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Quantitative analysis of the probability of introducing equine encephalosis virus (EEV) into The Netherlands

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ABSTRACT

Equine encephalosis is a midge-borne viral disease of equines caused by equine encephalosis virus (EEV, *Orbivirus, Reoviridae*), and closely related to African horse sickness virus (AHSV). EEV and AHSV share common vectors and show similar transmission patterns. Until now EEV has caused outbreaks in Africa and Israel. This study aimed to provide insight in the probability of an EEV outbreak in The Netherlands caused by infected vectors or hosts, the contribution of potential source areas (risk regions) to this probability, and the effectiveness of preventive measures (sanitary regimes).

A stochastic risk model constructed for risk assessment of AHSV introduction was adapted to EEV. Source areas were categorized in risk regions (high, low, and very low risk) based on EEV history and the presence of competent vectors. Two possible EEV introduction pathways were considered: importation of infected equines and importation of infected vectors along with their vertebrate hosts. The probability of EEV introduction (P_{EEV}) was calculated by combining the probability of EEV release by either pathway and the probability of EEV establishment.

The median current annual probability of EEV introduction by an infected equine was estimated at 0.012 (90% uncertainty interval 0.002–0.020), and by an infected vector at 4.0 10^{-5} (90% uncertainty interval 5.3 10^{-6}–2.0 10^{-4}).

Equines from high risk regions contributed most to the probability of EEV introduction with 74% on the EEV introduction by equines, whereas low and very low risk regions contributed 18% and 8%, respectively. International movements of horses participating in equestrian events contributed most to the probability of EEV introduction by equines from high risk regions (86%), but also contributed substantially for low and very low risk regions with 47% and 56%.

The probability of introducing EEV into The Netherlands is much higher than the probability of introducing AHSV with equines from high risk countries contributing most. The introduction by an infected equine is the most likely pathway. Control measures before exportation of equines showed to have a strong mitigating effect on the probability of EEV introduction. The risk of EEV outbreaks should be taken into account when altering these import regulations.

1. Introduction

Equine encephalosis is a vector-borne disease of equines that is proven endemic in South Africa, Ethiopia, Ghana, The Gambia (Oura et al., 2012), and Israel (Mildenberg et al., 2009; Aharonson-Raz et al., 2011; Wescott et al., 2013). Equine encephalosis is caused by equine encephalosis virus (EEV), belonging to the *Orbivirus* genus of the family *Reoviridae* (Viljoen and Huismans, 1989) and is closely related to African horse sickness virus (AHSV) and bluetongue virus (BTV) (Williams et al., 1993). EEV is transmitted by *Culicoides* spp. (midges).

Equine encephalosis has been described mostly as a subclinical disease (Paweska and Venter, 2004). Isolated clinical outbreaks were reported in South Africa during the late summers of 1967 and 1978 (Viljoen and Huismans, 1989), and in Israel in 2009 (Mildenberg et al., 2009). Occasionally, infected horses show clini-

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cal symptoms, such as fever, depression, and swelling of the eyelids, after an incubation period of 2–6 days (Mellor and Boorman, 1995; Oura et al., 2012). Donkeys and zebras are considered to be resistant to clinical disease, but not to infection with EEV. Therefore, these species might be a reservoir of EEV in endemic countries (Howell et al., 2002; Paweska and Venter, 2004). Mortality due to equine encephalosis is low in all equines and serological testing is required to identify current or past EEV infections (Williams et al., 1993; Howell et al., 2002; Crafford et al., 2003; Crafford et al., 2011).

Culicoides imicola and *Culicoides bolitinos* are the main vectors for EEV transmission in South Africa and Israel (Venter et al., 1999; Venter et al., 2002; Van der Rijt et al., 2008; Aharonson-Raz et al., 2011). *C. imicola* and *C. bolitinos* are also competent vectors for AHSV and BTV (Mellor and Boorman, 1995; Venter et al., 2002; Paweska and Venter, 2004). Although these *Culicoides* species have not been found in North-western Europe (Japin and G.A.Y., 2013), other species belonging to the Pulicaris and Obsoletus species complexes, were responsible for transmission of the related BTV in North-western Europe (Meiswinkel et al., 2008), and possibly also for AHSV transmission in Spain (Mellor et al., 1990). AHSV, BTV and EEV share common vectors in African endemic countries and hence the Pulicaris and Obsoletus species complexes should be also considered as potential vector for EEV in North-western Europe (Takken et al., 2008). EEV in Europe can thus not be excluded and might pose a threat to the horse industry (MacLachlan and Guthrie, 2010; Zimmerli et al., 2010).

The Netherlands is of special interest because the country experienced recent outbreaks of other *Culicoides* borne animal infections for which the source is currently still unknown (Elbers et al., 2008; EFSA 2013). Additionally it has a high horse density (De Vos et al., 2012). Furthermore, The Netherlands has an estimated horse population of 4.5×10^5 horses of which approximately 40% is for commercial purposes (Mourits and Saatkamp, 2010 in De Vos et al., 2012). An outbreak of EEV might have severe economic and socio-ethical impact due to imposed control measures such as movement standstills and export restrictions.

Large-distance spread of EEV in Africa might either have been due to dispersal of competent vectors or movement of infected hosts (Howell et al., 2008). It has been suggested that EEV reached Israel by infected vectors carried by wind from neighbouring countries (Aharonson-Raz et al., 2011; Mildenberg et al., 2009). Introduction by wind-borne spread of infected vectors of EEV into The Netherlands is unlikely to happen due to the large geographical distance to EEV-endemic countries.

AHSV and EEV have similar transmission patterns (Lord et al., 2002). In analogy with AHSV, introduction of EEV into areas free of infection might occur by translocation of infected hosts or infected vectors associated with large animal trade (De Vos et al., 2012; Faverjon et al., 2015). International movements of equines, including those participating in equestrian events, and ruminants should thus be considered as possible EEV introduction routes into The Netherlands.

The present research proposes a quantitative risk analysis to evaluate the probability of EEV introduction into The Netherlands. The introduction of EEV is defined as the release of the virus into an area free of EEV, followed by its establishment in the local host population. Two introduction pathways are evaluated: introduction of EEV by an infected equine (PW_{equine}) and introduction of EEV by an infected vector along with its vertebrate host (PW_{vector}). The aims of this research were to estimate the probability of introduction through both introduction pathways, to identify seasonal patterns in EEV introduction, and to evaluate the effectiveness of sanitary measures.

2. Material and methods

The stochastic risk model used in this study builds on previous risk models for the introduction of AHSV into The Netherlands (De Vos et al., 2012) and into France (Faverjon et al., 2015). The model was built in @Risk 6.3 (Palisade) and calculations are performed per month because of the seasonal effect on many input parameters. The results are the sum of all imports during a month for which the estimation is based on parameters with time unit in days.

Number of model iterations for each scenario was 10 000.

In this section the outline of the model (Fig. 1) is presented as well as the main calculations and input parameters. Details of the model are presented in the Supplementary information.

2.1. Probability of introduction

To estimate the probability of EEV introduction into The Netherlands, the probability of EEV release and the probability of subsequent establishment were calculated. It was assumed that all regions within The Netherlands have equal probability of EEV introduction and further local spread.

Potential source countries were categorized by epidemic risk and sanitary regime (Table 1). High, low, and very low risk regions for epidemic risk were distinguished according to EEV history and vector presence. High risk regions are regions in which the virus is presumed or known to circulate. Low risk regions are regions without known outbreaks but with a proven presence or likely presence of vector species. A likely presence was based on habitat suitability (Guichard et al., 2014) and proximity to regions in which the vector is currently known to be present. For example we did include countries in the Middle East and Caucasus, but not South-Eastern Asia and the Americas. Very low risk regions are all other countries. Furthermore, we assume a more suitable climate in low risk countries than in very low risk countries with an average temperature of 18° in low risk and 12° in very low risk countries. Given the low temperatures and absence of main vectors, we assume that

Table 1
Categorization of countries by risk regions and sanitary regime. The countries belonging to the different risk regions are given in the Supplementary information. Sanitary regimes are rigorous with quarantine, two sequential ELISA tests and clinical inspection, normal with quarantine and clinical inspection and easy with clinical inspection 48 h prior to transportation.

