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Review Article

Rapid Molecular Detection Methods for Arboviruses of Livestock of Importance to Northern Europe

Nicholas Johnson,[1] Katja Voller,[1] L. Paul Phipps,[1] Karen Mansfield,[1] and Anthony R. Fooks[1,2]

[1] *Animal Health and Veterinary Laboratories Agency, Woodham Lane, Addlestone, Surrey KT15 3NB, UK*
[2] *National Centre for Zoonoses Research, University of Liverpool, Leahurst Chester High Road, Neston CH64 7TE, UK*

Correspondence should be addressed to Nicholas Johnson, nick.johnson@ahvla.gsi.gov.uk

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Arthropod-borne viruses (arboviruses) have been responsible for some of the most explosive epidemics of emerging infectious diseases over the past decade. Their impact on both human and livestock populations has been dramatic. The early detection either through surveillance or diagnosis of virus will be a critical feature in responding and resolving the emergence of such epidemics in the future. Although some of the most important emerging arboviruses are human pathogens, this paper aims to highlight those diseases that primarily affect livestock, although many are zoonotic and some occasionally cause human mortality. This paper also highlights the molecular detection methods specific to each virus and identifies those emerging diseases for which a rapid detection methods are not yet developed.

1. Introduction

In 1983, Odend' Hal [1] published a short book listing the worldwide distribution of animal viruses. It reported the classification as well as host, historical movements, and diagnostic techniques available. Of the 110 viruses cited, 35 were arboviruses, those viruses that are transmitted primarily by an arthropod vector. This first attempt at mapping animal viruses provides a useful baseline for reviewing the current state of arboviruses of livestock. Arboviruses are mainly classified to the virus families *Bunyaviridae*, *Togaviridae*, *Reoviridae,* and *Flaviviridae* [2]. The majority of these virus families have a ribonucleic acid genome, with the clear exception of the *Asfarviridae* to which African swine fever virus belongs (Table 1).

In Table 2 an updated list of arthropod-borne virus pathogens of humans, livestock, and wildlife is provided. During recent years several pathogenic arboviruses have apparently dispersed to new locations. The most well-known cases have been the movement of West Nile virus from the Old world to the New World [3] and the introduction of bluetongue virus into northern Europe [4]. This has prompted a wide range of authors to review the potential viruses that could emerge in the UK and Europe and assess the risk of such emergence events in the future [5–9].

The cooler climate experienced in northern latitudes (above 50°) means that there are fewer species and less diversity among particular arthropod species and the viruses they harbour than found in subtropical and tropical regions. Currently, there are few arthropod-borne diseases of livestock in Europe and as a result, livestock in the UK and many areas of northern Europe may be highly susceptible to many arthropod-borne viruses listed in Table 2. The health impact of an emergence of one could likely be severe. An exception to this is the presence of louping ill virus, which is considered to be the only arbovirus of veterinary importance that is endemic within the UK. The virus has been present for hundreds of years and is restricted to moorland locations, particularly in Devon, Cumbria, Wales, and Scotland [10]. This tick-borne virus causes fatal encephalitic disease in sheep, although it has been reported in a range of other species. A number of reports have suggested that West Nile virus and two other mosquito-borne viruses had been introduced into the UK, although cases of disease in horses have not been reported [11]. This is in clear contrast to the situation in Italy where WNV has repeatedly emerged

Table 1: Details of selected arbovirus families.

Virus family	Enveloped	Genome (sense)	Segmentation (number)	Example virus
Bunyaviridae	Yes	RNA SS[1] (−)	3	Rift Valley fever
Flaviviridae	Yes	RNA SS (+)	Nonsegmented	Dengue virus
Reoviridae	No	RNA DS[2]	10–12	Bluetongue virus
Rhabdoviridae	Yes	RNA SS (−)	Nonsegmented	Vesicular stomatitis virus
Togaviridae	Yes	RNA SS (+)	Nonsegmented	Chikungunya virus
Asfarviridae	Yes	DNA DS	Nonsegmented	African swine fever virus
Orthomyxoviridae	Yes	RNA SS (−)	8	Thogoto virus

[1] Single-stranded.
[2] Double-stranded.

to cause neurologic disease in horses from the Tuscany Region [12]. Recurring outbreaks of disease have occurred in both livestock and humans suggesting the permanent establishment of this virus in the mosquito population [13]. This has made assessment of future climate change trends essential to understanding the impact on both the ecology of the UK and risk of vector-borne disease introduction and establishment [9]. Such changes might enhance the establishment of invasive arthropod species such as the Asian tiger mosquito (*Aedes albopictus*) that in turn could directly import an exotic virus. It could also boost the population of indigenous vectors that could in turn increase the numbers of biting events, enhancing the likelihood of virus transmission. Furthermore, increases in temperatures can shorten the extrinsic incubation period, the time between the vector taking a blood meal and becoming infectious to a new host, thus enhancing virus persistence in a new area.

A wide range of routes would enable arthropod-borne viruses to translocate into a disease-free area. These can be divided into those that are part of the normal ecology and are influenced by the environment and climate that are presumably occurring all the time. Avian migration is an example of this pathway. Avian species are known to harbour many pathogens [14] and certain viruses such as avian influenza and Newcastle disease virus are transmitted around the globe through bird migration. For arthropod-borne viruses, virus movement can occur via transportation of the vector [15] or through infection of the host, particularly a viraemic animal that is subsequently fed on by an arthropod in a disease-free destination. The range of Crimean-Congo haemorrhagic fever appears to be increasing slowly in south-east Europe associated with spread by its tick vector (*Hyalomma* spp.). Bluetongue virus transmission into the UK is believed to have resulted from direct introduction of its midge vector (*Culicoides* spp.) assisted by wind movements in 2007 [16]. It is likely that movements by these routes, if they happen, are occurring continuously and cannot realistically be controlled. Therefore, effort needs to be directed towards reducing the impact of introduction. The alternative to natural introduction is often mediated by the actions of man. Again, this could occur through passive introduction of the vector through the movement of humans, livestock, or trade goods between endemic and disease-free areas or by the movement of infected livestock between countries. Theoretically, these mechanisms of entry can be regulated and effort should be directed to prevention programs.

A key aspect in preparing for the emergence of arthropod-borne diseases is the establishment of tests capable of detecting them. Development of such tests needs to address a number of fundamental issues. These include key features such as sensitivity of the assay and its specificity for the target virus. The assay must also be validated to provide assurance of its reliability, or at least give an indication of what might be missed. The assay under development needs to compete with existing technologies in terms of cost and speed to deliver desirable benefits to encourage adoption. The application of a particular test needs to be considered. Some tests may be applied to surveillance for virus, in which case the test needs to be amenable to cost-effective delivery of high volumes of samples. This in turn can complement serosurveys for particular viruses or be applied to sampling arthropod vectors in order to provide early warning of potential disease incursion.

