegan JM, Khalil GM, Hoogstraal H, Adham FK, 1980. Experimental transmission and field isolation studies implicating *Culex pipiens* as a vector of Rift Valley fever virus in Egypt. American Journal of Tropical Medicine and Hygiene, 29(6):1405-1410.

Meegan JM, Yedloutschnig RJ, Peleg BA, Jaffa Shy, Peters CJ, Walker JS, Shope RE, 1987. Enzyme linked immuno-absorbent assay for detection of antibodies to Rift

Valley fever virus in ovine and bovine sera. American Journal of Veterinary Research, 48:1138-1141.

Metselaar D, Henderson BE, Kirya J, Tukei MP, de Geus A, 1974. Isolation of arboviruses in Kenya, 1966-1971. Transactions of the Royal Society of Tropical Medicine and Hygiene, 68:114-123.

Miller BR, Godsey MS, Crabtree MB, Savage HM, Al-Mazrao Y, Al-Jeffri MH, Abdoon AMM, Al-Seghayer SM, Shahrani AM and Ksiazek TG, 2002. Isolation and genetic characterization of Rift Valley fever from *Aedes vexans arabiensis*, Kingdom of Saudi Arabia. Emerging Infectious Diseases, 8(12):1492-1494.

Miller WS, Demchak P, Rosenberger CR, Dominik JW, Bradshaw JL, 1963. Stability and infectivity of airborne yellow fever and Rift Valley fever viruses. American Journal of Hygiene, 77:114-121

Mims CA, 1956. Absorption and multiplication of the virus. British Journal of Experimental Pathology, 37:110-119 and 37:120-128 and 37, Incomplete virus, 37:129-143.

Mims CA, 1956. Rift Valley fever in mice. General features of the infection. British Journal of Experimental Pathology, 37:99-109.

Mims CA, 1957. Rift Valley fever in mice. The histological changes in the liver in relation to virus multiplication. Australian Journal of Experimental Biology and Medical Science, 35:595-604.

Mims CA, Mason PJ, 1956. Rift Valley fever in mice. V. The properties of a haemagglutinin present in infective serum. British Journal of Experimental Pathology, 37:423-433.

Mitten JO, Remmele NS, Walker JS, Carter RC, Stephen EL, Klein F, 1970. The clinical aspects of Rift Valley fever virus in household pets. III. Pathological changes in the dog and cat. Journal of infectious Diseases, 121:25-31.

Morrill JC, Carpenter L, Taylor D, Ramsburg HH, Quance J, Peters CJ, 1991. Further evaluation of a mutagen-attenuated Rift Valley fever vaccine in sheep. Vaccine, 9(1):35-41.

Morrill JC, Czarniecki CW, Peters CJ, 1991. Recombinant human interferon-gamma modulates Rift Valley fever virus infection in the Rhesus monkey. Journal of Interferon Research, 11(5):297-304.

Morrill JC, Jennings GB, Johnson AJ, Cosgriff TM, Gibbs PH, Peters CJ, 1990. Pathogenesis of Rift Valley fever in rhesus monkeys: role of interferon response. Archives of Virology, 110(3-4):195-212.

Morrill JC, Knauert FK, Ksiazek JM, Meegan JM, Peters CJ, 1989. Rift Valley fever infection of rhesus monkeys. Implications for rapid diagnosis of human disease. Research in Virology, 140:139-146.

Morrill JC, Mebus CA, Peters CJ, 1997. Safety and efficacy of a mutagen-attenuated Rift Valley fever virus vaccine in cattle. American Journal of Veterinary Research, 58(10):1104-1109.

Morvan J, Rollin PE, Laventure S, Roux J 1992. Duration of immunoglobulin M antibodies against RVF virus in cattle after natural infection. Transactions of the Royal Society of Tropical Medicine and Hygiene, 86:675.

Morvan J, Saluzzo JF, Fontenille D, Rollin PE, Coulanges P, 1991. Rift Valley fever on the east coast of Madagascar. Research in Virology, 142(6):475-482.

Muller R, Poch O, Delarue M, Bishop DH, Bouloy M, 1994. Rift Valley fever virus L segment: correction of the sequence and possible functional role of newly identified regions conserved in RNA-dependent polymerases. Journal of General Virology, 75(Pt 6):1345-1352.