Risk regions	Description	Sanitary Regime		
		Rigorous	Normal	Easy
High risk (H_{risk})	EEV considered as endemic	Sub Saharan Africa	Israel	
Low risk (L_{risk})	No EEV history, but favourable climate or proven presence of the main vector (*C. imicola* and *C. bolitinos*) in part of the country.		North Africa, Western, Central and Southern Asia	Mediterranean Europe
Very low risk (Vl_{risk})	No EEV history, absence of the main vector or presence very unlikely according to climate			Northern and Eastern Europe, Americas, Oceania, South-Eastern Asia

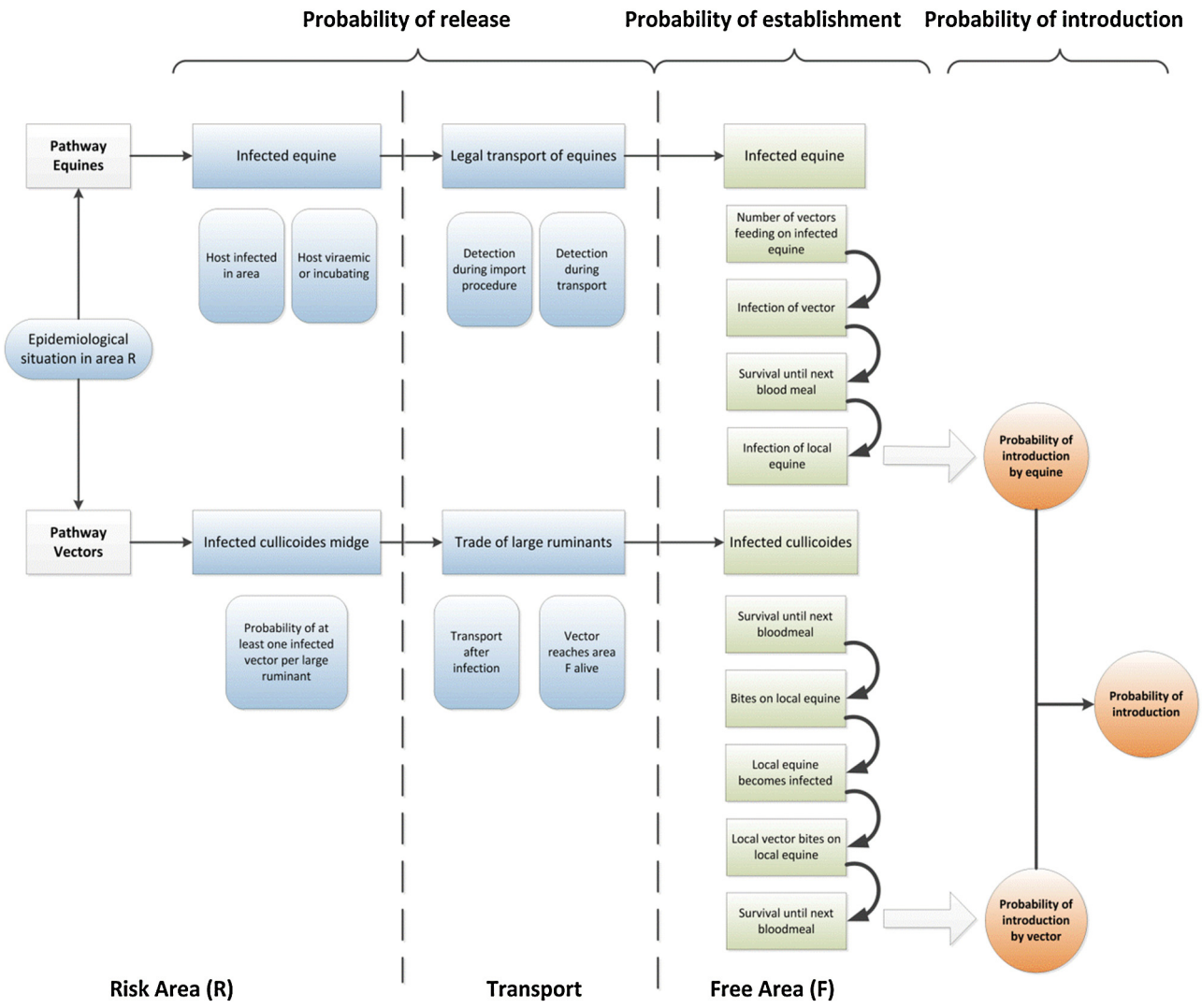

Fig. 1. Risk pathways for the introduction of EEV into The Netherlands (Free Area) describing all the necessary steps during EE transmission.

no introduction will take place through vectors from very low risk regions (Faverjon et al., 2015).

Regions with rigorous, normal, and easy sanitary regimes were distinguished according to EU legislation for importation of live animals (2009/156/EC; 2004/211/EC). The rigorous regime consists of quarantine with vector control, two ELISA tests and clinical inspection at embarkation, the normal regime comprises quarantine with vector control and clinical inspection at embarkation, and the easy regime consists of only clinical inspected 48 h prior to embarkation.

Two possible introduction pathways for EEV were distinguished in the risk model (Fig. 1), i.e., importation of an infected equine and importation of an infected *Culicoides* vector along with its vertebrate host.

EEV introduction into The Netherlands by both pathways was calculated by multiplying the probability of EEV release by the probability of establishment. The probability of EEV introduction via an infected equine $P_{eq\_int}(j, m)$ in month m from region j is calculated as the probability that at least one of the imported horses is infected ($P_{eq\_rel}(j, m)$) and that its importation results in establishment of the infection ($P_{eq\_est}(j, m)$):

$$P_{eq\_int}(j, m) = 1 - \left(1 - P_{eq\_rel}(j, m) \times P_{eq\_est}(j, m)\right)^{Neq_{j,m}} \quad (1)$$

where $Neq_{j,m}$is the number of imported horses in month m and region j.

The probability of EEV introduction via an infected vector $P_{vec\_int}(j, m)$ in month m from region j is calculated as the probability that at least one of the vectors travelling with imported ruminants is infected $P_{vec\_rel}(j, m)$ and that its importation results in establishment of the infection$P_{esteq}(j, m)$:

$$P_{vec\_int}(j, m) = 1 - (1 - P_{vec\_rel}(j, m) \times P_{vec\_est}(j, m))^{V_{m,j}} \quad (2)$$

where $V_{m,j}$ is the number of vectors imported on ruminants (i.e. number of imported ruminants, as we assume one vector per ruminant).

The annual probability of EEV introduction into The Netherlands (P_{EEV}) by either pathway was calculated as:

$$P_{EEV} = 1 - \prod_{j=1}^{5} \prod_{m=1}^{12} \left(\left(1 - P_{eq\_int}(j, m)\right) \times (1 - P_{vec\_int}(j, m))\right) \quad (3)$$

2.2. Probability of release

The probability of release depends on the number of vectors or equines transported along the pathway and the probability of being infected when entering The Netherlands. The number of vectors or equines and the probability of being infected when entering The Netherlands depend on the source region with its specific epidemic situation and sanitary regimes.

