UNIVERSITY OF SOUTHAMPTON

ABSTRACT

FACULTY OF SOCIAL AND HUMAN SCIENCES

Geography and Environment

Doctor of Philosophy

UNDERSTANDING THE RISKS AND FACTORS ASSOCIATED WITH THE INTRODUCTION OF CRIMEAN-CONGO HAEMORRHAGIC FEVER VIRUS INTO GREAT BRITAIN

By Marion Elizabeth England

The aim of the thesis was to assess the present (2010) and future (2080) risk of introduction of Crimean-Congo haemorrhagic fever virus (CCHFV) into Great Britain (GB). Migratory birds and horses were investigated as the most likely routes of introduction. Specifically, the numbers of CCHFV-infected unfed adult ticks in GB that could potentially bite and infect a human from these two pathways were predicted and compared.

Field work was carried out in Spain to estimate the prevalence of *Hyalomma* spp. ticks on migratory birds along the East Atlantic flyway, and also on horses. Data on the numbers of horses and birds entering GB from CCHFV-endemic countries were also collected. A spatial analysis of GB under both current and future climatic and land cover conditions predicted the areas of GB where imported *Hyalomma* spp. ticks could survive to the next life stage following detachment from imported horses or migratory birds now and in the future.

A total of 143 CCHFV-infected unfed adult *Hyalomma* spp. ticks was predicted to be present in GB as a result of importation on horses between 1st April and 31st July each year under current habitat conditions. Under a future climatic and land cover scenario, this number was predicted to be 121.8 ticks due to a predicted future reduction in suitable land cover type in areas with high densities of horse imports.

A total of 11 CCHFV-infected *Hyalomma* spp. adult ticks was predicted to be present in GB as a result of importation on migratory birds between 1st April and 31st July each year under current habitat conditions. Under a future climatic and land cover scenario, this number was predicted to be 12.9 ticks. Increased future temperatures and ranges for the five bird species studied contributed to the future increase in CCHFV-infected ticks present in GB.

Immature *Hyalomma* spp. ticks parasitize birds, while adult *Hyalomma* spp. ticks parasitize horses. The ability of mated adult female ticks to lay an average of 6,500 eggs following detachment in GB led to an overall greater number of CCHFV-infected ticks that could bite a human from the horse pathway than from the bird pathway. The findings of this thesis suggest for the first time that horses may have an important role in the geographic spread of CCHFV, where previous work has focused primarily on migratory birds. The areas of GB where habitat and climate may be suitable for the survival of imported *Hyalomma* spp. ticks, as predicted in this thesis, can be used to direct future surveillance and risk assessment of CCHFV in GB.

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Academic Thesis: Declaration Of Authorship

I, MARION ELIZABETH ENGLAND

declare that the thesis entitled,‘Understanding the risks and factors associated with the introduction of Crimean-Congo haemorrhagic fever virus into Great Britain’ and the work presented in this thesis are my own and has been generated by me as the result of my own original research.

I confirm that:

1. This work was done wholly or mainly while in candidature for a research degree at this University;
2. Where any part of this thesis has previously been submitted for a degree or any other qualification at this University or any other institution, this has been clearly stated;
3. Where I have consulted the published work of others, this is always clearly attributed;
4. Where I have quoted from the work of others, the source is always given. With the exception of such quotations, this thesis is entirely my own work;
5. I have acknowledged all main sources of help;
6. Where the thesis is based on work done by myself jointly with others, I have made clear exactly what was done by others and what I have contributed myself;
7. Either none of this work has been published before submission, or parts of this work have been published as: [please list references below]:

   Foley-Fisher, M., Phipps, P., Medlock, J. M., Atkinson, P. M., Atkinson, B., Hewson, R. & Gale, P. 2012. Ticks on northward migrating birds in southern Spain during Spring, 2011. *Journal of Vector Ecology,* 37**,** 478-480.

Signed: ……………………………………………………………………………

Date: ……………………………………………………………………………….

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Abbreviations

ºC	Degree Celsius
ºN	Degree North
AHVLA	Animal Health and Veterinary Laboratories Agency
a.s.l.	Above sea level
BADC	British Atmospheric Data Centre
BIP	Border Inspection Post
BTO	British Trust for Ornithology
BTV	Bluetongue virus
CCHF	Crimean-Congo haemorrhagic fever
CCHFV	Crimean-Congo haemorrhagic fever virus
CEH	Centre for Ecology and Hydrology
CFR	Case fatality rate
DEFRA	Department for Environment, Food and Rural Affairs
EBLEX	Organisation for the English beef and sheep industry
ECDC	European Centre for Disease Prevention and Control
ELISA	Enzyme-linked immunosorbent assay
EU	European Union
h	Hour
HadRM3	Hadley Centre Regional Climate Model 3
hPa	Hectopascal
GB	Great Britain
GIS	Geographical Information System
IPCC	Intergovernmental Panel on Climate Change
JPEG	Joint Photographic Experts Group
KDE	Kernel density estimation
km^2	Square kilometre
LCM1990	Land cover map 1990
LCM2000	Land cover map 2000
LCM2007	Land cover map 2007
m	Metre
MLE	Maximum likelihood estimation
mm	Millimetre
NBD	Negative binomial distribution

NDVI	Normalised difference vegetation index
NLogL	Negative log-likelihood
NOAH	National Office of Animal Health
OIE	World Organisation for Animal Health
PDF	Portable Document Format
PHE	Public Health England
ProMED	Programme for Monitoring Emerging Diseases
qRT-PCR	Real-time polymerase chain reaction
RSPB	Royal Society for the Protection of Birds
RT-PCR	Reverse-transcription polymerase chain reaction
spp.	Species
TOT	Trans-ovarial transmission
TRACES	Trade Control and Expert System
TST	Trans-stadial transmission
UK	United Kingdom
USA	United States of America

1 Introduction to Crimean-Congo haemorrhagic fever and the aims of this thesis

1.1 Introduction

Infectious disease epidemics of humans and animals can have devastating economic and health consequences (Kelly et al., 2012). The geographical spread of infectious diseases is facilitated by environmental and socio-economic factors, such as climate change and globalization (Hoek et al., 2012). There have been recent incursions of exotic vector-borne diseases into previously unaffected areas, such as the emergence of bluetongue virus in northern Europe in 2006 (Carpenter et al., 2009). Crimean-Congo haemorrhagic fever virus (CCHFV) is considered to be a major emerging disease threat to the European Union (EU) and, more specifically, a significant public health threat to southeastern Europe (Mertens et al., 2013). CCHFV causes the zoonotic disease Crimean-Congo haemorrhagic fever (CCHF) in humans with a case fatality rate of around 30% (Ergönül and Whitehouse, 2007). The arthropod vector for CCHFV is a tick and the genus of tick that has been implicated in the transmission of CCHFV is *Hyalomma,* with *Hyalomma marginatum* Koch being the main tick vector within Europe (Maltezou et al., 2010). The risk to the EU from CCHFV is anticipated to increase in the future as a result of climate change (Gale et al., 2009) and epidemics of CCHFV within the EU are considered to be more likely to occur in the future (Kelly et al., 2012). CCHFV is, therefore, a very important human pathogen and, indeed, is of worldwide public concern (Ergönül, 2012). This, together with the recent emergence of new endemic foci of CCHFV in Europe (particularly in Spain (Estrada-Peña et al., 2012b)), demonstrates an urgent need to understand the risks and factors associated with the potential introduction of CCHFV into Great Britain (GB). There are six pathways that have been identified as possible routes for the introduction of CCHFV into Western Europe (Hoek et al., 2012). These are human mobility, trade in livestock, migratory bird movements, trade and movements of mammal wildlife, trade in animal products and pet movements (Hoek et al., 2012). Expert opinion suggests that the pathways of migratory birds and livestock movements are the most important (Hoek et al., 2012). This thesis, therefore, investigates these two pathways as routes of introduction of CCHFV into GB to inform the current and future risk of CCHFV to humans in GB. Understanding the routes of entry of CCHFV-infected ticks into GB is key to assessing the risk of an incursion event or the risk of CCHFV becoming endemic. The pathways for the introduction of CCHFV-infected ticks into GB by migratory birds and horses are laid out in Figure 1.1. Birds have the potential to bring in immature *Hyalomma* spp. ticks from CCHF endemic areas and horses have the potential to bring in adult *Hyalomma* spp. ticks from CCHF endemic areas. Specifically, the first aim of this thesis was to quantify the number of CCHFV-infected *Hyalomma* spp. ticks that enter GB on migratory birds and imported horses. Many migratory birds that breed in GB, over-winter in Africa, some as far south as South Africa (e.g. *Hirundo rustica* Linnaeus, the barn swallow (RSPB)) and, therefore, are likely to come into contact with CCHFV-infected ticks. Indeed, CCHFV has recently been found in ticks on migratory birds in Morocco (Palomar et al., 2012). Horses were chosen as the ‘livestock pathway’ as the CCHFV tick vector has previously been found on a horse imported into GB (Jameson and Medlock, 2009). Also, there has been a recent decline in the number of live cattle and sheep imported into GB (DEFRA, 2009, EBLEX, 2012). Fieldwork was carried out in Spain (Chapters 3 and 4) to quantify the number of *Hyalomma* spp. ticks present on migratory birds and horses. The survival of CCHFV-infected *Hyalomma* spp. ticks after entry into GB was modelled under current and future habitat scenarios and the two risk pathways of birds and horses compared (Chapters 5 and 6). Areas of GB with the highest densities of CCHFV-infected *Hyalomma* spp. ticks were identified to direct further research and surveillance. The findings and conclusions of this thesis will be of importance for future studies that seek to fully assess the risk of CCHFV to GB. This thesis constitutes the release assessment of CCHFV into GB (specifically by migratory birds and horses) as part of a wider framework for risk analysis of CCHFV in GB.

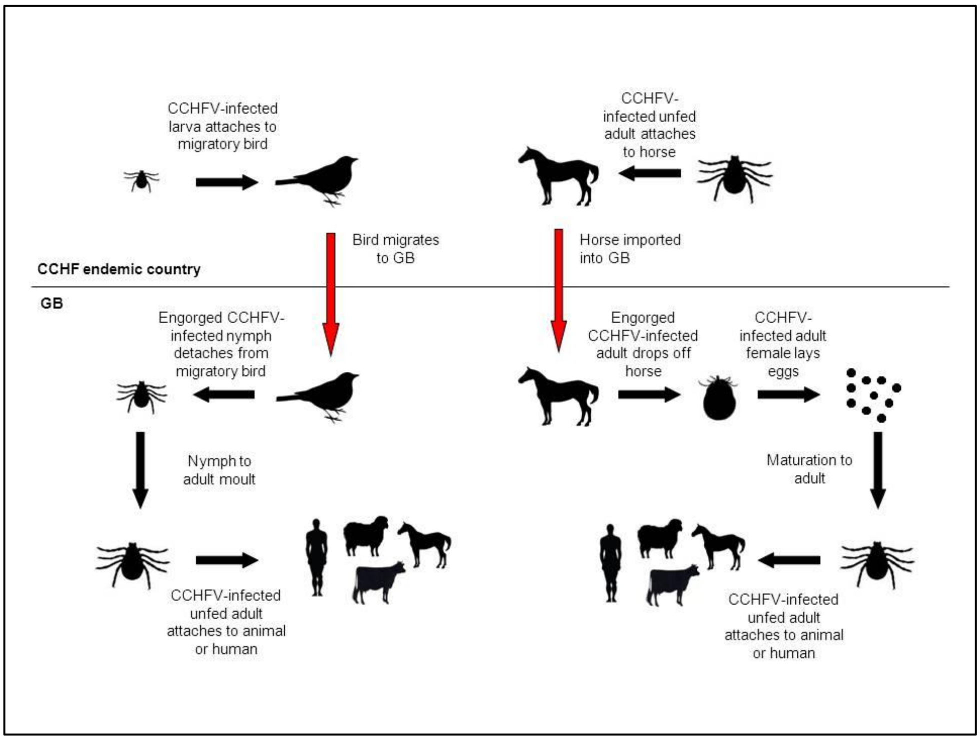


Figure 1.1: Pathways for incursion of CCHFV-infected ticks into GB on migrant birds and horses.

1.2 Background to CCHFV

The agent for CCHF is a virus of the genus *Nairovirus*, within the family Bunyaviridae (Flick, 2007). It is endemic to areas of Africa, southeastern Europe and Asia and is the second most widespread medically important arbovirus (literally arthropod-borne) after Dengue (Ergönül, 2006). The current distribution of CCHFV coincides with the geographic distribution of *Hyalomma* spp. ticks, which are not found north of the 47°N line of latitude (Estrada-Peña et al., 2011). There have been changes to the distribution of CCHFV over the last ten to fifteen years. Since 2000, the first human cases have been reported from Turkey, Greece and India and an increasing number of outbreaks have occurred in areas such as Kazakhstan, Kosovo, Iran and Afghanistan (ProMED-mail). Some of these trends may be attributed to better diagnostic capabilities and reporting, but new outbreaks are occurring and the disease is spreading into new areas. Indeed, prior to 2001, there were no reported cases of CCHF in Turkey, but to date there have now been a total of 4,441 human cases (ProMED-mail). To illustrate the current global distribution of human cases of CCHF and the distribution of *Hyalomma* spp. ticks, data were gathered from a range of sources (ProMED-mail, Hoogstraal, 1979, Ergönül and Whitehouse, 2007, Kolonin, 2009) to produce the map in Figure 1.2.

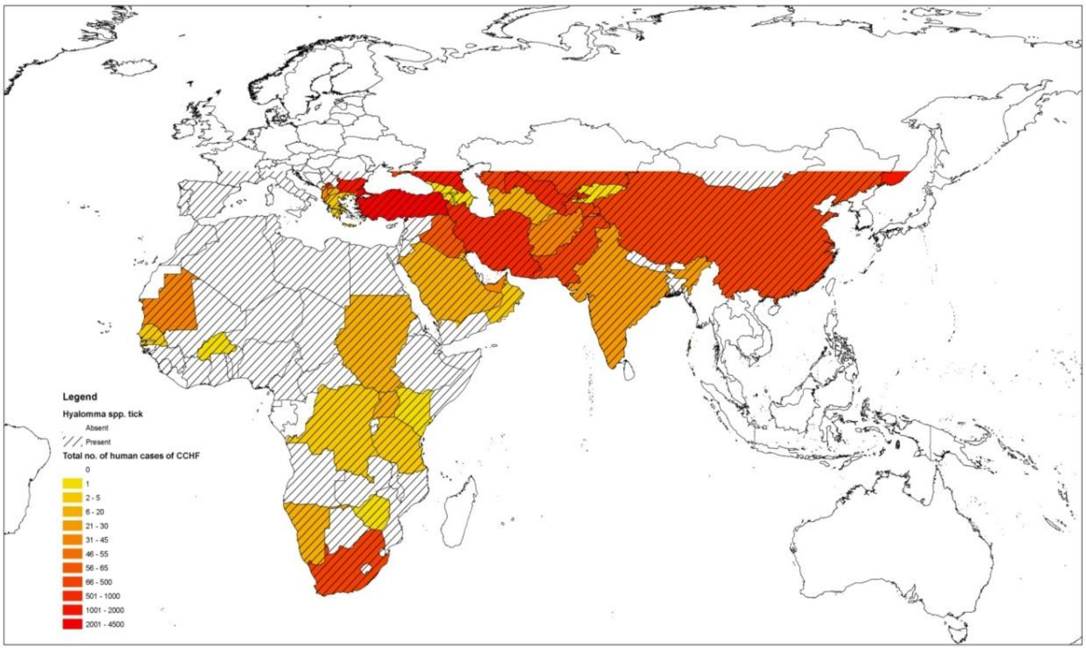


Figure 1.2: Total number of human cases of CCHF by country from 1945 to present and the geographical range of *Hyalomma* spp. ticks (www.promedmail.org, Hoogstraal, 1979, Ergönül and Whitehouse, 2007, Kolonin, 2009).

Currently, the tick vector is present in some western European countries such as Italy and Portugal, where there is currently no evidence for the presence of the virus. The absence of the virus would suggest that there are other as yet unidentified factors determining the transmission and establishment of the virus, as it is likely that the virus has been introduced, for example, through migratory birds (Hoek et al., 2012). In countries where the vector is present but there are no reported human cases, it may be that the endemic strain has low pathogenicity and, therefore, human infections go undetected particularly if there is no routine testing of blood for CCHFV antibodies. Alternatively, CCHFV may circulate in areas where human exposure to ticks is very low or does not occur.

Whilst animals and birds may develop antibodies to CCHFV, the infection is largely asymptomatic and subclinical. In humans, CCHFV induces symptoms such as nausea, diarrhoea, haemorrhages and enlargement of the liver and spleen (Swanepoel et al., 1989, Bakir et al., 2005, Ergönül, 2006). The case fatality rate (CFR) for humans with clinical symptoms of CCHF varies greatly between countries and between different outbreaks within a country. For example, in Turkey in 2009, the CFR was 5% (Ozkaya et al., 2010) whereas rates of 72.7% and 80% have been reported from China and the United Arab Emirates, respectively (Chinikar et al., 2010). Several factors influence the prognosis, such as the quality and availability of healthcare and the virulence of different strains of the virus.