Muller R, Saluzzo JF, Lopez N, Dreier T, Turell M, Smith J, Bouloy M, 1995. Characterization of clone 13, a naturally attenuated avirulent isolate of Rift Valley fever virus, which is altered in the small segment. American Journal of Tropical Medicine and Hygiene, 53(4):405-411.

Mulligan EJ, 1937. Rift Valley fever. Annual Report of the Veterinary Department, Nairobi, Kenya, pp. 60-62.

Mundel B, Gear J, 1951. Rift Valley fever. I. The occurrence of human cases in Johannesburg. South African Medical Journal, 25:926-930.

Munstermann LE, Conn JE, 1997. Systematics of mosquito disease vectors (Diptera: Culicidae): impact of molecular biology and cladistic analysis. Annual Review of Entomology, 42:351-369.

Nabeth P, Kane Y, Abdalahi MO, Diallo M, Ndiaye K, Bâ K, Schneegans F, Sall AA, Mathiot C, 2001. Rift Valley fever outbreak, Mauritania, 1998: seroepidemiologic, virologic, entomologic, and zoologic investigations. Emerging Infectious Diseases 7(6):1052-1054.

Niklasson B, Gargan TP, 1985. Enzyme-linked immunosorbent assay for detection of Rift Valley fever virus antigen in mosquitoes. American Journal of Tropical Medicine and Hygiene, 34:400-405.

Niklasson B, Grandien M, Peters CJ, Gargan TP, 1983. Detection of Rift Valley fever virus antigen by enzyme-linked immunosorbent assay. Journal of Clinical Microbiology, 17:1026-1031.

Niklasson B, Peters CJ, Grandien M, Wood O, 1984. Detection of human immunoglobulins G and M antibodies to Rift Valley fever virus by enzyme-linked immunosorbent assay. Journal of Clinical Microbiology, 19:225-229.

Ohder H, Lund LJ, Whiteland AP, 1970. Observations on the groth and development of bluetongue, Nairobi sheep disease and Rift Valley fever viruses by fluorescent antibody technique and titration in a tissue culture system. Archiv für die gesamte Virusforschung, 29:127-138.

OIE, 2004. Guidelines for Import Risk Analysis. Chapter 1.3.2. In: *Terrestrial Animal Health Code*. 13th edition. World Organisation for Animal Health, Paris, France.

OIE, 2004, Terrestrial Animal Health Code. 13th. Edition, World Organization for Animal Health, Paris, France.

OIE, 2004, Manual of Diagnostic Tests and Vaccines for Terrestrial Animals, 5th. Edition, World Organization for Animal Health, Paris, France.

Olaleye OD, Tomori O, Fajimi JL, Schmitz H, 1996. Experimental infection of three Nigerian breeds of sheep with the Zinga strain of the Rift Valley Fever virus. Revue d Elevage et de Medecine Veterinaire des Pays Tropicaux, 49:6-16.

Orlando MD, DeLauter RD, Riley JM, 1967. Effect of virus input multiplicity and tissue cell concentration on growth of Rift Valley fever virus. Applied Microbiology, 15:594-595.

Patrican LA, Bailey CL, 1989. Ingestion of immune bloodmeals and infection of *Aedes fowleri, Aedes mcintoshi,* and *Culex pipiens* with Rift Valley fever virus. American Journal of Tropical Medicine and Hygiene, 40(5):534-40.

Paweska JT, Barnard BJH, Williams R, 1995. The use of sucrose-acetone-extracted Rift Valley fever virus antigen from cell culture in an indirect enzyme-linked immunosorbent assay and haemagglutination-inhibition tests. Onderstepoort Journal of Veterinary Research, 62:227–233.

Paweska JT, Burt FJ, Anthony F, Smith SJ, Grobbelaar AA, Croft JE, Ksiazek T, Swanepoel R, 2003a. IgG-sandwich and IgM-capture enzyme-linked immunosorbent assay for detection of antibody to Rift Valley fever in domestic ruminants, Journal of Virological Methods, 113:103-112.