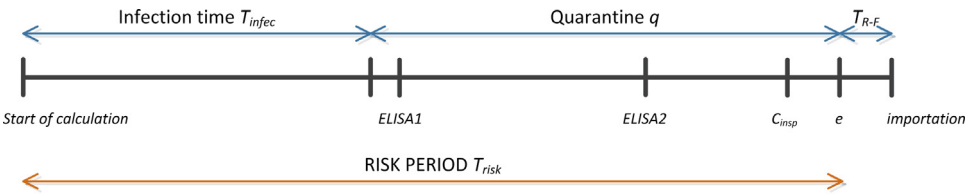

Fig. 2. Timeline of events defining the high risk period for EE detection during importation procedure. $T_{R\text{-}F}$ = time between transport and arrival in free area, ELISA1 and ELISA2 = ELISA test times, C_{insp} = time of clinical inspection, e = moment of inspection.
Source: Adjusted from De Vos et al., 2012

2.2.1. EEV release by importation of an infected equine

The probability of release by one single infected equine ($P_{eq_{rel}}(j, m)$) originating from region j in month m is the product of the probability that the imported equine is infected with EEV at the moment of embarkation ($P_{inf}(j, m)$), the probability that the imported equine is still incubating or viraemic at the moment of importation P_{vir}, and the probability that the infected horse is not detected by sanitary regime ($1 - P_{det}$).

The probability of release is different for equines infected at different periods of the high-risk period (T_{risk}) for the acquisition of the infection due to the length until embarkation and detection and prevention measures. These periods of T_{risk} are the pre-importation period (T_{infec}), the quarantine period (q), and the periods before, between and after clinical inspection (C_{insp}) or two subsequent ELISA tests (ELISA1 and ELISA2) (Fig. 2). Embarkation time e was arbitrarily set at the 12th day of each month (De Vos et al., 2012; Faverjon et al., 2015).

We assume equal probability of EEV infection for each day in each time period of T_{risk} by using uniform distribution for infection time. The overall probability of release is the T_{risk} weighted sum of the probabilities of release for each period of T_{risk}. For example we show the equation for the most elaborate importation process in a rigorous regime with $P\langle errorimage\rangle$'s indicating the probability of release when infected in a certain time period, q being the length of quarantine, $ELISA1$ and $ELISA2$ indicating time since start of quarantine and testing with ELISA, and C_{insp} being the time of clinical inspection and e the moment of embarkation:

$$P_{rel_{eq}} = \frac{P_q}{T_{risk} - q} + \frac{P_{q-ELISA1}}{ELISA1} + \frac{P_{ELISA1-ELISA2}}{ELISA2 - ELISA1} + \frac{P_{ELISA2-C_{insp}}}{C_{insp} - ELISA2} + \frac{P_{C_{insp}-e}}{C_{insp} - e} \quad (4)$$

The probability of release for each period depends on P_{inf}, $(1 - P_{det})$, and P_{vir}. Infection probabilities vary by month because of seasonal variations in EEV incidence in source countries. Preventive measures, such as vector-control, will reduce the probability of being infected at the moment of embarkation. The probability of release for each period x during T_{risk} is than the product of the probability of infection during that month, the probability of still being viraemic and the probability of not being detected.

$$P_{rel_{eq}}(j, m, x) = P_{\inf}(j, m) \times P_{vir}(x) \times (1 - P_{det}(x)) \quad (5)$$

For high risk regions T_{risk} was considered to start 30 days prior to the importation procedures, thus its total length equals 30 plus the length of the quarantine period. This period is assumed because most equines will seroconvert within 30 days (Mildenberg et al., 2009), and it is the same assumption as De Vos et al. (2012) making it comparable to the outcomes for AHS. For very low and low risk regions it is assumed that the second infected horse will be detected and the high risk period ends, because a known infection is occurring in this country. The risk of introduction in that situation is outside the scope of this risk assessment. This period is calculated as two times the incubation period of horses plus the extrinsic incubation period of vector, which is approximately the same length or longer than the assumed high risk period for endemic high risk regions.

The probability of an equine acquiring infection during T_{risk} differs per risk region and depends on the probability of EEV occurrence in each month (PO_m) and the monthly cumulative incidence of the infection (CI_m). For very low and low risk regions PO_m was estimated using outbreak reports (Mildenberg et al., 2009) whereas for high risk countries, which are assumed to be endemic, the PO_m was set to 1. CI_m was estimated using data from endemic areas (Lord et al., 2002; Aharonson-Raz et al.2012). For if quarantine (q) is applied before exportation of the equine, vector control will result in a reduction of the probability of acquiring the infection during the quarantine period (V_q).

Non-viraemic equines were considered to be not infectious. The probability of release is, therefore, determined by the probability of being viraemic, when arriving in the free area (P_{vir}). P_{vir} results from the length of incubation period in days (In), length of viraemic period in days (Vir), and the duration of transportation (T_{RF}),and the moment of infection.

Detection depends on the applied tests. For ELISA testing the probability of being detected by the first or second ELISA-test P_{ELISA1} and P_{ELISA2} were calculated assuming that only seroconverted animals can be detected, hence the time between infection and seroconversion (T_{sero}), the sensitivity (Se) of the ELISA test was taken into account. In the calculation of probability of detection by clinical inspection P_{clin} the length of In, Vir, and the sensitivity of clinical inspection (Se_{clin}), which was assumed to be 1 if the equine showed symptoms with a probability of 0.05, based on mortality rates (Aharonson-Raz et al., 2011). Except for this one observation, little is known about the incidence of disease, but varying the parameter within plausible range did not change the outcomes.

2.2.2. EEV release by infected culicoides spp

The probability of EEV release by pathway PW_{vector} from region j in month m ($P_{relvec}(j, m)$) was calculated taking into account the probability of disease occurrence in region j and month m ($PO_{j,m}$), the proportion of infected vectors in month "m" and region "j" ($Rv_{inf,m,j,}$), the probability that the infected vector is transported from risk regions to a free EEV area (F area)($P_{transRF}$), and finally the survival probability of the vector to survive until reaching the free area (i.e. The Netherlands) $P_{survRF,m}$.

In the default assessment (i.e. not the what-If scenarios) we assume that only infected vectors transported with non-susceptible hosts (i.e. ruminants) contribute to the introduction of EEV. Imports of ruminants are only allowed from FMD free countries, which results in no imports from high risk regions.

Transport on susceptible (equine) hosts might result in infection of the host and we assume that all infections occur prior to embarkation and no infected vectors are shipped with equines. To our knowledge there are no reports of infections or vectors on equines during transport. The effect of this assumption was tested in the what-if scenarios, where we included the possibility of vectors being transported with equines in the pathway of infected vectors.

In the calculation one infected vector per imported animal was assumed as by Faverjon et al. (2015).

2.3. Probability of establishment of EEV

After release of EEV into The Netherlands, the establishment and spread of the disease will only occur if local *Culicoides* spp. are able to transmit the virus to a local susceptible host (De Vos et al., 2012; Faverjon et al., 2015). Therefore, the probability of establishment of EEV is defined as the probability that at least one transmission has taken place from an indigenous vector to an indigenous host or vice versa (Fig. 1). The probability of establishment thus depends on the initial local spread of the disease (reproduction number), which is reported separately. In the model, we assume that local vectors equally efficient in transmitting EEV as vectors from endemic areas.

The reproduction number (R_m) is calculated as the number of newly infected hosts by one infected host for the conditions of month *m*.

$$R_m = \frac{P_{infvh} \times P_{infhv} \times P_{survm}}{R_{re} + 1} \times \mathrm{Nv_m} \quad (6)$$

Where P_{infhv} is the probability that a local vector gets infected after feeding on an infectious equine and$P_{infvh}$is the probability that an equine is infected after having been bitten by an infected vector, $P_{survm+1}$ is the probability that this vector survives until the first next blood meal after completing the extrinsic incubation period (*EIP*), which further depends on the length of the gonotrophic cycle and the mortality rate, which are all temperature dependent (Gubbins et al., 2008). The ratio $\frac{1}{R_{re}+1}$ is the probability that the infectious vector bites a susceptible host instead of a non-susceptible host, based on the ruminant-equine ratio (R_{re}), where an equal preference of midges for ruminants and equines is assumed, and $\mathrm{Nv_m}$ is the number of vectors in The Netherlands feeding on one equine during its entire viraemic period (Meiswinkel et al., 2004; Wilson and Mellor, 2009; De Vos et al., 2012).

