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A Qualitative Risk Assessment for the Incursion of Bluetongue Disease and African Horse Sickness into London Zoo

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ABSTRACT

Background

Bluetongue disease (BTV) and African horse sickness (AHSV) are two closely related animal viruses vectored by *Culicoides* biting midges. They are considered exotic to the United Kingdom (UK), however the 2007 outbreak of BTV in the UK highlights their potential for introduction and onward transmission. Given the susceptibility of some animals kept in zoo collections to vector-borne diseases, the risk of BTV and AHSV to animals in London Zoo was assessed.

Methods

A qualitative risk assessment for the introduction of BTV and AHSV to London Zoo was performed using OIE's Import Risk Assessment Framework, Gale et al. (2016)'s estimation of risk pathway probability, and the European Food Safety Authority's qualitative probability definitions. The determination and likelihood of risk pathways were analysed using available literature and data on the transmission and epidemiology of the diseases.

Results

Three BTV and two AHSV risk pathways were determined to have a non-negligible probability of resulting in the infection of an animal in the Zoo collection, and these were investigated in detail. The probability of BTV infection ranged from *low* to *low to medium,* with the greatest risk posed by the long-distance spread of infected-*Culicoides* being carried by wind across the English Channel. The probability of AHSV infection was *very low* and *very low to low*, with the probability of an infected equine imported to the UK posing a slightly higher risk than that of an infected zoo import.

Conclusion

The proximity of ongoing disease events in mainland Europe and proven capability of transmission to the UK places London Zoo at higher risk of BTV transmission than AHSV. However, given the recent long-range expansion of AHSV to Thailand and the ability of closed related viruses to replicate in temperate climates, AHSV continues to pose a non-negligible threat to animals in London Zoo collection.

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ABBREVIATIONS

AHSV	African Horse Sickness
BTV	Bluetongue disease
CFR	Case fatality rate
EIP	Extrinsic incubation period
EU	European Union
NAME	Numerical Atmospheric-dispersion Modelling Environment
OIE	World Organization for Animal Health
R_0	Basic reproduction number
TRACES	Trade Control and Expert System
UK	United Kingdom

1. INTRODUCTION

Vector-borne diseases, or diseases carried by arthropod vectors, are an increasing threat to the health of humans and animals. Vector-borne diseases of livestock carry enormous economic consequences for the agricultural industry, resulting in lost productivity and herd mortality during an outbreak. In recent years, diseases previously relegated to the tropics have emerged in mainland Europe. Historically, the United Kingdom (UK) has been buffered from such disease incursions through its geographic isolation. However, climate change, urbanization and extensive global travel and trade networks, render the UK at risk for introduction and circulation of exotic vector-borne diseases. Two vector-borne diseases with the potential for import into the UK, and subsequently London Zoo, will be looked at in depth: bluetongue disease and African horse sickness.

1.1 Bluetongue disease

Bluetongue disease is caused by bluetongue virus (BTV), a member of the genus *Orbivirus* in the Reoviridae family and causes disease in ruminants. While severe clinical symptoms are most often seen in sheep, infection can occur in deer, goats, buffalo, and camelids, with cattle considered the main livestock reservoir of the virus(1–3). There are 29 known serotypes of the virus, with varying geographical distributions(1,4). Clinical outcomes range from acute to severe depending on serotype, host, and environment(5). Symptoms commonly include fever, reddening of mucosal membranes, sores on the nose and mouth, swelling of the tongue and face, breathing difficulties, lameness, and birth abnormalities(4).

1.1.1 Transmission of BTV

BTV is transmitted between domestic and wild ruminants through *Culicoides* biting midges. After a midge has bitten an infected ruminant, the virus enters a 6-8 day replication period during which it disseminates to the salivary glands before onward transmission to another host(5). Once infected, adult midges remain infectious for their entire lifespan(6). Transmission is facilitated by conditions which enable midge activity and viral replication, such as precipitation, temperature, wind speed, elevation, habitat, and livestock density(3). In Europe, this occurs most frequently in late summer and early autumn(2). *Culicoides* preferentially bite at dawn and dusk and can travel several kilometres per day on their own, or much farther distances if they are carried by strong winds(2). The first incursion of BTV into northern Europe was in 2006 and exhibited the efficient vectorial capacity of Palaearctic *Culicoides,* with six species identified as the putative vectors: *Culicoides obsoletus, Culicoides* *scoticus, Culicoides dewulfi* and *Culicoides chiopterus* (the "*Culicoides obsoletus* complex"), and *Culicoides pulicaris* and *Culicoides punctatus* (the "*Culicoides pulicaris* complex")(7). The outbreak demonstrated the potential for the virus to persist over winter, since it has been found that viral replication within the vector stops below 12°C but can resume when temperatures are higher(1).

1.1.2 Global distribution and epidemiology of BTV

Bluetongue has historically been a disease associated with tropical climates, however its distribution has recently expanded to more temperate regions (Figures 1 and 2). Recent expansion into northern Europe is thought to have been facilitated by the movement of infected livestock and passive dispersal of infected midges on the wind(1). Key differences have been found in serotypes adapted to temperate climates, which define their epidemiology(9). These include marked seasonality, with clinical cases typically occurring July-December, frequent vertical transmission in pregnant ruminants, and higher morbidity and mortality in cattle(9).

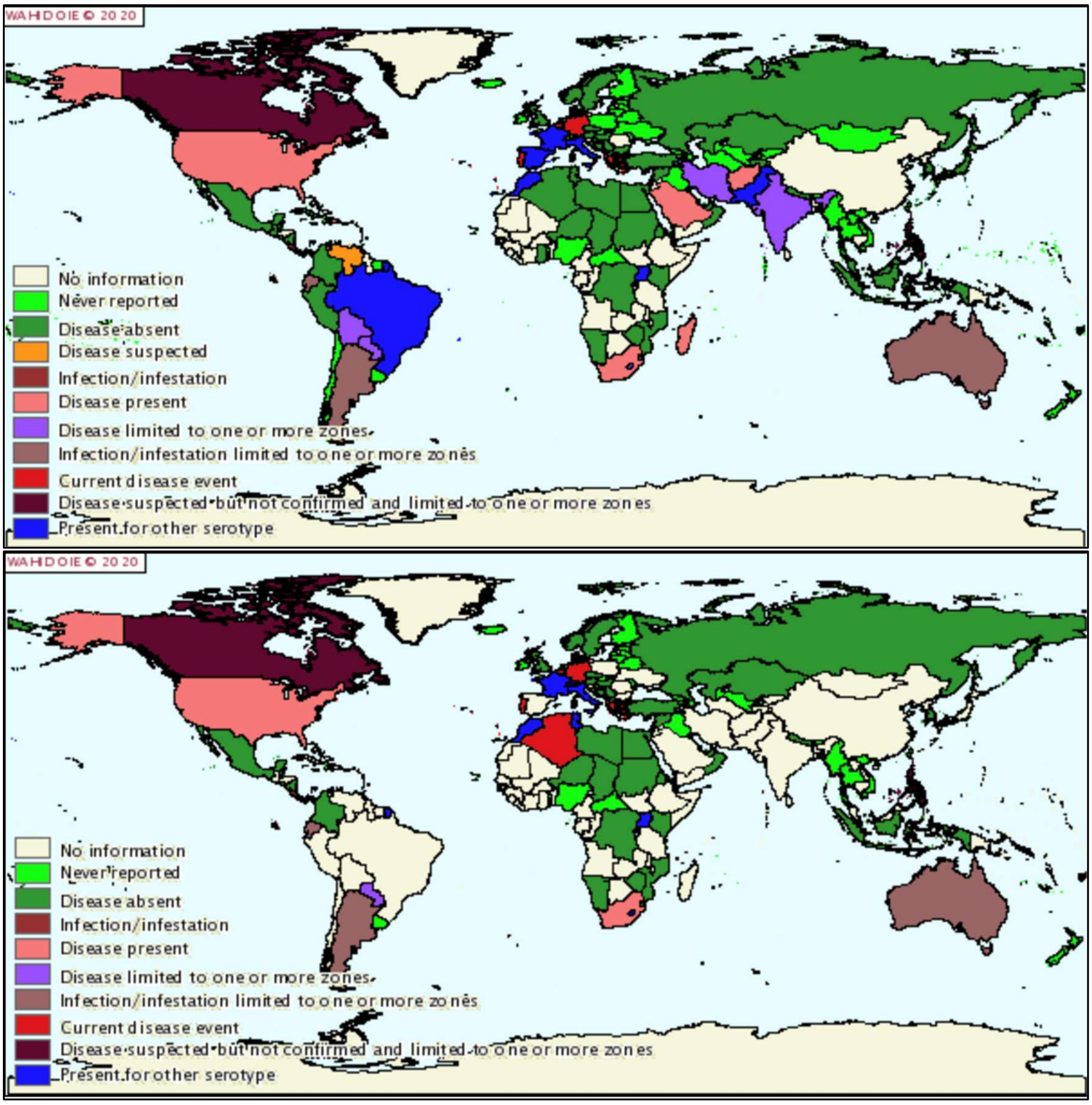

Figure 1: Global distribution of bluetongue disease in 2019 in January-June (top) and July-December (bottom)(10).

In August 2006, BTV was first reported outside of Maastricht in the Netherlands and quickly spread to neighbouring countries(11). Phylogenetic studies have identified the strain (BTV-8) as originating in sub-Saharan Africa, so it is postulated that the incursion occurred after the importation of an infected animal from this region, movement of infected vectors, or the illegal importation of a live-attenuated vaccine strain(9). The disease spread to the UK in August and September of 2007(13,14). The BTV-8 outbreak was contained by 2008 in the UK and 2009 in mainland Europe through mass vaccination campaigns using a new inactivated BTV-8 vaccine(1,15). The UK has been registered as officially disease-free since July 2011 and post-import testing has been implemented since September 2015, in response to an outbreak in France(16). In total, the outbreak is estimated to have cost the European agricultural industry more than £800 million(17).

In recent years, two serotypes of BTV have been reported in northern Europe. In 2018, both BTV-8 and BTV-4 were reported in France(18). Subsequently, BTV-8 cases were reported in border regions of Switzerland and Germany, spreading to Belgium by early 2019(18–20)(Figure 2). This spread is thought to have occurred through the movement of infected midges between the areas, as movement restrictions on livestock have been in place since 2015. In 2020 thus far, two subclinical cases have been reported in Switzerland through routine health exams, and cases have been reported from Belgium in January and February(21).

