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Pathways for entry of livestock arboviruses into Great Britain: Assessing the strength of evidence.

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Summary

The emergence of bluetongue virus and Schmallenberg virus in Great Britain (GB) during the last decade has highlighted the need for understanding the relative importance of the various pathways of entry of livestock arboviruses so as to help focus surveillance and mitigation. This paper summarises what is known for the main routes of entry and assesses the strength of the current evidence for and against. Entry through infected arthropod vectors is considered at the level of each life cycle stage for tick-, biting midge- and mosquito-borne viruses, and while there is evidence this could happen through most tick and mosquito stages, strong evidence only exists for entry through adult midges. There is also strong evidence that entry through immature midge stages could not happen. The weight of supporting evidence is strongest for importation of viraemic livestock including horses. While there is some indication of a common pathway for midge-borne viruses from sub-Saharan Africa to GB via Continental Europe, other factors such as maternal transmission in dogs and sheep need to be considered in the light of recent findings.

Introduction

Arthropod-borne viruses (arboviruses) typically cycle between an arthropod vector (typically a biting midge, tick or mosquito) and a vertebrate host(s) and many infect humans and livestock causing serious disease in some cases. Well-known arboviruses that infect livestock and/or horses and are transmitted by biting midges include bluetongue virus (BTV), African horse sickness virus (AHSV), and Schmallenberg virus (SBV). Rift Valley fever virus (RVFV), Venezuelan equine encephalitis virus (VEEV), West Nile virus (WNV) and Japanese encephalitis virus (JEV) are transmitted by mosquitoes and are examples of arboviruses which infect humans as well as livestock and/or horses. African swine fever virus (ASFV), Louping ill virus (LIV) and Crimean-Congo haemorrhagic fever virus (CCHFV) are transmitted by ticks. With the potential for expansion in range and increase in abundance of many important vector species (Estrada-Pena et al, 2012a; Mardulyn et al, 2013) together with the emergence of both well-known and previously unknown arboviruses in northern Europe, an understanding of the relative importance of the various pathways of entry of these viruses into Great Britain (GB) is central to focusing surveillance and mitigation.

This paper summarises the strength of the evidence for and against the entry of livestock arboviruses through various pathways into GB and draws on evidence from the viruses listed above. Following the OIE Terrestrial Animal Health Code definition (OIE, 2010) for import risk analysis, a risk assessment includes three steps, namely entry assessment, exposure assessment and consequence assessment. The entry assessment refers to introduction of the pathogenic agent into a particular environment but does not include what happens to that pathogenic agent or whether it establishes once it has entered. The specific aims are to identify whether a key or common pathway is currently emerging, and to consider what further information is needed to assess the risks of entry in the light of new data on virus transmission. In addition, to address whether resource should be spent on gathering data on routes for which there is little evidence regarding entry, the importance of those routes in terms of onward transmission to livestock in GB is briefly discussed here. This work does not set out to assess or rank the probabilities of entry.

Overview of pathways

Information, including vector species, vertebrate hosts, and known risk factors, was gathered from numerous references covering a wide range of arboviruses and used to produce an overview of the strength of the evidence for the known and potential routes of transmission (Table 1). The pathways include entry of infected vector, contaminated animal products, live animals and infected humans as set out in de Vos et al, (2013). The presence of arbovirus in semen of infected livestock is well-documented and, for example, SBV RNA has been detected in semen of experimentally infected bulls (Schulz et al, 2014). However, biological material (vaccines, serum, semen and ova) is imported from animals kept in approved centres and is not considered here.

Entry of infected vector. The relationship of each life cycle stage of the vector with both its vertebrate host and environment is an important consideration for the entry of virus via the vector. A recent modelling study (England, 2013), for example, has shown that, in terms of the potential to generate unfed, CCHFV-infected adult *Hyalomma* ticks that could infect a human or livestock animal within GB, surveillance in GB should focus more on adult ticks on imported horses and perhaps less on nymphal ticks entering on migrant birds. This reflects the fact that, although more nymphal ticks may enter GB on birds per year, each gravid female tick on a horse may generate several thousand eggs some of which would be infected through transovarial transmission (TOT).

There is strong evidence that BTV and SBV entered GB through adult midges carried on the wind from continental Europe (Burgin et al, 2013; Sedda and Rogers, 2012). It has also been documented that adult mosquitoes have entered GB in cabins of aircraft (Hutchinson et al, 2005). In addition, adult midges or mosquitoes could be transported within 8 hours at temperatures of 10-15°C (Van der Hulst, 2004) into northern Europe and GB in packed flowers from sub-Saharan Africa. Kenya is the main exporter of flowers to GB from RVFV-endemic areas, for example, with the United Kingdom (UK) importing 17% of Kenya's output (Hornberger et al, 2007). The flower growing regions are close to Nairobi International airport (Hornberger et al, 2007) and flower packaging operations continue into the night in Kenya with the bright lights attracting flying insects (Chris Oura, The Pirbright Institute, personal communication, 2011). The number of adult mosquitoes and midges being packed with the flowers, therefore, and exported to GB by aircraft per year cannot be assumed to be negligible and a proportion could be infected with arbovirus. It is concluded that the evidence suggests entry of arboviruses could occur through this route (Table 1).