2.3.1. Establishment of EEV when released by an infected equine

The probability of establishment for PW_{equine} per risk region *j* and month *m* ($P_{esteq}(j, m)$) is defined as the probability that at least one indigenous equine is infected by the imported infected equine via an indigenous vector. The probability of establishment of EEV after its release into The Netherlands via importation of an infected equine was calculated assuming a binomial process (De Vos et al., 2012):

$$P_{esteq}(j, m) = 1 - (1 - \frac{P_{infvh} \times P_{infhv} \times P_{survm+1}}{R_{re} + 1})^{\mathrm{Nv_m}} \quad (7)$$

$\mathrm{Nv_m}$ is the number of vectors in The Netherlands feeding on one equine during its entire viraemic period and is calculated as (De Vos et al., 2012):

$$\mathrm{Nv_m} = \mathrm{Nve_{F,m}} \times \mathrm{Vir} \times \frac{1}{\mathrm{G_{tmF}}} \quad (8)$$

Where, $Nve_{F,m}$ is the daily number of vectors per equine in each month, *Vir* is the length of viraemic period, and G_{tmF} is the length of the gonotrophic cycle of the vector dependent on the temperature month *m*.

2.3.2. Establishment when released by an infected vector

The probability of establishment for the vector pathway per risk region *j* in a month *m* ($P_{estvec}(j, m)$) is defined as the probability that at least one local vector is infected by feeding on a local equine that in its turn acquired its infection from the imported infected vector.

The probability of establishment of EEV after its release into The Netherlands via importation of an infected vector was calculated assuming a binomial process (Faverjon et al., 2015):

$$P_{estvec}(j, m) = P_{svF,m} \times \left(1 - \left(1 - \frac{P_{infvh} \times P_{in\,fhv} \times P_{surv_{m+1}}}{R_{re} + 1}\right)^{\mathrm{Nv_m}}\right) \quad (9)$$

Where,$P_{svF,m}$ is the probability that the imported infected vector survives in The Netherlands until its next blood meal and completes the EIP given the temperature in month *m*. Implicitly, we assume that an infected imported vector will at most infect one local equine (De Vos et al., 2012; Faverjon et al., 2015). Establishment of EEV after importation of an infected vector is, thus, more difficult, because it requires the imported vector to survive until the first blood meal after completing the EIP.

3. Input

3.1. Importations of equines and ruminants

The importation data were derived by combining several publically available sources, because none of these sources alone could be used for these data.

The *total* annual number of equines (horses, donkey, mules and hinnies) and ruminants (cattle, goats and sheep) imported from each risk region was obtained from Statistics Netherlands over the period 2008–2012 (CBS, 2014). No information was available on monthly importations. From the statistical office of the European Union (EUROSTAT) we obtained monthly import volumes per kilogram (and thus not number of animals). The monthly *number* of imports was therefore estimated by combining the data of total number of importation per country per year (CBS, 2014) and volume of imports per month in the period 1999–2008 (Eurostat, 2014).

Next to regular importations of equines we considered two groups of equines participating in equestrian events: horses owned and housed in The Netherlands that participate in equestrian events outside The Netherlands and horses owned and housed outside The Netherlands that participate in equestrian events in The Netherlands. These data are not available from CBS and Eurostat databases and were therefore derived from the Royal Dutch Equestrian Federation (KNHS, 2010).

In the model calculations, the number of imported animals from risk region *j* in month m ($N_{j,m}$) was simulated assuming a Poisson process, with a conservative estimate of lambda (Vose, 1997; De Vos et al., 2012):

$$N_{j,m} = \frac{\sum_{y=1}^{n} N_{j,m,y} + 1}{n} \quad (10)$$

where $N_{j,m,y}$is the number of imported animal from risk region *j* in month *m* in year *y*, and *n* is the number of years with importation data available.

When few animals were imported from risk region *j*, the monthly estimation was calculated as 1/12 x average annual number of imported animals to avoid an overestimation of the total number of animals imported (De Vos et al., 2012).

An overview of average annual equine and ruminant importations into The Netherlands is given in Table 2. Details on monthly importation data are given in Supplementary Table 1.

3.2. Vector population

The monthly number of *Culicoides* per host ($Nve_{F,m}$) was estimated using seasonal data of *Culicoides* abundance in The Netherlands based on trap collections from 2007 (Meiswinkel et al., 2014) (Supplementary Fig. 2). In the model calculations, the number of vectors per host was simulated using a truncated normal distribution with average monthly values of Culicoides collected per trap and their standard deviations as μ and σ, and the minimum and maximum values observed as lower and upper bounds of the distribution.

Table 2
Estimated annual number of equines and ruminant importation from risk regions into The Netherlands. Sources: CBS, 2014; Eurostat, 2014; De Vos et al., 2012.

Imported animals	High risk region	Low risk regions	Very low risk regions	Total
Number of imported equines from regions with rigorous sanitary regime	5.4	–	–	5.4
Number of imported equines from regions with normal sanitary regime	0.4	150.6	–	150.9
Number of imported equines from regions with easy sanitary regime	–	518.5	6709.0	7227.5
Number of "foreign" horses participating in equestrian events in The Netherlands	23.9	1742.0	4414.6	6180.4
Number of "Dutch" horses participating in equestrian events outside The Netherlands	2.0	1199.6	4739.6	5941.2
Number of ruminants	–	42806.8	20122.5	62929.3
Total	31.6	46417.5	15863.2	82434.8

3.3. Biological parameters

Host parameters included in the model were the mortality rate of infected equines (*MR*), the length of the incubating period (*In*), the length of the viraemic period (*Vir*) and the time period in days between infection and seroconversion (T_{sero}). *MR* was fixed to a value of 0.05 for all equines (DEFRA, 2009; Aharonson-Raz et al., 2011). *In*was modelled as a Pert (2,4,6) distribution based on AHSV (DEFRA, 2009; De Vos et al., 2012). *Vir* was modelled as a Pert (7,19,30) distribution based on length of illness reported in horses during the EEV outbreak in Israel (Mildenberg et al., 2009). No information on T_{sero} was available and it was therefore modelled as a Uniform (*In*, *Vir*) distribution which results in an on average seroconversion 11.5 days after infection.

Vector parameters included in the model were the mortality rate of the vector (μ_m), the length of the gonotrophic cycle (G_{tmF}), the length of the extrinsic incubation period (EIP_m), the number of *Culicoides* per host ($Nve_{F,m}$), and the fraction of the vector population infected during an outbreak ($Rv_{inf,m}$). All parameters are temperature dependent and vary per season and were therefore calculated on a monthly basis. Vector parameters $Nve_{F,m}$, μ_m, $G_{tmF}$and EIP_m are temperature-dependent and vary over seasons. Monthly average temperature values from De Bilt, The Netherlands, from 1979 to 2013 were used to simulate monthly temperatures in the model (T_m) (KNMI, 2014). Temperature values were simulated as a truncated normal distribution with average monthly values and their standard deviations as μ and σ, and the 1st and 99th percentile of the observed values as lower and upper bounds of the distribution (Supplementary Fig. 1).

Calculations of the mortality rate of the vector (μ_m) assumed an exponential increase with temperature (Wittmann et al., 2002; Backer and Nodelijk, 2011). The length of the gonotrophic cycle (G_{tmF}) and the length of the extrinsic incubation period (EIP_m) decrease with higher temperatures (Wittmann et al., 2002).

At temperatures below 9.6 °C vector activity was assumed to arrest, and establishment and local initial spread of EEV does not occur (De Vos et al., 2012).