Paweska JT, Smith SJ, Wright IM, Williams R, Cohen AS, Van Dijk AA, Grobbelaar AA, Croft JE, Swanepoel R, Gerdes GH, 2003b. Indirect enzyme-linked immunosorbent assay for the detection of antibody against Rift Valley fever virus in domestic and wild ruminant sera. Onderstepoort Journal of Veterinary Research, 70:49-64.

Paweska JT, Burt F, Swanepoel R, 2005a. Validation of enzyme-linked immunosorbent assay for the detection of IgG and IgM antibody to Rift Valley fever virus in humans. Journal of Virological Methods, 124:173-181.

Paweska, JT, Mortimer E, Leman PA, Swanepoel R, 2005b. An inhibition enzyme-linked immunosorbent assay for the detection of antibody to Rift Valley fever virus in humans, domestic and wild ruminants. Journal of Virological Methods, 127:10-18.

Pelissier A, Rousselot R, 1954. Enquête sérologique sur l'incidence des virus neurotropes chez quelques singes de l'Afrique équatoriale française. Bull Soc Pathol Exot, 47:228–230.

Peters CJ, 2003. Rift Valley fever virus: today's needs, and future prospects. In: VRC Symposium on Viral Hemorrhagic Fevers, Vaccine Research Center, NIAID, NIH, DHHS, Bethesda Maryland.

Peters CJ, Meegan JM, 1981. Rift Valley fever. In: G Beran (ed) CRC Handbook Series in Zoonoses. Sect B1. CRC Press, Boca Raton, Florida, USA. 403-420.

Peters CJ, Anderson GW, 1981. Pathogenesis of Rift Valley fever. Contributions to Epidemiology and Biostatistics, 3:21-41.

Peters CJ, Ennis WH, Turell MJ, Niklasson B, 1989. Rapid detection of Rift Valley fever antigen in the serum of infected lambs. Res. Virol., 140:43-46.

Pini A, Lund LJ, Davies FG, 1970. Detection of Rift Valley fever virus by the fluorescent antibody technique in organs of experimentally infected animals. Research in Veterinary Science, 11:82-85.

Pittman PR, Liu CT, Cannon TL, Makuch RS, Mangiafico JA, Gibbs PH, Peters CJ, 1999. Immunogenicity of an inactivated Rift Valley fever vaccine in humans: a 12-year experience. Vaccine, 18(1-2):181-189.

Portefield JS, Della-Porta AJ, 1981. Bunyaviridae: Infections and diagnosis. In: E Kurstak, C Kurstak (eds) Comparative diagnosis of viral disease. New York: Academic Press IV, 479-500.

Prehaud C, Lopez N, Blok MJ, Obry V, Bouloy M, 1997. Analysis of the 3' terminal sequence recognised by the Rift Valley fever virus transcription complex in its ambisense S segment. Virology, 227:189-197.

Pretorious A, Oelofson MJ, Smith MS, van der Ryst E, 1997. Rift Valley fever, a sero-epidemiological study of small terrestrial vertebrates in South Africa. American Journal of Tropical Medicine and Hygiene, 57:693-698.

Randall R, Binn LN, Harrison VR, 1964. Immunisation against Rift Valley fever virus. Studies on the immunogenicity of lyophilised formalin inactivated vaccine. Journal of Immunology, 92:293-299.

Reinert JF, 2000. New classification for the composite genus *Aedes* (Diptera: Culicidae: Aedini), elevation of subgenus *Ochlerotatus* to generic rank, reclassification of the other subgenera, and notes on certain subgenera and species. Journal of the American Mosquito Control Association, 16(3):175-188.

Rice RM, Erlick BJ, Rosato RR, Eddy GA, Mohanty SB, 1980. Biochemical characterisation of Rift Valley fever virus. Virology, 105:256-260.

Rippy MK, Topper MJ, Mebus CA, Morrill JC, 1992. Rift Valley fever virus-induced encephalomyelitis and hepatitis in calves. Veterinary Pathology, 29(6):495-502.

Robert V, Lhuillier M, Meunier D, Sarthou JL, Monteny N, Digoutte JP, Cornet M, Germain M, Cordellier R, 1993. Yellow fever virus, dengue 2 and other arboviruses isolated from mosquitos, in Burkina Faso, from 1983 to 1986. Entomological and epidemiological considerations. Bulletin de al Societe de Pathologie Exotique, 86(2):90-100.