The larvae of midges and mosquitoes are not haematophagous and thus, unlike tick larval/nymph stages, cannot be infected in the absence of TOT and transstadial transmission. TOT has been documented for mosquito-borne viruses such as RVFV and WNV. Entry of desiccation-resistant eggs of certain *Aedes* spp. mosquitoes in imported car tyres into Europe has occurred (Becker et al, 2011) and a proportion could be infected with arbovirus, depending on their origin. A route of entry for larvae of exotic mosquitoes is via ornamental plants, e.g. Lucky Bamboo (*Dracaena sanderiana*) which are transported in containers with standing water, making them an "ideal insectary in transit". Multiple introductions of *Aedes albopictus* to the Netherlands in commercial horticultural greenhouses were traced to intensive trade of this plant (Becker et al, 2011). Car tyres are more likely to be imported to GB from China or Japan than from Africa (African Business Pages, 2011) and Lucky Bamboo is exported from southeast Asia including China and Taiwan where JEV, for example, is endemic (Huang et al, 2010). The available evidence suggests entry of mosquito-borne livestock viruses to GB through import of larvae and eggs could happen (Table 1).

Most evidence suggests that midge-borne livestock viruses, notably BTV (Wilson et al, 2008), Akabane virus (Allingham and Standfast, 1990) and AHSV are not transmitted by TOT in midges. This is strong evidence that entry of arboviruses in eggs, larvae or pupae of midges could not occur (Table 1). However, Larska et al, (2013) present indirect evidence for the possibility of TOT of SBV in *Culicoides* midges in Poland, and cite a 2005 study which reported the BTV genome in over 10% of midge larvae collected in endemic sites in Colorado. There is no evidence that eggs, larvae or pupae of midges are imported into GB (Table 1). Indeed, they are found in maize silages and cattle dung (Zimmer et al, 2008) which are not likely to enter GB in large amounts.

Jameson and Medlock (2009) reported the entry of an adult *Hyalomma marginatum* tick, the vector for CCHFV, on a horse imported from Portugal, and with the recent detection of CCHFV in ticks in central Spain (Estrada-Pena et al, 2012b) it is concluded that there is strong evidence that arbovirus-infected adult ticks could enter GB (Table 1). Indeed, CCHFV-infected ticks have been imported to Egypt from Somalia on camels (Chisholm et al, 2012). Similarly, there is strong evidence that tick-borne viruses could enter GB in ticks on migratory birds (Jameson et al, 2012) and CCHFV-infected *H. marginatum* have been found on migrating birds in Morocco in spring (Palomar et al, 2013). CCHFV has recently been detected in *Hyalomma aegyptium* on tortoises in Turkey and Syria, although importation of wild tortoises to GB is illegal (Phipps et al, 2014). While entry to GB of *H. aegyptium* (on tortoises destined for the pet trade) and *H. marginatum* (on migrant birds) was documented by Martyn (1988), entry to GB of *Ornithodoros* ticks (the vector for ASFV) was not reported. Indeed, entry of ASFV-infected ticks for example on imported pigs, to GB would be most unlikely because live pigs would not be imported from endemic areas, and *Ornithodoros moubata* ticks, a vector for ASFV, probably complete feeding in 30 minutes from finding a host (Paul Phipps, AHVLA, personal communication, 2014). Some arthropod vectors, most notably the *H. marginatum* tick are predicted to expand their range northwards in the western Palearctic because of climate trends (Estrada-Pena et al, 2012a) and two infestations of cattle with adults of *H.m. rufipes* were recorded for the first time in central Europe in 2011 (Hornok and Horvath, 2012). Moreover, with livestock variants of tick-borne encephalitis virus (TBEV) in Europe and CCHFV emerging in the Balkans, pet dogs and cats could bring infected ticks into GB. Tick treatment for companion animals entering GB from continental Europe is no longer compulsory (Wall, 2012) although under the Pet Travel Scheme (Anon, 2014) pet owners are reminded that it is considered good practice to regularly treat their animals against a range of ticks.

Infected meat and animal products. The spread of ASFV to pigs by the transportation of pig meat is well documented (Costard et al, 2013) and AHSV has been transmitted to dogs through consumption of infected meat (Mellor and Hamblin 2004). The fluids and tissues from CCHFV- or RVFV-viraemic livestock contain high titres of virus. Indeed, direct contact with animal tissues, blood or other body fluids appeared to play a greater role in RVFV transmission to humans in South Africa than mosquito bite (Archer et al, 2013) highlighting the potential significance of meat and animal products as a route of entry. It is concluded that there is evidence that arboviruses could enter GB through import of infected meat (Table 1) both through legal and illegal routes, with the probability of entry for a given arbovirus depending on the prevalence in the country of origin. Thus entry of RVFV in livestock meat (illegally) carried by aircraft passengers from sub-Saharan Africa could occur, while entry via this route from within Europe is very unlikely because RVF is not endemic.