The ratio of ruminants to equines in The Netherlands (R_{re}) was calculated based on reports of the number of ruminants in The Netherlands, which is 12 times the number of horses (5.5×10^6 ruminants against 4.5×10^5 horses) (Mourits and Saatkamp, 2010 from De Vos et al., 2012). The R_{RE} was simulated by a Uniform (0, 12) distribution (De Vos et al., 2012), the lower bound representing the situation in which equines and ruminants do not mix, and the upper bound the situation that all ruminants and equines in The Netherlands are perfectly mixed.

The probability that an equine is infected when bitten by an infected vector (P_{infvh}) was modelled by a Beta (6, 2) distribution with a mean value of 0.75 based on an experiment with BTV (Baylis et al., 2008). The probability that a competent vector after feeding on an infectious equine gets infected (P_{infhv}) was modelled by a Beta (1.05, 39.6) distribution with a mean value of 0.04 based on experiments with *Culicoides* and AHSV (Paweska et al., 2003; Venter and Paweska, 2007).

3.4. Risk regions

The probability of disease occurrence ($PO_{highrisk}$) varies according to risk regions. High risk regions are endemic region, thus $PO_{highrisk}$ was set to 1. In low and very low risk regions $PO_{highrisk}$ was estimated based on EEV epidemic events reported.

One outbreak in a new area (i.e. Israel) was observed in 47 years since the first outbreak. EEV was likely to be circulating for seven years at the moment of detection (Wescott et al., 2013), therefore the probability of an undetected outbreak during the risk period, $PO_{lowrisk,m}$ for low risk regions was modelled as a Gamma [T_{risk} *7, $1/(47 \times 365)$] distribution where T_{risk} is the length of the risk period in days. For very low risk regions we assume that the risk based on at least 47 free year and thus with Gamma [T_{risk}, $1/(48 \times 365)$]. In the low and very low risk regions, we assume that outbreaks are not possible between January and July based on AHS in Portugal and Spain (Rodriguez et al., 1992; Mellor, 1993).

The monthly cumulative incidence (CI_m) was based on force of infection calculated for different regions of South Africa based on the age distribution of seroprevalence (Lord et al., 2002). The monthly cumulative incidence (CI_m) was derived by dividing the yearly cumulative incidence by 12. For high risk regions, the yearly cumulative incidence rate chosen was 35% which is approximately the average between the rate in a high incidence area in South Africa (Lord et al., 2002) and between summer 2010 and spring 2011 in Israel (Aharonson-Raz et al., 2012). For low and very low risk regions, we base our estimate as De Vos et al. (2012) on the outbreak of AHS in Portugal and Spain, but take into account that the force-of-infection of EEV is 2.5–3.0 times higher (Lord et al., 2002). The average yearly cumulative incidence rate for low and very low risk countries was thus $5{\cdot}10^{-4} - 6{\cdot}10^{-4}$.

The fraction of the vector population which is infected ($Rv_{inf,m}$) during an outbreak in a month *m* was simulated as a Uniform (0.014, 0.015) distribution, which corresponds to 0.5% AHSV prevalence in wild-caught vectors (Venter et al., 1999) times 2.5–3.0, which is the difference in force-of-infection between AHS and EEV in South Africa (Lord et al., 2002)

3.5. Sanitary regimes and transportation time

During quarantine in high risk regions, vector control is applied according to EU legislation 2009/156/EC and 90/425/EC. Vector control (V_q) was assumed to give a 50–90% reduction of the probability of infection during quarantine (De Vos et al., 2012; Faverjon et al., 2015), and hence V_q was modelled with a Uniform (0.5, 0.9) distribution.

The ELISA for EEV has 99% sensitivity (*Se*) was modelled with a Uniform (0.98,1.00) distribution and no cross-reaction and with other *Orbiviruses* such as AHSV and BTV (Crafford et al., 2003; Crafford et al., 2011). The sensitivity of clinical inspection depends on presentation of clinical signs. Since EEV infection in most equines does not result in clinical disease, the sensitivity of clinical inspection (Se_{clin}) was set at 0.05, which is equal to the disease induced mortality rate (*MR*).

Table 3
Relative contribution of imported equine per risk region to the risk of median annual probability of EEV release and introduction into The Netherlands by the equine pathway.

Equines	imports/year	%	Release probability	%	Introduction probability	%
High risk regions						
South Africa (rigorous regime)	5.37	17%	4.5E-03	10%	4.8E-04	9%
Israel (normal regime)	0.36	1%	2.6E-02	58%	1.8E-04	3%
Equestrian events inside The Netherlands	23.86	76%	7.3E-03	16%	4.6E-03	83%
Equestrian events outside The Netherlands	2.00	6%	6.8E-03	15%	2.7E-04	5%
Total (% of all regions)	*31.59*	*0%*	*4.4E-02*	*99.8%*	*5.5E-03*	*74%*
Low risk regions						
Africa (rigorous regime)	150.57	4%	6.9E-06	11%	2.0E-05	1%
EU-countries (easy regime)	518.54	14%	4.5E-05	70%	6.8E-04	51%
Equestrian events inside The Netherlands	1,741.96	48%	6.3E-06	10%	3.5E-04	26%
Equestrian events outside The Netherlands	1,199.62	33%	6.4E-06	10%	2.8E-04	21%
Total (% of all regions)	*3,610.69*	*19%*	*6.5E-05*	*0.1%*	*1.3E-03*	*18%*
Very low regions						
Non-EU countries (normal regime)	963.52	6%	1.0E-06	25%	2.2E-05	3%
EU-countries (easy regime)	5,745.46	36%	1.0E-06	25%	2.5E-04	40%
Equestrian events inside The Netherlands	4,414.56	28%	1.1E-06	26%	1.7E-04	27%
Equestrian events outside The Netherlands	4,739.61	30%	1.1E-06	25%	1.9E-04	29%
Total (% of all regions)	*15,863.15*	*81%*	*4.2E-06*	*0.0%*	*6.3E-04*	*8%*
Overall probability			2.7E-02		1.2E-02	

Transportation time of equines imported from high risk and low risk regions outside Europe was set to 1 day assuming transportation by plane (T_{RF}). Transportation time of equines imported from very low risk regions outside Europe, was modelled with a Uniform (1,2) distribution, assuming that transportation by plane will take 1–2 days. Transportation time of equines imported from EU-countries was modelled with a Uniform (1,3) distribution, assuming that transportation could either be by plane or over land depending on geographical distance (Faverjon et al., 2015).

4. Sensitivity analysis

Sensitivity analysis was performed to evaluate the impact of uncertain and variable parameters on the annual median probability of EEV introduction (P_{EEV}) using the sensitivity analysis tool in @Risk. The Spearman rank correlation test was used, which has the advantage that it is a non-parametric test. Correlations less than 0.05 were disregarded in the plots.

4.1. What-if scenarios

Seven what-if scenarios were used to evaluate the impact of sanitary measures and changes in numbers of equines imported on the annual probability of EEV introduction into The Netherlands. In each scenario one parameter was changed and all others kept constant. Output values of P_{rel} and P_{intro} of the what-if scenarios were compared to those of the default scenario.

Four scenarios were related to sanitary measures applied during importation procedures: a longer and shorter quarantine period, no use of ELISA testing, and regime quarantine for all equines originating from low risk and very low risk regions. Two scenarios were related to the number of equines imported: a two-fold increase of equines imported and no cross-boundary movements of horses participating in equestrian events. In addition, one scenario simulated the effect of importing infected vectors along with both ruminants and equines.

5. Results

5.1. Probability of release

The median annual probability of release (P_{eq_rel}) by infected equines was 0.027 (90% uncertainty interval 0.011–0.046). EEV release by infected equines imported into The Netherlands is thus expected approximately every 37 years. Monthly median probability of release ranges from 0.0005 in February to 0.0033 in November (Fig. 3A).

EEV release by infected vectors introduced into The Netherlands is very unlikely with a median annual probability of release (P_{vec_rel}) by infected vectors of 0.0009 (0.0003-0.0020). EEV release by infected vectors introduced into The Netherlands is very unlikely. Monthly median probability of release ranges from 3.5 10^{-6} before June to 1.5 10^{-4} after June (Fig. 3B).