For some technologies, the cost of individual tests is prohibitive for application to large numbers of samples or in resource-poor areas such as Africa. In each of these areas, molecular detection techniques have been very competitive as evidenced by the numerous tests developed in recent decades. Many of the assays reported in this paper provide a result considerably faster than more traditional detection methods such as virus isolation and plaque-reduction neutralization tests. Genetic variability of viruses is an inherent weakness in the use of molecular detection techniques with primer-mismatch being a constant problem. This has to some extent been overcome by the wealth of sequence data now available on many of the viruses that affect livestock.

Here we provide an overview of those arthropod-borne viruses that cause clinical disease in livestock and lead to economic losses. It will not consider important arboviruses that cause significant human disease with no livestock involvement such as yellow fever, dengue, Toscana virus, and chikungunya virus. Background information on the disease caused by particular viruses is described, the main arthropod vector and the reported current geographical distribution is provided for the viruses selected. This is followed by a brief review of reported rapid molecular tests that detect specific viruses or those tests that detect virus groups that contain numbers of animal pathogens.

Table 2: Pathogenic arboviruses (viruses in bold are dealt with in greater detail later in the paper).

Virus	Classification: family genus	Vector	Animals affected	Disease (SFI[1], HF[2], E[3])	Endemic presence	OIE* listed
African horse sickness virus	***Asfarviridae Asfivirus***	**Midge (*Culicoides* spp.)**	**Horses**	**SFI/HF**	**Africa**	**Yes**
African swine fever virus	***Reoviridae Orbivirus***	**Ticks (*Argasid* spp.)**	**Pigs**	**HF**	**Africa**	**Yes**
Akabane virus	***Bunyaviridae Orthobunyavirus***	**Mosquito (*Aedes* spp.) and midges**	**Cattle, sheep, horse,**	**Congenital abnormalities**	**East Asia, Turkey, South Africa**	**No**
Bluetongue virus	***Reoviridae Orbivirus***	**Midge (*Culicoides* spp.)**	**Cattle, sheep, goat**	**HF**	**Americas, Africa, Asia**	**Yes**
Bovine ephemeral fever virus	***Rhabdoviridae Ephemerovirus***	**Midge/mosquito**	**Cattle**	**SFI/respiratory**	**Africa, Asia, Australia**	**No**
California encephalitis virus	*Bunyaviridae Orthobunyavirus*	Mosquito	Humans, small mammals	E	Americas	No
Chikungunya virus	*Togaviridae Alphavirus*	Mosquito (*Aedes* spp.)	Humans	SFI	Africa, Asia, Europe	No
Colorado tick fever virus	*Reoviridae Coltivirus*	Tick (*Dermocentor andersonii*)	Humans, small mammals	SFI	North America	No
Crimean-Congo haemorrhagic fever virus	***Bunyaviridae Nairovirus***	**Ticks (*Hyalomma* spp.)**	**Humans**	**SFI/HF**	**Africa, Asia, Europe**	**No**
Dengue virus	*Flaviviridae Flavivirus*	Mosquitoes (*Aedes* spp.)	Humans	SFI/HF	Africa, Asia, Americas, Europe	No
Dugbe virus	*Bunyaviridae Nairovirus*	Ticks	Humans, cattle	SFI	Africa	No
Eastern equine encephalitis virus	***Togaviridae Alphavirus***	**Mosquitoes (*Culex* spp.)**	**Humans, equine**	**E**	**Americas**	**Yes**
Epizootic haemorrhagic disease virus	***Reoviridae Orbivirus***	**Midge (*Culicoides* spp.)**	**Cattle, deer**	**HF**	**Americas, Africa, Asia**	**Yes**
Equine encephalosis virus	***Reoviridae Orbivirus***	**Midge (*Culicoides* spp.)**	**Equine**	**E**	**Southern Africa, Israel**	**No**
Getah virus	*Togaviridae, Alphavirus*	Mosquito (*Culex* spp.)	Equine	SFI/E	Asia	No
Inkoo virus	*Bunyaviridae Orthobunyavirus*	Mosquito (*Aedes* spp.)	Cattle	SFI	Finland	No
Japanese encephalitis virus	***Flaviviridae Flavivirus***	**Mosquito (*Culex* spp.)**	**Humans, pigs, horses**	**E/abortion**	**Asia**	**Yes**
Kemerovo virus	*Reoviridae Orbivirus*	Tick (*Ixodes* spp.)	Humans, rodents, birds	SFI	Asia	No
Kyasanur Forest virus	*Flaviviridae Flavivirus*	Ticks (*Haemaphysalis* spp.)	Humans, small mammals	E	India	No
La Crosse virus	*Bunyaviridae Orthobunyavirus*	Mosquito (*Aedes* spp.)	Humans, Small mammals	E	North America	No
Louping ill virus	***Flaviviridae Flavivirus***	**Ticks (*Ixodes ricinus*)**	**Sheep, cattle**	**E**	**British Isles**	**No**
Murray Valley encephalitis virus	*Flaviviridae Flavivirus*	Mosquito (*Culex annulirostris*)	Humans, horse, cattle	E	Australia, Indonesia	No
Nairobi sheep disease virus	***Bunyaviridae Nairovirus***	**Tick (*Ripicephalus appendiculatus*)**	**Sheep, goats**	**HF/gastroenteritis**	**East Africa**	**Yes**
Omsk Haemorrhagic fever virus	*Flaviviridae Flavivirus*	Tick (*Dermacentor reticulatus*)	Humans	HF	Asia	No
Palyam virus	*Reoviridae Orbivirus*	Mosquitoes, midges	Cattle	Abortion	Africa, Asia and Australia	No
Peruvian horse sickness virus	*Reoviridae Orbivirus*	Mosquitoes	Horses	E	South America	No
Powassan virus	*Flaviviridae Flavivirus*	Mosquito (*Aedes*/*Anopheles* spp.)	Small/medium sized mammals		North America, Russia	No

Table 2: Continued.