1.3 The tick vector

In this thesis, the number of CCHFV-infected *Hyalomma* spp. ticks entering GB on migratory birds and horses is predicted in Chapter 3 and Chapter 4. Species of tick other than *Hyalomma* are likely to be attached to horses and migratory birds upon entry into GB, but *Hyalomma* spp. ticks have long been implicated in the transmission of CCHFV (Hoogstraal, 1972, Wilson et al., 1991, Ergönül and Whitehouse, 2007) and, thus, are of particular interest. Studies have isolated CCHFV from a range of other hard ticks (family Ixodidae) including *Rhipicephalus* spp., *Dermacentor* spp., *Haemaphysalis* spp., *Ixodes* spp. and *Amblyomma* spp. (ProMED-mail, Ergönül and Whitehouse, 2007) but the isolation of the virus from a particular tick species does not mean that the species is a competent vector of the virus. Vector competency studies show whether the virus can replicate within a tick species and whether onwards transmission is possible. *Hyalomma* spp. ticks have been experimentally infected with CCHFV and their ability to transmit the virus has been tested and demonstrated (Okorie and Fabiyi, 1980, Logan et al., 1989, Logan et al., 1990, Wilson et al., 1991, Gonzalez et al., 1992, Dohm et al., 1996). Within Europe, *H. marginatum* has been the main vector responsible for CCHF outbreaks in Europe (Avsic-Zupanc, 2007, Vatansever et al., 2007). In Africa and Asia other *Hyalomma* spp. ticks, such as *Hyalomma asiaticum* Schulze and Schlottke in China, are the main vectors for CCHFV (Gao et al., 2010).

1.3.1 Lifecycle of *Hyalomma* spp. ticks

To predict the survival within GB of imported CCHFV-infected *Hyalomma* spp. ticks (as in Chapter 4 and Chapter 6), it is necessary to understand the life-cycle of the tick. All ticks have three life stages (larval, nymphal and adult) with some ticks remaining on a single host for all three stages and others using two or three different hosts. *Hyalomma* spp. ticks (especially *H. marginatum* and *Hyalomma rufipes* Koch) prefer to use a ‘two-host’ tick strategy whereby they feed on two different hosts during their life cycle. Immature ticks feed twice on the same individual host, once as a larva and once as a nymph. Thus, the moult from larva to nymph takes place on the host. After dropping off the host, the nymphs moult to adults before finding a larger host on which to feed (Figure 1.3)

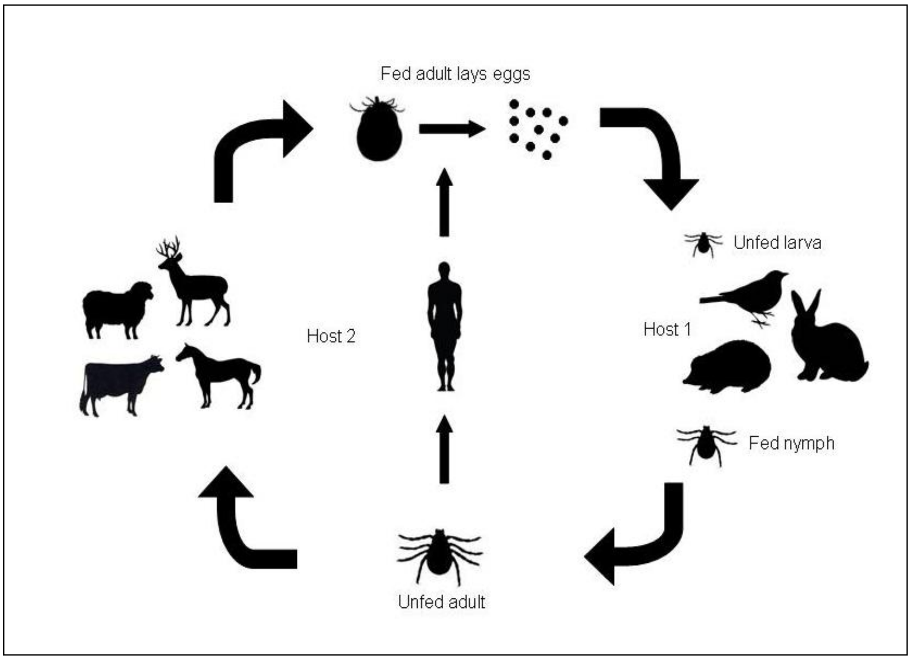


Figure 1.3: Lifecycle of a two-host tick.

When considering migratory birds as a route of introduction of CCHFV-infected ticks into GB, *H. marginatum* and *H. rufipes* are considered to be the species of greatest interest. *H. marginatum* ticks have been found on migrant birds entering GB (Jameson et al., 2012a) and *H. rufipes* ticks have been found on migrant birds entering Europe (Molin et al., 2011, Lindeborg et al., 2012). These species usually feed on a small mammal or bird as immatures while as adults they target a larger host, commonly wild herbivores and domestic livestock (Hillyard, 1996) and, less frequently, humans (Hoogstraal, 1981). Therefore, migratory birds import *Hyalomma* spp. nymphs (Jameson et al., 2012a) and horses import *Hyalomma* spp. adults (Jameson and Medlock, 2009). As two-host ticks, *Hyalomma* spp. ticks spend a long time on the host as immatures compared to three-host ticks, with *H. rufipes* immatures remaining on the host for an average of 22.8 days (Knight et al., 1978). This long period of attachment is important when considering the introduction of CCHFV-infected *Hyalomma* spp. ticks on migrating birds. Mating between *Hyalomma* spp. adults occurs on the second host after which both fed adults drop off. Males die soon after and females go on to lay an average of 6,500 eggs (Estrada-Peña et al., 2011). Both larvae and adults of *Hyalomma* spp. ticks use a ‘hunting’ strategy when questing for hosts (Estrada-Pena et al., 2011). They are cursorial, meaning that they are adapted for running and seek out potential hosts using signals such as carbon dioxide, shadows, heat and sweat using the Haller’s organ. This is a sensory structure located on the tip of the first tarsus of the first walking leg which can detect odour and humidity (Ruedisueli and Manship, 2011). The camel tick, *Hyalomma dromedarii* Koch is able to identify a camel host from a distance of 18 m (Kaltenrieder, 1990).

1.3.2 **Habitat of *Hyalomma* spp. ticks**

In this thesis, ‘habitat suitability’ for ticks in GB is defined as a combination of suitable temperature (‘climatic suitability’) and land cover. The habitat suitability for *H. marginatum* in GB under current and future conditions will be modelled to determine the probability of tick survival after entry on a migratory bird or horse (Chapter 5 and Chapter 6). A variety of *Hyalomma* spp. ticks parasitize migratory birds (*H. marginatum* (Jameson et al., 2012a) and *H. rufipes* (Hoogstraal et al., 1961, Molin et al., 2011)) and horses (e.g. *Hyalomma anatolicum* Koch and *Hyalomma lusitanicum* Koch (Walker et al., 2003, Apanaskevich et al., 2008)) and, therefore, may enter GB on both birds and horses. In Chapter 5 and Chapter 6, the habitat suitability for *H. marginatum* will be used as a proxy for all *Hyalomma* spp. ticks potentially being imported into GB to determine the probability of tick survival. It is, therefore, assumed that species of *Hyalomma* that parasitize birds and horses have broadly similar habitat preferences.

Research to understand the ecological niche of *H. marginatum* has focused on Mediterranean populations. Estrada-Peña and Venzal (2007) looked at the climatic niche of several species of tick that occur around the Mediterranean, including *H. marginatum* and found that warmer temperatures and lower rainfall increased habitat suitability for this tick. Estrada-Peña and Venzal (2007) state that between the latitudes of 33 °N and 47 °N, spring temperatures are sufficiently high and, thus, development rates are such that *H. marginatum* can feed early in the season and have completed their moult before the cold winter temperatures begin. Above 47 °N the cooler spring and summer temperatures mean slower development rates, such that *H. marginatum* ticks are unable to complete their moult before the low winter temperatures begin, and, therefore, there is 100% mortality of these ticks. Tick populations tend not to be sustainable if engorged stages are entering the winter unmoulted (Estrada-Peña et al., 2011). Similarly, below 33 °N the temperatures are high and development rates fast, so ticks have moulted early and, therefore, have to then cope with a long period of water stress and dehydration before the winter. Estrada-Peña and Venzal (2007) calculated from their models that the optimum temperature for survival of *H. marginatum* was between 3000 °C and 4000 °C (accumulated daily temperatures over one year). This was the limiting factor for northerly populations. They found the optimal water vapour deficit to be at or below 15 hPa and this was the limiting factor for southerly populations of *H. marginatum*. It is important to remember that there are many other factors that determine the success or failure of a tick population, such as the availability of hosts and vegetation cover. The habitats of other tick species have been mapped using remote sensing, including the use of the normalised difference vegetation index (NDVI) which is related to relative humidity and rainfall (as reviewed in Estrada-Peña (2001)).

Hyalomma spp. ticks are known to be better adapted to dry conditions than other tick genera, although they may still be able to survive in wet conditions as well (Estrada-Peña and Venzal, 2007). It is unsurprising that suitability for *H. marginatum* increases if temperatures are warmer, as tick development rate and activity increases with temperature (Randolph and Rogers, 2007). A population model has been developed to describe the distribution and seasonal dynamics of *H. marginatum* (Estrada-Peña et al., 2011). The model simulates the effects of temperature and water vapour deficit on tick survival rates, including questing and moult success (Estrada-Peña et al., 2011) and has provided an insight into the ecological limitations of *H. marginatum*. Parts of this life-cycle model (nymph to adult moult and egg production) along with land cover data for GB were used to predict the probability of survival of imported *Hyalomma* spp. ticks in GB in Chapter 5 and Chapter 6.

1.3.3 Immature *Hyalomma* spp. ticks on birds

All *H. marginatum* immatures that have been found in GB have been found on birds, namely sedge warbler (*Acrocephalus schoenobaenus* Linnaeus), whitethroat (*Sylvia communis* Latham), whinchat (*Saxicola rubetra* Linnaeus), wheatear (*Oenanthe oenanthe* Linnaeus) and redstart (*Phoenicurus phoenicurus* Linnaeus) (Martyn, 1988, Jameson et al., 2012a). Birds are regarded as important in maintaining *Hyalomma* spp. population densities (Hoogstraal, 1972). Hoogstraal (1972) suggested that the transportation of *H. marginatum* and *H. rufipes* between Eurasia and Africa by birds may have played a role in the intercontinental spread of CCHF. A recent study in Morocco found CCHFV-infected *H. marginatum* ticks on migratory birds (Palomar et al., 2012). GB migrants commonly use the East Atlantic flyway, crossing from Morocco into Spain at the Strait of Gibraltar, the narrowest stretch of water between Africa and Western Europe. Around 16 million birds migrate into GB each year (Eccleston, 2008) and in Chapter 3, the number of CCHFV-infected *Hyalomma* spp. ticks carried into GB on five bird species will be predicted. The five bird species that are considered to be the most likely to carry *Hyalomma* spp. ticks into GB will be selected.

There have been several studies carried out that provide quantitative data on the number of *Hyalomma* spp. ticks carried on migratory birds. Hoogstraal et al. (1961) carried out a study in Egypt during the spring migration along the East Mediterranean flyway, trapping birds and removing the ticks from them. They trapped a total of 959 migrants, of which 128 were found to be carrying a total of 349 ticks. All but two of these ticks were *Hyalomma* spp. immatures. It is difficult to identify *Hyalomma* spp. immatures to species level (Phipps, P., AHVLA, personal communication) but it was believed that the *Hyalomma* spp. ticks were all *H. rufipes* (Hoogstraal et al., 1961).

Kaiser and Hoogstraal (1974) found similar results in Cyprus when checking northward migrants for ticks in the spring. They found that 177 of 22,015 birds that they checked had ticks, of which 95% were immature *H. rufipes*. Molin et al. (2011) studied ticks on migratory birds on the islands of Capri (Italy) and Antikythera (Greece), where birds migrate along the central Mediterranean flyway into central European countries in the spring. A total of 7,453 migrants were caught in mist nets of which 2.7% carried ticks. A total of 386 ticks were removed and 367 of these were *Hyalomma* spp. immatures. Sequencing data for 10 of these ticks showed nine of them to be *H. rufipes* and one to be *H. marginatum*.

1.3.4 Adult *Hyalomma* spp. ticks on horses

Adult *H. marginatum* feed on a range of domestic animals, including dogs (Kotti et al., 2001), cattle (Georges et al., 2001, Aktas et al., 2004) and horses (Moltmann et al., 1983, Jameson and Medlock, 2009). Horses are imported into GB on ferries and by air for private riding, horse racing, polo and for trading. Horses, therefore, have the potential to carry ticks from one country to another during transportation. Horses from CCHFV-endemic countries may transport infected ticks into GB.

No previous studies have quantified the number of ticks that are brought into GB on horses. However, Jameson and Medlock (2009) report that an adult male *H. marginatum* was found on a horse near Dartmoor 48 hours after it had been imported from Portugal. Ticks were collected from companion animals in the Netherlands and an adult *H. rufipes* tick was removed from a horse (Nijhof et al., 2007). In Chapter 4, the number of *Hyalomma* spp. ticks present on horses in Spain (a *Hyalomma* spp. endemic country) will be investigated through field work that surveys horses for ticks. The collection of these data is necessary to predict the number of CCHFV-infected *Hyalomma* spp. ticks entering GB on horses.

1.4 Transmission cycles of the virus

The transmission cycles for CCHFV between the tick vector and its hosts are shown in Figure 1.4. The green arrows show the possible routes of virus transmission through the tick life cycle. For CCHFV to be endemic, some or all of these transmission routes must be exploited. An unfed larval tick may be infected with CCHFV if the virus has been passed from an infected adult female to the developing eggs (‘trans-ovarial transmission’), by feeding on a viraemic host (‘host to tick transmission’) or by feeding on a host alongside infected ticks (‘co-feeding’). A nymph may be infected with CCHFV if it was infected as a larva (‘trans-stadial transmission’), by host to tick transmission or by co-feeding on a host with infected ticks. An adult tick may be infected with CCHFV by trans-stadial transmission if it was infected as a nymph, host to tick transmission, co-feeding on a host with infected ticks or through mating with an infected tick. Hosts (including humans) may become infected with CCHFV if they are parasitized by an infected tick. Transmission of CCHFV to humans can occur through infected tick bite, from the blood of an infected animal or through nosocomial transmission (human to human in a healthcare setting). This is illustrated by the red arrows in Figure 1.4. When modelling the survival of imported CCHFV-infected ticks in GB in Chapter 5 and Chapter 6, estimates for the probability of CCHFV transmission between life-stages of the tick will be required to determine if the tick is still infected when it is able to bite a human. The specific types of transmission of interest are now described.

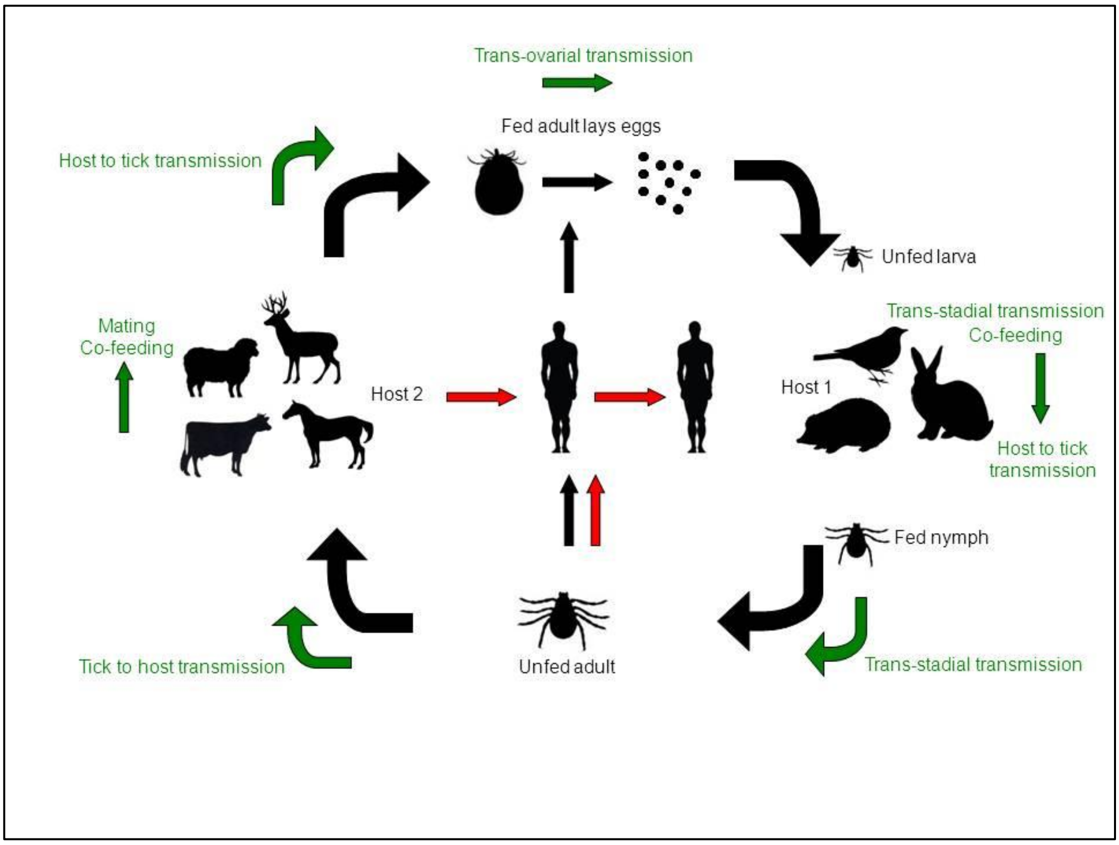


Figure 1.4: Transmission cycles of CCHFV in *Hyalomma marginatum* ticks and their hosts. Green arrows show all transmission routes and red arrows show transmission routes of CCHFV to humans (Gale et al., 2009a, Gale et al., 2009b, Randolph and Rogers, 2007).