For both pathways, the low estimate of the probability of release for the first half of the year results from the assumption that outbreaks in very low and low risk areas do not occur before July due to low temperatures. Further fluctuations are due to monthly variations in the importations of equines and ruminants (Supplementary Table 1), which are far more pronounced for equines than for ruminants in The Netherlands.

Equine importations from different risk regions and sanitary regimes differ in their probability of EEV release. Equines originating from high risk regions posed the highest risk to The Netherlands, although this is a minority of the imported equines. Especially the horses from Israel pose a risk for release into the Netherlands. Equines entering The Netherlands or staying outside The Netherlands for equestrian events determine a substantial proportion (10%–26%) of the probability of EEV release depending on the risk region (Table 3).

Very few importations of large animals from high risk regions were registered, resulting in a negligible risk of this region for the import of vectors.

5.2. Probability of establishment

The median monthly probability of EEV establishment into The Netherlands given a release of an infected equine ($P_{eq\_est}(j,m)$) peaks at 0.95 in August. Between November and April establishment is not expected to occur, because temperatures are too low. The monthly median probability of EEV establishment by PW_{vector} ($P_{vec\_est}(j,m)$) also peaked during the summer with 0.17 in July. The large difference in the probability of establishment between both pathways is explained by the low probability of survival of the imported vector during transport to and the establishment phase The Netherlands.

The probability of establishment for an infected vector $P_{vec\_est}(j,m)$ varies between high and low risk regions due to the length transportation time (e.g., in July, the median value of

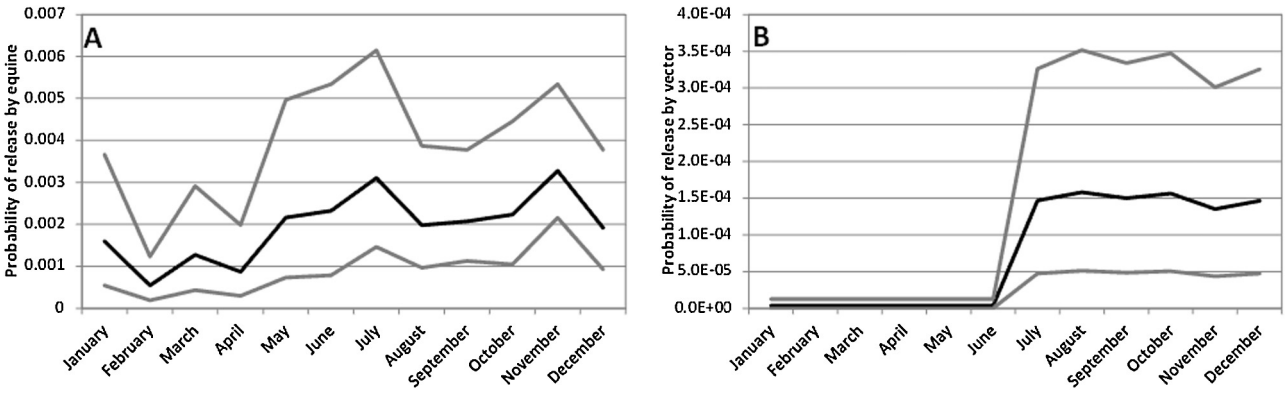

Fig. 3. Monthly probability of release of EEV. Black line indicates the median and the gray lines depict the 5% and 95% quantile of the simulations. A: Probability of release by infected equines. B: Probability of release by infected vectors.

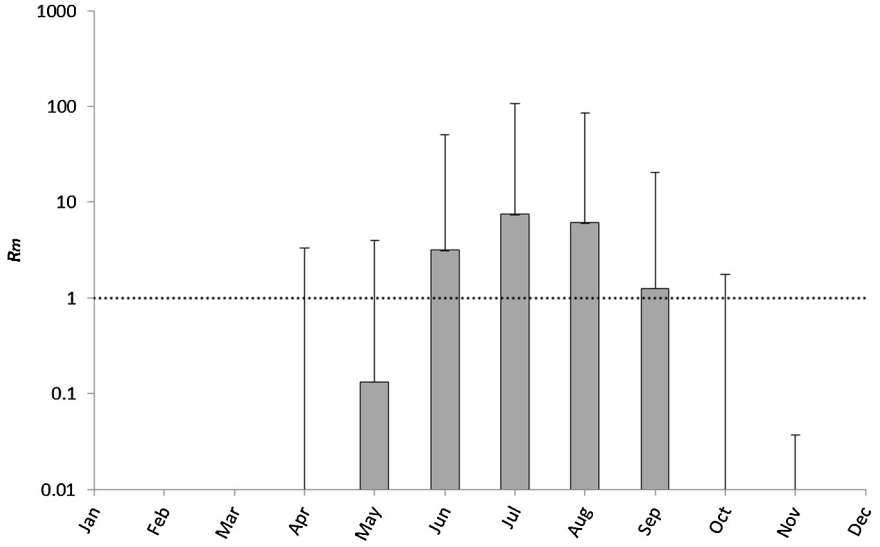

Fig. 4. Seasonality of R values in the F area on a log-scale, which is at risk of introduction (i.e. The Netherlands). Lower limit of the error bar is invisible, because it almost coincides with expected value in June – July, or in the rest of the year the lower limit is 0.

$P_{vec\_est}(j, m)$ equals 0.10 for high risk regions and 0.08 for low risk regions).

Establishment is possible if the reproduction number is higher than one. Only in the period June-September, prolonged spread is possible, because the threshold value of 1 is exceeded in the period from June (R_m = 3.1) to September (R_m = 6.2), with a maximum value in July (R_m = 7.6). The magnitude indicates the potential local spread after establishment. Large uncertainty for reproduction numbers was observed (Fig. 4).

5.3. Probability of EEV introduction into The Netherlands

The median annual probability of EEV introduction into The Netherlands by either pathway (P_{EEV}) is 0.012 (0.002–0.020). The monthly median probability of introduction peaks in July. Introduction is very unlikely between October and May with a median probability less than 0.001.

The probability of introduction via the pathway of infected equines (P_{eq_int}) contributes most to the P_{EEV} with a median value of 0.012 (0.002–0.020), indicating an entry of EEV resulting in spread every 83 years (50–500). P_{eq_int} has a distinct seasonal pattern and peaks with 0.003 (0.001–0.005) in July (Fig. 5A).

The probability of introduction via infected vectors (P_{vec_int}) is much lower than P_{eq_int} with a median value of 4.0 10^{-5} (5.3 10^{-6}–2.0 10^{-4}), indicating a very unlikely introduction by vectors. P_{vec_int} also has a seasonal pattern and peaks with 1.2 10^{-5} (1.4 10^{-6}–6.5 10^{-5}) in August (Fig. 5B). Equestrian events with horses from high risk areas contribute most to P_{EEV} with 61% of P_{eq} being explained by these equines. Almost 9% is due to equine importations from EU member states by definition having an easy sanitary regime (Table 3). If we assume substantially higher cumulative incidences given an outbreak in low and very low countries, the role of low risk EU countries becomes almost half of the risk (see Supplementary information). Second, very low risk countries were responsible for a marginal part of P_{eq}, where again equine importations from EU member states with an easy sanitary regime contributed most.

5.4. Sensitivity analysis

Sensitivity analysis showed that the probability of EEV introduction by infected equines (P_{eq_int}) is very sensitive to the effectiveness of vector control (V_q), followed by the time until seroconversion (T_{sero}) Both parameters are related to control of the export of infected equines and thus to the probability of release. The probability of infection from host to vector ($P_{inf_{hv}}$), followed by the length of the viraemic (Vir_h) and the incubation period (In_h) and the yearly cumulative incidence (IC_y) are important parameters.