Virus	Classification: family genus	Vector	Animals affected	Disease (SFI[1], HF[2], E[3])	Endemic presence	OIE* listed
Rift Valley fever virus	***Bunyaviridae Phlebovirus***	**Mosquito (*Aedes* spp.)**	**Humans, sheep, goats, camels**	**SFI/HF/abortion**	**Africa**	**Yes**
Russian spring-summer encephalitis virus	*Flaviviridae Flavivirus*	Tick (*Ixodes persulcatus*)	Humans, cattle, goats	E	Eurasia	No
Sandfly fever virus	*Bunyaviridae Phlebovirus*	Sandfly (*Phlebotomus perniciosus*)	Humans	SFI	Europe	No
Sindbis virus	*Togaviridae, Alphavirus*	Mosquito	Wildlife, avian	SFI	Africa, Europe, Asia	No
St. Louis encephalitis virus	*Flaviviridae Flavivirus*	Mosquito (*Culex* spp.)	Humans, avian	E	Americas	No
Semliki Forest virus	*Togaviridae Alphavirus*	Mosquito (*Aedes* spp.)	Equine	E	Africa	No
Tahyna virus	*Bunyaviridea Orthobunyavirus*	Mosquito (*Aedes* spp.)	Humans, pigs	SFI	Africa, Europe	No
Tick-borne encephalitis virus	*Flaviviridae Flavivirus*	Tick (*Ixodes* spp.)	Humans, wildlife	E	Europe, Asia	No
Thogoto virus	*Orthomyxoviridae Thogotovirus*	Tick (various spp.)	sheep	Abortion	Africa	No
Usutu virus	*Flaviviridae Flavivirus*	Mosquito	Avian, humans	E	Africa, Europe	No
Venezuelan equine encephalitis virus	***Togaviridae Alphavirus***	**Mosquito**	**Humans, horse**	**E**	**Americas**	**Yes**
Vesicular stomatitis virus	***Rhabdoviridae Vesiculovirus***	**Mosquito/sandfly/ midges/blackfly**	**Humans, cattle, horse, pigs**	**Mucosal vesicles**	**Americas**	**Yes**
Wesselsbron virus	***Flaviviridae Flavivirus***	**Mosquito (*Aedes* spp.)**	**Humans, sheep, cattle**	**HF**	**Africa, Asia**	**No**
West Nile virus	***Flaviviridae Flavivirus***	**Mosquito (*Culex* spp.)**	**humans, cattle, horse, avian**	**E**	**Africa, Eurasia, Americas**	**Yes**
Western equine encephalitis virus	***Togaviridae Alphavirus***	**Mosquito**	**Humans, cattle, horse**	**E**	**Americas**	**Yes**
Yellow fever virus	*Flaviviridae Flavivirus*	Mosquito (*Aedes* and *Haemagogus* spp.)	Humans, primates	HF	Africa, South America, Caribbean	No

[1]SFI: systemic Febrile Illness; [2]HF: haemorrhagic fever; [3]E: encephalitis.
*Office International des Epizooties (World Organisation for Animal Health).

2. Viruses Associated with Transmission by Midges

2.1. Bluetongue Virus

2.1.1. Clinical Disease. Bluetongue (BT) is a disease of ruminants with sheep being the most susceptible and affected species and cattle being affected to a lesser extent. Asymptomatic infections have been recorded in various other species such as deer, alpaca, llamas, and goats [17–19]. Since the emergence of BTV serotype 8 in northern Europe, significant clinical disease in cattle herds and goats has been reported [20]. The incubation period from exposure to disease is between two and fifteen days, averaging around 6 days [21]. In sheep, disease is first characterised by a fever and salivation, which develops into a more severe form causing mucopurulent discharge from the nasal passages and oedema of face and lips. The tongue may become cyanotic hence the name bluetongue and haemorrhages can form on the coronary band. Oral lesions can become haemorrhagic and ulcerative [21]. In affected cattle, crusts and erosions are visible on the nasal and oral mucosa, in or around the nostrils and lips. Other signs include salivation, fever, conjunctivitis, muscle necrosis, and stiffness in limbs.

2.1.2. Geographical Distribution. BTV is endemic throughout the world with the total of 24 serotypes circulating across the globe. In 2007, a new *Orbivirus*, Toggenburg virus, was isolated from goats in Switzerland and is now characterised as a tentative 25th serotype of bluetongue virus [22]. A further serotype has been detected in the Middle East [23].

Bluetongue virus was first reported in Africa when the disease was described in European Merino sheep that had been introduced into the Cape Colony [21]. Historically, the disease has predominantly been found between latitudes 40°N and 35°S until 1998 when the virus entered Europe and caused repeated outbreaks involving mainly five serotypes (BTV-1, BTV-2, BTV-4, BTV-9, and BTV-16) around the Mediterranean Basin affecting 12 European countries, three North African countries, and Israel [24]. In 2006, BTV-8

emerged in northern Europe with outbreaks in most western European countries and as far north as Denmark and southern Sweden [25]. BTV-8 emerged in south-east England in 2007 and spread to locations throughout the UK. However, the UK government introduced a voluntary vaccination programme in 2008, which controlled the disease and no further outbreaks have been reported within the UK [26]. In 2008, small localised outbreaks of BTV serotype-6 were reported in cattle herds in the Netherlands and Germany [27]. This was the second serotype to bypass southern Europe and emerge directly to northern Europe.

2.1.3. Vector. The only known biological vectors of bluetongue virus are the biting midges of the *Culicoides* genus. The most widespread vector is Afro-Asiatic *C. imicola*, which is distributed in Asia, the Middle East, most of Africa, and in southern and eastern Europe [28]. The absence and scarce distribution of *C. imicola* during the bluetongue outbreaks in Italy in 2000 onwards led to a search for novel vectors for virus transmission. Light-trapping and RT-PCR experiments identified the *C. pulicaris* and *C. obsoletus* complex (*C. obsoletus ss.*, *C. chiopterus*, *C. dewulfi*, *C. scoticus*) as potential vectors [29]. Later, field studies during the 2006 outbreak in Northern Europe also found no *C. imicola* in traps, but identified *C. dewulfi*, *C. obsoletus,* and *C. scoticus* as possible vectors [30]. In addition, these species have a palearctic distribution and they are known to be widespread throughout Europe. The high parity rates of *C. dewulfi* and *C. obsoletus* complex observed in Netherlands further supports the possibility of these species playing a role in bluetongue transmission [31]. These observations suggest that more than one species of midge are responsible for bluetongue virus distribution.

2.1.4. Molecular Diagnosis. Since the early 1990's, a range of RT-PCR assays targeting different BTV genome segments have been developed with a common drawback that they were only able to detect a limited number of serotypes [32–37]. Furthermore, most of these methods required the use of agarose gel electrophoresis for nucleic acid detection, which made them laborious and unpractical for diagnostic purposes. However, the spread of BTV in Northern Europe has seen the reporting of a number of conventional [38] and real-time RT-PCR assays detecting all 24 serotypes simultaneously [39–41]. These assays have been designed for diagnostic requirements; they are all rapid, reliable, and sensitive, enabling high throughput testing, which can be applied directly to clinical samples. The genome of bluetongue virus consists of ten double-stranded RNA segments coding for seven structural and three nonstructural (NS) proteins [42]. Several of these genome segments are highly conserved within serotypes, which make them potential targets for molecular detection. All recently published real-time RT-PCR and traditional RT-PCR assays use different BTV genome segments as a target. Orrù and coworkers [37] designed an assay targeting genome segment 10 (encodes NS3) using a stem-loop Molecular Beacon (MB) fluorescent probe. The probe can be used for both real-time RT-PCR detection and quantification purposes. Two groups have published duplex assays using two primer sets, where one assay targets BTV genome segment 7 (encoding the main BTV-specific antigen, VP7) and the other targets the segment 1 (encoding the viral polymerase, VP1) [39]. Using genome segment 1 as a target not only allows detection of all 24 serotypes, but will also detect geographic variants within individual serotypes by differentiating the samples to eastern (Middle-East, Asia, Australasia) and western (Africa, The Americas) genotypes [39]. Considering the high genetic variability through reassortment and mutations of RNA viruses, it is possible that current real-time RT-PCR assays might fail to detect some strains of BTV in the future. To address the problem of the genetic variability of BTV, the most recent real-time RT-PCR assay is based on the primer-probe energy transfer (PriProET) which is characterised by its tolerance towards mutations in probe region [42]. This assay is designed to detect all 24 serotypes.