1.4.1 Trans-ovarial transmission

Trans-ovarial transmission (TOT) is the passage of the virus from the adult female tick to the developing eggs. TOT is an important part of the mechanism by which an infected adult female tick introduced to GB on a horse could transmit CCHFV to hosts within GB. Data on rates of TOT enable the number of infected eggs produced by an infected adult female tick, after entry into GB on a horse, to be estimated (Chapter 6). TOT is considered to be important in the maintenance of the CCHF virus in tick populations, as adults feed on a separate host to larvae and nymphs and, therefore, infection of larvae and nymphs is often dependent on TOT (Maltezou and Papa, 2010).

1.4.2 Trans-stadial transmission

Trans-stadial transmission (TST) occurs when the CCHF virus is retained in the body of the tick when it moults from one life stage to the next. In this way the virus is able to persist in the body of the tick throughout its life cycle. This is an important mechanism such that the virus can be present within a tick for as long as the tick survives in a new area. TST is, therefore, incorporated into the models of tick survival following importation into GB in Chapter 6. As with TOT, experimental studies have shown a range of TST rates in various species of *Hyalomma* (for example, Gordon et al. (1993)) which will be used to estimate the probability of infection in the tick as it develops from one life-stage to the next in Chapters 5 and 6.

1.5 CCHF and climate change

In Chapter 6, the number of CCHFV-infected ticks entering GB on migratory birds and horses will be predicted under both current habitat conditions and under a future climatic and land cover scenario. Ticks are sensitive to changes in their environment during their non-parasitic (‘environmental’) phases (Gray et al., 2009), but hosts as well may expand their range as a result of changes to their environment and carry ticks to new areas. As discussed above, there are latitudinal constraints on the distribution of *H. marginatum* and these are related to the inability of the tick to moult at lower temperatures. The climate of Europe is predicted to change in the future to one where the south of Europe will be drier with increased temperatures (IPCC, 2007). Climate change may also have an impact on land-use change and this too can alter a habitat from being unsuitable to suitable for a particular arthropod species.

A northward expansion of habitat suitable for *H. marginatum* into northern France is predicted under future climate predictions, mainly due to increased temperatures and reduced rainfall (Estrada-Peña and Venzal, 2007). However, it is difficult to predict how populations of *H. marginatum* will react to changes in climate, as different populations are limited by different environmental variables. As long as temperatures are warm enough during the spring, summer and autumn for *H. marginatum* to complete its moult to the next life stage, they can survive the winter. If temperatures are not warm enough during this part of the year, then the tick will not survive the winter as moult is not completed (Gray et al., 2009). Changes in development rates are predicted to permit changes in seasonal activity and the expansion of ticks into new areas. Indeed, the impacts of climate change are likely to be most clearly seen at the geographical limits of both the vector and the disease (Gray et al., 2009). Gale et al. (2009) elicited expert opinion on the impact of climate change on CCHFV and four other vector-borne viruses. They found that the risk of CCHFV through vector and wildlife entry into new areas would be increased by climate change. The risk of incursion of CCHFV in livestock in Europe through introduction of CCHFV-infected *H. marginatum* ticks on migratory birds was predicted to increase in northern Europe in the future as a result of climate change (Gale et al., 2012). However, whilst climate change increased the likelihood of nymph to adult moult in imported ticks, the abundance of migratory birds across Europe was predicted to decrease by 34% (Gale et al., 2012). Therefore, climate change may have a detrimental effect on host species, whilst increasing the survival potential of ticks. Thus, in Chapter 6, the number of CCHFV-infected ticks entering GB on migratory birds and horses will be predicted for the future (2070’s) as well as the subsequent survival of these ticks under a future habitat scenario. Current and future predictions will then be compared to help inform current and future risk to humans in GB from CCHFV-infected ticks imported by birds and horses.

1.6 The OIE risk analysis framework

As mentioned above, this thesis forms the release assessment part of a wider risk analysis framework. The number of CCHFV-infected ticks in GB arising from migratory birds and horses (as will be predicted in Chapter 5 and Chapter 6) is related to the risk to humans of CCHFV in GB. Risk can be broken down into two parts; the probability of something happening and the consequences if it does happen (Murray et al., 2004). The World Organisation for Animal Health (OIE) has adopted the Covello and Merkhofer (1993) model for risk analysis and the framework for this type of risk analysis is laid out in Figure 1.5.

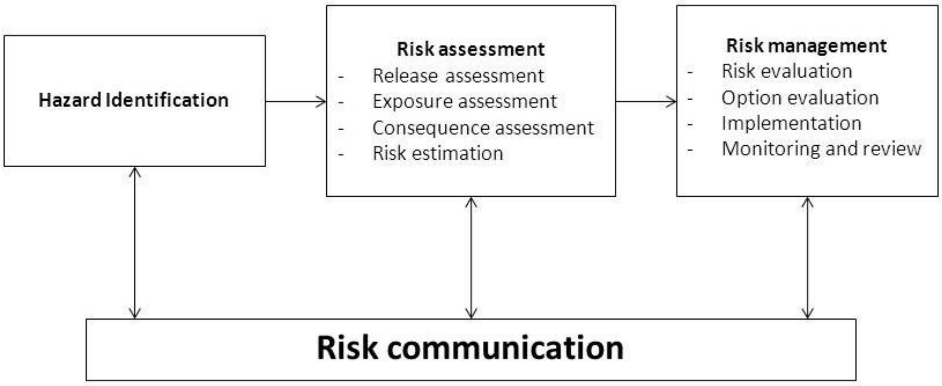


Figure 1.5: The structure of the OIE risk analysis process (Murray et al., 2004).

In this thesis, the hazards are identified as the CCHF virus itself as well as the tick vector. As can be seen in Figure 1.5, one element of the risk analysis process is risk assessment, which is the process of determining the likelihood and consequences of entry of the hazard (Murray et al., 2004).

The first part of the risk assessment is the release assessment and in this thesis, the release of CCHFV-infected *Hyalomma* spp. ticks into GB by migratory birds and horses is investigated. Subsequent exposure of humans to the CCHFV pathogen in GB is not addressed here, but the probability of survival of the tick following release into GB is predicted, as previously mentioned. Indeed, this thesis quantifies the number of CCHFV-infected unfed adult *Hyalomma* spp. ticks present in GB, as a result of tick importations on migratory birds and horses, as it is these ticks that could attach to a human and infect them with CCHFV, given human exposure to the tick. It is, thus, anticipated that the results and conclusions of this thesis will be used in subsequent risk analysis for CCHFV in GB.

1.7 Summary of the aims of this thesis

This thesis aims to understand the risks and factors associated with the introduction of CCHFV into GB through two different risk pathways. Firstly, the role of migratory birds in the importation of exotic ticks into GB is explored. Birds over-winter in and migrate through countries where *Hyalomma* spp. ticks are endemic and, therefore, have the potential to transport this tick into GB when they return to breed in the spring. Some birds migrate to GB from CCHFV endemic countries and, consequently, may import CCHFV-infected immature ticks into GB. In Chapter 3, the number of CCHFV-infected *Hyalomma* spp. nymphs entering GB on migratory birds each spring (between 1st April and 31st July) is predicted. The release pathway of CCHFV-infected ticks entering GB on birds is outlined in Figure 1.6.

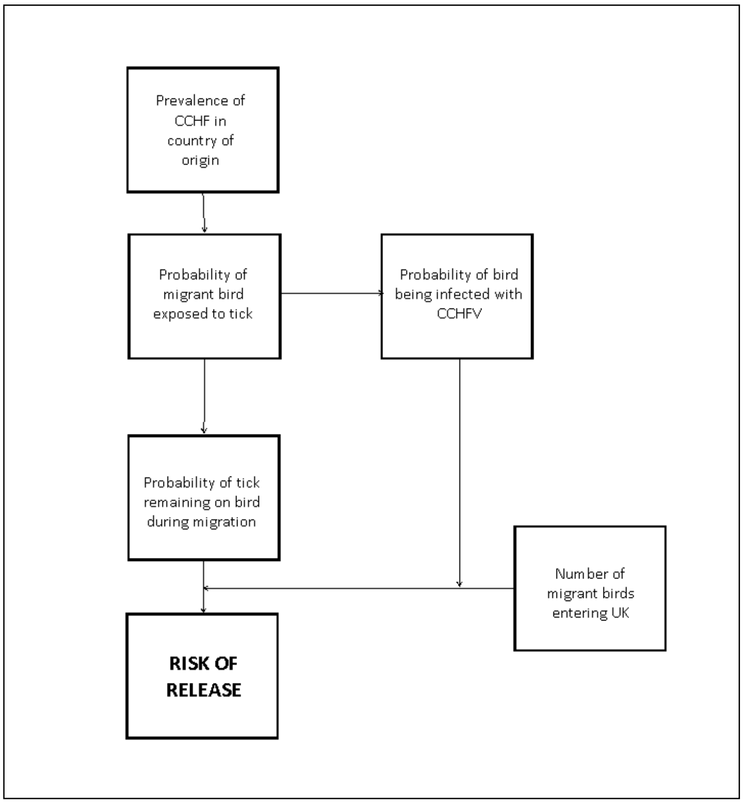


Figure 1.6: Release pathway for CCHFV entering GB on migrant birds.

Secondly, horses have also been shown to import *Hyalomma* spp. ticks into GB and, therefore, may present a route of incursion for CCHFV. In Chapter 4, the number of CCHFV-infected *Hyalomma* spp. adults entering GB on horses between 1st April and 31st July each year will be predicted. The release pathway of CCHFV-infected ticks entering GB on horses is outlined in Figure 1.7.

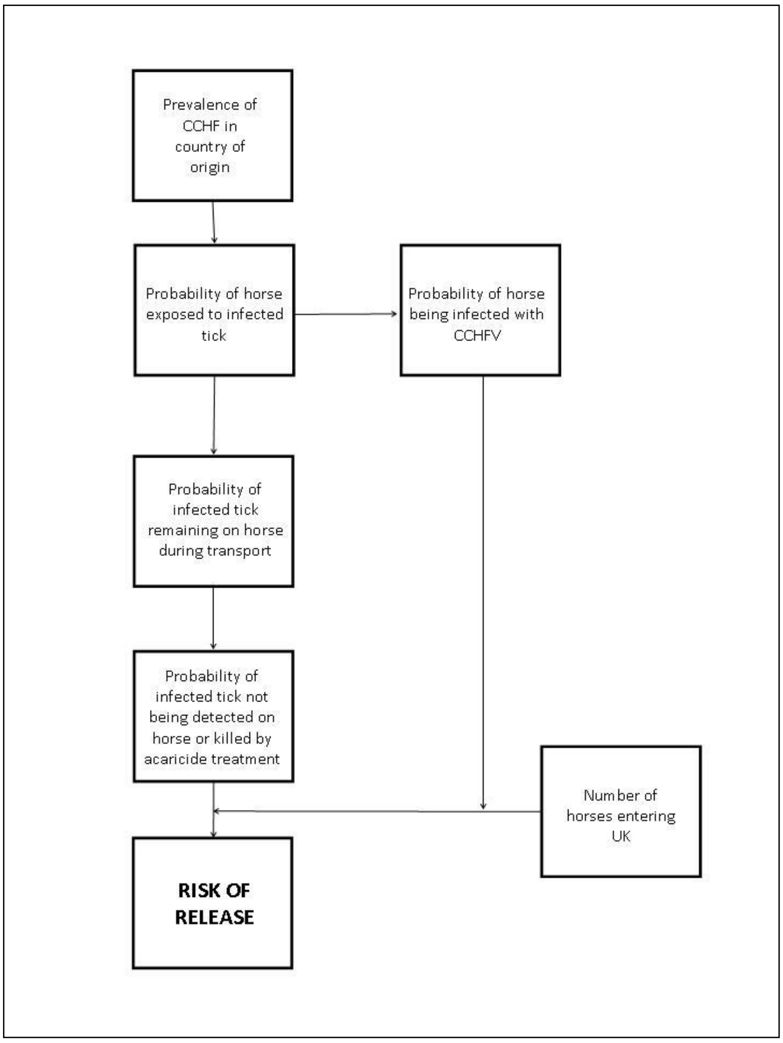


Figure 1.7: Release pathway for CCHFV entering GB on imported horses.

A working assumption in this thesis is that indigenous GB ticks, such as *Ixodes ricinus* Linnaeus, are not able to transmit CCHFV. Therefore, it was considered that a case of CCHF could only arise in humans from an imported CCHFV-infected *Hyalomma* spp. tick biting a human. This requires imported ticks to survive (moult to the next life-stage and/or reproduce) and these processes will be modelled under both current and future land cover and climatic scenarios in Chapter 5 and Chapter 6. The areas of GB where CCHFV-infected *Hyalomma* spp. ticks are predicted to be able to survive both now and in the future were mapped. The number of CCHFV-infected unfed adult ticks present in GB arising from horses and migratory birds were predicted under current and future habitat scenarios.

2 Review of methodologies

2.1 Introduction

The multi-disciplinary nature of the release assessment for CCHFV-infected ticks into GB carried out in this thesis will require the application and implementation of a range of techniques and methodologies. In this chapter, these will be described and explained. In Chapters 3 and 4, fieldwork will be carried out in southern Spain to collect ticks from their hosts. Here, the process of selection of the study site and the collection techniques employed are reviewed. The data collected from Spain will be analysed and then input into the models used in Chapters 5 and 6. A stochastic modelling approach will be used that accommodates the uncertainty and variability of the model parameters. The probability distributions used in the stochastic modelling process are described and reviewed in this chapter. The spatial modelling techniques that will be used in Chapters 5 and 6 to analyse the spatial data and present the results will also now be described.

2.2 Tick collection and study sites

Fieldwork carried out for this project will involve the collection of ticks from birds and horses. There are many different tick survey techniques including flagging, carbon dioxide-baited traps and direct collection from hosts (Ginseng and Ewing, 1989). Different collection methods are used for different genera and species of ticks, depending on their questing behaviour. For example, flagging is an appropriate collection method for *I. ricinus* as these ticks quest on vegetation for hosts (Daniel et al., 2003, Materna et al., 2005, Danielova et al., 2006). Potential hosts brush against the vegetation and *I. ricinus* ticks ‘grab’ on to the passing hosts. Flagging involves dragging a white sheet or blanket (the ‘flag’) over vegetation along a transect and, thus, *I. ricinus* ticks are collected on the ‘flag’. This method of tick collection is not appropriate for collecting *Hyalomma* spp. ticks as they actively seek hosts by running along the ground, responding to visual cues, heat and carbon dioxide (Latif and Walker, 2004). Appropriate collection methods for *Hyalomma* spp. ticks are those that mimic the presence of a host, such as carbon dioxide-baited traps, or direct collection from animals (Latif and Walker, 2004)

Tick collection will be carried out to determine the number of *Hyalomma* spp. ticks infesting birds on the East Atlantic flyway (Chapter 3) and the number infesting horses in a *Hyalomma* spp. endemic area (Chapter 4). Southern Spain was chosen as the location of the fieldwork as it is a ‘gateway’ into Europe for British breeding migratory birds (Birdlife International). Thus, it is a possible entry route into north-west Europe of CCHFV-infected *Hyalomma* spp. ticks originating from CCHFV-endemic countries in Africa. The East Atlantic flyway is the flyway that is used by British breeding birds that overwinter in Africa (Birdlife International). When the fieldwork was initially carried out, CCHFV had not yet been identified in ticks in Spain. Countries along the East Atlantic flyway that were known to be CCHFV-endemic were all located in Africa. Any CCHFV-infected ticks entering GB on migrant birds along the East Atlantic flyway would, therefore, be of African origin and be entering Europe through Southern Spain. Sampling at this location, therefore, provides data that are relevant to all countries in Europe on the East Atlantic flyway, including GB. In Chapter 4, the infestation rates of *Hyalomma* spp. ticks on horses in a *Hyalomma* spp.-endemic area will be determined. As Spain has endemic populations of *Hyalomma* spp. ticks, it is an appropriate location to survey horses for *Hyalomma* spp. ticks.

Ticks will be collected from migratory birds in Spain by first trapping the birds using mist nets. This is a standard method of trapping birds, as outlined by the British Trust for Ornithology (BTO). Birds will then be inspected for ticks and any ticks found will be removed. Further details of the collection method are given in Chapter 3. Many other studies have collected ticks from birds in various countries including Egypt (Hoogstraal et al., 1964), Cyprus (Kaiser and Hoogstraal, 1974), Norway (Hasle et al., 2009a), South Africa (Hasle et al., 2009b) and the UK (Jameson et al., 2012a). The choice of locations of mist netting for birds in this project will be directed by the movements of the local bird ringing group. All sites to be visited are those regularly used by the local ringing group and cover a wide range of different habitats. Site selection will be dictated by weather as well as time since the previous visit. Therefore, the locations where ticks will be collected from birds represent a range of habitats used by a wide variety of bird species.