Importation of viraemic livestock, including horses. The risks of arbovirus entry through this route for cattle, sheep and goats to GB are low (due to import bans in restricted zones, and testing of imports from disease-free areas during an outbreak in Europe) but not negligible, particularly in the early stages of an outbreak in Europe. There is strong evidence entry has occurred through this route (Table 1). For example, a BTV-infected cow was imported into GB during the incursion in northern Europe in 2007 (Anon, 2007). Movement of sheep has been associated with dispersal of LIV, a variant of TBEV, in GB and may have resulted in introduction of LIV to Scandinavia from GB in the 19th century (McGuire et al, 1998). The arbovirus typically associated with movement of viraemic livestock is RVFV (Sherman, 2011). Indeed, Antonis et al, (2013) have recently shown that RVFV can be transmitted vertically in sheep in the absence of detectable maternal viraemia. Thus in terms of a route of entry, an infected developing foetus may not be detected by monitoring of the ewe, and some arboviruses, e.g. RVFV and CCHFV, do not show clear clinical symptoms in livestock. Fooks et al, (2014) recently reported the presence of antibodies specific for WNV in a horse in the UK with neurological disease. The horse had left Cyprus some two weeks earlier and was transported to the UK by sea and road through WNV endemic areas in Europe. Although WNV RNA was not detected, this provides strong evidence that entry of WNV to GB can occur through importing of horses from Europe (Table 1). It should also be noted that there is a potential risk of viraemic horses (e.g., infected with WNV or VEEV) returning to GB from Brazil after the 2016 Olympics.

Natural movement and migration of viraemic wildlife. Many wild mammal species are known reservoirs for arboviruses. However, the English Channel is a natural barrier to the migration of most mammals. Viraemic birds are a potential route for introduction of WNV and other flaviviruses (Malkinson et al, 2002). Overall 2.4% of migrant birds captured in southeastern Sweden in 2005/06 tested positive for antibodies against WNV (Jourdain et al, 2011). No such data are available for GB, although it should be noted that all dead birds, including some migratory species, selected for testing by targeted surveillance in GB were negative by PCR for WNV between 2002 and 2009 (Brugman et al, 2013). In general, migratory birds are less important for entry of tick-borne viruses such as CCHFV because with the exception of the ostrich (*Struthio camelus*), many bird species are refractory to CCHFV (Capua, 1998). Although grouse are an avian reservoir host for LIV they are not migratory. While Oropouche virus (which is transmitted by midges to humans) infects birds in addition to mammals in South America (Mourao et al, 2009), wild birds in general do not migrate from South America to GB or Europe. Thus it is concluded that there is evidence that entry of mosquito-borne viruses could occur in GB through this route, while the evidence seems to be against entry of tick-borne or midge-borne viruses (Table 1).

Importation of viraemic exotic wildlife. The zebra (*Equus quagga*) is considered to be the natural vertebrate host and reservoir of AHSV and levels of viraemia are lower than in horses (Mellor and Hamblin, 2004). The outbreak of AHSV in central Spain in 1987 was due to importation of a number of sub-clinically infected zebra from

Namibia to a safari park near Madrid (Mellor and Hamblin, 2004). There is anecdotal evidence that since 2006 exotic ruminants, including small antelope, have been imported from sub-Saharan Africa through airports in continental Europe for private animal collectors (Helen Roberts, AHVLA, personal communication, 2014). To the authors' knowledge there is no strong evidence for entry of arboviruses to GB through this route although it could occur (Table 1). Illegal importation of exotic wildlife is more difficult to assess and there are many examples of smuggled live birds entering European airports (Van den Berg, 2009).

Importation of viraemic pets. There is evidence that arboviruses could enter GB in viraemic pets (Table 1). Thus, dogs are known to have been infected naturally and/or experimentally with a number of livestock arboviruses including WNV (Austgen et al, 2004), VEEV (Coffey et al, 2006), CCHFV (Nalca and Whitehouse, 2007), RVFV (Keefer et al, 1972), AHSV (Mellor and Hamblin, 2004), SBV and other orthobunyaviruses (Sailleau et al, 2013). However, there are relatively few studies on arbovirus prevalence in companion animals. Thus Adams et al, (2012) reported all of 6 dogs (6-8 months old) tested in a study in 2008-2009 in Mexico were seropositive for VEEV. Data are also available for the Everglades virus, which is zoonotic and although related to VEEV is not known to infect horses with 4% of dogs in Florida reported as seropositve (Coffey et al, 2006). A dog was confirmed TBEV-positive by ELISA in Belgium (Roelandt et al, 2011). Of interest is the potential maternal transmission of SBV in dogs (Sailleau et al, 2013).

Travel of viraemic humans. By far the greatest global transport of mammals is that of humans and their health is not checked on entry to GB. Although not a livestock disease, Chikungunya virus emerged in Italy where the index case was a visitor from India (Tilston et al, 2009). There is strong evidence for entry by this route (Table 1) and cases of humans infected with mosquito-borne or tick-borne viruses entering GB have been reported e.g. Eastern equine encephalitis virus (EEEV) (Harvala et al, 2009) and CCHFV (Lumley et al, 2014). To date, Oropouche virus is the only arbovirus primarily transmitted by *Culicoides* midges to humans (Carpenter et al, 2013). This is taken as strong evidence against the entry to GB of humans infected with midge-borne viruses which also infect livestock (Table 1).