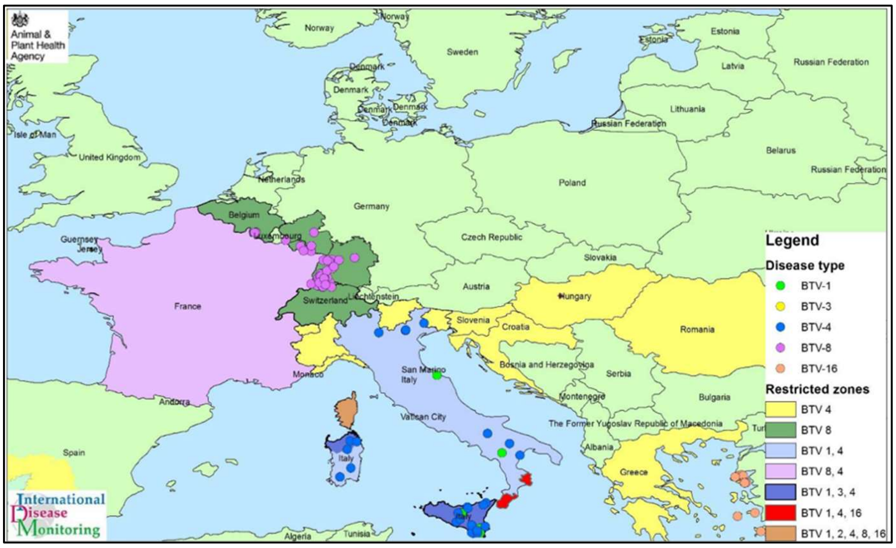

Figure 2: Locations of bluetongue disease reports and restriction zones in Europe, January-June, 2020(20).

1.1.3 Treatment and control BTV

Treatment for BTV is non-specific, involving the provision of rest and good husbandry, and treatment of complications or secondary infections during the recovery period(22). Preventative vaccination can be utilized, but current vaccines are serotype-specific and only available for limited serotypes(4). The UK's control strategy focuses on good biosecurity, monitoring of the disease situation in Europe and internationally, responsible sourcing of animals, and ensuring appropriate import testing(2). Currently, under the UK control policy, if a suspected infection arises, a restriction notice is issued to the premises prohibiting the movement of ruminants and animals may be culled(2). If more widespread circulation is suspected, a restricted zone may be declared in which the movement of susceptible animals, semen, ovum, or embryos is banned, except under license(2). The restriction zone consists of a control zone of at least 20km around the initial cases, a protection zone of at least 100km, and a surveillance zone of an additional 50km (2). The UK requires live animals dispatched for trade to undergo a veterinary check within 24 hours prior to departure, with an accompanying health certificate(2). If the risk of introduction is perceived to increase, document and identity checks, pre-export and post-import testing may be conducted(2). Given the limited effect of vector control activities on midge populations and the economic importance of livestock trade, vaccination campaigns provide the most viable control strategy(11).

1.2 African horse sickness

African horse sickness is a vector-borne disease caused by African horse sickness virus (AHSV), closely related to bluetongue virus(23,24). There are nine known serotypes of the virus, all of which occur in Africa(25). The disease primarily infects horses, donkeys, zebra, and other equids, but antibodies have been found in camels, African elephants, black and white rhinoceroses, and dogs(25,26). Zebra and African donkeys are considered to act as reservoir hosts of the virus (23,25). There are four main forms of clinical disease, classified according to clinical presentation, which vary according to prior exposure and host species(23). All four forms of the disease can occur during an outbreak, and in rare cases a nervous form may also occur(23,25). Overall, the mortality rate in horses is 70-95%, ~50% in mules, 5-10% in European and Asiatic donkeys, and rare in zebra or African donkeys(25). It is therefore one of the most lethal known viral infections in horses(23).

1.2.1 Transmission of AHSV

Culicoides vectors are responsible for the vast majority of transmission and the virus is considered to be non-contagious(25). *Culicoides imicola* appears to be the most important species for transmission, as it is present in high abundance across the majority of Africa, southern Europe, the Middle East, and southern Asia, and has proven vectorial capacity(27). *Culicoides bolitinos* has also been implicated in transmission in cooler, more mountainous regions of Africa (27). Additionally, *Culicoides variipennis* was shown to be an efficient vector under laboratory conditions, but its role in transmission in the field is unknown(25). AHSV was isolated from pools of Palearctic species of *Culicoides* during the 1987-1991 outbreak of AHSV-4 in Spain(27). The virus appears to occasionally be vectored by several species of mosquitoes (*Culex, Aedes, Anopheles*), ticks (*Hyalomma, Rhipicephalus*), and large biting flies (*Stomoxys, Tabanus*) (25), however their importance in transmission is considered to be low(27).

1.2.2 Global distribution and epidemiology of AHSV

AHSV is endemic is large parts of sub-Saharan Africa (Supplementary Figure S1). In 1966, an AHSV-9 epizootic in northwest Africa resulted in spread to Spain through the Straits of Gibraltar(23,28). The virus overwintered for the first time in recorded history in North Africa before its European incursion, but was swiftly eradicated in Spain through a rapid vaccination and slaughter campaign(23,28). The virus did not appear in Europe again until 1987, when an outbreak of AHSV-4 occurred for several months after the importation of infected zebra from Namibia(28). More recently, in 2007 AHSV-2 and AHSV-7 were reported in West Africa, expanding closer to North Africa and the Mediterranean basin(28). There is currently an ongoing outbreak of serotype 1 in Thailand that was first identified in March 2020, thought to have been caused by long-distance spread from Africa(30). This is the first time AHSV has occurred in East Asia and demonstrates the ability of the virus to be transmitted long distances to new foci.

AHSV exhibits both a seasonal occurrence, associated with vector activity, and an epizootic cycle, involving outbreaks following drought conditions succeeded by heavy rains(24,25). The El Niño phase of the El Niño/Southern Oscillation is strongly related to large epizootics in southern Africa(24). The presence of the plains zebra has also been found to be a major driver of the pattern of distribution and persistence of the disease(27).

1.2.3 Treatment and control of AHSV

Treatment of AHSV in horses is largely non-specific, involving rest and treatment of secondary infections or complications(28). Control measures include movement restrictions involving quarantining of animals from endemic/epidemic regions, euthanasia or isolation of viraemic animals, stabling with limited outdoor activity and insecticide treated housing, insecticide and repellent spraying of animals, and vaccination in the face of an outbreak(31). Vaccination is used widely across Africa in both endemic and epidemic regions(23). However, concerns about the possible reversion to virulence, transmission by vectors, and reassortment with wild strains of the currently available live-attenuated vaccines restrict their usage outside of Africa (23). Vaccination has been used to combat the current AHSV outbreak in Thailand and appears to be succeeding in controlling the outbreak (S. Carpenter, personal communication).

1.3 Impact of BTV and AHSV on zoos

Animals kept in zoo collections are at risk of vector-borne diseases, such as BTV and AHSV, and in some cases can be highly susceptible to severe manifestations of disease due to a lack of previous exposure to certain pathogens and increased potential exposure to the vectors(1). Zoos, particularly in urban areas where stocking is dense, may facilitate cross-species disease spread by the presence of a diverse community of susceptible animals and through the inadvertent creation of attractive vector breeding habitats. London Zoo is situated in Regents Park in the centre of London, an international hub and the largest city in the UK. In the event of a UK outbreak of BTV or AHSV, the surrounding farmland and wildlife could act as transmission reservoirs, enabling spill-over transmission to animals at the zoo. Of 49 zoos in northern Europe deemed at risk during the 2006 BTV outbreak due to their proximity to infected premises, clinical disease was reported in 62 susceptible animals with a case fatality rate of 69%(17).

1.4 Import risk assessment

The potential risk of transmission of exotic vector-borne diseases can be assessed using the World Organization for Animal Health's (OIE) Import Risk Analysis framework(33). The risk assessment comprises of four main components: hazard identification, release assessment, exposure assessment, and consequence assessment(33). The results are then integrated into a risk estimation, measuring overall risks associated with the hazards identified(33). While quantitative risk assessments assign a numerical value or probability of risk, either through deterministic or stochastic modelling, qualitative risk assessments present all available evidence on the risk of disease importation in a clear and transparent manner(34). They are especially useful for emerging diseases, as they do not require statistically powerful data and can be updated as the evidence base expands(34). They are the most widely used form of risk assessment for routine decision-making(33).

2. AIM AND OBJECTIVES

2.1 Overall aim

As both BTV and AHSV are exotic to the UK, a qualitative risk assessment of potential importation pathways to London Zoo will enable an understanding of the risk posed to animals in the zoo collection and inform preventative policies. The aim of this paper is to answer the following risk questions:

1. What is the probability that a susceptible animal at London Zoo can become infected with BTV?
2. What is the probability that a susceptible animal at London Zoo can become infected with AHSV?

2.2 Specific objectives:

1. Investigate the risk pathways by which BTV and AHSV could enter the London Zoo and cause infection within the zoo collection.
2. Determine the probability of occurrence for each of the identified risk pathways.
3. Compare the risk to London Zoo that each disease poses and highlight key areas for future research.

3. MATERIALS & METHODS

To determine the likelihood of entry into the UK and exposure of susceptible zoo animals, a qualitative risk assessment was performed using the OIE's Import Risk Assessment Framework (2019) and the European Food Safety Authority's (EFSA) qualitative probability definitions (Table 1)(35–37). The risk assessment focused on the risk of entry of BTV and AHSV to the UK and the subsequent risk of onward transmission to a susceptible zoo animal.

Initially, a traditional literature review was conducted using PubMed, OVID Medline, and Google Scholar to gather information on BTV and AHSV; their epidemiology in the UK, Europe, and globally; and their *Culicoides* vectors. This was used to construct the hazard identification for BTV and AHSV, presented in the "Background" section of this paper. Potential risk pathways for release and exposure to a London Zoo animal were identified based on proven and hypothesized transmission routes from the literature. Risk pathways with a non-negligible probability for release into the UK and onward transmission to London Zoo were determined. Pathways were determined to be negligible if the probability of them occurring was indistinguishable from zero, based on current distribution and knowledge of transmission(37).

Data were gathered on disease distribution (using OIE's WAHIS interface and ProMed), animal imports (from the European Union's (EU) Trade Control Expert System (TRACES) and London Zoo import records) and midge incursions to the UK (provided by the Met Office) (10,25,38–40). Using this data and existing literature for the non-negligible risk pathways, each step was assigned a qualitative probability of occurrence, following the EFSA scale(Table 1)(37). The overall risk pathway's qualitative probability was then determined given the probabilities of the steps necessary along the pathway and their weighted importance in determining the overall outcome of the pathway. Serotypes of the virus were not considered separately, and the probabilities reflect incursion of any serotype of the disease. A consequence assessment was not included within the scope of this paper.

Table 1: Definitions of qualitative probability categories(36,37,41).

Risk Probability	Definition
Negligible	Event is so rare that it does not merit consideration
Very low	Event is very rare but cannot be excluded
Low	Event is rare but does occur
Medium	Event occurs regularly
High	Event occurs very often
Very high	Event occurs almost certainly

4. RESULTS

4.1 Bluetongue disease

Risk Pathway #1: BTV-infected animal imported directly to the zoo and onward transmission within the zoo.

Estimation of P_1: Probability of BTV-infected animal entering the zoo.