In previous studies, ticks have also been collected from other hosts, including humans (Karaer et al., 2011, Papa et al., 2011a). Most commonly ticks have been collected from cattle (Bouattour et al., 1996, Razmi et al., 2007, Hornok and Horváth, 2012) and sheep (Milne, 1943, Pavlidou et al., 2008). Horses are less commonly surveyed for ticks although some studies have taken place in Brazil (Labruna et al., 2001, Labruna et al., 2002, Oliveira et al., 2003) and in the USA (Carroll and Schmidtmann, 1986). The horses that will be surveyed in this project are those that are due to be visited by a veterinarian for the purpose of artificial insemination. Selection of horses will, therefore, be dictated by the daily schedule of the veterinarian. All horses surveyed will be healthy as they will have previously received health checks prior to artificial insemination. However, there exists variation in the conditions of their accommodation and upkeep. Thus, they represent a cross-section of horses from those that live full-time out-of-doors and are rarely groomed, to those that live indoors and are frequently groomed. Horses will be checked for ticks primarily under the tail and around the perineum, as well as the legs, axillae and along the mane as this is where ticks tend to attach to horses (New Mexico Livestock Board). Any ticks found will be removed and stored for later identification. Detailed collection methods are given in Chapter 4.

2.3 Probability distributions

When carrying out risk assessments, it is necessary to identify uncertainty and variability in the model parameters. Variability is the natural variation that one would expect to observe in a biological system, for example, the natural variation in the number of ticks on each bird. As various factors influence the number of ticks on a bird, such as the location and behaviour of both the bird and the tick and environmental factors such as the weather, it would be impossible to state the exact number of ticks present on a bird (without looking). The number of ticks follows a probability distribution and varies from bird to bird. As with the number of ticks on birds, natural variation would be expected in the number of ticks infesting horses and in the number of birds and horses entering GB per month and per year. Uncertainty associated with parameters can be described as the variation around the parameter value that is due to lack of knowledge and/or measurement error (Vose, 2000). For example, uncertainty in an estimate of the average number of ticks per bird may be due to sampling only a small number of birds; the more birds that are sampled, the more certain we are of the average. In this thesis, a population model for *H. marginatum* developed by Estrada-Peña et al. (2011) is used to predict the survival of imported *Hyalomma* spp. ticks (Chapters 4 and 6). There is uncertainty associated with the use of the equations developed by Estrada-Peña et al. (2011). The coefficients of temperature and water vapour deficit and the constant in each of the equations are the results of regression analysis carried out on empirical data. Since the publication of the *H. marginatum* population model (Estrada-Peña et al., 2011), the equations have been further refined (A. Estrada-Peña, personal communication). Some of these refinements are included in the models used in this thesis, but as more empirical data are collected so the equations become further refined and the uncertainty associated with them decreases.

Probability distributions can be used to incorporate the uncertainty and variability within models. Stochastic simulation is a way of randomly sampling the model variables that are described by probability distributions. The stochastic simulation method used in this thesis is Monte Carlo simulation where a large number of random samples (realisations) are obtained from the input distributions (Vose, 2008). In this way, the output distribution will represent possible combinations of the input variables. Monte Carlo simulations were run using the computer package, MATLAB R2012a.

The characteristics of a probability distribution should match the characteristics of the variable that is being modelled, for example, the data may be discrete or continuous (Vose, 2008). By looking at the data, an appropriate probability distribution can be selected. It may be that more than one distribution is appropriate and, therefore, a goodness-of-fit test can be used to determine which distribution is the best fit for the data. Theoretical distributions can be fitted to the data using maximum likelihood estimation (MLE) (Vose, 2008) in MATLAB R2012a. Maximum likelihood estimators of a distribution are the parameter values that maximise the likelihood of producing the observed data from the fitted distribution. Various probability distributions were used in this thesis and these are now described.

2.3.1 The Poisson distribution

The distribution of macroparasites (including ticks) on hosts is often highly aggregated with few individuals harbouring a large number of parasites and many having none or just a few (Shaw and Dobson, 1995). The Poisson distribution can be used to describe the distribution of tick counts per host. Thus, it can be used to express the probability that a given number of events (in this case the number of ticks on some bird species) occurs in a fixed interval of time if the average rate of occurrence is known. The average number of ticks on migratory birds entering GB each year (between 1st April and 31st July) will be determined from fieldwork studies (see Chapter 3).

2.3.2 The negative binomial distribution

Over-dispersion, where the variability in the dataset is greater than that predicted by the fitted distribution, is a common problem encountered when trying to fit the Poisson distribution to biological count datasets, such as tick counts on hosts. For example, over-dispersion can often occur when using the Poisson distribution to model data with a few really high counts and lots of zeros. As the Poisson distribution only has one parameter that defines both the mean and the variance of the distribution, the Poisson distribution may not provide a reasonable fit to such skewed data. In these situations, using a distribution with more than one parameter (to independently estimate the mean and variance) can provide a better fit. In the case of count data, such as tick counts on birds and horses, the negative binomial distribution (NBD) is often appropriate (Korch JR, 1994). In this thesis, the negative binomial distribution will be used to describe the number of ticks entering GB each year (specifically between 1st April and 31st July) on the total number of horses and on some bird species entering GB each year (between 1st April and 31st July).

2.3.3 The beta distribution

The beta distribution can be used to describe uncertainty associated with a probability, fraction or prevalence (Vose, 2008). It is used in this thesis to describe the variability in the probability of vertical transmission of CCHFV in ticks and in the probability that a bird will pass through a CCHFV-endemic country. Data on vertical transmission rates of CCHFV in ticks will be collected from the literature to quantify the number of ticks successfully being infected out of the total number of ticks studied. For the probability that a bird will pass through an endemic country, a success will be considered as a CCHFV-endemic country that the bird passes through from the total number of countries that are along the migration route.

2.3.4 The empirical distribution

In some cases it is not necessary to fit a distribution to the data, particularly if there are no biological reasons for using a certain distribution (Vose, 2008). This is the case for the data on the prevalence of CCHFV in ticks and on the number of eggs laid by female ticks. It was considered that the data available for these variables provided a random and representative set of observations. It was also considered that the variable would not take values that were lower or higher than those in the observed dataset. Therefore, an empirical distribution was constructed from the data and values from this distribution were sampled.

2.4 Spatial Modelling

Spatial analysis seeks to determine and understand the spatial distribution of a variable and is increasingly used for epidemiological studies. A Geographical Information System (GIS) is an appropriate tool to analyse and display spatial data (Graham et al., 2004). A GIS is defined as ‘a series of tools for the acquisition, storage, retrieval, analysis and display of spatial data’ (Graham et al., 2004). In the area of veterinary epidemiology, several previous studies have used spatial analysis and spatial modelling to predict future distributions of disease and disease vectors (Baylis et al., 2001, Brownstein et al., 2003, Diuk-Wasser et al., 2006).

For arthropod-borne diseases, risk is often associated with the density of the vector species. Spatial prediction and mapping of such densities is, thus, an important component of predicting risk and this approach has been used in several studies. Remotely sensed data such as NDVI, temperature, rainfall, humidity and elevation are factors which have been used to model and predict arthropod vector distributions (Baylis et al., 2001, Estrada-Peña, 2001). To predict the areas of the Mediterranean basin most at risk from Bluetongue virus (BTV), the abundance of the vector *Culicoides imicola*, a biting midge, was modelled (Baylis et al., 2001). The model was validated using known data on the distribution of *C. imicola*-borne diseases. Similarly, the abundance of several vector mosquito species were modelled in Connecticut, USA, to predict the risk of West Nile Virus (Diuk-Wasser et al., 2006). Both models used landscape variables derived from remotely sensed satellite sensor data. An alternative method uses interpolation to predict the distribution of a species based on spatial autocorrelation. Interpolation is a method of estimating an unknown quantity from adjacent known quantities. Ryan et al. (2004) used interpolation to predict the distribution of several species of mosquito in Queensland, Australia, and then validated the models with data from mosquitoes caught in light traps. They found that interpolation was a suitable method for predicting the spatial distribution of some species but not others as some had more random distributions with little spatial correlation. Brownstein et al. (2003) used landscape variables to predict the spatial distribution of the tick *Ixodes scapularis* Say whilst taking into account spatial autocorrelation. Spatial autocorrelation is the correlation among data from different locations, for example the probability of a tick being present in one area being correlated with tick presence in an adjacent area. Many studies do not take into account spatial autocorrelation (Baylis et al., 2001, Brewer et al., 2003, Estrada-Peña et al., 2012c) and it may be more relevant for vector species that move across greater distances than ticks are able to.

In this thesis, point data on the location of imported horses will be collected and used in the risk models (see Chapter 4). To estimate the density of horses per square kilometer of GB from the point data, kernel density estimation (KDE) will be used. KDE has previously been used to determine the home range of an animal when point location data are available (Powell, 2000). For example, KDE was used by Wood et al. (2000) to map the home ranges of albatrosses, as well as ascertaining where in their ranges foraging activities took place. Here, KDE smooths the data on imported horses allowing for movement of the horses around their post-import points of destination. The result is a map that shows the areas of GB where there are higher and lower densities of imported horses, which is more useful and realistic than the original spatially clustered point data.

The KDE method used in this thesis utilises the KDE tool in ESRI ArcGIS 10.0. This tool uses the quadratic kernel function as described in Silverman (1986) (ESRI ArcGIS). Data were collected over two years on the number of horses imported into GB and their end destinations. The data for both years show similar numbers of horses being imported into similar locations and, thus, the data can be said to exhibit ‘temporal stationarity’. Temporal stationarity is where the statistical properties of the data, such as the mean and total are constant (or very similar) over time (Nau, 2005).

2.5 Habitat Suitability

Habitat is the environment where an organism naturally occurs, providing the right conditions for breeding, feeding and surviving. The habitat requirements of a tick are a very important part of tick biology (Oliver, 1989). To predict the habitat suitability of an area for a particular tick species, the habitat characteristics must be quantified and then these can be extrapolated over a wider area (Estrada-Peña, 2001). Although ticks, as with all living organisms, have a niche and particular habitat preferences, it may be difficult to know which factors are correlative and which are causative. Further controlled experiments to test any causative hypotheses are often necessary (Cumming, 2002). In this thesis, two factors are considered to contribute to habitat suitability and these are climatic suitability and land cover suitability. Previous studies have used NDVI data to predict the suitable habitat of vectors, but in this thesis a combination of temperature data and land cover data is used.

2.5.1 Climatic suitability

A recent study has modelled the climatic requirements of *H. marginatum* based on empirical data (Estrada-Peña et al., 2011). Tick colonies were kept in a laboratory setting and data on rates of development and mortality were collected at different values of temperature ranging from 0 °C to 32 °C and values of water vapour deficit (derived from relative humidity) ranging from 2 hPa to 26 hPa. Equations were then derived that modelled the development and mortality of each life stage with temperature and water vapour deficit as variables (Estrada-Peña et al., 2011). These equations form the basis of the models in this study. Indeed, the population model derived by Estrada-Peña et al. (2011) has been used to assess the distribution of *H. marginatum* under current and future climate scenarios across the Mediterranean basin (Estrada-Peña et al., 2012c). More specifically, the equations developed by Estrada-Peña et al. (2011) were used to evaluate the environmental suitability of different regions of Spain for the survival of tick stages introduced by migratory birds (Bosch et al., 2012). It is in this capacity that they are used in this thesis to evaluate the temperature suitability for the survival of introduced *Hyalomma* spp. ticks in GB. As previously mentioned, it has been suggested that populations of *H. marginatum* are geographically constrained by temperature in the northern extent of their range (Gray et al., 2009). GB is further north than the current range extent of *H. marginatum* and, therefore, the limiting factor is assumed to be temperature.

Current and future temperature data were obtained for input into the equations developed by Estrada-Peña et al. (2011). Current temperature data had previously been obtained by AHVLA from the UK Meteorological Office for January 2007 to October 2010. Predicted temperature data for 2077 to 2080 were available for download from the British Atmospheric Data Centre (BADC). These had also been previously obtained by AHVLA.

2.5.2 Land cover suitability

The habitat preferences of *Hyalomma* spp. ticks can be approximated by land cover type, as well as temperature. The types of land cover where *Hyalomma* spp. ticks are found, particularly *H. marginatum*, will be investigated in Chapter 5. The geographical locations that are most suitable for the survival of *H. marginatum* within GB (based on the location of the preferred habitat of *H.* marginatum) will then be predicted. Land cover data have been produced for GB by the Centre for Ecology and Hydrology (CEH). The first digital land cover map for GB was produced in 1990 from satellite sensor imagery of GB (LCM1990). Land cover was divided into classes and for each 625 km^2 pixel of GB; a land cover class was assigned based on spectral characteristics (Fuller, 1995). In 2000, an updated map was produced (LCM2000) which included Northern Ireland. This map was also derived using satellite sensor imagery but used objects instead of regular pixels allowing for the irregular edges of land parcels, such as fields and lakes (Fuller et al., 2002). The land cover map used in Chapters 5 and 6 of this thesis, is the latest land cover map to be produced by CEH (LCM2007), released in July 2011. The new map offers greater accuracy than LCM2000 through the use of digital cartography, as well as using more up-to-date satellite sensor imagery (Morton et al., 2011). The land cover classifications used in LCM2007 are the UK Broad Habitats identified by the UK Biodiversity Group and described by the Joint Nature Conservation Committee (Morton et al., 2011). LCM2007 was available as a raster dataset at a spatial resolution of 1 km^2 and had previously been obtained by AHVLA.

There is no available GB land cover map for the future. Therefore, to predict the future land cover suitability for *Hyalomma* spp. ticks in GB, a future land cover map was created under a scenario of increased urbanisation and wetland expansion (see Chapter 6). This utilised a map created by Wetland Vision, which showed the extent of proposed wetlands for England in 50 years’ time. This is available from the Wetland Vision website (Wetland Vision) as an image within a Portable Document Format (PDF) file. It was converted to an image file (JPEG) which could then be imported into ESRI ArcGIS 10.0. A technique called georeferencing was used to translate the image onto a map of GB. ERSI ArcGIS 10.0 has a georeferencing tool that allows specific locations of the image to be matched to corresponding locations on a map of GB (Figure 2.1). Once georeferenced, the image was exported as a raster and could then be used to predict wetland expansion for the final future land cover map.

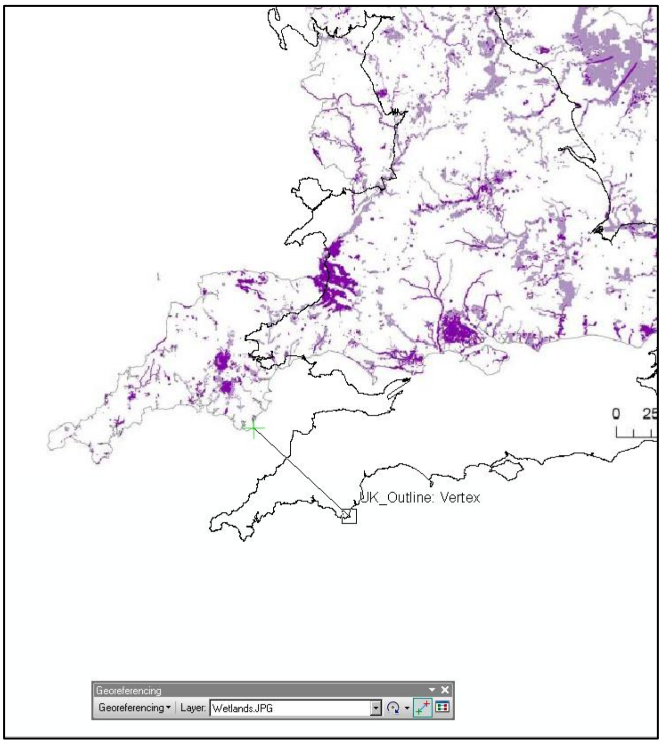


Figure 2.1: Georeferencing in ArcGIS 10.0 of the wetland vision image file.

3 Introduction of CCHFV-infected ticks into GB by migratory birds

3.1 Introduction

Migratory birds, together with small mammals are hosts for immature *Hyalomma* spp. ticks, particularly *H. marginatum* and *H. rufipes* (Hoogstraal, 1972). *Hyalomma* spp. ticks are two-host ticks (Ergönül and Whitehouse, 2007) and the larvae moult into nymphs while attached to the bird such that total time of host attachment is in the range of 12 to 26 days (Hueli, 1979). The long duration of host attachment enables immature *Hyalomma* spp. ticks and their associated pathogens to be carried across borders by migrating birds during the spring and autumn migration (Hoogstraal et al., 1961, Molin et al., 2011, Jameson et al., 2012a). Indeed, based on the duration of spring migration for some bird species, it is feasible for ticks to be transported from sub-Saharan Africa into northern Europe including GB. Recently Molin et al. (2011) recorded 367 *Hyalomma* spp. ticks on 7,453 spring migrants along the Central and Eastern Mediterranean flyways, specifically on migration stop-over points in Italy and Greece.

Many British breeding migrant birds overwinter in sub-Saharan Africa and, therefore, have the potential to introduce CCHFV-infected *Hyalomma* spp. ticks at breeding sites or *en route* during migration along the East Atlantic flyway. Ticks of the genus *Hyalomma* have been recorded on spring migrants trapped in the UK and Germany, but the origin of these ticks is unknown (Martyn, 1988, Rumer et al., 2011, Jameson et al., 2012a). Many migrants that breed in Western Europe and GB fly across the Strait of Gibraltar from Africa in the spring. Since *H. marginatum* is also endemic in Spain, Portugal and southern France (Maltezou and Papa, 2010), migrant birds may pick up ticks of this species at staging posts along the migration flyway in Europe in addition to in the wintering grounds in sub-Saharan Africa. The majority of GB migrants use oases in North Africa as staging posts after crossing the Sahara and then fly direct from Africa to GB (Banham, R., personal communication). However, some birds also stop-over in southern Spain after crossing the Strait of

Gibraltar, with bad weather sometimes causing a land fall of migrants (Banham, R., personal communication).