Discussion

In terms of the strength of evidence for entry of livestock arboviruses to GB (Table 1), the life cycle stage of the arthropod vector is an important consideration, particularly for midge-borne viruses. Thus, evidence for the absence of TOT strongly suggests that entry of arboviruses in the eggs and larvae of midges cannot occur. However, the evidence for entry of livestock arboviruses to GB through the vector is most compelling for adult midges, particularly where the disease has previously established in livestock in northern Europe. There is some indication for a common pathway for midge-borne viruses emerging in livestock in GB from sub-Saharan Africa, via north-west Europe. Indeed, there is suggestion that SBV was present in sheep in South Africa in July 2006 (Leask et al, 2013) and BTV8 is known to be linked to west Africa (Meiswinkel et al, 2007). The first step of this proposed pathway is the transport of viraemic exotic wildlife by aircraft from sub-Saharan Africa into north-west Europe where the virus establishes in the naïve livestock population and in the indigenous midge population. The ability of midges to fly unaided to GB given favourable wind conditions completes the route to GB. More information is needed on the first part of this proposed pathway, namely on the volume and origin of imports of exotic wildlife from Africa into north-western Europe. In the event of this pathway being closed through legislation, attention should focus on the numbers of adult midges (and mosquitoes) being transported from sub-Saharan Africa in packaged flowers, for example. To the authors' knowledge there is no definitive evidence for entry of livestock viruses to GB through infected mosquitoes, although evidence suggests it could occur through all life cycle stages of the mosquito (Table 1). Similarly entry of tick-borne viruses could occur through the larval and adult stages, although this is less of a concern to livestock than entry through infected adult midges or mosquitoes in that each tick stage has to moult before taking another blood meal. Thus only those tick species which can moult in the climate conditions in GB pose a potential risk to livestock. However, there is no longer the legal necessity to treat pets with acaricides on return to GB (Wall, 2012; Anon, 2014). There is currently little information on the numbers and species of ticks carried on imported horses and pets in Europe and the numbers and distribution of horses entering GB on ferries.

There is strong evidence for entry of mosquito-borne and biting midge-borne viruses through entry of imported viraemic livestock and horses to GB. The risk posed to GB livestock depends on the virus and the imported livestock species. For example, BTV-infected ruminants could infect indigenous GB midges. While horses are amplifying hosts for epizootic strains of VEEV (Taylor and Paessler 2013), they are dead-end hosts for WNV (Fooks et al, 2014) and Western equine encephalitis virus. Maternal transmission of arboviruses (e.g. RVFV) provides a mechanism for undetected entry through imported livestock. With the exception of mosquito-borne flaviviruses in viraemic migratory birds, there is strong evidence against entry through natural movement of viraemic wildlife. There is also strong evidence for entry of mosquito-borne and tick-borne livestock viruses through infected persons although viraemic humans are generally dead-end hosts with regard to infecting indigenous GB arthropod vectors and livestock. However, nosocomial transmission of CCHFV and RVFV occurs in endemic regions of the World and gathering data on the probability of entry to GB of arboviruses through infected humans is of greater importance for risks to public health than animal health.

The presumption of entry through many of the routes to GB is based on assumptions with the available evidence suggesting it could occur (Table 1). In particular, data are lacking for entry of arboviruses to GB through importation of meat and meat products, exotic wild animals and pets. Gathering evidence for entry of arboviruses through meat and meat products is judged not be high priority. First, the risks of exposure and hence transmission to livestock for most arboviruses through meat and meat products are minimised in GB by the EU Regulations which control animal by-products (EU 142/2011). Second, many arboviruses, for example SBV in cattle (Wernike et al, 2013), appear not to be readily transmissible to livestock through the oral route. The exception is ASFV in imported pork meat and Costard et al, (2013) have assessed the risk of entry of ASFV into GB through illegal importation of pork meat to be very high with a moderate risk of exposure of GB pigs given entry has occurred. Although competent tick vectors, namely *Ornithodoros* spp., for ASFV do not occur in GB, the virus is readily transmitted from pig-to-pig through secretions and fomites. With regard to arbovirus transmission to dogs through consumption of infected meat it is considered here that the focus should be on whether viraemic dogs are able to transmit the virus to arthropod vectors, rather than whether dogs are likely to be infected in GB through imported meat to GB. This is also a key data gap for assessing the potential risks from direct entry of viraemic pets. Thus, while it is reported that dogs are dead-end hosts for TBEV (Pfeffer and Dobbler, 2011), little is known about the ability of most livestock arboviruses to cause sufficient viraemia in dogs, cats or other pets for them to be able to infect an indigenous GB arthropod vector. There is some suggestion that viraemia levels for VEEV in naturally-infected dogs are sufficient to maintain the transmission cycle in mosquitoes (Coffey et al, 2006) although whether indigenous GB mosquitoes are competent vectors is not known. In this respect it should be noted that a single amino acid substitution in the envelope protein of VEEV enabled switching to a different species of mosquito vector (Brault et al, 2004). Peak viraemia levels for WNV observed in cats infected by mosquito bite may be high enough to infect mosquitoes at low efficiency, although this is unlikely with dogs (Austgen et al, 2004). Many exotic animals if viraemic could infect indigenous GB vectors. It is unlikely that exotic wild animals can be fully protected from exposure to indigenous arthropod vectors immediately after importation into GB (or northern Europe). Although quarantine may be effective against contact of imported vertebrates with indigenous ticks, midges are too small to be excluded by quarantine or isolation at zoos.