Rossi CA, Turell MJ, 1988. Characterization of attenuated strains of Rift Valley fever virus. Journal of General Virology, 69(4):817-823.

Roth JA, Spickler AR, 2003. A survey of vaccines produced for OIE list A diseases in OIE member countries. Developmental Biology (Basel), 114:5-25.

Sall AA, de A Zanotto PM, Zeller HG, Digoutte JP, Thiongane Y, Bouloy M, 1997. Variability of the NS(S) protein among Rift Valley fever virus isolates. Journal of General Virology, 78:2853-2858.

Sall AA, Thonnon J, Sene OK, Fall A, Ndiaye M, Baudes B, Mathiot C, Bouloy M, 2001. Single-tube and nested reverse transcriptase-polymerase chain reaction for the detection of Rift Valley fever virus in human and animal sera. Journal of Virological Methods, 91:85–92.

Sall AA, Zanotto PM, Sene OK, Zeller HG, Digoutte JP, Thiongane Y, Bouloy M, 1999. Genetic reassortment of Rift Valley fever in nature. Journal of Virology 73(10):8196-8200.

Saluzzo JF, Chartier C, Bada R, Martinez D, Digoutte JP, 1987. La fievre de la vallee du Rift en Afrique de l'Ouest. Revue d'Elevage et de Médecine Vétérinaire des Pays Tropicaux, 40:215-223.

Saluzzo JF, Digoutte JP, Cornet M, Baudon D, Roux J, Robert V, 1984. Isolation of Crimean-Congo haemorrhagic fever and Rift Valley fever viruses in Upper Volta. Lancet, 1(8387):1179.

Samany R, 1997. Rift Valley fever surveillance in Madagascar. Bulletin - Office International des Epizooties, 109(1):43-44.

Samui KL, Inoue S, Mweene AS, Nambota AM, Mlangwa JED, Chilonda P, Onuma M, Morita C, 1997. Distribution of Rift Valley fever among cattle in Zambia. Japanese Journal of Medical Science and Biology, 50(2):73-77.

Schultz KCA, 1951. The pathology of Rift Valley fever or enzootic hepatitis in South Africa. Journal of South African Veterinary Medical Assocation, 22:113-120.

Schwentker FF, Rivers TM, 1934. Rift Valley fever in man. Report of a fatal laboratory infection complicated by thrombophlebitis. Journal of Experimental Medicine, 59:305-313.

Scott GR, 1963. Pigs and Rift Valley fever. Nature, 200:920-921.

Scott GR, 1981. Fever and death in sheep infected with the viruses of Rift Valley fever and Nairobi sheep disease. Transactions of the Royal Society of Tropical Medicine and Hygiene, 75:329-329.

Scott GR, Coakley W, Roach RW, Cowdy NR, 1963. Rift Valley fever in camels. Journal of Pathology and Bacteriology, 86:229-231.

Scott GR, Heisch RB, 1959. Rift Valley fever and Rift Valley rodents. East African Medical Journal, 36:665-667.

Sellers RF, Pedgely DE, Tucker MR, 1977. Possible spread of African horse sickness on the wind. Journal of Hygiene, Cambridge, 79:279-298.

Sellers RF, Pedgley DE, Tucker MR, 1982. Rift Valley fever, Egypt 1977: disease spread by windborne insect vectors. The Veterinary Record, 110:73-77.

Shimshony A, Barzilai R, 1983. Rift Valley fever. Advances in Veterinary Science and Comparative Medicine, 27:347-425.

Shoemaker T, Boulianne C, Vincent MJ, Pezzanite L, Al-Qahtani MM, Al-Mazrou Y, Khan AS, Rollin PE, Swanepoel R, Ksiazek TG, Nichol ST, 2002. Genetic analysis of viruses associated with emergence of Rift Valley fever in Saudi Arabia and Yemen, 2000-01. Emerging Infectious Diseases, 8:1415-1420.

Shope RE, Meegan JM, Peters CJ, Tesh RB, Travassos da Rosa AA, 1981. Immunologic status of Rift Valley fever and approaches to its control. Bulletin of the World Health Organisation, 60:299-304.

Shope RE, Peter CJ, Davies FG, 1982. The spread of Rift Valley fever and approaches to its control. Bulletin of the World Health Organization, 60:299-304.