CCHFV has not yet been found in *H. marginatum* ticks in Spain, Portugal and southern France, but recently CCHFV was found in Spain in populations of *Hyalomma lusitanicum* (Estrada-Peña et al., 2012b). *H. lusitanicum* ticks do not parasitize birds or other migratory animals and instead feed on domestic and wild ungulates, rabbits, hares and hedgehogs. CCHFV may have entered Spain through the importation of infected domestic ungulates before being transmitted to local populations of *H. lusitanicum*. Alternatively, an infected *H. marginatum* tick may have been carried into Spain on a migratory bird. If this tick survived, the CCHF virus may have been transmitted to local populations of *H. marginatum* which, as adults, feed on similar host species to *H. lusitanicum.* The CCHF virus could, therefore, have been transmitted from *H. marginatum* to *H. lusitanicum* through feeding on the same individual host. In either case, it seems likely that the virus would also now be circulating in Spanish populations of *H. marginatum* but surveys to test this have not yet been carried out. The status of CCHFV in Spain and within which tick species it may be circulating is an important consideration in assessing the risk of introduction of CCHFV to GB. If the virus is circulating in *H. marginatum* ticks in Spain, migrant birds stopping over in Spain could pick up infected ticks and, thus, introduce the virus into GB either at the breeding sites or at migration stop-over points. As *Hyalomma* spp. are two-host ticks, they would detach from migratory birds in GB as fed nymphs.

Migratory birds as a route of introduction of CCHFV-infected *Hyalomma* spp. nymphs into GB was, therefore, investigated. The aim of this chapter was to predict the number of CCHFV-infected *Hyalomma* spp. nymphs entering GB in the spring of each year on migratory birds. To do this, the *Hyalomma* spp. tick infestation rate of migrant birds along the East Atlantic flyway (the migration route used by African-wintering GB migrants) was established by trapping migratory birds on their northward migration in Spain and checking them for ticks. These data, together with data previously collected from migratory birds in GB (Jameson et al., 2012a) were then used to estimate the number of *Hyalomma* spp. nymphs entering GB each year on the British population of five migrant bird species that breed in GB. Mathematical distributions were used to model the number of *Hyalomma* spp. ticks on each bird species. Thus, a stochastic model was constructed to predict the number of CCHFV-infected *Hyalomma* spp. nymphs entering GB each spring on each of the five bird species using data on the CCHFV-infection rate in populations of *Hyalomma* spp. ticks in CCHFV-endemic countries.

3.2 Method

It was considered that *H. marginatum* and *H. rufipes* were the most likely species of *Hyalomma* to be carried into GB on migratory birds through the East Atlantic flyway. Hereafter in this chapter, '*Hyalomma* spp.' refers to these two species of tick, unless otherwise specified. The number of CCHFV-infected *Hyalomma* spp. nymphs entering GB per year on each of the five chosen bird species ($\mathrm{n}_{\mathrm{infb}}$) is given by Equation (3.1):

$$n_{\mathrm{infb}} = n_{\mathrm{hyalomma}} \mathrm{\ x\ } p_{\mathrm{inf\_nymph}} \qquad (3.1)$$

where n_{hyalomma} is the number of *Hyalomma* spp. nymphs entering GB on migratory birds and $p_{\mathrm{inf\_nymph}}$ is the probability of CCHFV-infection in *Hyalomma* spp. nymphs on birds along the East Atlantic flyway (see also Table 3.2). For each of the five bird species to be studied, a Monte Carlo simulation was run using Equation (3.1) with 10,000 realisations using MATLAB R2012a. From the simulation, the mean number of CCHFV-infected *Hyalomma* spp. ticks imported into GB on each bird species (n_{infb}) was calculated. The 50th, 95th and 99th percentiles of n_{infb} were also determined for each species.

Figure 3.1 illustrates the route of introduction of CCHFV-infected nymphs into GB by migratory birds and the parameters for estimation. The method by which estimates were made for each parameter is now described.

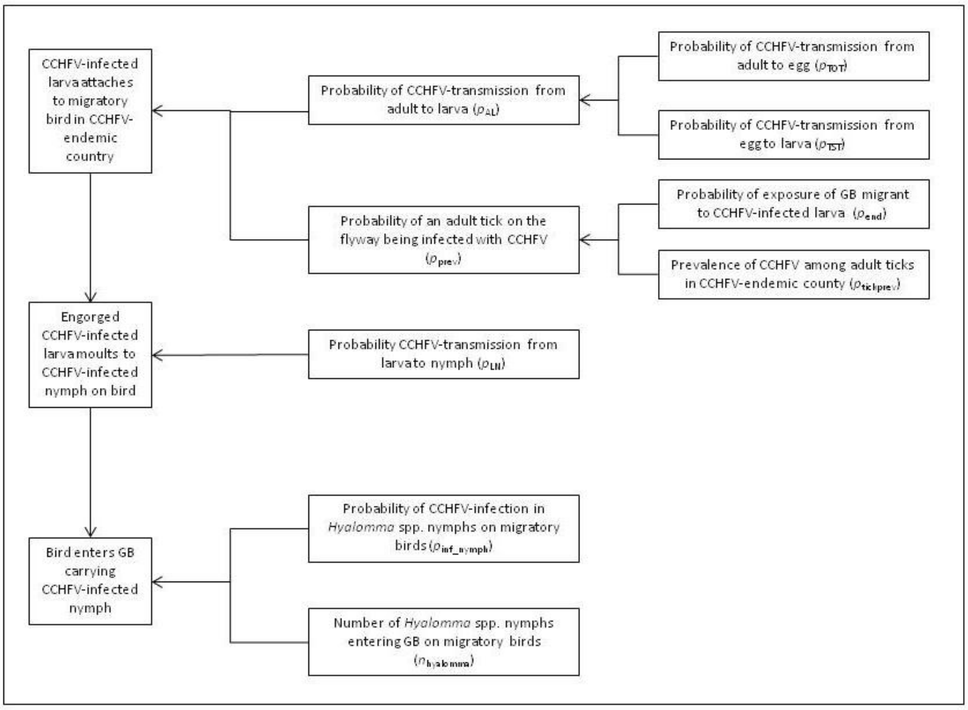


Figure 3.1: The route of introduction of CCHFV-infected *Hyalomma* spp. nymphs into GB by migratory birds and the parameters for estimation.

3.2.1 Estimation of the number of *Hyalomma* spp. nymphs entering GB on migratory birds

To estimate the number of *Hyalomma* spp. nymphs entering GB each year on migrant birds, information was needed on the number of birds of each species and their respective infestation rates (Gale et al., 2012). Fieldwork was carried out in Spain in spring 2011 and 2012 to determine the *Hyalomma* spp. infestation rate of migratory birds along the East Atlantic flyway. It was assumed that the ticks present on migrants in Spain would not detach prior to reaching GB. Five bird species were chosen as the most likely to bring *Hyalomma* spp. ticks into GB and, thus, the number of *Hyalomma* spp. ticks brought into GB by these birds each year was estimated. These five birds were selected on the basis of the empirical data from the fieldwork carried out in Spain and also from data presented by Jameson et al. (2012a) where birds were trapped and checked for ticks on the south coast of GB during the spring migration. For each of the chosen five species, the data collected on the *Hyalomma* spp. infestation rates for both years of this study (2011 and 2012) and from the study by Jameson et al. (2012a) were pooled as data on the *Hyalomma* spp. infestation rate of migrants using the East Atlantic flyway are scarce. The pooled

Hyalomma spp. infestation rate for each bird species was assumed to represent the infestation rate for the total breeding population in GB of each of the five selected bird species. Data on the GB breeding population of each of these bird species were obtained from Robinson (2005).

Statistical distributions were fitted to the data for each bird species using maximum likelihood estimation (MLE) using MATLAB R2012a. Initially, a negative binomial distribution (NBD) was chosen and in the case where this was not appropriate (when the mean was equal to or greater than the variance), a Poisson distribution was fitted. The goodness-of-fit of each distribution was calculated using the negative log-likelihood using MATLAB R2012a. For each bird species, 10,000 realisations were generated randomly from the fitted distributions using MATLAB R2012a and from these the mean number of *Hyalomma* spp. ticks imported each year on the GB population for each of the five bird species (n_{hyalomma}) was estimated. The 50th, 95th and 99th percentiles of n_{hyalomma} were also determined for each species.

The fieldwork carried out in Spain to determine *Hyalomma* spp. infestation rates on migratory birds along the East Atlantic flyway is now described.

3.2.1.1 Trapping of migratory birds

Birds were captured in mist nets at 10 different locations throughout the provinces of Cadiz and Malaga, southern Spain between 23rd April and 15th May 2011 and between 22nd April and 16th May 2012. The last two weeks of April and the first two weeks of May are the most important for migrant birds introducing *Hyalomma* spp. ticks into central Spain (Bosch et al., 2012). The provinces of Malaga and Cadiz are on the East Atlantic flyway which is the flyway that is used by GB migrants who overwinter in Africa. Therefore, migrants trapped in southern Spain can be used to estimate the tick load of birds coming into Europe which have overwintered in Africa. The 10 locations chosen were existing bird ringing sites regularly used by the local bird ringing group and represented different habitat types including grassland, woodland, riverine and coastal habitats (Table 3.1 and Figure 3.2). Birds were captured between 0600h-1300h and 1600h-1800h. Each captured bird was identified to species and the ears, eyes, beak, gape, nape and abdomen of each bird were checked for ticks (as in Molin et al., 2011).

Table 3.1: Localities of field sites used in southern Spain, spring 2011 and 2012.

Site code	Site name	Grid reference	Height ASL	Habitat
A2	Llanos de Tabanos	36°20’35.92"N 05°16´26.00"W	168m	Heathland
A3	Algeciras	36°07’46.47"N 05°26’54.60"W	24m	Urban
G1	Patera Guadacorte	36°11´53.98"N 05°26´04.22"W	48m	Forest
H1	Finca El Hornillo Rio Genal	36°30´08.22"N 05°17´13.83"W	108m	Low woodland and orchard
H2	Rio Hozgarganta	36°23’57.81”N 05°26’35.68”W	28m	Orchard and river
N1	Los Quejigales Sierra de las Nieves	36°41´29.07"N 05°02´39.87"W	1294m	Woodland and grassland
O1	Huerto de Guadadorte	36°12’09.03”N 05°25’51.67”W	14m	Citrus orchard
P1	Palmones	36°10’02.71”N 5°26’21.76”W	2m	Grassland and sand dunes
R1	Junta de los Rios	36°24´03.51"N 05°19´20.16"W	18m	Riverine reed beds and grassland
R2	Guadiaro estuary and Reserve Sotogrande	36°16´52.13"N 05°16´48.43"W	0m	Coastal reed beds

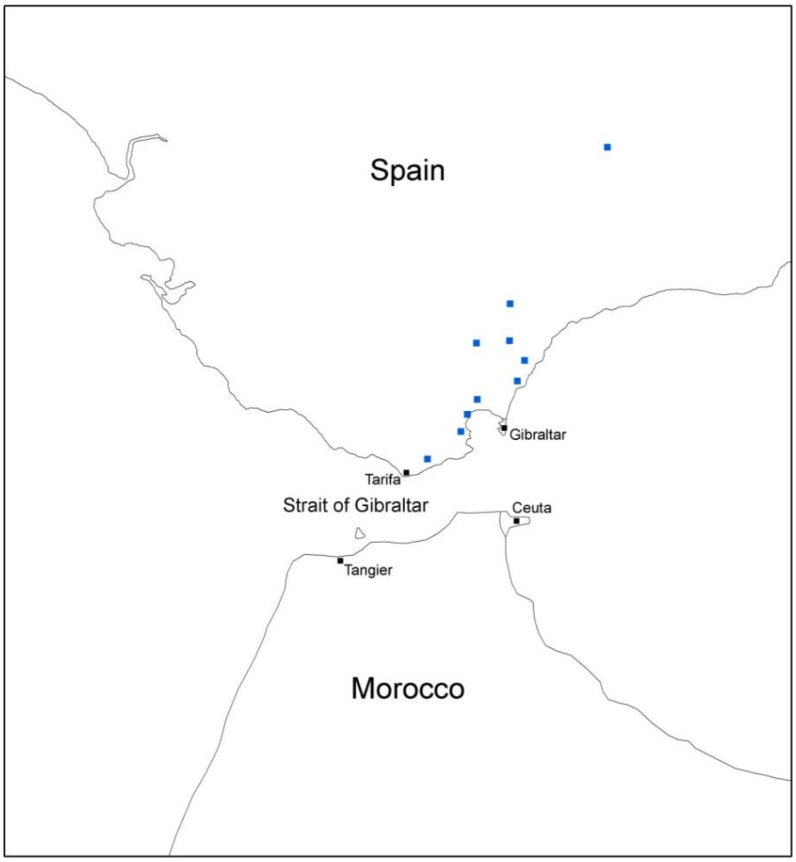


Figure 3.2: Locations in Spain where birds were surveyed for ticks in spring 2011 and 2012, denoted by the blue symbol.

3.2.1.2 Collection and analysis of ticks

Any tick observed was removed with tweezers and placed immediately into tick fixative consisting of 70% ethanol, 20% distilled water and 10% glycerol. Ticks were stored separately according to the individual birds from which they were removed. The ticks were aged and identified to genus morphologically using the method of Estrada-Peña et al. (2004). As *Hyalomma* was the genus of interest in this study, all *Hyalomma* spp. nymphs and larvae were then identified morphologically to species (identification provided by A. Bouattour, Institut Pasteur, Tunis), but facilities for molecular confirmation were not available. Immature *Hyalomma* spp. ticks were tested for CCHFV by Barry Atkinson at Public Health England, Porton Down, UK, using the RT-PCR developed by Atkinson et al. (2012) using a SuperScript III (SSIII) Platinum One-step qRT-PCR kit (Invitrogen). Birds were categorised as migrant or resident on the basis of species, or wing length in the case of *Sylvia atricapilla* Linnaeus (blackcap).

In southern Spain, some populations of *S. atricapilla* are migratory and some are resident. The individual birds can be distinguished by wing length, as those with a wing length between 66-72 mm are residents and those with a wing length between 72-80 mm are migrants (Banham, R. personal communication).

3.2.1.3 Statistical analysis of tick data

The number of migrant birds found to be carrying *Hyalomma* spp. ticks was calculated as a percentage of the total number of migrants trapped for each year (the *Hyalomma* spp. infestation rate). This was compared with that of Molin et al. (2011) using a Fisher’s exact test to see if any observed differences between the *Hyalomma* spp. infestation rate along the East Atlantic flyway and the Central and Eastern Mediterranean flyways were statistically significant at the 5% level. A higher rate along one flyway could be indicative of more CCHFV-infected *Hyalomma* spp. ticks were being carried into certain parts of Europe compared to others. The *Hyalomma* spp. infestation rate for migrants is not given in Molin et al. (2011) and, therefore, this had to be calculated from an estimate of the number of birds carrying *Hyalomma* spp. ticks using the average number of *Hyalomma* spp. ticks per bird.

3.2.2 Estimation of the probability of CCHFV-infection in *Hyalomma* spp. nymphs along the East Atlantic flyway

The probability of CCHFV-infection in imported nymphs was estimated using data on the CCHFV-prevalence in tick populations gathered from the literature. Several studies have been carried out in CCHFV-endemic areas, including Kenya (Sang et al., 2011), Bulgaria (Gergova et al., 2012), Turkey (Tonbak et al., 2006, Albayrak et al., 2010a, Albayrak et al., 2010b, Gargili et al., 2011, Gunes et al., 2011, Tekin et al., 2012), Russia (Yashina et al., 2003), Kosovo (Avsic-Zupanc, 2007), Iran (Fakoorziba et al., 2012) and Oman (Williams et al., 2000) to determine the CCHFV-infection prevalence in *Hyalomma* spp. populations in countries where CCHFV is endemic. Data on prevalence in adult *H. marginatum* and *H. rufipes* were collected from the literature, but data were excluded from studies where the *Hyalomma* spp. tested were not specified. Data were available on CCHFV-prevalence for other specified *Hyalomma* spp. (*Hyalomma aegyptium* Linnaeus*, H. anatolicum, H. asiaticum, Hyalomma detritum* Schulze*, H. dromedarii, Hyalomma excavatum* Koch*, Hyalomma* *truncatum* Koch and *Hyalomma turanicum* Pomerantsev) but these were also excluded as these species either do not parasitize birds or do not occur in countries along the East Atlantic flyway. The data on CCHFV-prevalence for adult *H. marginatum* and *H. rufipes* were collated from CCHFV-endemic countries (p_{tickprev}). These values were considered to be equally likely to occur and were assumed to represent the variation in prevalence rates within and between countries and tick populations. Thus, these data were described by a discrete uniform distribution (an empirical distribution).