2.2. African Horse Sickness Virus

2.2.1. Clinical Disease. African horse sickness virus (AHSV) is the causative agent of African horse sickness, a disease of *Equidae* with a high level of mortality. Horses are the most affected species whereas mules and donkeys show mild clinical signs or no signs at all, and zebras are considered a natural host and reservoir for AHSV [43]. Based on clinical and pathological findings, African horse sickness can manifest in four forms ranging from mild symptoms with no mortality to a severe disease with 95% mortality rate [43]. The mildest form, horse sickness fever, is characterised by mild to moderate fever lasting up to 5 days and affecting most commonly the African donkey. A cardiac (subacute) form is recognised by long-lasting fever, oedema of the head, neck, chest, or supraorbital fossae and petechial haemorrhages in the eyes and tongue with mortality rates around 50%. The most severe manifestation with highest mortality rates is a pulmonary (peracute) form, which is characterised by a rapid onset of disease. Death can occur without previous indication of illness or an animal can show signs of fever, depression and respiratory distress. The most commonly seen clinical presentation is a mixed (cardiac-pulmonary) form which can reach mortality rates as high as 70% 3–6 days after onset of fever.

2.2.2. Geographical Distribution. The virus is currently endemic in subtropical and tropical areas of Africa below the Saharan desert, which seems to provide a natural barrier against spread northwards [43]. Nine serotypes have been recognised which all have been reported in southern and eastern Africa. Serotypes 4 and 9 are found in western Africa and are the only serotypes that have caused outbreaks outside of Africa. The major outbreaks outside Africa have so far occurred in the Middle East, Spain, and Portugal. Spain has experienced five outbreaks of African horse sickness since 1966 [44]. The first outbreak in 1966 started from Gibraltar and was caused by serotype 9, which resulted in 637 animals dying or being slaughtered. In 1987, a number of subclinically infected zebras were imported into a Safari park near Madrid, which caused four further outbreaks between

1987 and 1990. However, these outbreaks were due to serotype 4 of AHSV. This was the first time that a serotype other than 9 had been recorded north of the Sahara desert. Before the eradication of the virus in Spain at the end of 1991, the outbreaks resulted in over 1300 horses dying or being destroyed and the virus spreading into Portugal, Tunisia, and Morocco [45].

2.2.3. Vector. Like bluetongue virus, African horse sickness virus is spread by biting midges of the *Culicoides* genus. The major vector for transmission is *C. imicola*, but also *C. bolitinis* has been shown to play a role in virus spread [46]. In 1998, over 100 horses died in an isolated population in Clarens Valley in South Africa and AHSV was isolated from the most locally abundant midge species, *C. bolitinis*, collected during light trap studies. It has been suggested that other *Culicoides* species might be involved, especially where low-grade cycling of virus is occurring.

2.2.4. Molecular Diagnosis. The first RT-PCR assays for AHSV detection and serogroup identification were time consuming and labour intensive procedures which involved either restriction fragment length polymorphism (RFLP) or dot-blot hybridisation assays [47–49]. These were followed by several conventional RT-PCR assays, which still required gel-based visualisation and took between four and six hours to complete [50–52]. However, a huge improvement in molecular diagnosis has occurred during recent years, resulting in numerous real-time RT-PCR assays that are able to detect, quantify, and discriminate the serotypes of AHSV in a short period of time [53–57]. Currently AHSV is only endemic in Africa, where laboratory conditions vary and real-time RT-PCR equipment can be too costly. Therefore, some studies have aimed to develop both real-time RT-PCR and conventional RT-PCR in parallel or solely improve conventional RT-PCR to provide better and faster diagnostic tools that are available in all circumstances [58]. These studies have shown high sensitivity and specificity of both conventional and real-time RT-PCR assays for all nine serotypes. Detection limit for both conventional and TaqMan real-time RT-PCR has been reported to be 1.2 $TCID_{50}$/mL [55]. More importantly new, improved RT-PCR assays can provide results within three hours of sample receipt.

2.3. Epizootic Haemorrhagic Fever Virus

2.3.1. Clinical Disease. Epizootic haemorrhagic fever virus (EHDV) causes a haemorrhagic disease in ruminants, especially in white-tailed deer in America and in cattle elsewhere. The clinical signs are often similar to those caused by bluetongue virus which complicates diagnosis. The clinical signs reported in cattle include reduction in milk production, fever, loss of appetite, weakness, excessive nasal and ocular discharge, oral ulcerations, discolouration of the udder, and oedema of hooves [59].

2.3.2. Geographical Distribution. There are currently ten serotypes of EHDV circulating throughout the world. EHDV-1 was first isolated in white-tailed deer in New Jersey in 1955 and it is still the most important infectious disease in deer in North America. All serotypes have caused clinical disease in cattle across the globe including North America, Africa (north and south), Australia [60], the island of Réunion [61], and Japan. Recent outbreaks have been reported around the Mediterranean including Morocco, Algeria, Israel in 2006, and Turkey in 2007 [62].

2.3.3. Vector. *Culicoides* spp. transmit the disease between ruminant hosts.

2.3.4. Molecular Diagnosis. The earliest EHDV RT-PCR assays have been based on American isolates, mainly on serotypes 1 and 2 targeting different genomic segments [63–65]. Since the spread of EHDV into new territories, the RT-PCR assays reported have been type-specific rather than serotype specific or even multiplex RT-PCR assays that can simultaneously detect both bluetongue and EHDV [66, 67]. A real-time RT-PCR has been reported that detects eight serotypes of EHDV [68].

2.4. Bovine Ephemeral Fever Virus

2.4.1. Clinical Disease. Bovine ephemeral fever virus (BEFV) causes disease in domestic cattle and water buffalo [69]. The disease is also known by the names 3-day sickness, stiff sickness, bovine epizootic fever, lazy man's disease, or dengue of cattle. The clinical outcome can vary from inapparent infection to death, but generally disease has four main phases. After an incubation period of between one and ten days, disease begins with a sudden fever that can be bi-, tri-, or polyphasic with peaks 12 to 18 hours apart. Fever lasts around half a day before the infected animal may become depressed and reluctant to move. Mucous discharge from the nose and profuse salivation can be observed and milk production is reduced or ceased altogether. This period of disability usually lasts between one and two days after which most animals start to recover. Although mortality rates as high as 30% have been observed, in most uncomplicated cases it is less than 2%. Sequelae include reduced milk production and other complications include pneumonia, mastitis, abortion in late pregnancy and temporary infertility of bulls [70].