In conclusion, there is evidence that entry of livestock arboviruses to GB could occur through most tick and mosquito stages, and there is strong evidence that entry has occurred through adult midges. There is also strong evidence that entry through immature midge stages could not happen. The weight of supporting evidence is strongest for importation of viraemic livestock including horses. The weakest evidence is for travelling companion animals returning to GB and for importation of exotic wild animals to GB. With regard to virology, more information is needed on viraemia levels in pets and whether or not they are dead-end hosts and also on maternal transmission of arboviruses in livestock and pets including dogs.

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References

Adams, A.P., R. Navarro-Lopez, F.J. Ramirez-Aguilar, I. Lopez-Gonzalez, G. Leal, J.M. Flores-Mayorga, A.P.A. Travassos da Rosa, K.D. Saxton-Shaw et al, 2012: Venezuelan Equine Encephalitis Virus Activity in the Gulf Coast Region of Mexico, 2003–2010. PLoS Negl Trop Dis 6(11): e1875.

African business pages, 2011: The market for tyres in East Africa (http://www.africa-business.com/features/tyres.html). Accessed 5 Aug 2014.

Allingham, P.G. and H.A. Standfast, 1990: An investigation of transovarial transmission of Akabane virus in Culicoides brevitarsis. Australian Veterinary Journal 67, 273-274.

Anon, 2007: Bluetongue virus imported in a cow. Vet Record 161, 831-832.

Anon, 2014: Pet Travel Scheme (https://www.gov.uk/pet-travel-information-for-pet-owners#countries-and-territories). Accessed 10 October 2014.

Antonis, A.F.G., J. Kortekaas, J. Kant, R.P.M. Vloet, A. Vogel-Brink, N. Stockhofe and R.J.M. Moormann, 2013: Vertical transmission of Rift Valley fever virus without detectable maternal transmission. Vector-borne and zoonotic diseases, 13, 8, 601-606.

Archer, B.N., J. Thomas, J. Weyer, A. Cengimbo, D.E. Landoh, C. Jacobs, S. Ntuli, M. Modsie, M.S. Mashishi, et al, 2013: Epidemiologic investigations into outbreaks of Rift Valley fever in humans, South Africa, 2008-2011. Emerg. Infect. Dis. 19, 1918-1925.

Austgen, L.E., R.A. Bowen, M.L. Bunning, B.S. Davis, C.J. Mitchell and G.J.J. Chang, 2004: Experimental infection of cats and dogs with West Nile virus. Emerg. Infect. Dis. 10, 82-86.

Becker, N., K. Huber, B. Pluskota and A. Kaiser, 2011: *Ochlerotatus japonicus japonicus* – a newly established neozoan in Germany and a revised list of the German mosquito fauna. European Mosquito Bulletin 29, 88-102.

Brault, A.C., A.M. Powers, D. Ortiz, J.G. Estrada-Franco, R. Navarro-Lopez and S. C. Weaver, 2004: Venezuelan equine encephalitis emergence: Enhanced vector infection from a single amino acid substitution in the envelope glycoprotein. *PNAS USA* **101**, 11344-11349.

Brugman, V.A., D.L. Horton, L.P. Phipps, N. Johnson, A.J.C. Cook, A.R. Fooks and A.C. Breed, 2013: Epidemiological perspectives on West Nile virus surveillance in wild birds in Great Britain. Epidem. Infect. 6, 1134-1142.

Burgin, L.E., J. Gloster, C. Sanders, P. Mellor, S. Gubbins and S. Carpenter, 2013: Investigating incursions of bluetongue virus using a model of long-distance Culicoides biting midge dispersal. Transbound. Emerg. Dis. 60, 263-272.

Capua, I., 1998: Crimean-Congo haemorrhagic fever in ostriches: a public health risk for countries of the European Union? Avian Pathology 27, 117-120.

Carpenter, S., M.H. Groschup, C. Garros, M.L. Felippe-Bauer and B.V. Purse, 2013: Culicoides biting midges, arboviruses and public health in Europe. Antiviral Research 100, 102-113.

Chisholm, K., E. Dueger, N.T. Fahmy, H.A.T. Samaha, A. Zayed, M. Abdel-Dayem and J.T. Villinski, 2012: Crimean-Congo haemorrhagic fever virus in ticks from imported livestock, Egypt. Emerg. Infect. Dis. 18, 181-182.

Coffey, L.L., C. Crawford, J. Dee, R. Miller, J. Freier and S.C. Weaver, 2006: Serologic evidence of widespread Everglades virus activity in dogs, Florida. Emerg. Infect. Dis. 12, 1873-1879.

Costard, S., B.A. Jones, B. Martınez-Lopez, L. Mur, A. de la Torre A, M. Martinez, F. Sanchez-Vizcaino, J.-M. Sanchez-Vizcaino et al, 2013: Introduction of African swine fever into the European Union through illegal importation of pork and pork products. PLoS ONE 8(4): e61104.

De Vos, C., M. Hoek, E. Fischer and A. de Koeijer, 2013: Risk assessment framework for emerging vector-borne livestock diseases. Central Veterinary Institute, Wageningen, Netherlands. (https://www.wageningenur.nl/upload_mm/5/f/8/d77e2ef6-cfe2-4b14-8cca-70bce8d355c5_RiskAssesmentFrameworkEmergingVectorBorneLivestock.pdf). Accessed 16 July 2004.