Not all countries along the East Atlantic flyway used by GB migrants for over-wintering or as staging posts are CCHFV-endemic. Here, it is assumed that the CCHFV-prevalence for adult *Hyalomma* spp. ticks on the flyway (p_{prev}) reflects the proportion of countries (that the birds would pass through or overwinter in) which are CCHFV-endemic (p_{end}). Thus:

$$p_{\text{prev}} = p_{\text{tickprev}} \times p_{\text{end}} \qquad (3.2)$$

The number of CCHFV-endemic countries that the five selected bird species would pass through or overwinter in, as a proportion of all countries that they would pass through or overwinter in, was estimated for p_{end}. A beta distribution was fitted to the data for p_{end} using MATLAB R2012a.

Hyalomma spp. ticks attach to birds during the larval stage of their life cycle and, therefore, the infection rate for larvae in CCHFV-endemic countries had to be estimated from the infection rate for adults. It was assumed that infection of larvae would occur by vertical transmission from adult ticks. Data on the vertical transmission of CCHFV from adults to eggs and from eggs to larvae were obtained from the literature to estimate of the probability that a larval *Hyalomma* spp. tick is infected with CCHFV given that the adult female tick is infected (p_{AL} for adult to larval transmission). This is based on trans-ovarial transmission (p_{TOT}) and trans-stadial transmission from egg to larvae (p_{TST}). The parameter, p_{AL} is a conditional probability (i.e. given the adult female *Hyalomma* spp. tick is infected with CCHFV) and is given by Equation (3.3):

$$p_{\text{AL}} = p_{\text{TOT}} \times p_{\text{TST}} \qquad (3.3)$$

As larval *Hyalomma* spp. ticks moult to nymphs on the host, vertical transmission from larval to nymphal stages was also included. Therefore, data on CCHFV-transmission between *Hyalomma* spp. larvae and nymphs were obtained from the literature to estimate the probability that a nymph is infected with CCHFV after moulting from a CCHFV-infected larva (p_{LN}). It was assumed that all ticks that attached to birds as larvae successfully moulted to nymphs whilst on the bird. Beta distributions were fitted to the data for each of the variables, p_{TOT}, p_{TST} and p_{LN}. The probability of a nymphal *Hyalomma* spp. tick being infected with CCHFV along the East Atlantic flyway ($p_{\mathrm{inf\_nymph}}$) was estimated by Equation (3.4):

$$p_{\mathrm{inf\_nymph}} = p_{\mathrm{prev}} \times p_{\mathrm{AL}} \times p_{\mathrm{LN}} \qquad (3.4)$$

The parameters used in Equation (3.1), (3.2), (3.3) and (3.4) are described in Table 3.2.

Table 3.2: Summary of parameters for estimation of the number of CCHFV-infected ticks imported into GB by migratory birds in spring each year (n_{infb}).

Parameter	Description
n_{hyalomma}	Predicted number of *Hyalomma* spp. ticks imported into GB each year in spring on migratory birds.
p_{tickprev}	Prevalence of CCHFV among adult *Hyalomma* spp. ticks in CCHFV-endemic countries.
p_{end}	Probability of exposure of GB migrants to CCHFV-infected ticks along the East Atlantic flyway.
p_{prev}	Probability that an adult *Hyalomma* spp. tick on the flyway would be infected with CCHFV.
p_{TOT}	Probability of CCHFV-transmission from adult ticks to eggs.
p_{TST}	Probability of CCHFV-transmission from eggs to larvae.
p_{AL}	Probability of CCHFV-transmission from adult ticks to larval offspring.
p_{LN}	Probability of CCHFV-transmission from larva to nymph.
$p_{\mathrm{inf\_nymph}}$	Probability of a nymphal *Hyalomma* spp. tick being infected with CCHFV along the East Atlantic flyway.
n_{infb}	Number of CCHFV-infected immature *Hyalomma* spp. ticks entering GB on the five chosen migratory bird species each year.

3.3 Results

3.3.1 Results of study of ticks on migratory birds in Spain, spring 2011

A total of 531 birds were caught in mist nets and checked for ticks. A total of 86 ticks were collected from 31 individual birds (5.83% of birds caught), with an average tick load of 2.77 ticks per infested bird. The majority of the ticks found (*n*=84) were larvae and nymphs of *Ixodes* spp. (Table 3.3). Of the birds caught, 202 were migrants and one of these, a single *Iduna opaca* Cabanis (western olivaceous warbler) carried two nymphal *H. marginatum* ticks (Figure 3.3 and Figure 3.4) giving a *Hyalomma* spp. infestation rate of 0.5% for migrants (with 95% binomial confidence interval of 0.01% - 2.73%).

A Fisher’s exact test was applied to determine if this percentage was significantly less than the number of *Hyalomma* spp. ticks found on migrant birds in Molin et al. (2011) migrating along the Central and Eastern Mediterranean flyways. Molin et al. (2011) found 386 ticks on 200 of 7,453 migrants checked, giving an average of 1.93 ticks per infested bird. Of the ticks found, 367 were *Hyalomma* spp. which, using the average of 1.93 ticks per bird, gives 190 birds infested with *Hyalomma* spp. ticks. The percentage of birds carrying *Hyalomma* spp. ticks was therefore calculated as 2.55% (190 of 7,453 migrants with 95% binomial confidence interval of 2.20% to 2.93%).

The result of the Fisher’s exact test was p = 0.0653 which shows that the difference along the two flyways was significant at 10% but not at the 5% level (Table 3.4). The total number of ticks found on migrant bird species was five, compared to 81 found on resident bird species and all ticks found were immatures (43 nymphs, 41 larvae) with the exception of two ticks that it was not possible to age due to damage during removal. The result of the RT-PCR test on the two *Hyalomma* spp. nymphs showed them to be negative for CCHFV.

Table 3.3: Bird species trapped during springtime migration 2011 that were infested with ticks. An additional 289 birds of 40 different species were also caught but none were found to be infested with ticks.

Species	Family	Resident (R) or Migrant (M) species	No. of birds	No. of ticks	No. of birds infested	Mean no. of ticks /infested bird	Mean no. larvae /infested bird	Mean no. of nymphs /infested bird	Genus of ticks
Carduelis chloris	Fringillidae	R	84	26	13	2	1.08	0.85	*Ixodes*
Erithacus rubecula	Muscicapidae	R	9	1	1	1	1	0	*Ixodes*
Iduna opaca	Acrocephalidae	M	8	2	1	2	0	2	*Hyalomma*
Luscinia megarhynchos	Muscicapidae	M	50	2	2	1	0.5	0.5	*Ixodes*
Parus major	Paridae	R	10	6	3	2	0.67	1.33	*Ixodes*
Serinus serinus	Fringillidae	R	47	1	1	1	0	1	*Ixodes*
Sylvia atricapilla	Sylviidae	M	11	1	1	1	0	1	*Ixodes*
Turdus merula	Turdidae	R	23	47	9	5.22	2.56	2.56	*Ixodes*

Table 3.4: A 2 x 2 contingency table showing the results of this study in 2011, the results from Molin et al. (2011) and the *p* value from the Fisher's exact test

Dataset	Number of birds infested with *Hyalomma* spp. ticks	Number of birds not infested with *Hyalomma* spp. ticks	Total number of migrants trapped	*p* value
2011	1	201	202	
Molin et al. (2011)	190	7263	7453	
Total	191	7464	7655	0.0653

Figure 3.3: *Iduna opaca* (western olivaceous warbler) with *H. marginatum* attached around the eye. Photo taken by M. England in Spain, 2011.

Figure 3.4: *Hyalomma* spp. ticks that were removed and stored in labeled tubes. Photo taken by M. England in Spain, 2011.

3.3.2 Results of study of ticks on migratory birds in Spain, spring 2012

A total of 564 birds were caught in mist nets and checked for ticks. A total of 65 ticks were collected from 26 individual birds (4.61% of birds caught), with an average tick load of 2.5 ticks per infested bird. As in 2011, the majority of the ticks found (*n*=52) were larvae and nymphs of *Ixodes* spp. (Table 3.5) although an adult female *Ixodes acuminatus* was found on a *Turdus merula* Linnaeus (blackbird). Of the birds caught, 228 were migrants of which five birds were infested with a total of 11 *H. marginatum* nymphs and one *H. marginatum* larva giving a *H. marginatum* infestation rate of 2.19% (with 95% binomial confidence interval of 0.72% - 5.04%). The birds that carried *H. marginatum* ticks were

Acrocephalus schoenobaenus Linnaeus (sedge warbler), *Sylvia communis* Latham (whitethroat), *I. opaca* and *Phylloscopus trochilus* Linnaeus (willow warbler). When this percentage was compared to the percentage for 2011 and to the percentage found on the Central and Eastern Mediterranean flyways (Molin et al., 2011) using a Fisher's exact test, the results were found to be p = 0.2206 and p = 0.8306 respectively (Table 3.6 and Table 3.7). Therefore, the difference in the *Hyalomma* spp. infestation rate between 2011 and 2012 and between 2012 and that of Molin et al. (2011) was found to be not significant at the 5% level. The number of ticks found on migrant bird species was 13 compared to 52 ticks found on resident bird species. Once again, the result of the RT-PCR test on the *H. marginatum* ticks showed them to be negative for CCHFV.

Table 3.5: Bird species trapped during springtime migration 2012 that were infested with ticks. An additional 538 birds of 42 different species were also caught but none were found to be infested with ticks.

Species	Family	Resident (R) or Migrant (M) species	No. of birds	No. of ticks	No. of birds infested	Mean no. of ticks /infested bird	Mean no. of larvae /infested bird	Mean no. of nymphs /infested bird	Mean no. of adults /infested bird	Genus of ticks
Acrocephalus schoenobaenus	Acrocephalidae	M	8	5	1	5	0	5	0	*Hyalomma*
Carduelis carduelis	Fringillidae	R	22	1	1	1	0	1	0	*Ixodes*
Carduelis chloris	Fringillidae	R	36	31	10	3.1	0.2	2.9	0	*Ixodes*
Fringilla coelebs	Fringillidae	R	10	1	1	1	0	1	0	*Ixodes*
Iduna opaca	Acrocephalidae	M	8	1	1	1	0	1	0	*Hyalomma*
Luscinia megarhynchos	Muscicapidae	M	43	1	1	1	1	0	0	*Ixodes*
Parus major	Paridae	R	7	1	1	1	0	1	0	*Ixodes*
Phylloscopus trochilus	Phylloscopidae	M	10	1	1	1	1	0	0	*Hyalomma*
Serinus serinus	Fringillidae	R	48	3	3	1	0	1	0	*Ixodes*
Sylvia communis	Sylviidae	M	12	5	2	2.5	0	2.5	0	*Hyalomma*
Turdus merula	Turdidae	R	31	15	4	3.75	0.5	3	0.25	*Ixodes*

Table 3.6: A 2 x 2 contingency table showing 2011 and 2012 results of this study and the *p* value from the Fisher's exact test

Dataset	Number of birds infested with *Hyalomma* spp. ticks	Number of birds not infested with *Hyalomma* spp. ticks	Total number of migrants trapped	*p* value
2011	1	201	202	
2012	5	223	228	
Total	6	424	430	0.2206

Table 3.7: A 2 x 2 contingency table showing 2012 results of this study, the results from Molin et al. (2011) and the *p* value from the Fisher's exact test

Dataset	Number of birds infested with *Hyalomma* spp. ticks	Number of birds not infested with *Hyalomma* spp. ticks	Total number of migrants trapped	*p* value
2012	5	223	228	
Molin et al. (2011)	190	7263	7453	
Total	195	7486	7681	0.8306

3.3.3 Selection of bird species for modelling the number of CCHFV-infected nymphs entering GB

The five most likely candidate bird species for bringing *Hyalomma* spp. into GB were chosen as *A. schoenobaenus, Oenanthe oenanthe* Linnaeus (northern wheatear)*, Phoenicurus phoenicurus* Linnaeus (redstart)*, P. trochilus* and *S. communis*. From the results of the fieldwork in Spain (Table 3.3 and Table 3.5), *P. trochilus*, *A. schoenobaenus* and *S. communis* were all found to be carrying *H. marginatum* ticks and were, therefore, chosen, but *I. opaca* which was also found to be carrying *H. marginatum* ticks was excluded as it does not breed in GB. A study conducted by Jameson et al. (2012a) where birds were trapped and checked for ticks on the south coast of GB during the spring migration also found *H. marginatum* ticks on *A. schoenobaenus* and *S. communis*, and additionally on *O. oenanthe* and *P. phoenicurus*. All empirical data were combined and are given in Table 3.8.

Table 3.8: Collated empirical data from the studies described here in Spain (Table 3.3 and Table 3.5) and from Jameson et al. (2012) in GB for each of the five chosen bird species.

Species	No. of birds trapped	No. of birds infested	No. of *Hyalomma* spp. ticks found	Mean no. of *Hyalomma* spp. ticks per bird
A. schoenobaenus	20	2	6	0.30
O. oenanthe	51	2	8	0.16
P. phoenicurus	30	1	1	0.033
P. trochilus	553	1	1	0.0018
S. communis	73	4	9	0.12

3.3.4 Estimation of the number of *Hyalomma* spp. nymphs entering GB on birds

The distribution of *Hyalomma* spp. ticks on individual birds for *A. schoenobaenus*, *O. oenanthe* and *S. communis* were best described by a negative binomial distribution (NBD). The Poisson distribution was also fitted to the data for tick counts per bird for these three bird species and a goodness-of-fit test (negative log-likelihood) was carried out to see which distribution was more appropriate. In each case, the negative log-likelihood was lower when an NBD was fitted than when a Poisson distribution was fitted, showing that the NDB was a better fit to the data (Table 3.9). A Poisson distribution was fitted to the data for *P. phoenicurus* and *P. trochilus* as for these variables the mean was equal to or greater than the variance such that MLE for the dispersion parameter ceased to exist when fitting an NBD (Al-Khasawneh, 2010). The parameter values of the fitted distributions, including the British breeding population of each bird species (Robinson, 2005) and the negative log-likelihood for different distributions fitted are shown in Table 3.9. The probability mass functions for the fitted distributions for each bird species are plotted in Figure 3.5 to Figure 3.9.

Table 3.9: The fitted distributions for the number of *Hyalomma* spp. nymphs on each bird species, the parameter estimations and the negative log-likelihood values (NLogL). The value of *n* was given by the British breeding population of each bird species (Robinson, 2005).

Bird species	Fitted distribution	MLE parameter values		Negative log likelihood	
A. schoenobaenus	Negative binomial	$r_{A.scho}$	0.0583	NLogL negbin	10.3575
		$p_{A.scho}$	0.1627	NLogL Poisson	18.0113
		$n_{A.scho}$	594,000		
O. oenanthe	Negative binomial	$r_{O.oena}$	0.0176	NLogL negbin	13.6348
		$p_{O.oena}$	0.1011	NLogL Poisson	29.3983
		$n_{O.oena}$	540,000		
P. phoenicurus	Poisson	$\lambda_{P.phoe}$	0.0323	NLogL Poisson	4.434
		$n_{P.phoe}$	202,000		
P. trochilus	Poisson	$\lambda_{P.troc}$	0.0018	NLogL Poisson	7.3154
		$n_{P.troc}$	4,000,000		
S. communis	Negative binomial	$r_{S.comm}$	0.0409	NLogL negbin	22.1332
		$p_{S.comm}$	0.2493	NLogL Poisson	32.4035
		$n_{S.comm}$	1,862,000		

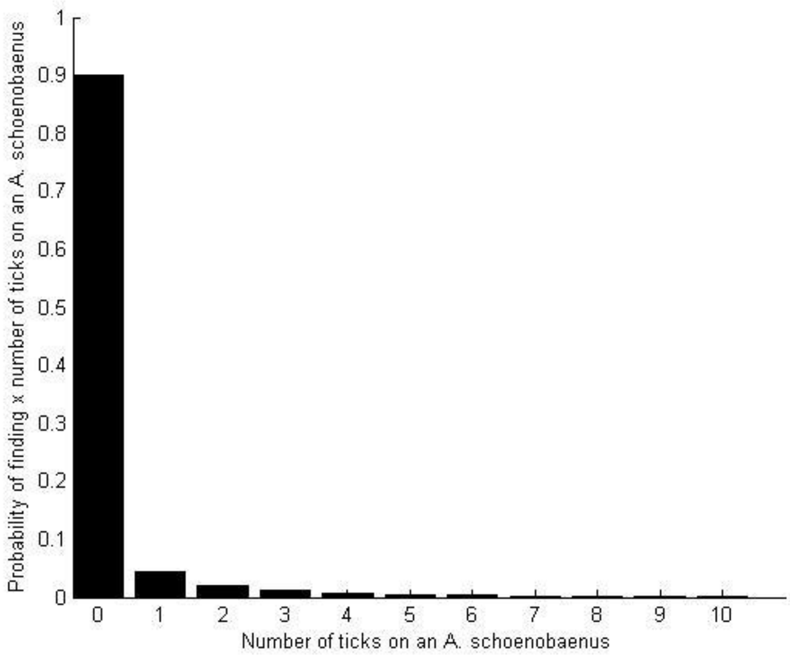


Figure 3.5: Probability mass function of the number of *Hyalomma* spp. ticks on an individual *A. schoenobaenus*.