2.4.2. Geographical Distribution. The bovine ephemeral fever was first recognised in Zimbabwe in 1906. The current distribution of BEFV includes all of Africa, the Middle East, Asia, and Australasia [71]. The outbreaks in the Middle East have occurred in Saudi Arabia and Israel [72, 73]. The disease has not been reported in Europe or the Americas.

2.4.3. Vector. Epidemiological studies indicate that BEFV is transmitted through flying insects. No arthropod vector has been shown to transmit the virus, but it has been isolated from *Culicoides* midges in Kenya and mosquitoes (*Culicine* mosquito species and *Anopheles bancroftii*) in Australia [74, 75].

2.4.4. Molecular Diagnosis. Currently, virus isolation seems to be the standard method for bovine ephemeral fever diagnosis and only two molecular diagnostic assays detecting BEFV have been reported. Real-time RT-PCR has been developed by Stram and coworkers [76], whereas Zheng and coworkers [77] reported the development of reverse transcription loop-mediated isothermal amplification (RT-LAMP) method. Both assays target the G gene and are highly sensitive assays, real-time RT-PCR being able to detect 10 BEFV genome copies in a sample [76]. The advantage of RT-LAMP is that no specialist equipment is required as there is no requirement for thermal cycling. However, a range of specialist apparatus is now available specifically for application with RT-LAMP such as lateral flow devices and turbidometers.

2.5. Akabane Virus

2.5.1. Disease. Akabane is a disease of ruminants. In adults the disease is generally asymptomatic with a transient viraemia occurring between one and six days after infection, which lasts for about six days. Occasional cases of encephalomyelitis have been observed in some infected animals [78]. The main economic impact of Akabane virus results from abortions, stillbirths, and congenital abnormalities that affect pregnant animals. Abnormalities vary depending on the trimester when infection occurs, although most are severely affected and euthanized shortly after birth.

2.5.2. Geographical Distribution. The disease occurs between latitudes 35°N and 35°S [79]. Serological evidence indicates that the virus is present throughout Africa, Asia, and the northern half of Australia. Disease has been observed in South Africa, Cyprus, the Middle East, and Japan.

2.5.3. Vector. Akabane virus has been isolated from *Culicoides* spp. in Australia (*C. brevitarsis* and *C. wadei)*, Africa (*C. milnei* and *C. imicola)*, Japan (*C. oxystoma*), and a number of mosquito species including *Aedes vexans*, *Culex tritaeniorhynchus* and *Anopheles funestus*.

2.5.4. Molecular Diagnosis. The genome of Akabane virus, like other orthobunyaviruses, is segmented, consisting of a small (s), medium (m), and large (l) segments. Standard RT-PCR assays have been described for detection of Akabane virus that target the S segment of the virus [78, 80]. A further development has been to incorporate detection of Akabane virus with that of Aino virus, a causative agent of congenital defects in cattle. This combined assay takes the format of a real-time multiplex RT-PCR that also targets the S segment [81]. The limit of detection is reported to be between 3 and 30 genome copies.

2.6. Equine Encephalosis Virus

2.6.1. Disease. Equine encephalosis virus (EEV) causes an acute disease in horses with a high fever and depressed appetite. A characteristic of the disease is the swelling of the lips and eyelids. Neurological disease is common and abortion can result from infection. Clinical features such as oedema are similar to those observed for AHSV and this should be considered in the diagnosis. However, fatalities are rare, particularly when supportive treatment is provided.

2.6.2. Geographical Distribution. The virus was originally isolated in South Africa and seven serotypes have been reported within the country [82]. A recent report has suggested the emergence of EEV in Israel [83].

2.6.3. Vector. Culicoides spp. are implicated in the transmission of this virus [84].

2.6.4. Molecular Diagnosis. No specific RT-PCR assays have been reported for equine encephalosis virus.

2.7. Vesicular Stomatitis Virus

2.7.1. Disease. Vesicular stomatitis virus (VSV) causes disease in cattle, horses, and pigs and is significant as it is clinically similar to foot and mouth disease (FMD). The initial incubation period is between two and eight days with a fever that often goes undetected. Early signs include drooling and frothing at the mouth. Blister-like lesions form in the mouth, on the dental pad, the tongue, the lips, the nostrils, the hooves, and the teats. Oral lesions can be sufficiently painful to cause the infected animal to refuse food and weight loss can occur. Infection is not fatal, although recovery from acute disease can take two weeks and ulceration can take months to heal.

2.7.2. Geographical Distribution. The virus is endemic in Central America and northern South America. Sporadic outbreaks occur in the USA and western regions of South America [85].

2.7.3. Vector. A range of haematophagus insects have been associated with transmission of VSV including Sand flies (Diptera: *Psychodidae*), black flies (Diptera: Simulidae), mosquitoes (Diptera: *Culicidae*), and *culicoides* midges (Diptera: *Ceratopogonidae*) [86]. Experimentally, only the sand fly (*Lutzomyia shannoni*) and the black fly (*Simulium vittatum*) have been shown to transmit the virus transovarially or to susceptible hosts. Serological evidence suggests that wild mammals can be infected with VSV but as yet there is no clear wildlife reservoir for the disease.

2.7.4. Molecular Diagnosis. Multiplex, real-time RT-PCRs have been devised to detect and differentiate different serotypes of VSV [86] and differentiate VSV from FMDV within the same assay [87].

3. Viruses Associated with Transmission by Mosquitoes

3.1. Eastern, Venezuelan, and Western Equine Encephalitis Virus

3.1.1. Disease. All three viruses cause disease in horses and humans [88]. This can range from asymptomatic infection

to acute, sometimes fatal, encephalitis. VEEV in particular has caused extensive epizootics in some regions of South America (Venezuala and Colombia).

3.1.2. Geographical Distribution. EEEV has been reported from the Eastern USA, Caribbean, South, and Central America. WEEV has been reported from North America and Cuba. VEEV is reported from many regions of South and Central America.

3.1.3. Vector

EEEV. The mosquito vector varies with climate and geography. In temperate zones, the ornithophagic *Culiseta melanura* is the main vector. In tropical regions, EEEV has been isolated from *Culex melanoconion*.

WEEV. In North America *Culex tarsalis* is considered the main vector of transmission between avian species. *Aedes* spp. have also been implicated in transmission to mammals. In South America *Aedes albifasciatus* has been reported as a vector.

VEEV. Culex melanoconion is associated with transmission of VEEV.

3.1.4. Molecular Diagnosis. TaqMan assays for North American EEEV and WEEV have been described [89]. Primer sets that detect a range of alphaviruses, including EEEV and VEEV have been described [90], however, this was linked to final detection using electrospray ionization mass spectrometry.