ECDC, 2013: *Hyalomma marginatum* (http://ecdc.europa.eu/en/healthtopics/vectors/ticks/Pages/hyalomma-marginatum-.aspx). Accessed 10 October 2014.

England, M., 2013: Understanding the risks and factors associated with the introduction of Crimean-Congo haemorrhagic fever virus into Great Britain. PhD thesis, University of Southampton, Southampton, UK.

Estrada-Pena, A., N. Sanchez and A. Estrada-Sanchez, 2012a: An assessment of the distribution and spread of the tick Hyalomma marginatum in the Western Palearctic under different climate scenarios. Vector-borne and Zoonotic Diseases 12, 758-768.

Estrada-Peña, A., A.M. Palomar, P. Santibáñez, N. Sánchez, M.A. Habela, A. Portillo, L. Romero, and J.A. Oteo, 2012b: Crimean-Congo haemorrhagic fever virus in ticks, southwestern Europe, 2012. Emerg. Infect. Dis. 18: 179-180.

EU 142/2011: Commission Regulation implementing Regulation (EC) No 1069/2009 of the European Parliament and of the Council laying down health rules as regards animal by-products and derived products not intended for human consumption and implementing Council Directive 97/78/EC as regards certain samples and items exempt from veterinary checks at the border under that Directive. (http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2011:054:0001:0254:EN:PDF). Accessed 17 July 2014.

Fooks, A.R., D.L. Horton, L.P. Phipps, K.L. Mansfield, F. McCracken, C.L. Jeffries, N. Johnson, S. Wrigley, R. Deuchande, B. Toth, and R. Alzola, 2014: Suspect West Nile encephalitis in a horse imported into the UK from Europe. Vet. Rec. Case. Rep. 2014;2:e000104. doi:10.1136/vetreccr-2014-000104.

Harvala, H., J. Bremner, S. Kealey, B. Weller, S. McLellan, G. Lloyd, E. Staples, F. Faggian and T. Solomon, 2009: Case report: Eastern equine encephalitis virus imported to the UK. Journal of Medical Virology 81: 305-308.

Hornberger, K., N. Ndiritu, L. Ponce-Brito, M. Tashu and T. Watt, 2007: Kenya's cut-flower cluster. Final paper for microeconomics of competitiveness (http://www.isc.hbs.edu/resources/courses/moc-course-at-harvard/Documents/pdf/student-projects/Kenya_Cut-FlowerCluster_2007.pdf). Accessed 5 Aug 2014.

Hornok, S. and G. Horvath, 2012: First report of adult Hyalomma marginatum rufipes (vector of Crimean-Congo haemorrhagic fever virus) on cattle under a continental climate in Hungary. Parasites and Vectors 5, 170.

Huang, J.-H., T.-H. Lin, H.-J., Teng, C.-L. Su., K.-H. Tsai, L.-C. Lu, C. Lin, C.-F. Yang, S.-F. Chang, T.-L. Liao, S.-K. Yu, C.-H. Cheng, M.-C. Chang, H.-C. Hu and P.-Y. Shu, 2010: Molecular epidemiology of Japanese encephalitis virus, Taiwan. Emerg. Infect. Dis. 16: 876-878.

Hutchinson, R.A., M.N. Bayoh and S.W. Lindsay, 2005: Risk of airport malaria in the UK. European Mosquito Bulletin. Journal of the European Mosquito Control Association 19, 12-15.

Jameson, L.J. and J.M. Medlock, 2009: Results of HPA tick surveillance in Great Britain. Vet Rec. 165, 154.

Jameson, L.J., P.J. Morgan, J.M. Medlock, G. Watola, and A.G.C. Vaux, 2012: Importation of *Hyalomma marginatum*, vector of Crimean-Congo haemorrhagic fever virus, into the United Kingdom by migratory birds. Ticks Tick-borne Dis. 3, 95-99.

Jourdain, E., B. Olsen, A. Lundkvist, Z. Hubalek, S. Sikutova, J. Waldenstrom, M. Karlsson, M. Wahlstrom, M. Jozan and K.I. Falk, 2011: Surveillance for West Nile virus in wild birds from Northern Europe. Vector-borne and Zoonotic Diseases 11: 77-79.

Keefer, G.V., G.L. Zebarth, and W.P. Allen, 1972: Susceptibility of dogs and cats to Rift Valley Fever by inhalation or ingestion of virus. Journal of Infectious Diseases 125: 307-309.

Larska, M., L. Lechowski, M., Grochowska and J.F. Zmudzinski, 2013: Detection of the Schmallenberg virus in nulliparous Culicoides obsoletus/scoticus and C. punctatus. The possibility of transovarial virus transmission in the midge population and a new vector. Veterinary Microbiology 166: 467-473.

Leask, R., A.M. Botha and G.F. Bath, 2013: Schmallenberg virus – is it present in South Africa. Journal of the South African Veterinary Association 84, E1-4.

Lumley, S., B. Atkinson, S.D. Dowall, J.K. Pitman, S. Staplehurst, J. Busuttil, A.J. Simpson, E.J. Aarons et al, 2014: Non-fatal case of Crimean-Congo haemorrhagic fever imported into the United Kingdom (ex Bulgaria), June 2014. Euro Surveill 19:30.