A previous serological study of zoological animals imported from Africa to the US found that 13/30 species had antibodies to BTV(48). Between 2017-2020, London Zoo imported 52 animals (Supplementary Table S1)(H. Jenkins, personal communication). One of these imports was a Sumatran tiger from Ebeltoft, Denmark in January 2019. While it is unclear the role a tiger may play in onward transmission of BTV, asymptomatic infections have previously been reported in big cats and other carnivores(9). Antibodies have been found in dogs, cats, cheetahs and lions, with suggestions they may become infected by oral ingestion of infected meat or through vector feeding(49,50). In 2017, BTV was absent in all import countries except Switzerland, but no susceptible animals were imported from Switzerland. In 2018 and 2019, BTV was present in France, Canada, and Germany, but once again no susceptible animals were imported(10). It is unlikely that the Sumatran tiger imported from Denmark would have been exposed to the BTV circulating in Germany at the time prior to export. France and Germany have set up restriction zones and encouraged voluntary vaccination for BTV-8 and BTV-4(38,51). Vaccination is mandatory in Switzerland, but this may only be enforced for breeding and export stock(38). The UK currently requires all animals from France to be vaccinated(38).

BTV has been isolated from the blood of infected cattle for up to 49 days, and from sheep for 11-21 days post-infection(52). This creates a plausible time window for import into London Zoo. Given that antibodies have been found in other carnivore species, an infected tiger may be asymptomatic, increasing the chance of undetected viraemia prior to importation(49). However, given the provisions for the control and eradication of bluetongue in the EU outlined in Council Directive 2000/75/EC, including animal movement restrictions from affected areas to non-infected regions, as well as strict border checks at both the UK border and within the zoo, the likelihood of an infected animal going undiagnosed during the importation process is low(53).

The lack of susceptible imported animals from countries with BTV transmission over the last few years, the low probability for an infected animal to pass border checks in its country of origin and the UK, as well as veterinary inspection at the zoo greatly reduce the probability of a BTV-infected animal entering the zoo. However, given the potential for asymptomatic animals to be imported the probability is classified as *very low*.

Estimation of P_2: Probability of BTV-infected Culicoides in the zoo.

Between June 2014-June 2015, England et al. (2020) collected 5,768 *Culicoides* from London Zoo, comprising 25 different species(54). The majority of the total catch (97.8%) was made up by the putative vectors of BTV in northern Europe, *C. obsoletus, C. scoticus, C. dewulfi, C. chiopterus, C. pulicaris* and *C. punctatus*. After bloodmeal analysis, *C. obsoletus/C. scoticus* specimens (the females of which cannot be morphologically distinguished) from London Zoo and Whispande Zoo were found to have fed on: Asian elephants (*Elephas maximus*), Alpaca/Llama (*Vicugna pacos*/*Lama glama*), Bactrian camels (*Camelus bactrianus*) and Przewalski's horse (*Equus przewalskii*). The Bactrian camels and Alpaca/Llamas are present at London Zoo, and therefore constitute susceptible hosts.

The average length of viremia in a host which is able to infect a feeding *Culicoides* is 21 days, so an infected animal entering the zoo would likely be fed upon by multiple midges during viremia (assuming adult *Culicoides* activity), increasing the probability of onward transmission(52). The species composition at the zoo reflects what is commonly found at livestock farms in northern Europe(55). Previous BTV outbreaks in northern Europe have demonstrated that these *Culicoides* species are successfully able to transmit the virus within and between farms.

In the event that an infected animal is imported to London Zoo, the species composition and feeding preferences of the zoo *Culicoides* populations render the probability of BTV-infected *Culicoides* in the zoo as *medium to high*.

Estimation of P_3: Probability of zoo animal becoming infected with BTV.

Of the 19,035 total animals at London Zoo, 22 are susceptible to BTV infection (Table 2)(42). During the 2006-2008 outbreak, clinical disease was reported in 62 of over 1000 susceptible animals held in zoos, with a case fatality rate (CFR) of 69% in Bovidae(6). This is considerably higher than the CFR seen in cattle and sheep during the outbreak, which were 11% and 51% respectively(57). The transmission rate of BTV is dependent on temperature, as this directly affects the extrinsic incubation period (EIP) of the virus and the activity of adult *Culicoides*. Below 12°C, the transmission rate is zero because the virus is not able to replicate within the *Culicoides*(58). Average temperatures in London exceed 12°C from approximately April to October, enabling both adult *Culicoides* activity and virus replication(61).

The feeding host preferences of the *Culicoides* at London Zoo were discussed in the previous section, but it is important to also note that by far the most *Culicoides* at London Zoo were caught in the trap located near the Bactrian camels(54). Blood-meal analysis suggests that of all the susceptible animals in the zoo, the camels are at the highest risk of BTV infection. With the exception of wild birds, the zoo *Culicoides* population appears to feed almost exclusively on zoo animals, which combined with the small geographic size of the zoo and the close proximity of the animals to each other, greatly increases the risk of transmission to susceptible zoo animals from infected *Culicoides*.

Table 2: ZSL London Zoo animals at risk of BTV infection(42)(H. Jenkins, personal communication).

Scientific Name	Common Name	Total No. of animals
Camelus bactrianus domestic	Bactrian camel (domestic)	**2**
Muntiacus reevesi	Chinese muntjac	**2**
Giraffa camelopardalis	Giraffe	**3**
Okapia johnstoni	Okapi	**3**
Capra hircus domestic nigerian	Nigerian goat (domestic)	**4**
Capra hircus domestic west_africa_pygmy	West African pygmy goat (domestic)	**3**
Cephalophus natalensis	Red forest duiker	**2**
Lama glama	Llama	**2**
Vicugna pacos	Alpaca	**1**
Total		**22**

Assuming there are BTV-infected *Culicoides* in the zoo, the probability of a zoo animal becoming infected is *medium to very high*, given the availability of susceptible zoo animals kept in close proximity and the demonstrated host feeding preferences of the *Culicoides* populations in the zoo.

Risk Pathway #1 Conclusion: The overall probability of a BTV-infected animal imported directly to the zoo resulting in the infection of a susceptible animal is ***low*,** given that the probability a BTV-infected/viremic animal enters the zoo is low and this is an essential step in initiating a transmission.

Risk Pathway #2: BTV-infected livestock imported to the UK resulting in BTV infection in zoo animals.

Estimation of P_4: Probability of BTV-infected livestock entering the UK.

In October 2017, post-import testing on a consignment of 32 animals from France destined for two farms in England and two farms in Scotland, identified BTV-8 positive animals(16). BTV was again detected in late Autumn 2018 in French livestock imports(38). The importations occurred in periods of low vector activity and strict movement restrictions were put into place on all detected farms, so no onward transmission occurred(16,17). Between 2018-2020, 102,515 susceptible animals were imported to the UK (Supplementary Table S2)(40). Of these, 13,960 were imported from countries with BTV circulation(10). Given previously mentioned length of viremia, an imported animal could be capable of onward transmission upon arrival(52). Spain is the only country that has reported using vaccines to OIE, however, voluntary vaccination is encouraged in France and Germany, and restriction zones have been set up within those countries(38). Vaccination is mandatory in Switzerland and enforced in the export industry(38). After the detection of cases in 2017, compliance issues with the vaccination status of cattle in the area of France were uncovered(16). Current EU legislation allows the movement of unvaccinated animals during periods of low vector activity, but they must be accompanied by a health certificate and are subject to further random checks at their final destination(62,63). Additionally, the UK initiates risk-based post-import checks of susceptible ruminants of EU-origin in accordance with Directive 90/425/EEC, as well as documentary, identity, and physical checks of animals from non-EU countries at border inspection posts(2). The overall probability of infected animals passing border checks both pre- and post-import is low.

The current BTV-8 outbreak in northern Europe is causing a wide range of non-specific symptoms and may therefore be difficult to differentiate from other common diseases. Cases are frequently mild or asymptomatic, with animals usually making a full recovery(38). Without post-import testing, BTV could enter the UK and remain undetected for some time, facilitating onward transmission to the zoo.

The probability of BTV-infected livestock entering the UK is *low*. Border checks and post-import testing appear to be working well, however, the proximity and frequency of imports of susceptible animals from countries with ongoing BTV circulation render the entry of an infected animal a constant threat.

Estimation of P_5: Probability of BTV entering native Culicoides populations.

The 2006-2009 BTV-8 outbreak in northern Europe demonstrated the vectorial capacity of Palearctic *Culicoides* species, namely members of the *C. obsoletus* and *C. pulicaris* complexes. In laboratory tests, *C. obsoletus* from different geographic regions of the UK were found to have BTV infection rates from 0.4-7.4%, whereas *C. pulicaris* specimens from Keele were found to have a 13% infection rate(59). Carpenter et al. (2006) found some populations of Palearctic species could reach infection rates of up to 26% using membrane and pad-feeding, exceeding those recorded for BTV's putative vector in Africa, *Culicoides imicola*(59). Given the large populations of *Culicoides* throughout the UK, with traps collecting thousands of individuals in a single night, these infection rates would enable substantial transmission(64). Livestock density and land use has been linked to *Culicoides* abundance, with larger populations in areas with higher livestock density(65). BTV spreads to *Culicoides* more effectively in warmer conditions, when populations peak due to more rapid life cycles(60). However, BTV is believed to persist in a latent phase in infected *Culicoides* for long periods in cold temperatures, resuming replication once the temperatures increase(60).

Biting rates of vector *Culicoides* on livestock can be extremely high and have been observed to be in excess of one bite per minute on cattle(66). Therefore, there is a significant window of opportunity for the virus to enter local *Culicoides* populations, depending on the timing of import and the subsequent housing of the infected animal.

Given the large abundance of members of the *C. obsoletus* and *C. pulicaris* complexes throughout the UK, their proven vectorial capacity, and their proximity to livestock, the probability that BTV enters native *Culicoides* populations from an imported infected animal is *medium.*

Estimation of P_6: Probability of spread of BTV to London Culicoides populations.

The movement of infected livestock has been found to establish new foci of BTV outbreaks, but this only accounts for a small proportion of transmission and cannot sustain an outbreak on its own(67). Midge dispersal on the other hand has been found to be the principal mode of transmission between farms(67). Sedda et al. (2012) referred to this phenomenon of midge dispersal as a 'stepping stone effect,' in which a sequence of short-range infections result in what appears to be a long-distance transmission(68). Analysing the 2006-2009 BTV-8 outbreak in northern Europe, Sedda et al. (2012) found 54% of the outbreaks occurred over distances up to 5 km, 92% over distances up to 31 km, and only 2% over distances greater than 31 km. If infected livestock were imported to a farm in the UK, this 'stepping-stone effect' could potentially carry the infection to London *Culicoides* populations, with proximity of the initial farm to London determining the time scale. Additionally, the species composition on farms surrounding London is suitable for BTV transmission, with vector species present on farms in Hertfordshire, Essex, Kent, Berkshire and Surrey (M. England, unpublished data)(69,70). However, the proximity of susceptible livestock to London may be a limiting factor for *Culicoides* dispersal. The density of cattle and sheep is low in the London area (Figure 3), rendering it less likely for an infected *Culicoides* to disperse into London(43,44). There is, however, a relatively high density of goats in some parts of the Greater London area, with 2-25 animals per km^2(45).

The probability for the spread of BTV to London *Culicoides* populations is *low.* While an infection could plausibly spread to London from infected farms through midge dispersal and competent vector species are present in London, the risk is limited by the low densities of cattle and sheep.