3.2. Japanese Encephalitis Virus

3.2.1. Disease. Japanese encephalitis virus (JEV) is asymptomatic in adult pigs but causes abortion, still-birth, and birth defects including central nervous system defects resulting in economic loss [91]. The virus also causes encephalitic disease in humans with over 50,000 cases reported annually [92]. Occasional cases in equines have been reported [93].

3.2.2. Geographical Distribution. JEV is found throughout Asia from Pakistan to Japan [94]. There is evidence that the virus is dispersing westwards through Asia.

3.2.3. Vector. The main vector of JEV is *Culex tritaeniorrhyncus*, which favours breeding in rice paddies. *C. gelidus* complex mosquitoes enable transmission to birds which assists in maintaining the virus in the environment [95].

3.2.4. Molecular Diagnosis. A real-time RT-PCR [96] that targets the 3 untranslated regions has been reported with detection to 112 $TCID_{50}$/mL. A multiplex real-time RT-PCR for detection of JEV, yellow fever virus, West Nile virus, dengue virus (serotypes 1–4), and St. Louis encephalitis virus has been reported [97] with a sensitivity of 2 PFU/mL. Real-time RT-LAMP [98, 99] assays have been developed for detection of JEV with detection levels as low as 0.1 PFU.

3.3. Rift Valley Fever Virus

3.3.1. Disease. Rift Valley fever virus (RVFV) affects ruminants with susceptibility influenced by age. Newborn animals are highly susceptible with adults showing less severe disease [100]. The incubation period ranges from one to three days followed by fever, recumbency, and haemorrhagic diarrhoea. Mortality can reach 70%. High rates of abortion are also associated with epidemics of Rift Valley haemorrhagic fever (RVHF), often described as abortion storms. The virus causes disease in humans ranging from uncomplicated influenza-like illness to haemorrhagic fever with liver damage and occasionally encephalitis.

3.3.2. Geographical Distribution. The virus is considered endemic throughout much of Africa, although clinical disease occurs infrequently. However, sudden outbreaks with high livestock mortality occur in many regions often following flooding. The disease was first reported in East Africa (Kenya and Tanzania) but is believed to have expanded its range north, west, and south, characterised by sudden epidemics [101]. There has been a well-documented outbreak of RVHF in the Arabian Peninsula associated with livestock movements from East Africa [102].

3.3.3. Vector. Aedes spp. are the principal virus vector for livestock and are believed to maintain the virus between epidemics although other species are capable of acting as bridge vectors enabling transmission during epidemics, such as *Culex* spp.

3.3.4. Molecular Diagnosis. Numerous real-time RT-PCR methods have been developed for rapid detection of RVFV [103–106]. The detection limit of these assays is typically between 10 and 100 genome copies. An alternative approach is the use of reverse transcription loop-mediated isothermal amplification (RT-LAMP) technique [107, 108]. The detection limits of RT-LAMP assays are comparable to real-time RT-PCR.

3.4. Wesselsbron Virus

3.4.1. Disease. Wesselsbron virus (WSLV) causes infection in sheep and goats and is associated with abortion and congenital abnormalities [109–111]. In adult animals, infection is usually subclinical, although in newborn animals clinical disease can result after a one to three day incubation period with fever and anorexia. Mortality can reach as high as 27%. Infection in humans has been reported following laboratory exposure and causes a mild influenza-like illness.

3.4.2. Geographical Distribution. Virus isolation and seroprevalence studies suggest that the virus is present across Africa [112, 113].

3.4.3. Vector. Aedes spp. are considered the main vector for Wesselsbron virus. In a recent study over 50 isolates of WSLV were isolated from *Aedes vexans* collected in Mauritania and Senegal [113].

3.4.4. Molecular Diagnosis. No specific RT-PCR tests have been reported, although the Wesselsbron virus genome has been published (NCBI Reference Sequence: NC_012735). Wesselsbron virus can be detected by RT-PCR using universal flavivirus primers and sequencing [114].

3.5. West Nile Virus

3.5.1. Disease. West Nile virus (WNV) causes encephalitic disease in horses, humans and some avian species [115]. In horses early disease consists of fever that is usually inapparent. Subsequent disease is neurological including ataxia, paresis and limb paralysis leading to recumbence. Muscle tremor and muscle rigidity may be observed. Mortality rates vary and may be particularly high in the USA reaching over 55%.

3.5.2. Geographical Distribution. Until recently, WNV was an Old World disease present throughout Africa and Asia with occasional incursions into Europe around the Mediterranean Basin [116]. However, in 1999, the virus emerged in the north east USA and spread throughout the Americas [117] and has remained endemic since that time [118].

3.5.3. Vector. The principal vector for WNV is the ornithophilic mosquito *Culex pipiens*. However, a wide range of mosquito species have been shown to support virus replication and transmit virus to mammalian species [119].

3.5.4. Molecular Diagnosis. Molecular detection of WNV has been reviewed recently by Shi and Kramer [120]. Such assays usually detect between 40 and 100 genome copies, although some suggest that sensitivity can be even lower [121]. Further developments have enabled multiplexing with other arboviral diseases [122, 123] and improved assay sensitivity [124] suggesting that the detection limit is 0.07 genome copies/mL. RT-LAMP assays have been developed for WNV [125] and used to detect the virus in mosquito samples [126]. The sensitivity limit of these assays has been reported to be approximately 0.1 PFU.

4. Viruses Associated with Transmission by Ticks

4.1. African Swine Fever Virus

4.1.1. Clinical Disease. African swine fever virus infects warthogs and bush pigs in Africa with no clinical disease, but domestic pigs can succumb to severe infection with an incubation period of five to fifteen days [127]. The disease can manifest in any one of four forms depending on the virulence of a strain. Highly virulent strains cause peracute and acute infections with clinical signs of high fever, anorexia, diarrhoea, recumbency, and general reddening of skin or discoloration on the ventral chest and abdomen, tips of the ears or tail, and on distal limbs. Death can occur within a day, sometimes before obvious clinical signs. The less virulent strains cause subacute and chronic infection. The subacute form manifests as a mild illness with an intermittent fever lasting approximately one month although pregnant animals might abort. In chronic cases, low fever, pneumonia and swelling of joints may occur. Some animals infected with low virulence strains can seroconvert without any clinical symptoms. Morbidity rates can reach close to 100% in herds that are naïve for ASFV whereas mortality rates vary, but can be as high as 100%, depending on virulence of the strain. Those animals that have survived acute or chronic disease can become persistently infected and act as carriers for the virus.