Malkinson, K., C. Banet, Y. Weissman, S. Pokamunski, R. King, M.T. Drouet and V. Deubel, 2002: Introduction of West Nile virus in the Middle East by migrating white storks. Emerg. Infect. Dis. 8, 392–397.

Mardulyn, P., M. Goffredo, A. Conte, G. Hendrickx, R. Meiswinkel, T. Balenghien, S. Sghaier, Y. Lohr and M. Gilbert, 2013: Climate change and the spread of vector-borne diseases: using approximate Bayesian computation to compare invasion scenarios for the bluetongue virus vector *Culicoides imicola* in Italy. Molecular Ecology 22, 2456-2466.

Martyn, K.P., 1988: *Provisional Atlas of the Ticks (Ixodoidea) of the British Isles*. Swindon: Biological Records Centre, Natural Environment Research Council.

McGuire, K., E.C. Holmes, G.F. Gao, H.W. Reid and E.A. Gould, 1998: Tracing the origins of louping ill virus by molecular phylogenetic analysis. Journal of General Virology 79, 981-988.

Medlock, J.M., K.M. Hansford, F. Schaffner, V. Versteirt, G. Hendrickx, H. Zeller and W. van Bortel, 2012: A review of the invasive mosquitoes in Europe: Ecology, public health risks, and control options. Vector-borne and Zoonotic Diseases 12, 435-447.

Meiswinkel, R., P. van Rijn, P. Leus and M. Goffredo, 2007: Potential new Culicoides vector of bluetongue virus in northern Europe. Vet Rec. 161, 564-565.

Mellor, P.S. and C. Hamblin, 2004: African horse sickness. Vet Res. 35, 445-466.

Menzies, F. D., S. J. McCullough, I.M. McKeown, J.L., Forster, S. Jess, C. Batten, A.K., Murchie, J. Gloster et al, 2008: Evidence for transplacental and contact transmission of bluetongue virus in cattle. Vet Rec. 163, 203-209.

Mourao, M.P.G., M.S. Bastos, J.B.L. Gimaque, B.R. Mota, G.S. Souza, G.H.N. Grimmer, E. Arruda, and L.T.M. Figueiredo, 2009: Oropouche fever outbreak, Manaus, Brazil, 2007-2008. Emerg. Infect. Dis. 15, 2063-2064.

Nalca, A. and C.A. Whitehouse, 2007: Introduction. In: Ergonul, O. and C.A. Whitehouse (eds) *Crimean-Congo Hemorrhagic Fever – A global perspective* pp 155 - 165. Springer, Dordrecht, Netherlands.

OIE (World organisation for animal health), 2010: Terrestrial animal health code. Import risk analysis (http://www.oie.int/fileadmin/Home/eng/Health_standards/tahc/2010/chapitre_import_risk_analysis.pdf). Accessed 10 October 2014.

Palomar, A. M., A. Portillo, P. Santibanez, D. Mazuelas, J. Arizaga, A. Crespo, O. Gutierrez, J.F. Cuadrado and J.A. Oteo, 2013: Crimean-Congo hemorrhagic fever virus in ticks from migratory birds, Morocco. Emerg. Infect. Dis. 19, 260–263.

Pfeffer, M. and G. Dobbler, 2011: Tick-borne encephalitis virus in dogs – is this an issue? Parasites and Vectors 4, Article 59.

Phipps, L.P., N. Johnson, P. Gale, L. Shickle, H. Roberts, A.R. Fooks and R. Quest, 2014: Potential pathway for Crimean Congo haemorrhagic fever virus to enter the UK. Vet Record 195, 100-101.

Roelandt, S., P. Heyman, M. De Filette, S. Vene, Y. Van der Stede, A.B. Caij, P. Tavernier, A. Dobly, H. De Bosschere et al, 2011: Tick-borne encephalitis virus seropositive dog detected in Belgium: Screening of the canine population as sentinels for public health. Vector-borne and Zoonotic Diseases 11, 1371-1376.

Sailleau, C., C. Boogaerts, A. Meyrueix, E. Laloy, E. Breard, C. Viarouge, A. Desprat, D. Vitour, V. Doceul et al, 2013: Schmallenberg virus infection in dogs, France, 2012. Emerg. Infect. Dis. 19, 1,896-1,897.

Schulz, C., K. Wernike, M. Beer and B. Hoffmann, 2014: Infectious Schmallenberg virus from bovine semen, Germany. Emerg. Infect. Dis. 20, 338-340.

Sedda, L. and D.J. Rogers, 2012: The influence of the wind in the Schmallenberg virus outbreak in Europe. Scientific Reports 3, Article number 3361.

Sherman, D.M., 2011: The spread of pathogens through trade in small ruminants and their products. Rev. Sci. Tech. Off. Int. Epiz. 30, 207-217.

Taylor, K.G. and S. Paessler, 2013: Pathogenesis of Venezuelan equine encephalitis. Vet. Microbiol. 167, 145-150.

Tilston, N., C. Skelly and P. Weinstein, 2009: Pan-European Chikungunya surveillance: designing risk stratified surveillance zones. International Journal of Health Geographics, 8, 61.

Van den Berg, T., 2009: The role of the legal and illegal trade of live birds and avian products in the spread of avian influenza. Rev. Sci. Tech. Off. Int. Epiz. 28, 93-111.