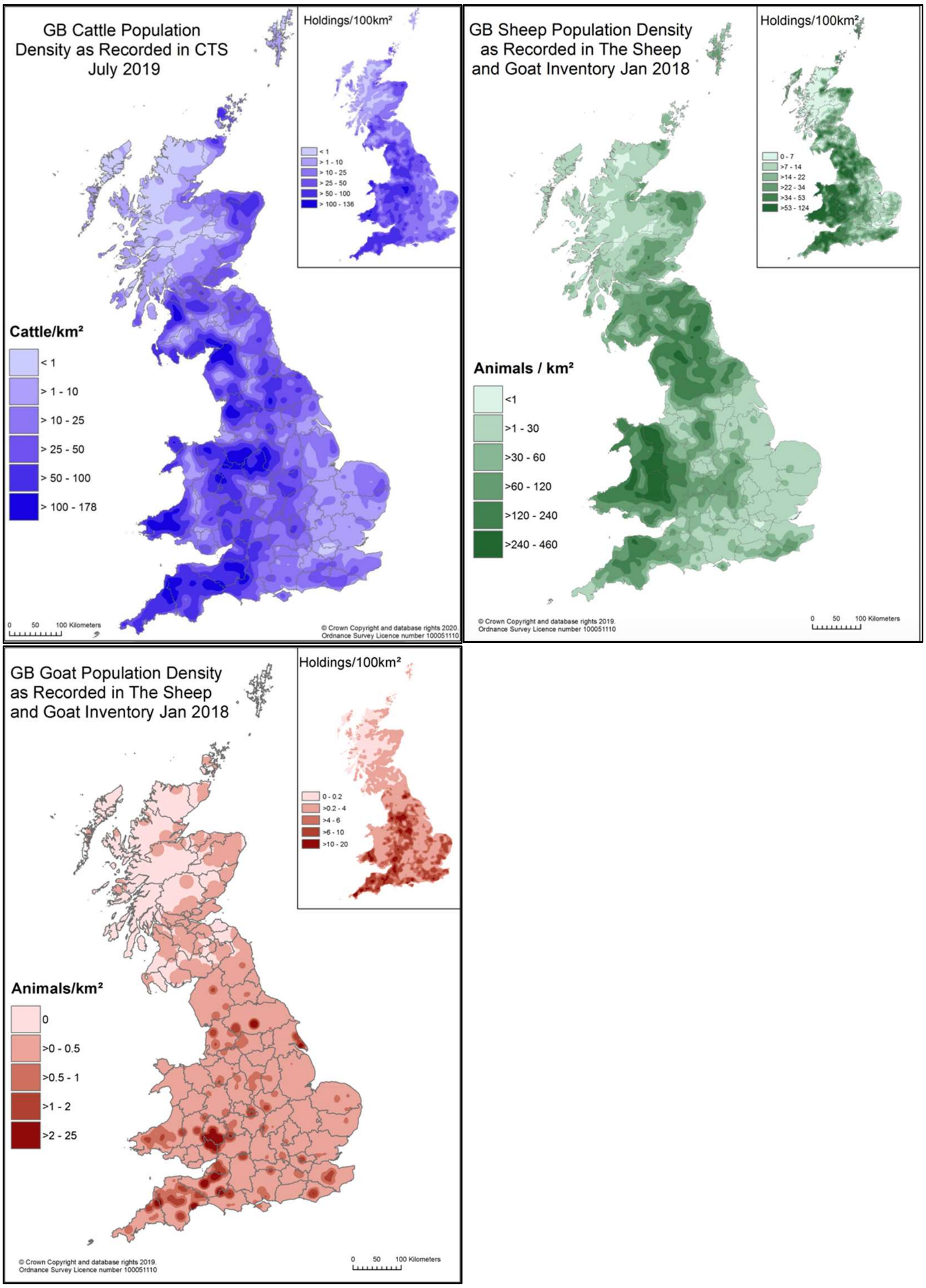

Figure 3: Livestock densities in the UK. The cattle population was recorded in July 2019 and the sheep and goat populations were reported in December 2017(43–45)**.**

Estimation of P_3: Probability of zoo animal becoming infected with BTV.

As shown in Risk Pathway #1, the probability of a zoo animal becoming infected with BTV is ***medium to very high****.*

Risk Pathway #2 Conclusion: The overall probability of a BTV-infected animal being imported to a farm in the UK resulting in the infection of a susceptible animal in the zoo is ***low.*** The probability for BTV-infected livestock to be imported to the UK undetected is low, as is the combined probabilities of the subsequent steps necessary.

Risk Pathway #3: Long-distance spread of BTV-infected midges across the English Channel resulting in BTV infection in zoo animals.

Estimation of P_7: Probability of windborne BTV-infected Culicoides entering the UK.

During the 2006-2008 northern Europe outbreak of BTV-8, it is largely believed the incursion into the UK occurred through long-distance wind dispersal of infected *Culicoides* from continental Europe(67). The small body size of *Culicoides* (1-3mm in length) enables their passive dispersion over great distances by wind(39). The UK Met Office's Numerical Atmospheric-dispersion Modelling Environment (NAME) models the spread of BTV through windblown midges, by analysing meteorological data and data on *Culicoides* populations (39). According to routine outputs from the NAME model, there were approximately 211 incursions of windblown midges from continental Europe in 2017, 205 in 2018, and 224 in 2019 (Figures 4-5, Supplementary Table S3)(39,72). Cuéllar et al. (2018) found *C. obsoletus* accounted for 83% of *Culicoides* trapped in nine EU countries between 2007-2013, and increased in density toward northern latitudes(56). Therefore, competent vectors are likely present along the coast of continental Europe. Based upon the available data, it is assumed that BTV was present in France and Germany during all three years of the model output and present in Belgium in 2019-2020(38). It is highly likely that infected *Culicoides* would survive after entry into the UK, particularly since incursions would likely occur during a period of high vector activity, in order for them to be caught by the wind(64).

The probability of windborne BTV-infected *Culicoides* entering the UK is *medium*, given these incursions take place over 200 times each year and the likelihood of infection in *Culicoides* populations of European origin.

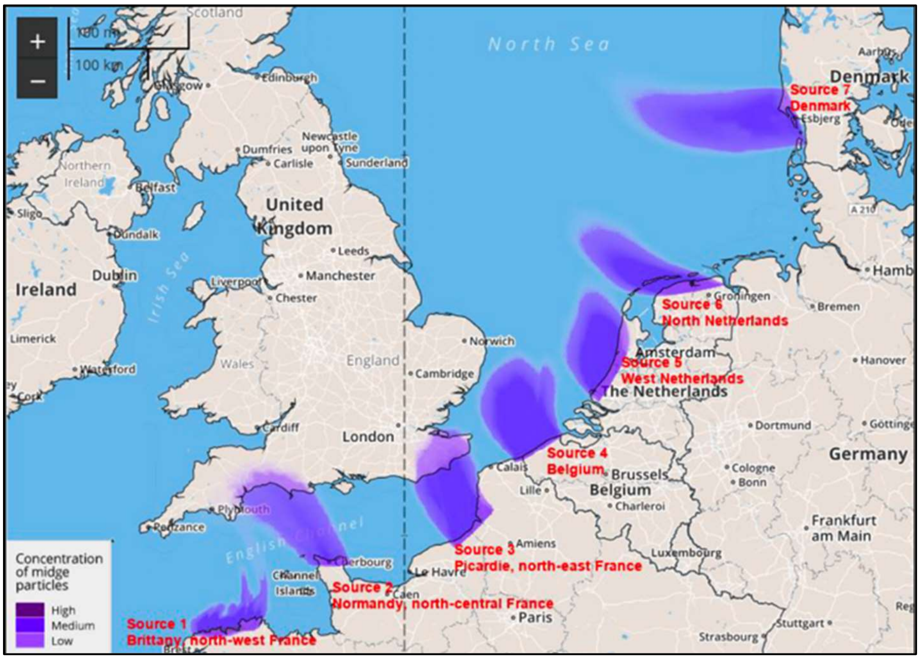

Figure 4: European source locations of infected Culicoides and modelled Culicoides dispersal over water(39).

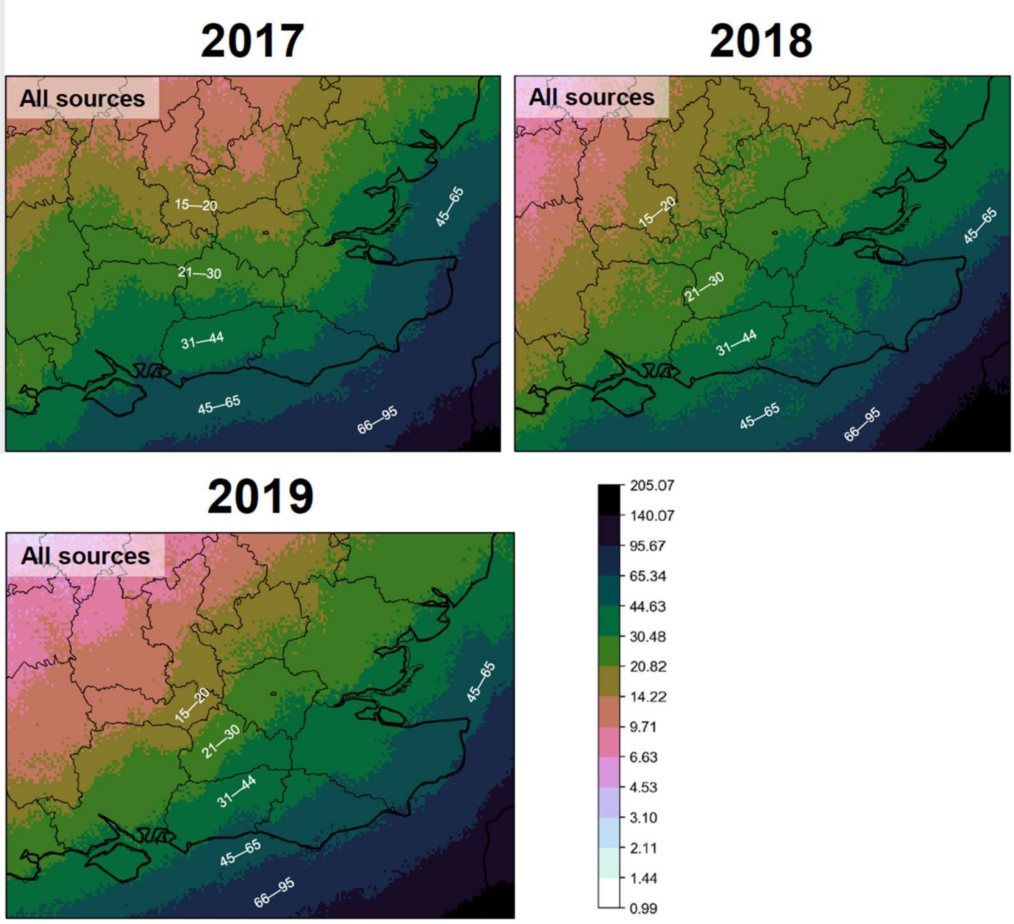

Figure 5: NAME-modelled Culicoides incursions from all European sources to the UK(39).