4.1.2. Geographical Distribution. African swine fever has been reported in Africa since the 1890's and is endemic in most parts of sub-Saharan Africa where transmission appears in three different forms; a sylvatic cycle, a domestic pig cycle and a pig-tick cycle [128, 129]. In 1958, ASFV emerged for the first time in Europe in Portugal before spreading to Spain in 1960, where it caused several outbreaks until the disease was finally eradicated [130]. In 1967, virus was detected in Italy and in 1978 ASF outbreaks occurred simultaneously in Malta and Sardinia. Whereas disease was eradicated from Malta and mainland Italy, ASF remains endemic in Sardinia where it is established in free-range pigs and wild boars. In 1998, Madagascar reported the first case of African swine fever [131], and from the year 2000 onwards virus has continued to spread into new territories including Georgia, Iran, and Mauritius [132–134]. The Georgian outbreak in 2007 demonstrated perfectly the emerging and transboundary characteristics of ASFV, as within a year virus spread from Georgia to several neighboring countries including Armenia, Azerbaijan, and the Russian Republic of Chechnya [132]. In the following year, virus spread to northwestern Iran where it caused an outbreak in wild boar [133]. Other short-lived, sporadic outbreaks have been reported from France, Belgium, Netherlands, Caribbean, and South America.

4.1.3. Vector and Transmission. The subclinical infection of ASFV in warthogs and bush pigs is maintained by soft ticks of the genus *Ornithodoros*. In Africa, the main vector is *O. moubata,* whereas in Southern Europe ASFV is transmitted by *O. erraticus* [135]. *O. moubata* is most abundant in eastern and southern Africa particularly in Cameroon, Central African Republic, and Sudan. The most prevalent viral hosts include warthogs, domestic pigs, and man. Although, ticks do play a part in virus transmission, the epidemiological role is thought to be low, especially in those areas where tick populations are small [134]. The more likely route of virus spread is through direct oronasal contact with an infected animal or indirectly via fomites and contaminated pig feed. The most recent outbreaks in previously ASFV-free areas could have been transmitted through feed products containing infected pig meat.

4.1.4. Molecular Detection. As there is no cure or vaccine for ASFV, slaughter is the only tool for control and eradication of this highly contagious virus in infected areas. Therefore, fast and reliable laboratory diagnosis is required to limit the socio-economic burden of outbreaks. Furthermore, highly specific, differential diagnosis of disease is necessary,

as clinical signs of African swine fever may resemble those of other infections, especially classical swine fever. The current OIE manual recommends Taqman real-time PCR such as the assay developed by King and coworkers [136]. As real-time PCRs are high throughput, sensitive, specific, and quick to run, recent development of these assays has focused on improvement of existing real-time assays by applying varying PCR chemistries. This has led to several modifications of real-time PCR including use of minor groove binding probes or molecular beacon assays [137, 138]. A recent method has been further developed with field diagnostics in mind. Two assays, a LAMP method and a linear-after-the-exponential PCR (LATE-PCR), have both been adapted for use in field conditions [139, 140]. These methods could considerably shorten the time between infection and diagnosis as there is no requirement for samples to be transported to the laboratory.

4.2. Crimean-Congo Haemorrhagic Fever Virus

4.2.1. Disease. Crimean-Congo haemorrhagic fever (CCHF) is one the most important and widespread diseases caused by tick-borne viruses. The causative agent, Crimean-Congo haemorrhagic fever virus does not cause disease in livestock, but vertebrates play a role in virus transmission as part of a tick-vertebrate-tick enzootic cycle [141]. Although there is no evidence of clinical disease in animals, contact with viremic animals, tick bite, or crushing ticks taken from infected animals can lead to human infection. In humans, CCHF virus causes a disease with four phases: incubation, prehaemorrhagic, haemorrhagic, and convalescence phases [141]. Following the short incubation period (3–7 days), sudden onset of fever, headaches, myalgia, and dizziness occur [142, 143]. A few days later, a rapidly developing haemorrhagic period occurs, with haematomas appearing on the skin and mucous membranes with mortality rates ranging from 3% to 30%. The surviving patients will go through the convalescence period lasting 9-10 days which can include variable symptoms such as tachycardia, temporary hair loss, poor vision, and loss of appetite. Infected humans can spread CCHF via close contacts resulting in community and nosocomial outbreaks [144–148]. Furthermore, individuals in certain occupations such as health carers, veterinarians, farmers, and abattoir workers are at increased risk of contracting Crimean-Congo haemorrhagic fever [141, 143, 149].

4.2.2. Geographical Distribution. The geographic distribution of CCHF virus is the widest amongst all tick-borne diseases. Currently CCHFV is endemic in Africa, Asia, Balkan countries, and Middle and Far East [142, 145, 150]. During the last ten years, CCHFV has been rapidly introduced into new, previously nonendemic areas; especially into eastern and southeastern Europe including Greece and Turkey [149, 151–153].

4.2.3. Vector. *Hyalomma* spp. are the vector and reservoir for CCHFV, particularly *Hyalomma marginatum*. The geographical distribution of these ticks closely match the distribution of CCHFV and covers southern Europe, southern Russia extending to southern Asia, and most of Africa [154]. The host range of these ticks varies from domestic animals (cattle, horse, sheep, and goats) for adults to small wild animals and birds for larvae and nymphs.

4.2.4. Molecular Detection. Since the mid 1990's several nested and real-time RT-PCR assays, which all target genomic S segment of the tripartite genome. These assays have been developed to achieve fast initial and differential laboratory diagnosis of CCHFV [104, 155–158]. Both the nested and real-time RT-PCR assays are comparable in terms of sensitivity, but the results can be achieved in two hours with one-step real-time RT-PCR, whereas it takes 4-5 hours to run nested RT-PCR without gel electrophoresis. Due to the high genetic variability of CCHFV isolates, the first real-time RT-PCR assay detecting CCHFV was based on a Sybr Green method that intercalates to any double stranded DNA and requires identification of the positive product using gel electrophoresis [104]. More specific assays can be developed by designing primers and probes directed at strains of interest, for example, those circulating in geographically defined areas. Strains from the Balkan region have been successfully identified by using a one-step real-time RT-PCR that is based on the fluorescence resonance energy transfer (FRET) probe technology employing the endonuclease ($5' \rightarrow 3'$) activity of *Taq* polymerase enzyme [158]. Furthermore, this assay can also be used for detection of CCHFV from ticks.

4.3. Nairobi Sheep Disease Virus

4.3.1. Disease. Nairobi sheep disease virus (NSDV) causes acute haemorrhagic gastroenteritis in sheep and goats. This is a severe and fatal disease with mortality rates reaching over 90 percent in non-immune animals. The short incubation period (2–4 days) is followed by high fever, diarrhoea, and collapse [159]. Watery to mucoid and bloody diarrhoea is seen 2–4 days after the onset of fever which itself can last up to 8 days. As the disease progresses nasal discharge and conjunctivitis are common features [160]. In pregnant animals, the infection can lead to abortion. In fatal cases, death often occurs in the early stages of a disease. A variant of NSDV, Ganjam virus has been isolated in India [161]. This virus is highly pathogenic in exotic and crossbred sheep and goats causing a disease with high fever, dullness, depression, and loss of appetite [162].