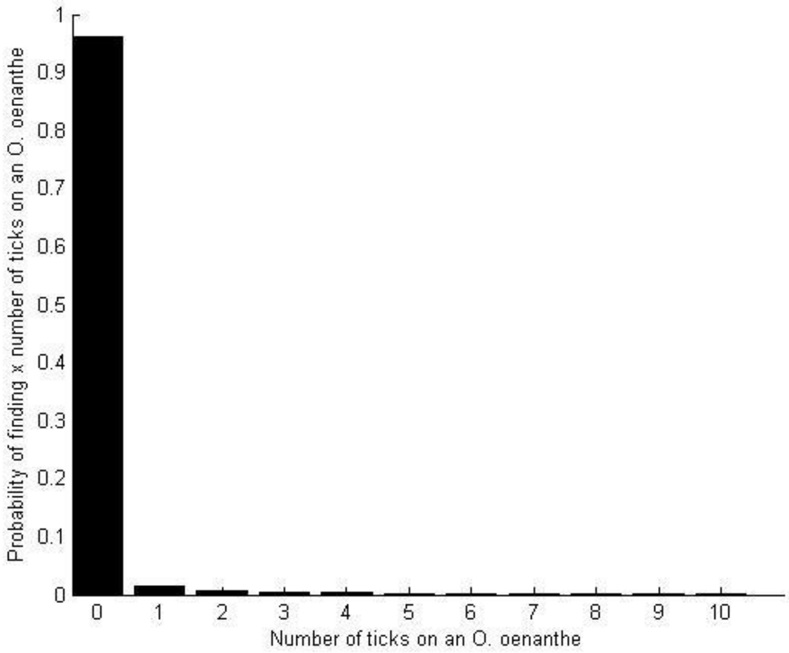


Figure 3.6: Probability mass function of the number of *Hyalomma* spp. ticks on an individual *O. oenanthe.*

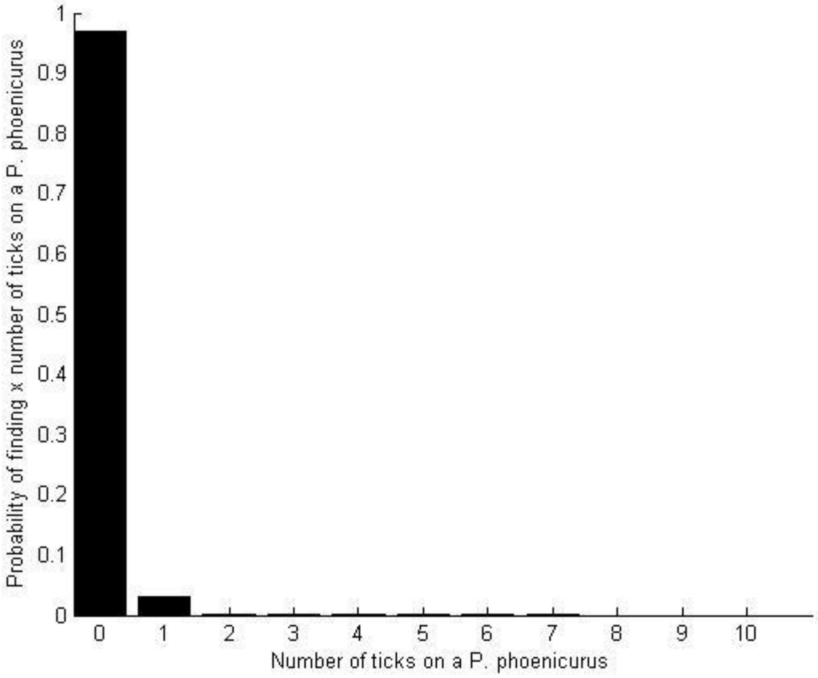


Figure 3.7: Probability mass function of the number of *Hyalomma* spp. ticks on an individual *P. phoenicurus.*

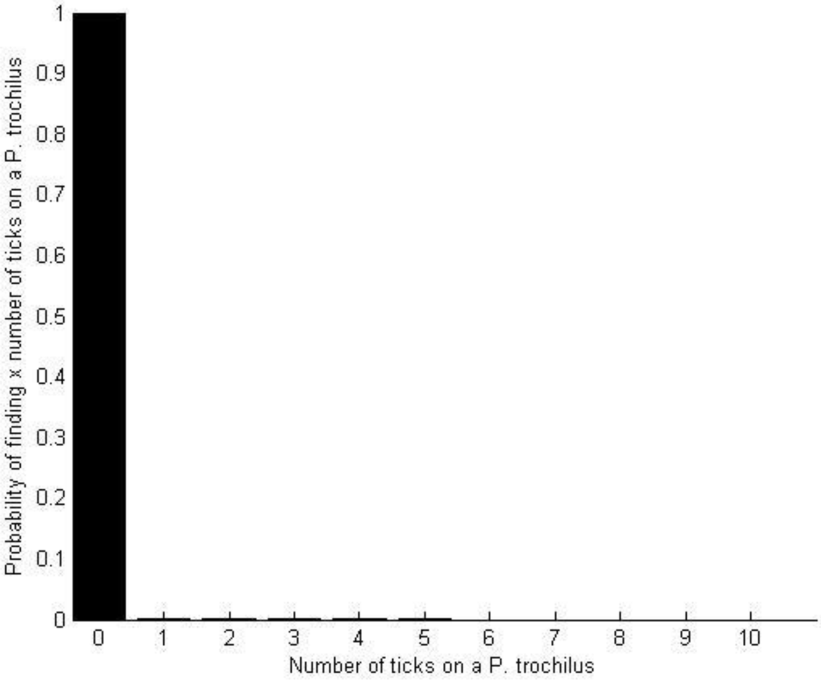


Figure 3.8: Probability mass function of the number of *Hyalomma* spp. ticks on an individual *P. trochilus.*

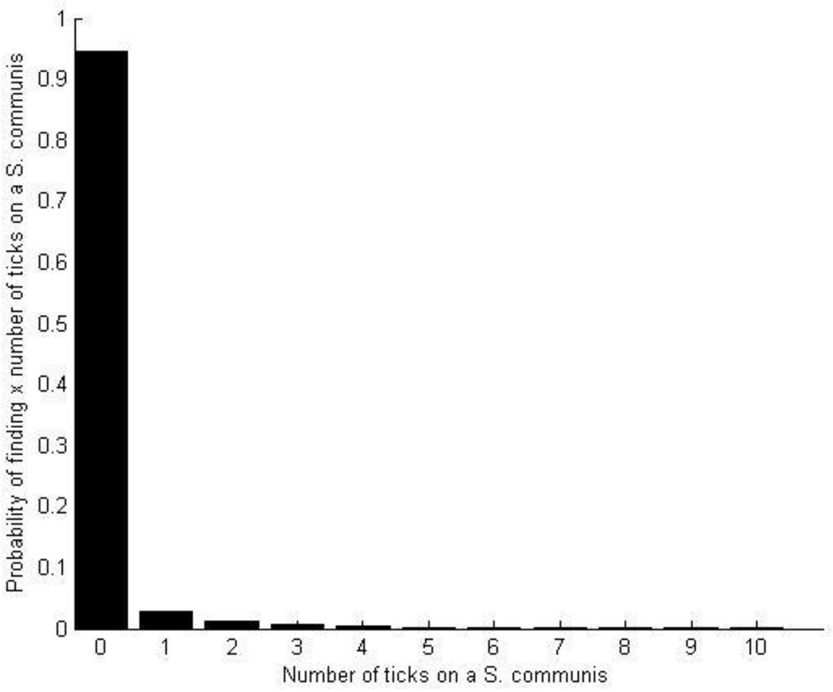


Figure 3.9: Probability mass function of the number of *Hyalomma* spp. ticks on an individual *S. communis.*

Using the fitted distributions for the number of *Hyalomma* spp. ticks per bird and the total British breeding population of each bird species (Table 3.9), it was possible to estimate the number of *Hyalomma* spp. nymphs predicted to enter GB on the whole population of each bird species in the spring of a single year using a Monte Carlo simulation with 10,000 realisations of the number of ticks per bird (Table 3.10). *S. communis* was predicted to carry the most *Hyalomma* spp. ticks into GB (mean=215,620) and *P. phoenicurus* was predicted to carry the fewest (mean=6,181). Although *P. trochilus* had the largest population of British breeding birds at 4,000,000 individuals, the number of *Hyalomma* spp. ticks per bird was small ($\lambda_{P.troc}$=0.0018) and hence the predicted number of *Hyalomma* spp. ticks entering GB per year on this species was relatively small compared to the other species. The mean number of *Hyalomma* spp. ticks predicted to be carried by all species (n_{hyalomma}) was found to be 506,223 ticks.

Table 3.10: Number of *Hyalomma* spp. nymphs predicted to enter GB on each species of bird during spring each single year. British breeding population data from (Robinson, 2005).

Species	British breeding bird population	Mean no. of *Hyalomma* spp. ticks predicted to enter GB per year	50th percentile of simulation	95th percentile of simulation	99th percentile of simulation
A. schoenobaenus	594,000	194,200	0	1,188,000	4,158,000
O. oenanthe	540,000	80,622	0	0	2,160,000
P. phoenicurus	202,000	6,181	0	0	202,000
P. trochilus	4,000,000	9,600	0	0	0
S. communis	1,862,000	215,620	0	1,862,000	5,586,000
Total	7,198,000	506,223	0	3,050,000	12,106,000

3.3.5 Estimation of the number of CCHFV-infected *Hyalomma* spp. nymphs entering GB on birds

Data on the CCHFV-infection rates of *H. marginatum* and *H. rufipes* were collected from 13 different studies carried out in CCHFV-endemic areas and the results are given in Table 3.11. All ticks tested in all studies were adults. In the study by Sang et al. (2011), 696 pools of unspecified *Hyalomma* spp. were tested for CCHFV. The authors state that individuals of *H. rufipes* were among those tested (and found to be positive) and, therefore, it was decided to include the *Hyalomma* spp. infection rate found by Sang et al. (2011) as it is the only study that has tested field populations of *H. rufipes*, a tick which could be transported into GB on migratory birds. The study by Sang et al. (2011) also had a very large sample size of 5,104 *Hyalomma* spp. ticks and is, therefore, the most extensive study that has been carried out to test CCHFV-infection rates.

Table 3.11: Data on CCHFV-infection rates in *H. marginatum* and in *Hyalomma* spp. in the case of Sang et al. (2011).

Country	*Hyalomma* spp. CCHFV-infection rate	*H. marginatum* CCHFV-infection rate	Reference
Oman	-	0.00%	Williams et al. (2000)
Turkey	-	0.93%	Gargili et al. (2011)
Turkey	-	2.04%	Gunes et al. (2011)
Turkey	-	3.23%	Tonbak et al. (2006)
Kenya	3.30%	-	Sang et al. (2011)
Turkey	-	3.51%	Tekin et al. (2012)
Bulgaria	-	4.93%	Gergova et al. (2012)
Turkey	-	7.08%	Albayrak et al. (2010a), Albayrak et al. (2010b)
Russia	-	10.24%	Yashina et al. (2003)
Iran	-	12.00%	Fakoorziba et al. (2012)
Kosovo	-	15.80%	Avsic-Zupanc (2007)
Turkey	-	28.00%	Bursali et al. (2011)
Mean CCHFV-infection rate		7.59%	

The two studies by Albayrak et al. (2010a) and Albayrak et al. (2010b) tested the same set of ticks for CCHFV but in Albayrak et al. (2010a) ticks were tested using Antigen enzyme-linked immunosorbent assay (ELISA) and in Albayrak et al. (2010b) a nested RT-PCR was used. These two tests yielded slightly different results and, therefore, the mean of these two results was taken (Table 3.11). The mean CCHFV-infection rate across all studies (p_{tickprev}) was found to be 7.59% (Table 3.11).

The studies in Table 3.11 give a range of values for p_{tickprev} and provide the best data available on CCHFV-infection rates in *Hyalomma* spp. ticks and were described by a discrete uniform distribution. However, they may represent a preferential sample of CCHFV-infection rates whereby the authors have specifically sampled in areas where CCHFV is present. This was addressed at the country level by estimating the exposure of birds to CCHFV-infected ticks. Data on the regional variation in the presence or absence of *Hyalomma* spp. tick populations and CCHFV-infection rates in *Hyalomma* spp. ticks do not exist. Therefore, within each CCHFV-endemic country, p_{tickprev} was considered to be a worst case scenario. The exposure of migratory birds to CCHFV-infected ticks was estimated at the country level for which there are data and this is now described.

There are 23 countries on the East Atlantic flyway (south of GB) that are used by the five British breeding migrants in this study (*A. schoenobaenus, O. oenanthe, P. phoenicurus, P. trochilus* and *S. communis*) as overwintering grounds, staging posts or passage countries (Wernham et al., 2002). Of these 23 countries, six are CCHFV-endemic (Table 3.12) suggesting that these birds are in, or travel through, CCHFV-endemic countries 26.1% of the time during autumn, winter and spring. The probability of exposure of the five chosen bird species to CCHFV-infected ticks (p_{end}) was described by a beta distribution denoted by $X \sim \text{Beta}(7,18)$.

Table 3.12: Countries on the East Atlantic flyway in which the five chosen bird species overwinter or fly through on migration. Those that are CCHFV-endemic are shaded.

Countries on the East Atlantic flyway in which the five chosen bird species overwinter or fly though on migration
Algeria
Benin
Cameroon
Central African Republic
Congo
Cote d'Ivoire
Democratic Republic of Congo
Equatorial Guinea
France
Gambia
Ghana
Guinea
Guinea-Bissau
Liberia
Mali
Mauritania
Morocco
Nigeria
Portugal
Senegal
Sierra Leone
Spain
Western Sahara

Transovarial transmission rates of *Hyalomma* spp. ticks were determined from the literature. According to Gonzalez et al. (1992), there is a transmission rate of 50% from CCHFV-infected adult female ticks to eggs (p_{TOT}) and 16.67% transmission from eggs to larvae (p_{TST}) (from experiments using *H. truncatum*). Another study on *H. truncatum* found a CCHFV transmission rate of 16.67% from adult females to eggs (Wilson et al., 1991). The probability of transovarial transmission was described by a beta distribution, $X \sim$ Beta(5,13) and the probability of vertical transmission from egg to larva was described by $X \sim$ Beta(2,6).

For the ticks to still be infected with CCHFV when they enter GB as nymphs, vertical transmission must occur between the larval and nymphal stages whilst attached to the bird host. A review of the literature suggests that this rate (p_{LN}) is 0% in *H. truncatum* according to Gonzalez et al. (1992) and 14.3% in *H. rufipes* according to (Zeller et al., 1994b).The variable, p_{LN}, was, therefore, described by a beta distribution, $X \sim$ Beta(2,22).

A simulation to estimate n_{infb} was run for each bird species and the mean, 50th, 95th and 99th percentiles were estimated. These are presented in Table 3.13.

Table 3.13: The results of the stochastic model for n_{infb}, the number of CCHFV-infected *Hyalomma* spp. nymphs imported into GB on the five selected bird species each spring. The mean, 50th, 95th and 99th percentiles of the output for each bird species are given.

Bird species	Mean of n_{infb}	50th percentile	95th percentile	99th percentile
A. schoenobaenus	24.9	0	54.5	539.2
O. oenanthe	12.5	0	0	205.3
P. phoenicurus	0.8	0	0	21.5
P. trochilus	0.8	0	0	0
S. communis	33.9	0	9.7	722.5
Total	72.9	0	64.2	1488.5

The mean number of CCHFV-infected immature *Hyalomma* spp. ticks entering GB on the five chosen migratory bird species (n_{infb}) each year was predicted to be 72.9 ticks. However, due to the skewed distribution of ticks on birds, the average of n_{infb} is better described by the median, which in this case is 0 ticks. The simulation shows that more than half the time, there are no CCHFV-infected ticks predicted to enter GB on the five bird species considered here. Indeed, for the bird species considered here, the number of infected ticks entering GB is predicted to be 0 until at least the 95th percentile. However, very occasionally (0.01% of the time) as many as 1488.5 CCHFV-infected ticks are predicted to enter GB. *S. communis* was predicted to carry the most CCHFV-infected ticks into GB (mean = 33.9) and *P. trochilus* and *P. phoenicurus* were predicted to carry the least (mean = 0.8 for both).

3.4 Discussion

Eighty-six ticks were found on 531 birds between late April and mid May 2011 and 65 ticks were found on 564 birds between late April and mid May 2012 at sites in the provinces of Cadiz and Malaga, Spain. The majority of ticks were found to be *Ixodes* spp. (97.7% for 2011 and 81.5% for 2012) collected from resident birds with just two *Hyalomma* spp. ticks found on a single migrant during 2011 and 12 *Hyalomma* spp. ticks found on five migrants during 2012. Molin et al. (2011) found that *Hyalomma* spp*.* made up 95.6% of all the ticks that they collected from migrant birds in Antikythera, Greece and Capri, Italy, with only seven *Ixodes* spp*.* ticks reported. This study focused on the movement of ticks on birds along the East Atlantic flyway in the western Mediterranean and in this respect complements the study of Molin et al. (2011) on ticks on migrants along the Central and Eastern Mediterranean flyways. In Greece and Italy, immature ticks (predominantly *Hyalomma* spp.) were recovered from some 20 species of birds including *Erithacus rubecula* Linnaeus (robin), *Ficedula hypoleuca* Pallas (pied flycatcher), *Sylvia borin* Boddaert (garden warbler), *Iduna pallida* Ehrenberg (eastern olivaceous warbler) and *Luscinia megarhynchos* Brehm (nightingale) (Molin et al., 2011).