Estimation of P_8: Probability of BTV-infection in native livestock.

The greatest risk for onward transmission of BTV after an incursion of infected *Culicoides* occurs in areas with high livestock densities close to the coast where rates of incursion are high. Comparing the livestock density maps in Figure 3 with the incursions listed in Figure 5, East Sussex and Kent are considered to be at the greatest risk for onward transmission(39,44). All five counties that experience incursions have similar densities of cattle and goats(43–45). The rate of transmission has been found to be highest on cattle-only farms, followed by sheep-only farms, and lowest on mixed farms(59). Given the similar densities of cattle in all five counties, the higher densities of sheep in East Sussex and Kent will still increase their risk for infection. Sumner et al. (2013) found that there was a high chance of disease spread beyond the initial site of incursion in the absence of vaccination(14). Additionally, they found incursions occurring in September resulted in smaller outbreaks with less geographical spread than incursions occurring in May. Incursions occurring earlier in the year have more time for disease spread, taking full advantage of the adult *Culicoides* active season.

The probability of BTV-infection in native livestock following a windborne incursion of an infected *Culicoides* is *medium.* The presence of unvaccinated cattle, sheep and goats in counties with frequent incursions throughout the year presents a highly susceptible population. However, the associated dependence on seasonal factors reduce the overall probability to *medium*.

Estimation of P_5: Probability of BTV entering native Culicoides populations.

As shown in Risk Pathway #2, the probability of BTV entering the native *Culicoides* populations is ***medium***.

Estimation of P_6: Probability of spread of BTV to London Culicoides populations.

As shown in Risk Pathway #2, the probability of BTV spreading to London *Culicoides* populations is ***low****.*

Estimation of P_3: Probability of zoo animal becoming infected with BTV.

As shown in Risk Pathway #1, the probability of a zoo animal becoming infected with BTV is ***medium to very high****.*

Risk Pathway #3 Conclusion: The overall probability of a long-distance spread of BTV-infected midges across the Channel resulting in the infection of a susceptible animal is ***low to medium***, given that the probability for a windborne BTV-infected *Culicoides* to arrive in the UK is *medium,* but the probability of infection in native livestock is *low* in three of five coastal counties with midge incursions, which limits the probability of subsequent steps. However, this probability is *medium* in two of five coastal counties with midge incursions, so the risk varies.

Table 3: Estimated qualitative probabilities for the incursion of BTV.

Probability	**Qualitative Probability**	**Key Assumptions and Uncertainties**
Risk Pathway 1		
BTV-infected/viraemic animal enters zoo (P_1)	Low	• No exposure on the journey • Information accurately reported to OIE • EU/Canadian restrictions are being fully carried out • Sumatran tiger is able to be infected and transmit BTV
BTV-infected *Culicoides* in the zoo (P_2)	Medium to high	• Length of viremia transmittable to *C. sonorensis* (21 days) can be assumed for *C. obsoletus* and *C. pulicaris* • Assumption that vectors are active and temperatures suitable
Zoo animal infected with BTV (P_3)	Medium to very high	• Susceptible animals are bitten by *Culicoides* • EIP found for *C. sonorensis* can be assumed for *C. obsoletus* and *C. pulicaris*
Overall	Low	
Risk Pathway 2		
BTV-infected livestock enter the UK (P_4)	Low	• No exposure on journey • Information accurately reported to OIE • Assumption that imported livestock have come into contact with BTV in the country of origin if BTV is present there • Lack of reporting in France and Germany
BTV enters native *Culicoides* populations (P_5)	Medium	• No information on *Culicoides* populations at exact incursion sites • Infection rates are calculated using laboratory feeding techniques

Spread of BTV to London *Culicoides* populations (P_6)	Low	- No information on *Culicoides* populations in suburban/urban areas around London
Zoo animal infected with BTV (P_3)	Medium to very high	- See above
Overall	Low	
Risk Pathway 3		
Windborne BTV-infected *Culicoides* enter the UK (P_7)	Medium	- Disease presence equates to infection in coastal *Culicoides* populations in Europe - NAME only accurately predicts midge movement over water - Lack of reporting in France and Germany
BTV-infection in native livestock (P_8)	Low- Hampshire, W. Sussex, Essex Medium- Kent, E. Sussex	- Accurate reporting of livestock densities - Similar livestock density between 2017-2020
BTV enters native *Culicoides* populations (P_5)	Medium	- See above
Spread of BTV to London *Culicoides* populations (P_6)	Low	- Assumption of the “stepping-stone effect” for overland spread
Zoo animal infected with BTV (P_3)	Medium to very high	- See above
Overall	Low to medium	

4.2 African horse sickness

Risk Pathway #1: AHSV-infected animal imported directly to the zoo and onward transmission within the zoo.

Estimation of P_1: Probability of AHSV-infected animal entering the zoo.

Plains zebra (*Equus quagga*) act as reservoir hosts for AHSV, driving its distribution and persistence in endemic regions of Africa(25,27). The presence of plains zebra in zoos across the UK and northern Europe, therefore, present a route for introduction through an asymptomatic zebra import. The export of AHSV-infected zebra from Namibia to Spain in 1987 caused an outbreak that lasted for three years(29). Zebra are viraemic for up to 40 days, so it reasonable that an asymptomatic zebra would have a transmissible infection upon entry into the UK(25,33). Between 2017-2020, London Zoo only imported one potentially susceptible animal, a Sumatran tiger from Ebeltoft, Denmark in January 2018 (H. Jenkins, personal communication). It is unknown whether a Sumatran tiger is susceptible to AHSV, but given that asymptomatic infections can occur in carnivores, in particular big cats, it may be possible. The domestic dog is the only non-equid species known to exhibit severe disease and it has recently been suggested that natural infection could occur via a non-oral, vector-mediated route(46). AHSV has never been reported in any of the countries from which animals were imported to London Zoo over the last three years(73). No import countries reported the use of any vaccine doses to OIE, and vaccination is only recommended in countries that experience endemic or epidemic transmission(27,28,74). Susceptible equids undergo pre- and post-import testing in the UK and available tests are highly sensitive and specific, so are likely to pick up presence of the virus(54,75).

Since June 2004, horses in the UK and EU are required to be accompanied by passports with recommended veterinary certificate confirming AHSV-status(33,76). However, Robin et al. (2013) found that between 2005-2010, 95% of passports checked through local authorities were noncompliant with UK regulations(76). The current situation is Thailand is hypothesized to be the result of the importation of an infected equid from an AHSV-endemic country. This shows that despite having appropriate precautions in place, it may still be possible for infected equids to enter a country, either illegally or through incorrect certification. Additionally, post-import testing is only carried out on equids.

The probability of an infected/viraemic animal entering the zoo is *very low,* given that AHSV has never been reported in any of the import countries and no equids were imported into the zoo over the last years three years. The risk is not *negligible* due to the potential for importation of an asymptomatic zebra to London Zoo a part of a breeding programme and the potential for asymptomatic infection in non-equids

Estimation of P_2: Probability of AHSV-infected Culicoides in the zoo.

The main vector of AHSV is *C. imicola*, which is found in high abundance across AHSV's known distribution(27). During the 1987-1991 outbreak in Spain, AHSV was isolated from pooled samples containing *C. obsoletus, C. pulicaris,* and lacking *C. imicola*(27). After similar patterns in Portugal, it was postulated that transmission was driven by *C. imicola* and the coinciding high abundances of *C. obsoletus* and *C. pulicaris* allowed the infection to enter these species(77). These findings support the theory that *C. obsoletus* and *C. pulicaris* could become infected in the absence of *C. imicola*, as is the case with BTV(27). Prevalence of AHSV infection in *Culicoides* is often less than 10%, so transmission relies on large populations and high biting pressure(27). As previously discussed for BTV, England et al. (2020) found large populations of *C. obsoletus* and *C. pulicaris* at London Zoo(54). Following bloodmeal analysis, the *C. obsoletus* population at London Zoo was found to have fed on the Bactrian camels in the greatest abundance, suggesting a potential transmission route. Additionally, England et al. (2020) confirmed *C. obsoletus* in the zoo as non-specific opportunistic feeders, who would likely be competent vectors for AHSV facilitating transmission between an import equid and susceptible zoo animals, such as the Asiatic donkey(42).

The probability of AHSV-infected *Culicoides* in the zoo following the importation of an infected animal is *low to medium,* given the large populations of potential AHSV vectors in the zoo and their proven feeding on a wide range of hosts.

Estimation of P_3: Probability of a zoo animal becoming infected with AHSV.

As shown in Table 6, there are 15 susceptible animals currently kept in the London Zoo collection(42). The small geographic size of the zoo and the close proximity of the animals would allow rapid transmission to take place in the event of an incursion. After the introduction of an infected zebra to a wildlife park in Spain in 1987, widespread transmission occurred to other local equids and resulted in an outbreak encompassing three countries (48). The transmission rate is largely dependent on temperature and seasonal variations in *Culicoides* population abundance(47). Given endemic regions in Africa are extremely warm year-round, summer months in London are likely the only months capable of supporting transmission. In unvaccinated horses in endemic areas, the basic reproduction number (R_o) is equal to 2.6(47). The lower average temperatures in London would likely slow the rate of viral replication and EIP in the midges, so the R_o would likely be lower. Studies in the Netherlands and France have found *Culicoides* species to be highly attracted to horses(78,79).

Table 4: ZSL London Zoo animals at risk of AHSV infection(40)(H. Jenkins, personal communication).

Scientific Name	**Common Name**	**Total**
Lycaon pictus	African hunting dog	**7**
Equus asinus domestic	Donkey (domestic)	**2**
Equus quagga burchelli	Burchell’s zebra	**2**
Equus quagga chapmani	Chapman’s zebra	**2**
Camelus bactrianus domestic	Bactrian camel	**2**
Total		**15**

The probability of a zoo animal becoming infected with AHSV is *medium*, due to the availability of susceptible zoo animals and demonstrated feeding preferences of *Culicoides* populations in the zoo and across northern Europe.

Risk Pathway #1 Conclusion: The overall probability of an AHSV-infected animal imported directly to the zoo resulting in the infection of a susceptible animal is ***very low***, given there have been no recent importations of equids, or animals from AHSV-endemic areas to the zoo for the last three years.

Risk Pathway #2: AHSV-infected equid imported to the UK resulting in AHSV infection in zoo animals.

Estimation of P_4: Probability of AHSV-infected equid entering the UK.

The current AHSV outbreak in Thailand demonstrates AHSV's ability to overcome enormous geographical barriers(30). Between 2018-2020, the UK imported 16,380 equids from EU countries and 4,254 equids from non-EU countries between 2018-2019 (Supplementary Table S4)(40). In 2018 and 2019, AHSV was absent in all countries that exported animals to the UK(73). As of July 2020, there was an ongoing outbreak in Thailand and no information available for any of the other import countries, so we can assume no outbreaks occurred. No vaccination use was reported to OIE by any of the import countries between 2018-2020(74). In EU countries, AHSV has been a notifiable disease since December 1982 according to Council Directive 82/894/EEC, and EU countries are required to have contingency plans in operation with restriction and surveillance zones (Council Directive 92/35/EEC)(80). Outside of the EU, most non-endemic countries require import testing and quarantine of equids and similar action plans if an infection is detected(81). In endemic countries, which neighbour a few of the export countries (such as Morocco and Tunisia), live attenuated vaccines are routinely used and movement restrictions are employed in the case of an outbreak(81). Once in the UK, the probability an infected equid passes border checks is low, due to strict pre- and post-import testing required(54). The OIE Terrestrial Code defines the infective period as 40 days for domestic horses and donkeys are viraemic up to 17 days(82). Horses tend to exhibit severe symptoms which would likely be detected during routine veterinary checks at border posts.