4.3.2. Geographical Distribution. Nairobi sheep disease has been reported since 1910 when it was first identified near Nairobi in Kenya [163]. NSDV is now mostly enzootic in Kenya with the majority of indigenous sheep and goats having protective antibodies, and outbreaks tend to occur when flocks from uninfected areas are brought into enzootic areas [164]. Further outbreaks have been reported from other countries in east and central Africa. Serosurveys suggest that the virus may be more widespread in countries of Southern Africa [165]. Ganjam virus, a variant of NSDV, circulates across southern India [166].

4.3.3. Vector. In Africa, the main vector for NSDV is the three-host Ixodid tick *Rhipicephalus appendiculatus*, which

is found between its northern limits of southern Sudan and Ethiopia through Eastern, central and Southern Africa. Livestock hosts for this tick include cattle, sheep, goats, and horses [164]. In wildlife, antelopes and buffalo are frequently infested. Ganjam virus has been isolated from *Haemaphysalis* spp. ticks, which have been collected from sheep and goats in India [161].

4.3.4. Molecular Detection. Primers for NSDV amplification have been reported [166, 167]. The complete genome sequences of the tripartite genome of NSDV have been published. No real-time RT-PCR has been reported.

4.4. Louping Ill Virus

4.4.1. Disease. The main forms of tick-borne encephalitis virus (TBEV) found in Europe and Asia do not cause clinical disease in livestock although human cases are on the increase in Europe [168]. However, a number of closely related variants of TBEV cause disease in livestock. These include louping ill virus (LIV), Spanish sheep encephalitis virus (SSEV), Greek goat encephalitis virus (GGEV), and Turkish tick-borne encephalitis virus (TTEV). Of these, LIV is the most studied virus as the cause of disease in sheep and red grouse. In red grouse, LIV causes rapid and fatal encephalitis whereas in sheep, disease is biphasic, especially in young nonimmune animals [169]. The initial clinical signs of louping ill include fever and weakness before animals develop neurological signs of disease. A range between 5 and 60% of infected animals develop clinical signs and death can occur between 24 and 48 hours following the development of neurological signs. During the neurological phase, the most damage is caused to the cerebellum. Clinically, the second phase is characterized by cerebral ataxia, hyperexcitability, and progressive paralysis [169]. Occasionally, natural infection of louping ill has also been reported from other species such as dogs, roe and red deer, and llamas [170–173]. The other variants are genetically distinct, but closely related to louping ill. They are much less studied, but appear to all cause disease that resembles louping ill [174].

4.4.2. Geographical Distribution. Tick-borne encephalitis virus is found in many areas throughout Europe and Asia. Transmission to humans is mainly through tick bites but can occasionally result from consumption of unpasteurized milk [175]. LIV is endemic within upland areas in the UK, particularly in Scotland, Wales, the northwest and southwest of England [169]. There have also been reports of LIV in Ireland and Norway [176]. Sheep infected with SSEV have been reported from the Basque region of Spain [174, 177]. The first isolation of GGEV was made in the village of Vergina in northern Greece [178]. Subsequent studies have suggested that GGEV circulates in the *I. ricinus* population in northern Greece, although at low density [179]. TTEV was first reported from northwestern Turkey (Anatolia) as encephalitis in sheep and was subsequent confirmed by nucleotide analysis to be related to tick-borne encephalitis virus [180–182].

4.4.3. Vector. Both TBEV and its variants are transmitted by the three-host hard tick *Ixodes ricinus* in Europe. These ticks are the most common species of ticks in northwest Europe and are also known as the common sheep tick, castor bean tick or wood tick. In addition to sheep and red grouse, the hosts for *I. ricinus* in the UK include mountain hares, red deer, roe deer and small mammals [183]. In the Far East of Europe and throughout Asia, TBEV is transmitted by *Ixodes persulcatus*.

4.4.4. Molecular Detection. The first nested one-step RT-PCR for louping ill detection was developed in mid-1990s. This assay, which targets the envelope (E) and membrane (M) genes of the virus genome, can be used for virus identification in ticks and other species [184]. As the E gene is the most conserved of the three LIV structural genes, a one-step Taqman RT-PCR assay has been developed with primers for this protein [185]. Brain and spinal cord samples were tested and detection limit of 1 PFU/mL was achieved, making this assay as sensitive as the traditional plaque assay. In recent years, real-time RT-PCR assay detecting eight different tick-borne flaviviruses (including louping ill) and West Nile virus has been developed in the UK [186]. The assay employs degenerate primers targeting nonstructural protein 5 and is based on incorporation of Sybr green. The assay is not as sensitive as species specific assays, but makes it an ideal choice when a single virus cannot be specified before testing [186]. A recently reported assay uses one-step real-time RT-PCR with pyrosequencing which enables the rapid differentiation between TBEV subtypes [187]. Although, this assay was developed to differentiate between the main TBE subtypes, it is also able to detect louping ill virus. Specific detection assays for SSEV, TTEV, and GGEV have not been reported.

5. Conclusions

The majority of the viruses reviewed have been thoroughly characterized and there are a wide range of molecular diagnostic tests available. These include bluetongue virus, AHSV, VSV, WNV, and ASFV. These tend to be those viruses that cause the greatest impact either to livestock health or as a consequence of spillover into the human population as a zoonotic agent. New serotypes and variants emerge and techniques may require modification or augmentation in response to genetic differences. Other viruses have molecular tests available, but these are not widely in use, either because many countries do not consider them necessary or do not have suitable containment facilities to handle the virus. This group includes EHFV, BEFV, Akabane virus, the equine encephalitides, JEV, RVFV, CCHFV, and variants of tick-borne encephalitis virus. Further effort is needed to establish molecular tests for these viruses more widely in preparation for potential outbreaks. Finally, there is a small group for which no molecular diagnostic tests have been reported. This includes equine encephalosis virus, Wesselsbron disease virus and Nairobi sheep disease virus. These viruses require some test development.

Test development and implementation could take two forms. The first option is to develop specific tests that detect

a single virus, often at high sensitivity. The alternative is the development of assays that detect a range of related pathogens in single step. This has been applied to groups including the flaviviruses, alphaviruses, and phleboviruses. The latter option has the benefit of screening for more than one pathogen. However, this approach is often less sensitive and may require further analysis to identify the exact virus involved.

Currently, rapid detection methods are dominated by nucleic acid amplification methods, particularly the polymerase chain reaction. By linking nucleic acid amplification to sequencing, it is possible to rapidly identify a large number of potential pathogenic viruses [114, 188]. This approach has been applied to detection of flaviviruses using universal primers ([186], and references therein). New technologies such as microarray [189] and next generation sequencing [190] are being applied to the investigation of infectious viruses. However, these technologies are expensive to establish and maintain for purely diagnostic or surveillance purposes. Future development is required in this area to make these technologies more accessible and affordable for use in the detection of arthropod viruses of livestock.

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