Van der Hulst, J., 2004: Cool chain management for cut flowers. FlowerTECH 7, 49-51.

Van der Sluijs, M.T.W., D.P.H. Schroer-Joosten, A. Fid-Fourkour, M.P. Vrijenhoek, I. Debyser, V. Moulin, R.J.M. Moormann and A.J. de Smit, 2013: Transplancental transmission of bluetongue virus serotype 1 and serotype 8 in sheep: Virological and pathological findings. PLOS ONE 8, 12, e81429.

Wall, R., 2012: A ticking clock for tickborne disease? Vet Rec. 170, 326-328.

Wernike, K., M. Eschbaumer, H. Schirrmeier, U. Blohm, A. Breithaupt, B. Hoffmann and M. Beer, 2013: Oral exposure, reinfection and cellular immunity to Schmallenberg virus in cattle. Veterinary Microbiology 165, 155-159.

Wilson, A., K. Darpel and P.S. Mellor, 2008: Where does bluetongue virus sleep in the winter? PLoS Biol 6(8): e210. doi:10.1371/journal.pbio.0060210.

Zimmer, J.-Y., E. Haubruge, F. Francis, J. Bortels, G. Simonon, B. Losson, B. Mignon, J. Paternostre, R. de Deken, G. de Deken and I. Deblauwe, 2008: Breeding sites of bluetongue vectors in northern Europe. Vet Rec. 162, 131

Table 1: Summary of known and potential routes of entry of livestock arboviruses into GB; -, Strong evidence does not happen; 0, no evidence; +, evidence could happen; ++, strong evidence does happen.

Route of Entry	Tick-borne viruses	Biting midge-borne viruses	Mosquito-borne viruses
Entry of infected vector - Egg	0 Tick eggs could be infected, but laid in soil (ECDC, 2013) and hence not likely to be imported to GB	- Not likely to be infected and eggs not likely to be imported to GB	+Imported car tyres (Medlock et al, 2012)
Entry of infected vector -Larva	+Entry of larvae/nymphs on migrant birds, mammals/game birds imported, pets (dogs in particular) (Jameson et al, 2012) and some larval ticks on birds shown to be infected in Morocco (Palomar et al, 2013)	- Not likely to be infected and larvae not likely to be imported to GB	+Entry in stagnant water (lucky bamboo) (Medlock et al, 2012)
Entry of infected vector - Pupa	N/A	- Not likely to be infected and pupae not likely to be imported to GB	+presumably could occur through metamorphosis of larval stage, must remain in water
Entry of infected vector - Adult	+Imported pets (Wall, 2012) and livestock/horses e.g. adult *Hyalomma* spp. on a horse imported to England (Jameson and Medlock, 2009). Illegally imported tortoises (Phipps et al, 2014)	++Direct flight from Europe (Burgin et al, 2013; Sedda and Rogers, 2012); +Imported in aircraft, e.g. potential through packed in flowers from Africa	+Cabins of aircraft from Africa (Hutchinson et al, 2005), luggage holds, and potential through packed flowers from Africa
Infected meat and meat products	+ASFV entry through meat; CCHFV present in livestock tissues/fluids	+Note SBV not transmitted to cows orally (Wernike et al, 2013)	+RVFV present in livestock tissues/fluids but not endemic in EU. Could occur from illegal importation from endemic areas outside EU
Importation of viraemic	+Flaviviruses in TBEV group including LIV albeit in 18th century from GB	++ BTV-infected cow imported into GB from Germany in	++ Horse with neurologic disease and seropositive for WNV imported into GB

livestock including horses		December 2007 (Anon, 2007). Note, maternal transmission routes for certain BTV serotypes in cattle (Menzies et al, 2008) and sheep (Van der Sluijs et al, 2013)	through Europe in 2013 (Fooks et al, 2014). Note possible undetectable maternal transmission route for RVFV in ewes (Antonis et al, 2013)
Natural entry of viraemic wildlife	-English Channel is natural barrier to migration of most mammalian reservoir hosts and most birds are refractory to CCHFV or do not migrate in the case of LIV reservoirs	-English Channel is natural barrier to migration of most mammalian reservoir hosts	+Birds and flaviviruses. Example of WNV in Israel (Malkinson et al, 2002), although dead wild bird surveillance in UK did not detect WNV (Brugman et al, 2013)
Importation of viraemic exotic animals	+Note, quarantine likely to be more effective at excluding indigenous ticks than flying insects	+AHSV in zebras in zoo near Madrid (Mellor and Hamblin, 2004), SBV, BTV-8 potentially introduced in north-west Europe through import of exotic ruminants from Africa	+Flaviviruses through illegal importation of wild birds
Importation of viraemic pets	+CCHFV endemic in south east Europe	+SBV, possible maternal transmission of SBV in dogs	+WNV in cats. RVFV unlikely as currently not enzootic in Europe or in non-EU listed countries[1]. VEEV in dogs in New World
Travel of viraemic humans	++Two cases of CCHFV to GB between 2012 and 2014 (Lumley et al, 2014)	-only one midge-borne virus known to infect humans	++one case of EEEV to GB to date (Harvala et al, 2009)

[1]United Arab Emirates is a listed non-EU country (Anon, 2014) next to Saudi Arabia which reported outbreak of RVFV in 2000.