The probability of an infected imported equid entering the UK is *very low*, due to the absence of the disease in all but one of the countries that have exported equids to the UK in the last two years, as well as the strict control measures in place both pre- and post-import into the UK. However, the large number of susceptible equids imported into the UK every year and the global nature of horse travel, does create a non-negligible risk.

Estimation of P_5: Probability of AHSV entering native UK Culicoides populations.

As previously mentioned, vectorial competence for AHSV of the Palearctic species *C. obsoletus* and *C. pulicaris* has been suggested(27,77,78). These species are widespread across the UK in high abundance, comprising between 93.5-97% of specimens caught on farms, with traps in some locations catching thousands of specimens in a single night

(64,71,78). Given the large populations found in the UK and the relatively high rate of infection in endemic region *Culicoides* species compared to BTV, it is likely AHSV circulation could occur. As previously discussed, studies in France and the Netherlands determined *C. obsoletus* and *C. pulicaris* will bite horses, so onward transmission would likely occur after initial importation of an infected equid, assuming the adult vectors are active(78,79). The destination of imported horses has been found to cluster in South-East England, where temperatures could support transmission during the summer months (Supplementary Figure S2)(46).

The probability of AHSV entering native *Culicoides* populations is *low to medium,* given the large populations of potentially competent *Culicoides* species and suitability of summer temperatures at the destinations of the majority of imported equids.

Estimation of P_6: Probability of spread of AHSV to London Culicoides populations.

Given the relatedness of AHSV and BTV, it is likely that if conditions allowed for AHSV circulation within UK *Culicoides* populations, the outbreak would follow a similar ‘stepping stone’ pattern, with small jumps between equine holdings(68). The species composition in London is likely suitable for AHSV transmission, given that studies at London Zoo caught mainly members of the *C. obsoletus* and *C. pulicaris* complexes(8). However, the spread of AHSV into London populations may be limited by the low density of horses within the immediate London area(47). Unfortunately, there is limited data on the distribution of horses in the UK. Lo Iacono et al. (2013) modelled the potential spatio-temporal transmission rates of AHSV in Great Britain using ambient temperatures during the year, seasonal abundance of *Culicoides,* and the distribution of other hosts. Their model found the patterns of transmission were mainly influenced by the abundance of *Culicoides,* the distribution of horses, and the presence of non-susceptible hosts (sheep and cattle). They produced an estimate of horse density across Great Britain (Figure 6) and there appears to be a ring formed around London with limited transmission potential (47).

The probability of AHSV spreading to the London *Culicoides* populations is *very low*, given the limited host distribution in the immediate London area.

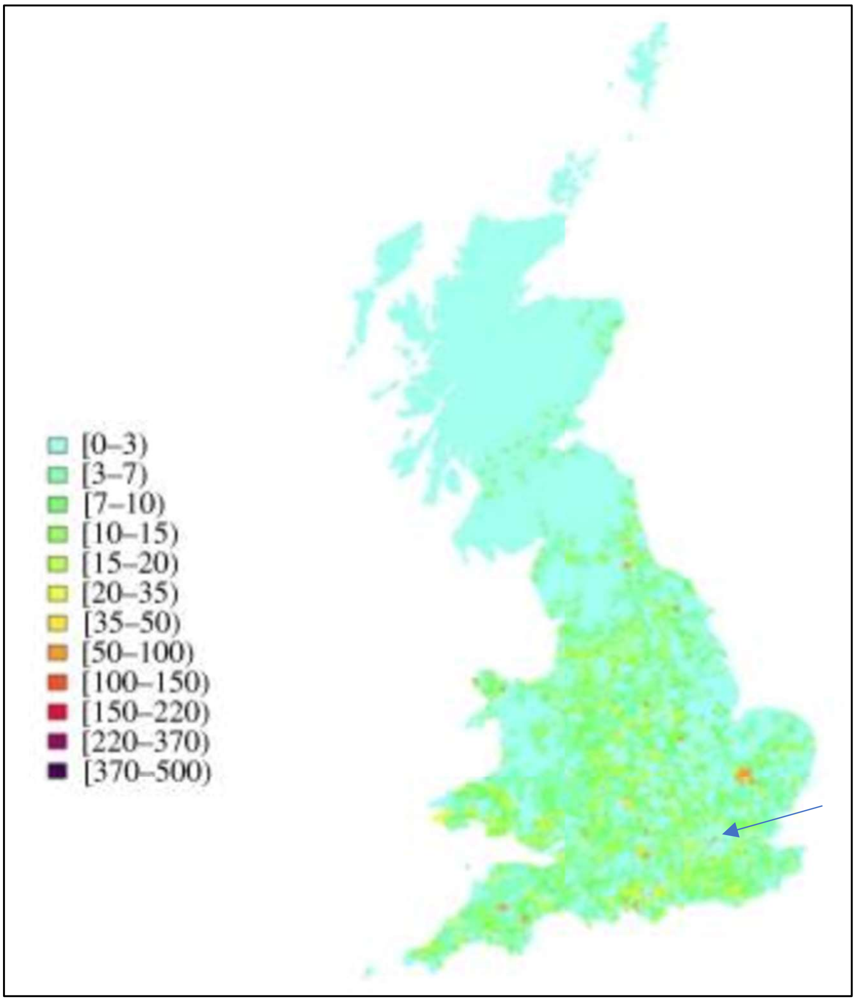

Figure 6: Modelled distribution of the density of horses per 1km. The blue arrow indicates the Greater London area(47).

Estimation of P_3: Probability of a zoo animal infected with AHSV.

As shown in Risk Pathway #1, the probability was determined to be ***medium.***

Risk Pathway #2 Conclusion: The overall probability of a AHSV-infected equid imported to the UK resulting in the infection of a susceptible zoo animal is ***very low to low*****,** given that the probability a AHSV-infected/viremic animal enters the country is very low, as well as the ultimate probability the infection enters the native and subsequent London *Culicoides* populations.

Table 5: Estimated qualitative probabilities for the incursion of AHSV.

Probability	**Qualitative Probability**	**Key Assumptions and Uncertainties**
Risk Pathway 1		
AHSV-infected animal enters zoo (P_1)	Very low	- No exposure on the journey - Information accurately reported to OIE - Sumatran tiger is susceptible to infection
AHSV-infected *Culicoides* in zoo (P_2)	Low to medium	- *C. obsoletus* and *C. pulicaris* complexes are assumed to be competent vectors
Zoo animal infected with AHSV (P_3)	Medium	- *C. obsoletus* and *C. pulicaris* are assumed to have a lower EIP in temperate conditions - Susceptible animals are bitten by *Culicoides*
Overall	Very low	
Risk Pathway 2		
AHSV-infected equids enter UK (P_4)	Very low	- Illegal importations have not been considered - No exposure on journey - Information accurately reported to OIE - Assumption that equids have come into contact with AHSV in the country of origin if AHSV is present there
AHSV enters native *Culicoides* populations (P_5)	Low to medium	- *C. obsoletus* and *C. pulicaris* are competent vectors
Spread of AHSV to London *Culicoides* populations (P_6)	Very low	- Based on Lo Iacono et al. (2013) R_0 model predictions
Zoo animal infected with AHSV (P_3)	Medium	- See above
Overall	Very low-low	

5. DISCUSSION

The relatedness of the African horse sickness and bluetongue viruses, as well as their shared *Culicoides* vector, render their potential pathways for incursion into the UK and on to London zoo extremely similar. The major divergence in risk between the two diseases appears to come from BTV's established prevalence in temperate northern Europe. BTV's 2006-2009 outbreak in northern Europe and on to the UK proved its ability to replicate in temperate conditions, be transmitted by Palearctic *Culicoides* species, and sweep through naïve livestock populations(13,15,17,63). Its continued presence in northern Europe leaves the UK at continual risk for accidental introduction(10,38). Contrastingly, AHSV has never been reported in temperate areas and has only made a few incursions into southern Europe (29,73,83). Very little is known about its potential to spread in temperate conditions via Palearctic *Culicoides,* and its geographic distance from the UK lessens the risk of accidental introductions(47,77,84). The current status of scientific knowledge and limited distribution of AHSV is remarkably similar to that of BTV before its breakthrough to northern Europe.

Therefore, whilst the greatest risk of the two viruses to London zoo is currently posed by BTV, the potential for an AHSV outbreak should not be discounted.

The most likely pathway of BTV introduction into the UK and on to London zoo appears to be from long-distance windborne incursion of infected *Culicoides* from France, Belgium, or the Netherlands. Evidence suggests windborne incursions are responsible for the 2007 BTV-8 outbreak in the UK(67). The continued presence of the disease in countries known to be the origins of hundreds of windborne *Culicoides* incursions annually render this pathway a constant risk(10,38,39). The second two risk pathways considered here for BTV both have the potential to occur, and if this incursion coincided with the active vector season in the UK, onward transmission is likely. The probability an infected zoo or livestock import would pass both pre-import testing in its country of origin and post-import testing within the UK is low, given the widespread knowledge of the disease and strict restrictions on susceptible animal movements(2,53). While infected cattle have passed through France's pre-import testing undetected, the rapid identification of these cases after arrival in the UK highlight the effectiveness of the UK's post-import surveillance(16). Additionally, the zoo has imported very few susceptible animals in the past few years (H. Jenkins, personal communication).

The introduction and onward transmission of AHSV on the other hand, appears relatively unlikely through either an infected animal imported directly to the zoo or an infected equid imported to the UK. The consequences associated with the severe clinical disease often seen in horses, have forced strict pre- and post-import checks that reduce the likelihood that an infection would go undetected(54,80,81). Greater knowledge of the disease's manifestation and potential for transmission in non-equid hosts would increase the accuracy of this risk assessment.

5.1 Key assumptions and uncertainties

From previous studies, the active vector season is assumed to begin in late April and end in late October/early November at London Zoo(54). For this risk assessment, it has been assumed that all vector species are equally active throughout the season and temporal variation in risk throughout the year has not been considered. However, Sanders et al. (2011) sampled *Culicoides* at 12 sites across the UK and found general *Culicoides* abundance exhibited bimodal seasonality, with populations peaking in April/May and then again in September/October(65). There was variation between the sites, but lowered abundance was noted across all sites in June, and *C. punctatus* and *C. pulicaris* emerged earliest and were continually caught later than other species. Bimodal peaks were most pronounced in *C.*

punctatus and *C. pulicaris*, however they were also noticed in *C. dewulfi* and *C. obsoletus.* These variations would directly impact the risk of BTV and AHSV introduction, as transmission would be less likely in periods with lower population abundance. Therefore, the greatest risk for onward transmission would occur in April/May and September/October, but this is not reflected in this risk assessment.