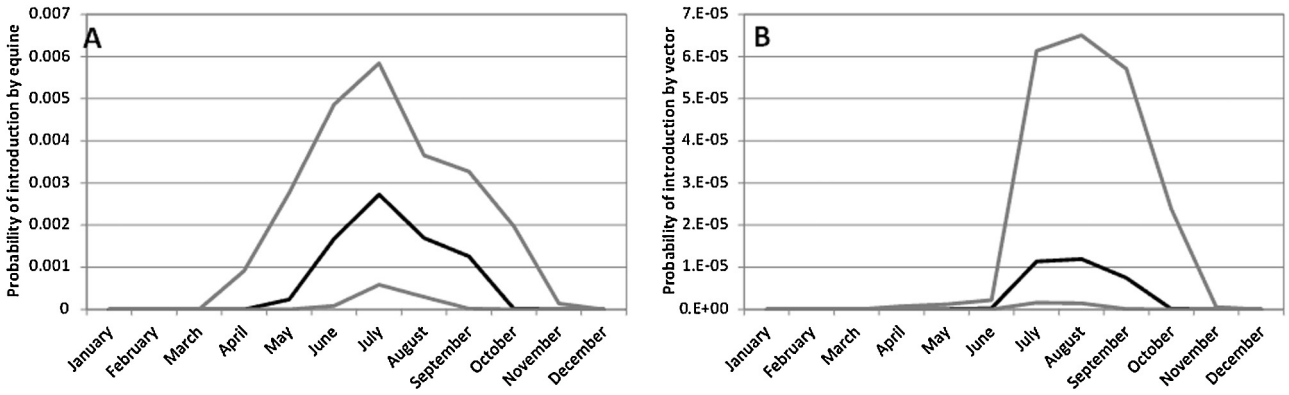

Fig. 5. Monthly probability of introduction of EEV. Black line indicates the median and the gray lines depict the 5% and 95% quantile of the simulations. A: Probability of introduction by infected equines. B: Probability of introduction by infected vectors.

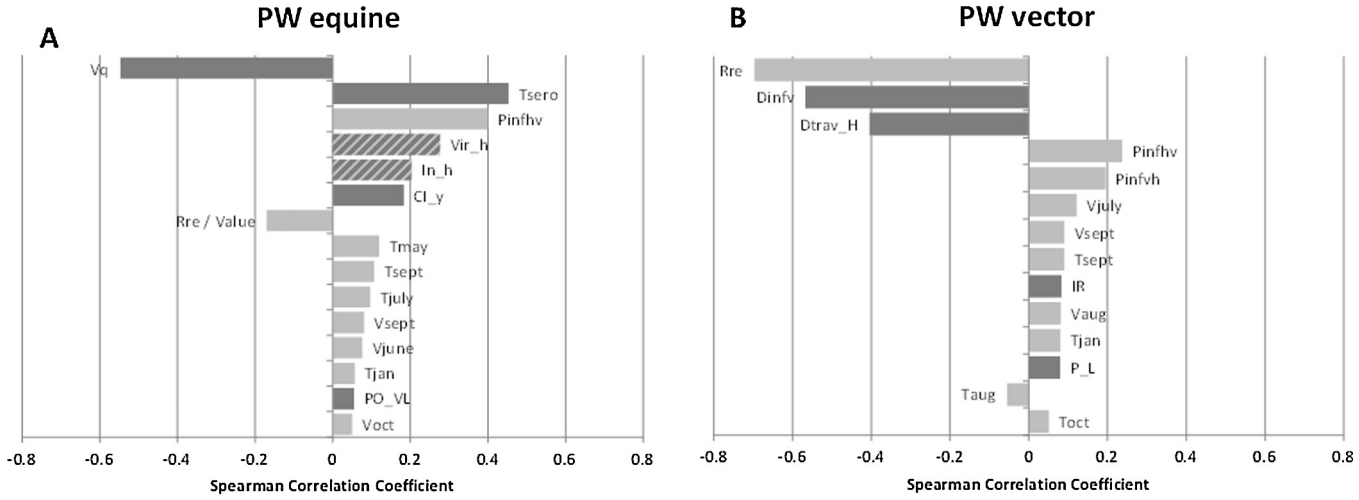

Fig. 6. Sensitivity of parameters determined by Spearmann rank correlation test. Dark grey bars depict parameters which only affect release and light grey those parameters affecting release. The shaded bars depict those parameters affecting both release and establishment probabilities. A: Pathway of introduction by infected equines. B: Pathway of introduction by infected vectors.

Other parameters for which P_{eq_int} is sensitive are mainly related to the establishment phase, such as transmission probabilities and the temperature (Fig. 6A).

Sensitivity analysis showed that the probability of EEV introduction by infected vectors (P_{vec_int}) is mostly sensitive to the ruminant-host ratio (R_{RE}), which is an important parameter in the establishment of the infection. On the other hand, the outcomes are sensitive to parameters that determine the survival of the vector until release, i.e. day of infection before embarkation (D_{infv}) and length of the transport for high risk areas (D_{trav_H}) Also the transmission probabilities and temperatures determine the establishment, and the outcomes were sensitive to these parameters (Fig. 6B).

5.5. What-if scenarios

The number of equines entering The Netherlands by importation or through equestrian events, and the length of the quarantine period have a strong effect on the median P_{eq_int} (Table 4). Abandoning ELISA testing as control measure increased the median P_{eq_int} and will highly increase the probability of introduction, while applying quarantine before importing equines from all low and very low risk regions can mitigate P_{eq_int} only marginal. Doubling the number of imported equines only raises P_{eq_int} with 18.8% (Table 4).

Assuming that EEV-infected vectors would not only be imported along with ruminants but also with equines increased the median P_{vec_int} substantially from 0.0009 to 0.0013, but this is still much lower than P_{eq_int} (Table 4).

6. Discussion

This paper is the first to estimate the probability of introduction of equine encephalosis virus (EEV) into The Netherlands. Introduction of EEV into The Netherlands might occur every 83 (50–500.0) years. The pathway of importing infected equines contributed much more to this probability than the pathway of importing infected vectors. The annual probability of introduction by infected vectors imported on ruminants is very unlikely (0.0009 per year (0.0003–0.0020)).

The introduction probability of EEV (P_{EEV}) is substantially higher than previous estimates for AHSV (less than once in 1000 years) for The Netherlands or France (De Vos et al., 2012; Faverjon et al., 2015). The much higher introduction probability of EEV than AHSV is explained by the mostly subclinical course of infection in equines and low case fatality rate (DEFRA, 2009) which is very different from AHS with disease in almost all infected horses with a case fatality rate varying from 70 to 95% (Mellor and Hamblin, 2004). Hence, EEV is less likely to be detected during quarantine or transport, and more equines will survive transport and arrival in The Netherlands.

Table 4
Median values for the annual probability of EEV release (P_{rel}) and EEV introduction (P_{eq_int} or P_{vec_int}) and for seven what-if scenarios and their relative change (%) compared with the default scenario.

What-if scenarios	P_{rel}	Relative Change (%)	P_{intro}	Relative Change (%)
Pathway Equines				
Default	0.0267		0.0118	
Excluding horses participating in equestrian events	0.0062	−76.8%	0.0022	−81.0%
20 day quarantine	0.0540	+102.3%	0.0244	+107.2%
60 day quarantine	0.0182	−31.6%	0.0009	−92.1%
No ELISA testing	0.1103	+313.6%	0.0499	+324.2%
Quarantine for all equines imported from low and very low risk regions	0.0250	−6.3%	0.0113	−4.1%
Two-fold increase in equine importations	0.0327	+22.5%	0.0140	+18.8%
Pathway Vectors				
Default	0.0009		$4.0\ 10^{-6}$	
Infected vectors travelling on equines and ruminants	0.0013	+40.1%	0.0001	+42.4%

+Denotes increase of probability, −denotes decrease of probability.