Shope RE, Peters CJ, Walker JS, 1980. Serological relation between Rift Valley fever virus and the viruses of the Phlebotomus fever serogroup. Lancet, 1:886-887.

Smithburn KC, 1949. Rift Valley fever: the neurotropic adaptation of the virus and the experimental use of this modified virus as a vaccine. British Journal of Experimental Pathology, 30: 1-16.

Smithburn KC, Haddow AJ, Gillett JD, 1948. Rift Valley fever, isolation of the virus from wild mosquitoes. British Journal of Experimental Pathology, 29:107-121.

Smithburn KC, Mahaffy AF, Haddow AJ, Kitchen SF, Smith JF, 1949. Rift Valley Fever accidental infections among laboratory workers. Journal of Immunology, 62:213-227.

Swanepoel R, 1976. Studies on the epidemiology of Rift Valley fever. Journal of South African Veterinary Assocation, 3:42-52.

Swanepoel R, 1981. Observations on Rift Valley fever in Zimbabwe. Contributions to Epidemiology and Biostatistics, 3:42-52.

Swanepoel R, Blackburn NK, Efstratiou S, Condy JB, 1978. Studies of Rift Valley fever in some African murids (Rodentia: Muridae). Journal of Hygiene, Cambridge, 70, 183-196.

Swanepoel R, Coetzer JAW, 2004. Rift Valley fever. In: JAW Coetzer, RC Tustin (eds). Infectious diseases of livestock. Volume 2. 2nd ed. Oxford University Press, Oxford, UK. 1037-1076.

Swanepoel R, Struthers JK, Erasmus MJ, Shepherd SP, McGillivray GM, Erasmus BJ, Barnard BJH, 1986. Comparison of techniques for demonstrating antibodies to Rift Valley fever virus. Journal of Hygiene, Cambridge, 97:317-329.

Swanepoel R, Struthers JK, Erasmus MJ, Shepherd SP, McGillivray GM, Shepherd AJ, Hummitzsch DE, Erasmus BJ, Barnard BJH, 1986. Comparative pathogenicity and

antigenic cross-reactivity of Rift Valley fever and other African phleboviruses in sheep. Journal of Hygiene, Cambridge, 97:331-346.

Tesch RB, Peters CJ, Meegan JM, 1982. Studies on the antigenic relationships among Phleboviruses. American Journal of Tropical Medicine and Hygiene, 31:149-155.

Theiler M, 1957. Action of sodium desoxycholate on arthropod-borne viruses. Proceedings of the Society for Experimental Biology and Medicine, 96:380-382

Thiongane Y, Thonnon J, Fontenille D, Zeller H, Akapo JA, Gonzalez JP, Digoutte JP, 1997. Epidemio-surveillance de la fievre de la vallee du Rift au Senegal. Epidemiologie et Sante Animale, No. 31-32.

Thonnon J, Picquet M, Thiongane Y, Lo M, Sylla R, Vercruysse J, 1999. Rift Valley fever surveillance in the lower Senegal River Basin: update 10 years after the epidemic. Tropical Medicine and International Health, 4(8):580-585.

Tomori O, 1979. Clinical, virological and serological response of the West African dwarf sheep to experimental infection with different strains of Rift Valley fever virus. Research in Veterinary Science, 26:152-159.

Traoré-Lamizana M, Fontenille D, Diallo M, Bâ Y, Zeller HG, Mondo M, Adam F, Thonon J, Maïga A, 2001. Arbovirus surveillance from 1990 to 1995 in the Barkedji area (Ferlo) of Senegal, a possible natural focus of Rift Valley fever virus. Journal of Medical Entomology, 38(4):480-492.

Trouillet J, Ba Y, Traore-Lamizana M, Zeller HG, Digoutte JP, Fontenille D, 1995. Phlébotomes (Diptera: *Psychodidae*) du Sénégal. Peuplement du Ferlo. Isolement d'arbovirus. Parasite, 2:289-296.

Turell MJ, 1989. Effect of environmental temperature on the vector competence of *Aedes fowleri* for Rift Valley fever virus. Research in Virology, 140(2):147-154.