Another assumption for this risk assessment was that UK *Culicoides* species are capable of transmitting AHSV. This assumption was based off their ability to transmit the closely-related bluetongue virus(15,91). Depending on the actual vector competence of UK species, the risk of transmission of AHSV could dramatically increase or decrease. There is an immediate need to understand their vector competence, in order to avoid an outbreak resembling the 2007 BTV-8 epidemic. Additionally, the length of the EIP for UK vectors had to be assumed based on laboratory work performed on *C. sonorensis*(60,92).

The distribution and abundance of livestock and equid populations was assumed to remain fairly constant. The exact distribution of equids was particularly uncertain, given the National Equine Database records only the address of the owner not the animal, so additional information was taken from Lo Iacono et al. (2013)'s model(47). It was also assumed without livestock or equid hosts within the immediate London area, it would be less likely for the virus to be transmitted to populations of *Culicoides* within the zoo.

The Sumatran tiger was assumed to be susceptible to both BTV and AHSV based on evidence of antibodies in other big cats and carnivores(9). There is additional levels of uncertainty around this assumption, given that it is unknown whether they could be infected by vector feeding or solely through oral transmission, and whether onward transmission would be possible(49). It is unclear whether an infection would be asymptomatic or present as clinical, and what the length of the incubation and viraemic periods would be. Without evidence of infection in tigers, their role in transmission can only be assumed based on evidence in related species.

Several uncertainties arise given the research and data currently available. Much of the disease distribution and vaccination status of animals' statistics were drawn from OIE databases. The accuracy of countries' reporting to OIE is uncertain, given countries such as France with restriction zones are not required to report disease events. The accuracy of the livestock and equine import data is also uncertain, as a third party enters the information into the TRACES system. There will always be variation between years in import trends, so the exact risk will vary accordingly between and across years. Additionally, the NAME model used to determine windborne incursions to the UK adds uncertainty. The nature of a model itself carries inherent uncertainty, since it uses observed trends to predict into the future. The NAME model is only able to predict the transport of midges across water, but once over land it is unable to determine where they will land. Therefore, it was assumed areas with higher livestock densities would attract more midges, but what triggers *Culicoides* to land in certain locations is unknown. The survival of the midges arriving is also uncertain, given the model treats them as particles and the exact survival rate is unknown.

5.2 Negligible risk pathways

The aforementioned risk pathways were analysed as they were determined to be the most likely pathways for the incursion of BTV and AHSV into the UK and cause onward transmission to an animal in London Zoo. The selection of these pathways was limited by the availability of research on the various elements of each pathway and current scientific understanding of the disease. It is therefore critical to mention a few additional risk pathways deemed to pose a negligible risk at this time, but whose importance may increase in the future.

For BTV, it has been suggested that disease introduction could occur through the importation of infected midges with cargo, such as cut flowers. Initially, the 2006-2009 BTV-8 outbreak in northern Europe was thought to have originated via this pathway, since initial cases occurred in Maastricht, an international plant trading hub(93). This was later disproved through the discovery of earlier infections on farms nearer to Belgium(94). Elements of this pathway are largely based on anecdotal evidence. However, Nie et al. (2005) surveyed international ships arriving in Qinhuangdao Port, China during the summer of 2003 and found 29 of 70 ships inspected contained live midges, including species of *Culicoides*(95). The UK imports 17% of Kenya's flower exports (an endemic country), creating ample opportunity for this pathway to occur(10,96). However, flowers are grown in specific growing areas near to Nairobi airport, from there they are shipped directly via airplane at low temperatures to the UK, and then directly on to supermarkets (J. Stokes, personal communication). Given the lack of susceptible livestock at either end of this pathway, as well as the conditions of travel, the risk of this occurrence is considered negligible.

Another risk pathway for BTV is the potential import of infected germplasm. Transmission is possible via either frozen or chilled germplasm and it is hypothesized that frozen cattle sperm from 2007 has caused the resurgence of BTV-8 in France(38). The risk of disease importation to the UK is currently negligible via this pathway given the testing measures in place at semen collection centres and the restriction of specimens from restricted areas(38).

Finally, a proven risk pathway for AHSV incursion occurs through the long-distance spread of infected midges via wind movements. In Africa, winds from endemic regions have caused outbreaks in naïve equid populations in non-endemic areas(23). The maximum possible distance for this sort of dispersal has been postulated as 700km over water or 150km over land(24,25). The risk to the UK is therefore negligible, due to the lack of AHSV presence within this range(73).

5.3 Further work

Further studies and data collection are required to increase the accuracy and reduce the uncertainties of the risk estimates. In particular, there is a need for vector competence studies to clarify whether UK species of *Culicoides* could have a role in AHSV transmission in the event of an incursion. The temperature requirements for AHSV replication within the midge have been previously studied in *C. sonorensis* but have not been investigated for *C. obsoletus* or *C. pulicaris* complexes. Studies that investigate how *Culicoides* fly over land, and what drives them select a place to land after arriving in the UK would also be useful to increase the predictability of the NAME model, and therefore increase the accuracy when estimating risk from wind-borne incursions.

The risk to the UK from both BTV and AHSV is to a large extent dependent on the disease situation in northern Europe. In the case of BTV, increased reporting of cases to establish the precise location of outbreaks would inform future risk assessments. For AHSV, the risk of incursion into Europe should also be assessed, particularly with reference to zoo populations due to the transportation of animals between zoos. Additionally, it would be interesting to investigate how zoo animals respond to AHSV-infection in endemic countries. This would help to clarify which species are most at risk from incursion, and also those that may have the potential to carry the virus across borders when being transferred between zoo collections.

6. CONCLUSIONS & RECOMMENDATIONS

Bluetongue disease and African horse sickness are two closely related *Culicoides* midge-borne viruses, which incur immense economic consequences and disrupt global trade. After careful analysis of their risk of introduction to the UK and onward transmission to the London zoo through the assessment of the most likely risk pathways, BTV appears to pose the greater threat, but the uncertainty surrounding AHSV may underestimate its potential. Overall, the probability of BTV infecting a zoo animal was determined as low to medium risk, with the greatest threat posed by the windborne introduction of infected *Culicoides* from mainland Europe. The combined probability of a zoo animal becoming infected with AHSV was determined to be very low to low.

In order to combat the threat of BTV or AHSV introduction into the zoo collection and potential onward transmission, there are several mitigation strategies the zoo can undertake. Stringent post-import testing on ruminants and equids for BTV and AHSV should continue, and potentially expand to encompass the previously mentioned species found to have antibodies when imported from countries with known disease. The zoo should ensure it has access to a sufficient quantity of vaccines, particularly for BTV-8, and in the event of an incursion of BTV to the UK, should vaccinate all susceptible animals with a serotype-specific vaccine. Vaccination for AHSV would depend on amendments to current licensing. While vector control for *Culicoides* is largely ineffective, continued surveillance should be performed of zoo- and London-based *Culicoides* populations. This will ensure the risk of transmission is adequately understood based on host preferences, species composition, and species abundances. In the event of an outbreak in the UK, efforts to reduce vector-host contact should be enacted, such as protective housing, and restricting outdoor access to periods of low vector activity (midday).

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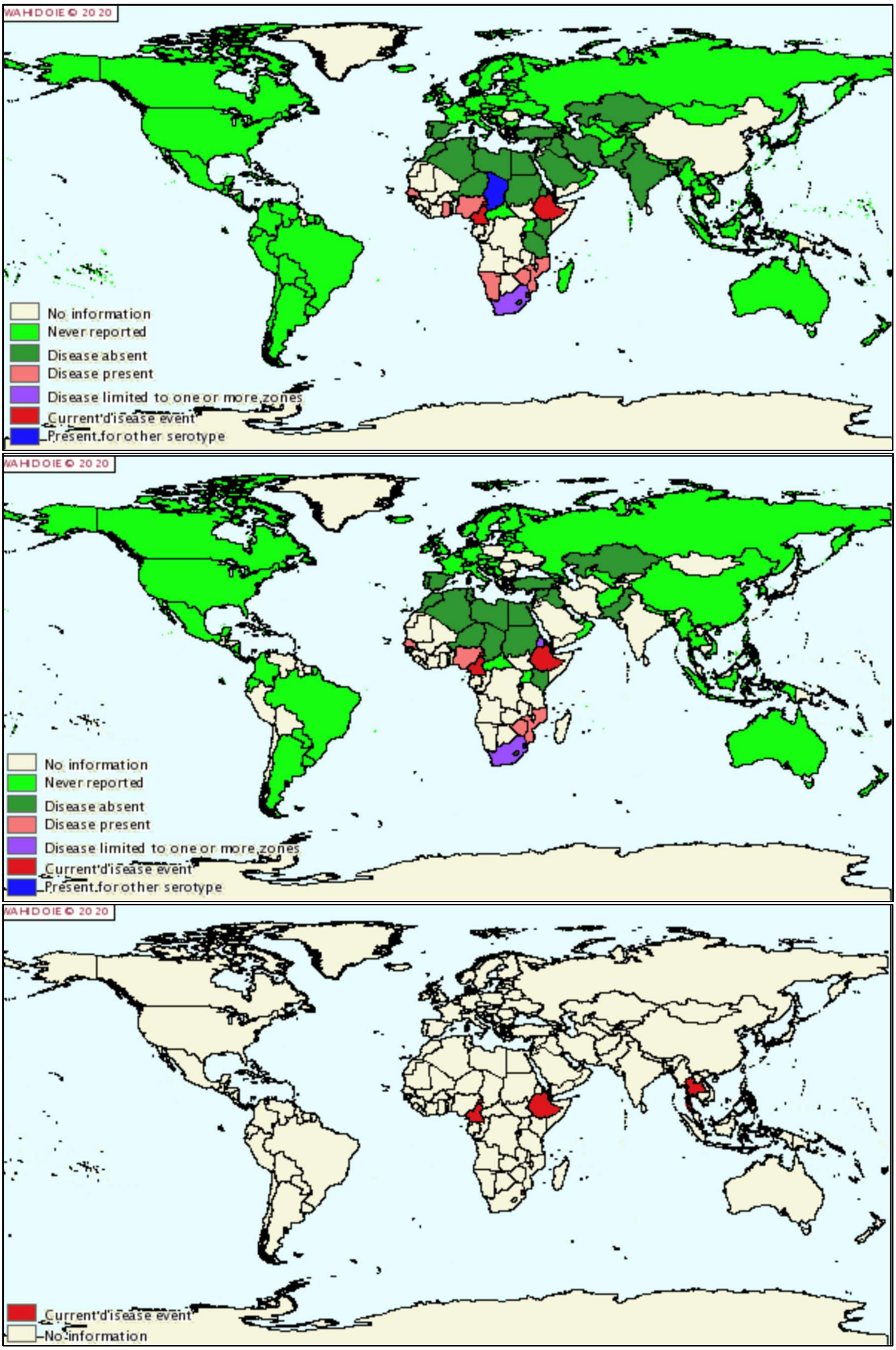
8. APPENDICES



Supplementary Figure S1: Countries reporting cases of African horse sickness from January 2020 to present (73).