Furthermore, prevalence of infection in hosts and vectors is much higher for EEV than for AHSV in high risk areas (Howell et al., 2002; Howell et al., 2008). This results in a larger number of infectious hosts and vectors being transported to The Netherlands than for AHSV.

The impact of an outbreak of EEV in The Netherlands is, however, expected to be less than for AHSV because of milder disease compared to AHS or even absence of clinical disease and a much lower case fatality rate (Mellor and Hamblin, 2004). Control measures for EEV are also likely to be less rigorous than for AHSV although an outbreak of EEV might also result in trade bans. The consequences of an EEV outbreak might thus be much smaller even given the high probability of such an outbreak. A formal impact assessment is, however, required to substantiate this statement.

High risk regions contribute most to P_{EEV}, due to horses attending equestrian events in the Netherlands. If however, we would assume higher cumulative incidence (0.075 and 0.055 instead of 0.0055) in an outbreak in low or very low risk countries, imports from these countries become more important contributions to the risk (see Supplementary material). Countries neighbouring Israel, especially Lebanon and Jordan, were now categorized as low risk countries, but might be closer in risk to Israel although no EEV infection are reported. Therefore, it is of importance that also low and very low risk countries have good early warning system, such that after incursion The Netherlands and other countries can take measures. Currently, the almost absent measures in low risk regions will not mitigate the introduction to the Netherlands, and should be reconsidered.

High risk countries (Africa and Israel) substantially contribute to the estimated P_{EEV}, even with the small numbers of equines and rigorous sanitary regimes of the restrictions for imports from these countries. The major "leak" are the animals that attend to equestrian events, but also import equines do form 12% of the probability of introduction from high risk regions. This might be overestimated, because the cumulative incidence of South Africa was based on high endemic areas, while only horses from the Western Cape Export Region are allowed in which the cumulative incidence is thought to be lower. The cumulative incidence of EEV infections was lower than other regions of South Africa in the 1980′s, but in a smaller sample size in the 1990′s all 18 samples were positive in the Western Cape indicating a high cumulative incidence (Lord et al., 2002). Altering these sanitary regimes by removing ELISA testing or shortening the quarantine period showed to have substantial effect on the probability of introduction.

In our model, we assumed that all high risk countries have a year round cycling of EEV. The contribution from high risk countries might be overestimated due to the asynchrony in vector-season between the southern and northern hemisphere.

Low risk countries were assigned to this risk category (Table 1) based on expected presence of competent vectors. Therefore, whole countries, such as France and Italy, were included. This results in an overestimation of the probability of introduction from these countries, which is already not very substantial. Furthermore, we included Mediterranean countries and Asian countries, that do not have a proven presence of known EEV-vectors. These countries were expected based on climate to have either non-detected *C. imicula* or C. *bolitinos* populations, or be favourable because of their climate (Guichard et al., 2014), and close to the currently known presence of the vector. The effect of this assumption is deemed to be very small, because equine import from these countries is very little.

The registered number of equines imports may differ from the real number, for instance illegal equine movements is always possible (Chevalier et al., 2010; De Vos et al., 2012). The increase of 2-fold of equine importation from risk areas results in an increment of 18.8% the probability of EEV introduction. Thus, an accurate registration of equine movements of equines within the EU and from outside the EU other than import would improve the risk assessment and management of equine exotic diseases such as EE.

One of the routes on which the data are scarce and control limited, are horses participating in equestrian events. The movements contribute highly to the risk of EEV incursion, given that the probability of release is mainly due to horses participating in equestrian events. The high risk posed by horses attending equestrian events is in accordance with the work on AHS by De Vos et al., (2012). Horses participating in equestrian events are hence a critical point for controlling the probability of EEV introduction into The Netherlands, and thus preventive measure can be improved and applied on those in order to decrease the probability of EEV (and AHSV) introduction. Movements of horses participating in equestrian events are not registered, and therefore the numbers were based on estimates from data of equestrian events, which is likely to be an underestimation of movements.

Introduction of EEV by vectors has a much lower probability than by infected equines. Basing these estimates on one vector per imported animals might either be an overestimation when on average less than one vector per host reaches the free area or as an underestimation, when vector leave the host during the transport (Faverjon et al., 2015). If EEV introduction by infected vector is possible by being transported together with an equine host, our result showed an increase of 42.4% in EEV introduction and an increment of 40.1% on EEV release. Still the median probability of introduction remains much smaller than that of the pathway of infected equines, though some uncertainty is present.

Even when considering both introduction by equines and by vectors, we might, however, still miss other routes and without suggesting illegal practices, the route of introduction for BTV and

Schmallenberg virus were never unravelled (Mintiens et al., 2008; Veldhuis et al., 2013). Both *Culicoides*-borne viruses were able to cause an outbreak and establish in a large area. This risk assessment does not take into account the possible introduction of the disease by wind-blown or flying midges from neighbouring countries, which might play a role after establishment in these countries.

Establishment of EEV requires the presence of one or more vector-species. We assumed all *Culicoides* species in The Netherlands to be competent vectors for EEV (as for AHSV in De Vos et al., 2012; Faverjon et al., 2015). This assumption may lead to an overestimation of EEV establishment and spread in The Netherlands. The BTV and AHSV outbreaks in Europe have, however, shown that *Culicoides* species, previously unknown to be a vector, are likely to act as vectors for these *Orbiviruses* (Meiswinkel et al., 2008; Mellor et al., 1990; Takken et al., 2008). Vector competence might, however, differ between *Culicoides* species (Venter et al., 1999) and within EEV serotypes (Paweska and Venter, 2004; De Vos et al., 2012).

Parameters in this study have degree of uncertainty or natural variation and vector parameters are strongly temperature-dependent which will determine the spread of the disease. This might change dramatically as a result of climate change, which has been described as a potential cause of global emerging of bluetongue (MacLachlan and Guthrie, 2010). Again this study showed that vector abundance plays an important role during establishment of a vector-borne disease and that uncertainty in this information determines the outcomes (Fischer et al., 2013; Faverjon et al., 2015; Hartemink et al., 2009). The use of trap data to estimate the host-vector might not be as accurate as using landing-catches (Elbers and Meiswinkel, 2015), but are, however, often the only source of information. Therefore, we used a wide-uncertainty distribution around these parameter values.

Recent study in the Netherlands shows that the attack rate of different *Culicoides*-species differ between a cow, shetland pony and an ewe. The cow was equally or more attractive in this study for the *Culicoides*-species, which would reduce the probability of establishment (Elbers and Meiswinkel, 2015). Additionally, a lack of information regarding the number of equines in The Netherlands and their location made an accurate estimation of the ratio between the equine host, and ruminants impossible. The presence of equines (highly competent host) and ruminants (incompetent host) at the same location would decrease the probability of disease transmission due to dilution effect (Schmidt and Ostfeld, 2001; De Vos et al., 2012; Elbers and Meiswinkel, 2015).

The absolute outcomes of introduction risk assessments cannot be considered as true, because of large uncertainties in parameter values. The values are also not fixed, because parameters are not static and might change due to environmental conditions (e.g. temperature, husbandry practices), number of animal importations into The Netherlands and the presence of competent vectors (De Vos et al., 2012). Nevertheless, the outcome of these kind of risk assessments allow for comparison between regions, periods and different diseases. Furthermore, the assessment pinpoints the parameters with high impact and allows to form hypotheses on changes in practices, such as control strategies.

7. Conclusions

The probability of introducing EEV into The Netherlands is substantial and much higher than the probability of introducing AHSV with importations of equines from low risk countries in the EU contributing most. The import of infected equines is the most likely pathway.

Generally speaking, high risk countries contribute most to the probability of introduction. Control measures before exportation of equines showed to have a strong mitigating effect on the probability of EEV introduction. The risk of EEV outbreaks should be taken into account when altering these import regulations.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.prevetmed.2016.07.005.

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