Turell MJ, Bailey CL, 1987. Transmission studies in mosquitoes (Diptera: Culicidae) with disseminated Rift Valley fever virus infections. Journal of Medical Entomology, 24(1):11-18.

Turell MJ, Bailey CL, Beaman JR, 1988a. Vector competence of a Houston, Texas strain of *Aedes albopictus* for Rift Valley fever virus. Journal of the American Mosquito Control Association, 4(1):94-96.

Turell MJ, Bailey CL, Rossi CA, 1984a. Increased mosquito feeding on Rift Valley fever virus-infected lambs. American Journal of Tropical Medicine and Hygiene, 33(6):1232-1238.

Turell MJ, Cynthia AR, 1991. Potential for mosquito transmission of attenuated strain of Rift Valley fever virus. American Journal of Tropical Medicine and Hygiene, 44:278-382.

Turell MJ, Faran ME, Cornet M, Bailey CL, 1988b. Vector competence of Senegalese *Aedes fowleri* (Diptera: Culicidae) for Rift Valley fever virus. Journal of Medical Entomology, 25(4):262-266.

Turell MJ, Gargan TP, Bailey CL, 1984b. Replication and dissemination of Rift Valley fever virus in *Culex pipiens*. American Journal of Tropical Medicine and Hygiene, 33(1):176-181.

Turell MJ, Kay BH, 1998. Susceptibility of selected strains of Australian mosquitoes (Diptera: Culicidae) to Rift Valley fever virus. Journal of Medical Entomology, 35(2):132-135.

Turell MJ, Linthicum KJ and Beaman JR, 1990. Transmission of Rift Valley fever virus by adult mosquitoes after ingestion of virus as larvae. American Journal of Tropical Medicine and Hygiene, 43:677-680.

Turell MJ, Perkins PV, 1990. Transmission of Rift Valley fever virus by the sand fly, *Phlebotomus duboscqi* (Diptera: Psychodidae). American Journal of Tropical Medicine and Hygiene, 42(2):185-188.

Turell MJ, Presley SM, Gad AM, Cope SE, Dohm DJ, Morrill JC, Arthur RR, 1996. Vector competence of Egyptian mosquitoes for Rift Valley fever virus. American Journal of Tropical Medicine and Hygiene, 54(2):136-139.

Turell MJ, Rossi CA, 1991. Potential for mosquito transmission of attenuated strains of Rift Valley fever virus. American Journal of Tropical Medicine and Hygiene, 44(3):278-282.

Turell MJ, Rossi CA, Bailey CL, 1985. Effect of extrinsic incubation temperature on the ability of *Aedes taeniorhynchus* and *Culex pipiens* to transmit Rift Valley fever virus. American Journal of Tropical Medicine and Hygiene, 34(6):1211-1218.

Turell MJ, Rossignol PA, Spielman A, Rossi CA, Bailey CL, 1984c. Enhanced arboviral transmission by mosquitoes that concurrently ingested microfilariae. Science, 225 (4666):1039-1041.

Turell MJ, Spielman A, 1992. Nonvascular delivery of Rift Valley fever virus by infected mosquitoes. American Journal of Tropical Medicine and Hygiene, 47(2):190-194.

Van Velden DJJ, Meyer JD, Oliver J., Gear JHS, McIntosh B, 1977. Rift Valley fever affecting humans in South Africa: a clinicopathological study. South African Medical Journal, 51:867-871.

Vaughan JA, Turell MJ, 1996. Facilitation of Rift Valley fever virus transmission by Plasmodium berghei sporozoites in *Anopheles stephensi* mosquitoes. American Journal of Tropical Medicine and Hygiene, 55(4):407-409.

Vinogradova EB, 2000. *Culex pipiens pipiens* mosquitoes: taxonomy, distribution, ecology, physiology, genetics, applied importance and control. Pemberley Books, Iver, UK.

Wasel M, Atia MM, Mohsen A, El Sayed M, Zaki K, 1990. Effect of Rift Valley fever virus on pregnant and non-pregnant local Barki ewes after artificial infection. Archiv für Experimentelle Veterinärmedizin, 44:645-648.

Weinbren MP, Williams MC, Haddow AJ, 1957. A variant of Rift Valley fever virus. South African Medical Journal, 31(38):951-957.