Supplementary Table S1: Imports of animals to London Zoo from outside of the UK (including Jersey) From January 2017-December 2019 (H. Jenkins, personal communication).

Country	**City**	**Animal Common Name**	**Animal Scientific Name**	**No. of Animals**	**Date of import**
Austria	Vienna	Charco Palma pupfish	*Cyprinodon veronicae*	1	14/12/2017
		Killifish	*Aphanius saldae*	1	14/12/2017
		La Palma Pupfish, Cachorrito de Charco Palmal	*Cyprinodon longidorsalis*	1	14/12/2017
		Potosi pupfish	*Cyprinodon alvarezi*	1	14/12/2017
Canada	Toronto	Big-headed turtle	*Platystemon megacephalum*	4	25/09/2018
		Malagasy cichlid	*Ptychochromic insolitus*	3	03/10/2019
		Panchax	*Panchypanchax amoulti*	1	03/10/2019
		Zonobe rainbowfish	*Rheocies vatosoa*	1	03/10/2019
Czech Republic	Praha	Moholi bushbaby	*Galago moholi*	1	12/04/2017
	Olomouc	Southern tamandua	*Tamandua tetradactyla*	1	07/09/2017
Denmark	Ebeltoft	Sumatran tiger	*Panthera tigris sumatrae*	1	28/01/2019
France	Lille Zo	Grey parrot	*Psittacus erithacus*	4	24/01/2018
Germany	Hamburg	Dwarf mongoose	*Helogale parvula undulatus*	2	19/09/2017
	Frankfurt	Grey slender loris	*Loris lydekkerianus grandis*	1	24/01/2018
	Koln	Abdim's stork	*Ciconia abdimii*	1	26/06/2018
Netherlands	Rotterdam	Hyacinth macaw	*Anodorhynchus hyacinthinus*	1	28/07/2018
Switzerland	Zurich	Galapagos tortoise	*Chelonoidis nigra*	8	28/02/2017
		Panther chameleon	*Furcifer pardalis*	2	19/12/2019
UK	Jersey	Round Island skink	*Leiolopisma telfarii*	16	21/07/2017
		Giant jumping rat	*Hypogeomys antimena*	1	19/12/2018

Supplementary Table S2: Ruminant imports from EU countries January 2018-July 2020 No imports occurred from non-EU countries in these years. Countries and years with reported BTV circulation are marked in red (40).

Country	***Species***	**2018 (No. of animals)**	**2019 (No. of animals)**	**2020 (No. of animals)**
Austria	*Bos taurus*	74	0	0
Belgium	*Bos taurus*	957	1621	33
Czech Republic	*Bos spp*	1	0	0
	Bos taurus	0	1	0
Denmark	*Bos taurus*	2962	3858	0
France	*Bos taurus*	1228	230	18
Germany	*Bos spp*	2	0	0
	Bos taurus	4683	6019	32
Ireland	*Bison spp*	0	2	0
	Bos taurus	29838	38930	6

Italy	*Bos taurus*	11	4	0
	Bubalus bubalis	48	25	0
Luxembourg	*Bos taurus*	601	629	0
Norway	*Bos taurus*	0	8	0
Poland	*Bos taurus*	0	1	0
Spain	*Bos taurus*	6	0	0
Sweden	*Bos taurus*	140	349	0
Netherlands	*Bos taurus*	7810	2394	0
Total		48355	54071	89

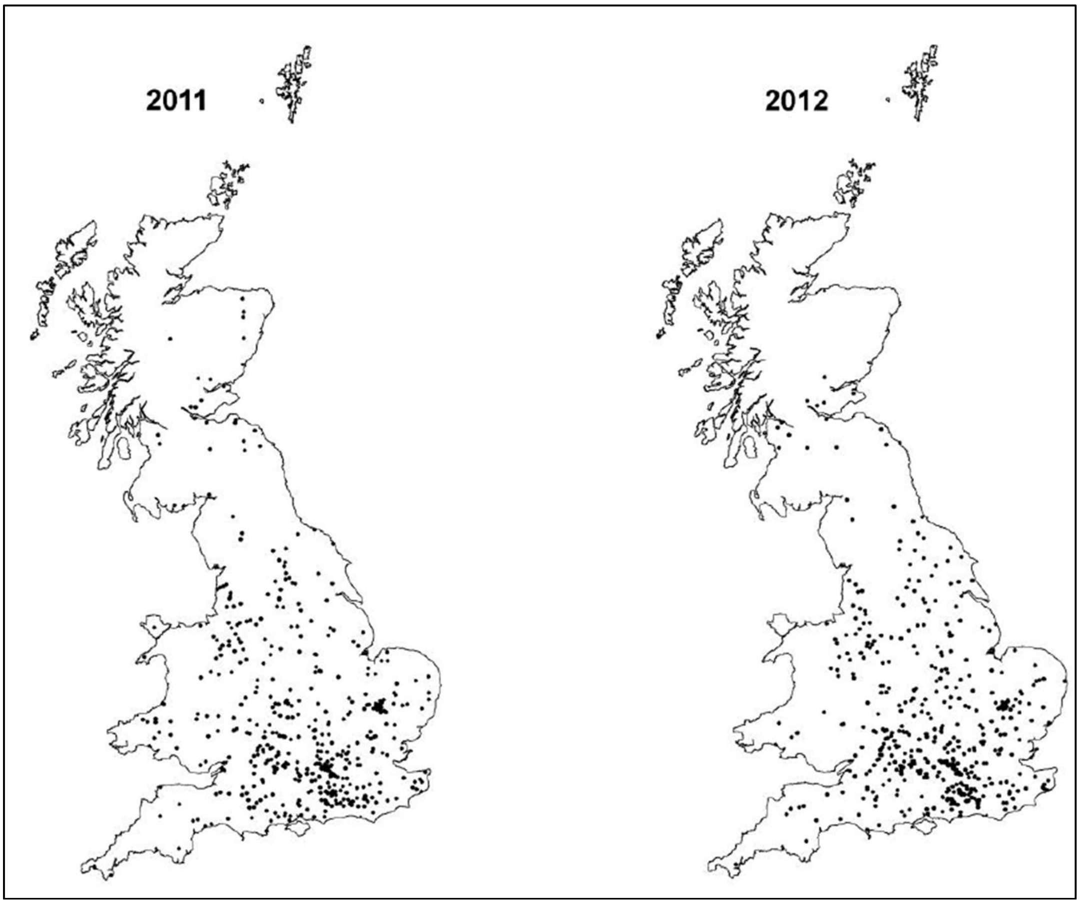
Supplementary Table S3: Total number of windborne incursions of *Culicoides* from northern Europe to the UK each year from routine NAME model runs. The number of incursion events are visual approximations from the model's graphical outputs. The numerical outputs refer to the number of potential incursion events into each county for each year (39).

UK County	EU Sources	2017 Incursions	2018 Incursions	2019 Incursions
Hampshire	1,2,3	39	31	31
W Sussex	1,2,3	42	35	45
E Sussex	1,2,3	45	37	57
Kent	3,4,5	57	65	55
Essex	4,5	28	37	36

Supplementary Table S4: Imported equids from non-EU countries from January 2018-December 2019. An additional 16,307 horses, 44 donkeys and 29 mules were imported from EU countries from January 2018-July 2020(40).

Country of Origin	2018 (No. of Horses)	2019 (No. of Horses)
Argentina	423	430
Australia	93	124
Bahrain	24	35
Barbados	2	0
Canada	35	52
Chile	8	4
China	14	6
Hong Kong	41	31
Iceland	5	18
Indonesia	10	0
Israel	2	5
Japan	13	18
Jordan	0	6
Korea, Republic of	1	2
Kuwait	8	18
Malaysia	2	6
Mauritius	142	87
Morocco	16	45
New Zealand	27	34
Oman	43	50
Peru	0	6
Qatar	35	37
Russian Federation	47	21
Saudi Arabia	3	16
Serbia	0	1
Singapore	3	9
Thailand	1	5
Tunisia	0	7
Turkey	0	3
Ukraine	4	17
United Arab Emirates	484	600
United States	559	498
Uruguay	16	2

Total	**2061**	**2193**



Supplementary Figure S2: The destination of imported horses between April 1, 2011-July 31,2012. The locations are based on the addresses of the importer, but the imported horses are likely housed nearby (46).

Student's Questionnaire

Candidate No: 200052

MSc: Control of Infectious Diseases

Project Supervisor: Dr. Marion England (Pirbright Institute) and Dr. Mary Cameron (LSHTM)

Project Title: A Qualitative Risk Assessment for the Incursion of Bluetongue Disease and African Horse Sickness into London Zoo

As part of our assessment procedure for student projects we are asking you to complete the following short questionnaire. Please tick the most appropriate statements. **A copy of this questionnaire should be included in your project when submitted.**

(*Please ensure you tick the correct box, if filling in electronically double click the check box to mark as checked*)

Who initiated the project?

☒ My supervisor
☐ Me

How much help did you get in developing the project?

☐ none: I decided on the design alone
☐ some: I used my initiative but was helped by suggestions from my supervisor
☒ substantial: My supervisor had most say, but I added ideas of my own
☐ maximal: I relied on the supervisor for ideas at all stages
☐ not applicable: the nature of the project was such that I had minimal opportunity to contribute to the design

How much help did you get in carrying out the work for the project?

☐ none: I worked alone with no supervisor input
☐ minimal: I worked alone with very little supervisor input
☒ appropriate: I asked for help when needed
☐ substantial: the supervisor gave me more assistance than expected
☐ excessive: the supervisor had to give me excessive assistance to enable me to get data

What was the degree of technical difficulty involved?

☐ slight: data easily obtained
☒ moderate: data were moderately difficult to obtain
☐ substantial: data were difficult to obtain

How much help were you given in the analysis and interpretation of any results?

☐ none
☒ standard: My supervisor discussed the results with the me and advised on statistics and presentation
☐ substantial: My supervisor pointed out the significance of the data and told me how to analyse it

How much help were you given in finding appropriate references?

☐ none
☒ some: only a few references were provided
☐ substantial: most references were given by my supervisor
☐ maximal: the supervisor supplied all the references used by me

How much help did you get in writing the report?

☐ none: my supervisor did not see the report until it was submitted
☐ minor: my supervisor saw and commented on parts of the report
☒ standard: my supervisor saw and commented on the first draft of the report
☐ substantial: my supervisor gave more assistance than standard

How much time was spent on the project?

☐ too little to expect adequate data*
☒ sufficient
☐ too much*

**if too little or too much, were there any reasons for it, e.g. unforeseen technical problems, lack of materials, etc.?*

During the course of the work was your contact with your supervisor

☐ Daily
☒ Weekly
☐ Monthly
☐ Varied but at regular intervals
☐ Never

Was this contact with your supervisor

☐ too infrequent
☐ infrequent but sufficient
☒ frequent but not excessive
☐ excessive

I really enjoyed working with both of my supervisors. I thought they provided excellent guidance and really made the project a wonderful learning experience.

THIS QUESTIONNAIRE MUST BE INCLUDED INTO YOUR PROJECT REPORT