Weiss KE, 1957. Rift Valley fever: a review. Bulletin of Epizootic Diseases of Africa, 5:431-458.

WHO, 2000. Outbreak news: Rift Valley fever, Saudi Arabia, August–October 2000. Weekly Epidemiological Record, 75(46):369–376.

WHO, 2003. Rift Valley fever in Egypt. http://www.who.int/csr/don/2003_09_02a/en/

Williams MC, Woodall JP, Corbet PS and Haddow AJ, 1960. An outbreak of Rift Valley fever occurring near Entebbe: Entomological studies and further isolations. East African Virus Research Institute Report, 10:24-25.

Wilson ML, Chapman LE, Hall DB, Dykstra EA, Ba K, Zeller HG. 1994. Rift Valley fever in rural northern Senegal: human risk factors and potential vectors. American Journal of Tropical Medicine and Hygiene, 50:663-675

Woodall JP, 1964. The viruses isolated from arthropods at the East African Virus Research Institute in the 26 years ending December 1963. Proc. of the East African Academy, 2:141-146.

Woods CW, Karpati AM, Grein T, McCarthy N, Gaturuku P, Muchiri E, Dunster L, Henderson A, Khan AS, Swanepoel R, Bonmarin I, Martin L, Mann P, Smoak BL, Ryan M, Ksiazek TG, Arthur RR, Ndikuyeze A, Agata NN, Peters CJ, and the WHO Haemorrhagic Fever Task Force. 2002. An Outbreak of Rift Valley Fever in Northeastern Kenya, 1997-1998. Emerging Infectious Diseases, 8:138-144.

Wright, P.F., Nilsson, E., Van Rooij, E.M.A., Lelenta, M., Jeggo, M.H., 1993. Standardisation and validation of enzyme-linked immunosorbent assay techniques for the detection of antibody in infectious disease diagnosis. Revue Scientifique et Technique Office International des Epizooties, 12:435-450.

Yedloutshnig R J and Walker J S. 1981. Rift Valley fever. in Current veterinary Therapeutics, Food Animal Practice; pp 568-571.

Yedloutschnig RJ, Dardiri AH, Walker JS, Peters CJ, Eddy GA, 1979. Immune response of steers, goats and sheep to an inactivated Rift Valley fever vaccine. Proceedings of the United States Animal Health Association 83rd Annual Meeting, 83:253-260.

Yedloutschnig RJ, Dardiri AH, Mebus CA, Walker JS, 1981a. Abortion in vaccinated sheep and cattle after challenge with Rift Valley fever virus. Veterinary Record, 109:383-384.

Yedloutschnig RJ, Dardiri AH, Walker JS, 1981b. The response of ponies to inoculation with Rift Valley fever virus. Contributions to Epidemiology and Biostatistics, 3:68-71.

Yedloutschnig RJ, Dardiri AH, Walker JS, 1981c. Persistence of Rift Valley fever virus in the spleen, liver, and brain of sheep after experimental infection. Contributions to Epidemiology and Biostatistics, 3:72-76.

Zaki SR, Greer PW, Coffield LM, Goldsmith CS, Nolte KB, Foucar K, Feddersen RM, Zumwalt RE, Miller GL, Khan AS, 1995. Hantavirus pulmonary syndrome. Pathogenesis of an emerging infectious disease. American Journal of Pathology, 146:552-579.

Zeller HG, Bessin R, Thiongane Y, Bapetel I, Teou K, Ala MG, Atse AN, Sylla R, Digoutte JP, Akakpo JA, 1995. Rift Valley fever antibody prevalence in domestic ungulates in Cameroon and several West African countries (1989-1992), following the 1987 Mauritanian outbreak. Research in Virology, 146(1):81-85.

Zeller HG, Fointeneille D, Traore Lamizana M, Thiongane Y, Digoutte JP, 1997. Enzootic activity of Rift Valley fever virus in Senegal. American Journal of Tropical Medicine and Hygiene, 56:265-272.

Zeller HG, Rakotoharinadrasana HT, Rakoto Andrianarivelo M, 1998. Rift Valley fever in Madagascar: infection risks for the abattoir staff in Antananarivo. Revue d'Elevage et de Médecine Vétérinaire des Pays Tropicaux, 51(1):17-20.

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