In this study, more resident birds (*n*=665) were trapped than migrants (*n*=430). The total number of all birds trapped, especially migrants, was smaller than in Molin et al (2011) (*n*=7,453) and, therefore, comparison with Molin et al (2011) has to be made with caution, due to their much larger sample size. The difference between the number of birds trapped in the studies could be due in part to the greater number of nets used to trap birds in Molin et al. (2011). Antikythera has an established bird observatory with approximately 40 nets (personal observation) whereas the fieldwork carried out in Spain for this study used approximately 10 nets.

Birds of the species *T. merula* were the most infested with ticks followed by *Carduelis chloris* Linnaeus (greenfinch) in 2011. The converse was true in 2012 although these two species were still the most infested compared to all other species trapped. Hasle et al. (2009b) found that most of the ticks they collected were from ground feeding birds, with particular reference to the family Turdidae. It is possible that ticks are more likely to infest ground feeding birds as the birds will come into contact with questing ticks among low vegetation in the case of *Ixodes* spp. and on the ground itself in the case of *Hyalomma* spp.. In total, 68 of the 151 ticks collected were from bird species that nest on or close to the ground and 70 of the 151 ticks collected were from bird species that feed on or close to the ground. However, there is variation in nesting and feeding strategies within bird species and, therefore, it is not possible to determine whether these factors had an impact on tick infestation rates in this study.

The four species in this study that were found to be carrying *H. marginatum* ticks were *I. opaca*, *A. schoenobaenus*, *S. communis* and *P. trochilus*. All these bird species are migrants in Spain and spend the winter in sub-Saharan Africa. In general, these species prefer dry, open scrub and grassland habitats in their wintering areas (Hagemeijer and Blair, 1997) and, therefore, it is unsurprising that they were parasitized by the xerophilic *Hyalomma* spp. tick. The *Hyalomma* spp. ticks removed from the birds in this study were all morphologically identified as *H. marginatum.* If this identification is correct, then the origin of the ticks could have been Spain or North Africa (Apanaskevich and Horak, 2008). If the ticks were not *H. marginatum*, then they would almost certainly have been *H. rufipes* picked up in Africa. A large number of *H. rufipes* have been found on African wintering migrants (Hoogstraal et al., 1961, Hoogstraal et al., 1964). Indeed Molin et al. (2011) found immature *H. rufipes* on birds in their study. If the *Hyalomma* spp*.* ticks recovered from the migrants in this study were confirmed to be *H. marginatum* by molecular identification, their specific origin would still remain uncertain. In some cases the nymphs removed were engorged suggesting they had been attached to the bird for some time, but it is not known when the individual birds arrived in Spain. Irrespective of their origin, the ticks tested negative for CCHFV. At present CCHFV has not been found in any other tick species in Spain other than *H. lusitanicum* which does not commonly parasitize birds (Apanaskevich et al., 2008). This suggests that any ticks picked up by passage migrants in Spain would currently pose no risk of entry of CCHFV into North West Europe. Further research on the prevalence of CCHFV in other *Hyalomma* spp. ticks, particularly *H. marginatum* in Spain is required to monitor the risk.

The percentage of migrant birds in 2011 infested with *Hyalomma* spp. was low in this study carried out along the East Atlantic flyway, at 0.5%. The percentage of migrants carrying *Hyalomma* spp. along the Central and Eastern Mediterranean flyways, the other migration routes into Europe from Africa, was found to be 5-fold higher at 2.55% (Molin et al., 2011). This difference was found to be significant to 10% (p=0.0660) such that initially it seemed that there was some evidence to suggest that the number of immature *Hyalomma* spp. ticks introduced per migrant bird was smaller on migrants coming into western Europe through the East Atlantic flyway compared to those entering central and eastern Europe. However, the data collected for 2012 in this study showed this not to be the case, with a *Hyalomma* spp. infestation rate for migratory birds of 2.19%. The difference between this percentage and that found by Molin et al. (2011) was not significant (p=0.8306). The between-year difference in the infestation rates that were found in this study were also not significant (p=0.2206) but may serve to demonstrate the importance of long-term sampling that would allow for between-year fluctuation of tick and host populations.

A study conducted at four bird observatories on the south coast of Norway (an area that falls within the East Atlantic flyway) over a period of three years found low infestation rates of *Hyalomma* spp. ticks (Hasle et al., 2009a). Of 9,768 passerine birds captured, seven nymphal *H. rufipes* were found on seven individual migrants, giving an infestation rate of 0.07% (with 95% binomial confidence interval of 0.03% - 0.15%). A recent two year study on the south coast of England found a *Hyalomma* spp. infestation rate of 0.67% (with binomial confidence interval of 0.25% - 1.45%), with 57% of all *Hyalomma* spp.

ticks found on *O. oenanthe* (Jameson et al., 2012a). These two studies again give a different rate of *Hyalomma* spp. infestation for birds migrating along the East Atlantic flyway and it would seem that longer-term studies are required to ascertain what results a ‘typical year’ would yield, or whether the importation rates consistently fluctuate greatly year-on-year. Similarly, Molin et al (2011) carried out sampling in the spring of a single year (2009) and, therefore, longer-term studies are also needed along flyways other than the East Atlantic flyway if a comparison is to be drawn between them.

In this study it was shown that different bird species may be more or less likely to bring ticks into GB. Based on the data collected in this study and from Jameson et al. (2012a), it would appear that *S. communis* is likely to bring larger numbers of *H. marginatum* ticks into GB compared to other bird species. This is partly due to the fact that they are hosts of *H. marginatum* and partly because they have a large breeding population migrating into GB each spring. The mean number of *H marginatum* ticks entering GB on the five chosen bird species each spring was predicted to be 506,223 ticks (5th and 95th percentiles of 0 and 3,050,000 ticks respectively). CCHFV was not found in any of the ticks recovered in this study but by using data from the literature on CCHFV-infection rates in endemic populations of *H. marginatum*, the mean number of CCHFV-infected *H. marginatum* entering GB on the chosen five bird species was estimated to be 72.9 ticks (5th and 95th percentiles of 0 and 64.2 ticks respectively). If the number of infected *H. marginatum* ticks on migrant birds entering GB is in the order of 72.9 importations per year, it is worth considering where these ticks might detach from their avian hosts and whether they are able to survive to the next life stage. Understanding what stimulates the tick to detach from its host such that this does not happen during flight may be important in predicting where the imported tick might be dispersed to within GB. The ticks may detach at a bird’s first landfall site in GB or when the bird has reached its final breeding ground. The habitat in each of these locations would also be important in determining the continuing survival of an imported tick and, therefore, its associated pathogens. Each bird species (and individual bird) arrives into GB at a slightly different time and it may be that species that leave their wintering grounds early (in February or early March) are unlikely to be carrying *Hyalomma* spp. ticks as the ticks will have not yet emerged from winter diapause. Birds that do not return to GB until May, therefore, may have higher *Hyalomma* spp. infestation rates and any transported ticks would be introduced to warmer temperatures which may aid their survival within GB.

Due to the highly skewed distribution of ticks on birds, as previously mentioned, an alternative measure of central tendency is the median, as the mean overestimates the central tendency of the data. For all bird species, the median of n_{infb} was 0. Some birds could carry a large number of ticks though, and this has implications for the sampling of *Hyalomma* spp. ticks on birds. It could be that just a few birds import a large number of ticks, and as can be seen from Table 3.10, 1% of the time (or for one in every 100 years), 12,106,000 *Hyalomma* spp. ticks could be imported between 1st April and 31st July each year. This suggests the need for long-term sampling over several years.

In this study, the British breeding population of each of the five bird species was considered. There may have been additional individuals of these species that are passage migrants which could import *Hyalomma* spp. ticks at staging posts in GB. However, those that breed in areas to the north of GB, such as Scandinavia, would generally migrate across Europe further east than GB and, therefore, would not occur in GB as passage migrants. The nominate race of *O. oenanthe* occasionally breeds as far north as Greenland, but the sub-species *O. oenanthe leucorhoa* is a passage migrant through GB to its breeding grounds in Iceland, Greenland and Canada. These birds are known to stop-over in GB especially in coastal areas, and they could potentially introduce *Hyalomma* spp. immatures. Similarly, there may be several other species of birds that migrate from Africa and southern Europe in the spring that have not been considered in this chapter. Therefore, the total number of CCHFV-infected *Hyalomma* spp. ticks imported into GB on all migratory birds may be larger than the number predicted here for the five chosen bird species.

A recent report from a workshop on CCHFV stated that “*Hyalomma* ticks, including CCHF virus, are probably introduced into western Europe by migrating birds every year, but the tick species and virus have apparently not persisted” (Hoek et al., 2012). This study on the prevalence of *Hyalomma* spp. ticks on birds migrating into Western Europe supports the supposition in Hoek et al. (2012) that it is possible that both the tick vector and the virus are being imported into western Europe (and, therefore, GB). Further research is required at different locations along the East Atlantic flyway to clarify where *Hyalomma* spp. ticks are being picked up by migratory birds that breed in North West Europe and whether these ticks are infected with CCHFV. Sampling strategies that account for the over-dispersion of ticks on birds as seen in this study would need to be implemented. This would help to more accurately evaluate the risk, if any, that this migration pathway poses to North West Europe and GB in terms of an incursion event of CCHFV.

4 Introduction of CCHFV-infected ticks into GB by imported horses

4.1 Introduction

Adult *Hyalomma* spp. ticks are known to preferentially parasitize large mammals including domestic livestock and horses (Lutsuk and Evtenko, 1980, Labruna et al., 2001, Ruedisueli and Manship, 2011). Horses are frequently transported across country borders for training, riding, polo and other competition events and it has been proposed that horses are involved in the dissemination of tick populations and their associated pathogens (Jameson and Medlock, 2009), including CCHFV. This chapter examines horses as a potential entry route of CCHFV-infected *Hyalomma* spp. ticks into Great Britain (GB). Temperature has been shown to affect the development rates and survival of *H. marginatum* (Estrada-Peña et al., 2011). A study by El Ghali and Hassan (2010a) showed that unfed *Hyalomma dromedarii* adult ticks survived for longer at lower temperatures and, therefore, it may be possible for individual imported exotic ticks to survive in locations beyond their current range, such as in GB. CCHFV antibodies have been found in horses (Ergönül, 2006), but the virus itself has not been isolated from horses and it is not known if they become viraemic if infected. It is not known whether horses can act as amplifying hosts for the virus and, therefore, whether they have a role in the transmission of the virus in some CCHFV-endemic areas.

An adult male *H. marginatum* was imported into GB on a horse from Portugal (Jameson and Medlock, 2009). This occurred in March 2009 in Dartmoor and is the only recorded importation of an adult *H. marginatum* tick into GB. However, there is considerable scope for ticks to be imported into GB on horses without being detected. It is difficult to check for ectoparasites on horses that have been in transit, as they can be bad tempered after travel (personal observation) and veterinary checks at Border Inspection Posts (BIP) may vary in thoroughness and consistency. Ticks are often attached under the tail of the horse and, thus, are not easy to detect (personal observation). The recorded importation of a *H. marginatum* tick on a horse into GB supports the need to assess the release of CCHFV-infected ticks into GB from imported horses.

The number of CCHFV-infected *Hyalomma* spp. ticks entering GB on horses is related to the number of *Hyalomma* spp. ticks attached to horses, the number of horses entering GB per year and the prevalence of CCHFV in the country of origin of the tick. The number of *Hyalomma* spp. ticks found on horses is a function of the exposure of horses to populations of *Hyalomma* spp. and the use and efficacy of acaricides (tick-killing treatments) on horses. This study aimed to predict the number of CCHFV-infected *Hyalomma* spp. ticks that are imported into GB between 1st April and 31st July each year on horses, reflecting the timing of peak tick activity and host questing in the northern hemisphere. In the southern hemisphere, the peak adult *Hyalomma* spp. questing period is from February to March and from October to November (Burt et al., 2007). Ticks do not actively quest for hosts in the winter months in the northern hemisphere as they undergo winter diapause (Bakirci et al., 2011, Estrada-Peña et al., 2011). Therefore, it was assumed that any ticks entering GB from the southern hemisphere during the winter months would be unable to survive. Data on the number of horses that are imported into GB between 1st April and 31st July each year from CCHFV-endemic countries and the prevalence of CCHFV in these ticks were obtained in order to quantify the number of CCHFV-infected *Hyalomma* spp. adult tick entering GB for this time period each year.

4.2 Method

4.2.1 Prediction of the density of CCHFV-infected *Hyalomma* spp. ticks per 1 km^2 entering GB on horses between 1st April and 31st July each year

The density of CCHFV-infected adult *Hyalomma* spp. ticks entering GB on horses per square kilometre between 1st April and 31st July each year (n_{infh}) was predicted using Equation (4.1):

$$n_{\mathrm{infh}} = n_{\mathrm{horse}} \times n_{\mathrm{tick}} \times p_{\mathrm{prev}} \qquad (4.1)$$

The parameters are defined in Figure 4.1. A Monte Carlo simulation with 5,000 realisations was run in MATLAB R2012a for each 1 km^2 cell of GB. For the simulation the average density of horses imported into GB from CCHFV-endemic countries per square kilometre (n_{horse}) was kept constant in each 1 km^2 cell. It was considered very difficult to quantify the uncertainty around the number of horses imported into GB, as well as uncertainty in the spatial distribution, due to the unrecorded and, therefore, unknown numbers of horses using various different routes of importation (this is discussed further in the Discussion). The number of realisations was sufficient as n_{infh} converged to the mean after 5,000 realisations. For each cell, the mean and a range of percentile values of n_{infh} were obtained and mapped in ERSI ArcGIS 10.0 for the whole of GB. The output of each cell was summed across GB to give a prediction of the total number of CCHFV-infected adults entering GB on horses between 1st April and 31st July each year. Figure 4.1 illustrates the route of introduction of CCHFV-infected adult *Hyalomma* spp. ticks into GB by horses and the parameters for estimation. The method by which estimates were made for each parameter is now described.

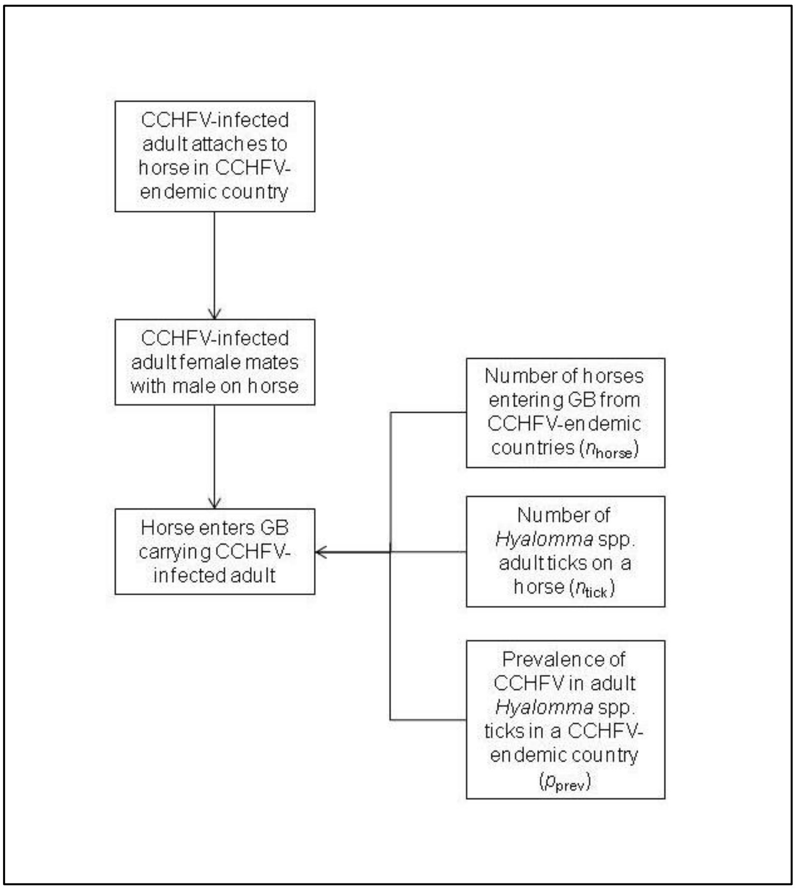


Figure 4.1: The route of introduction of CCHFV-infected Hyalomma spp. adults into GB by horses and the parameters for estimation.

4.2.2 Estimation of the number of horses imported into GB from CCHFV-endemic countries between 1st April and 31st July each year

It was assumed that ticks were not infected with CCHFV if they were imported from countries where CCHFV was not endemic. To construct a map of the density of imported horses annually per square kilometre in GB from CCHFV-endemic countries (n_{horse}), the online tool, TRAde Control and Expert System (TRACES), was accessed. TRACES is a veterinary certification tool maintained by the European Commission that facilitates, controls and records the importation and movement of live animals and animal products within the EU. Data on live horse importations into GB from 2009 to 2012 were collected from the online certificate database. The data included date of importation, country of origin, number of horses per consignment and the end destination within GB of each consignment. The geographical distribution of *Hyalomma* spp. ticks within CCHFV-endemic countries was not known and, thus, it was assumed that horses from CCHFV-endemic countries had an equal level of exposure to *Hyalomma* spp. ticks to one another. It was not known whether imported horses had received acaricide treatment at any stage and, therefore, it was also assumed that all horses were equally susceptible to parasitization by ticks. CCHFV-endemic countries were defined as those that had reported human cases of CCHF originating from the country since 1970, or the virus had been isolated from endemic tick populations since 1970 or livestock populations had tested seropositive for CCHFV antibodies since 1970 (ProMED-mail, Hoogstraal, 1979, Mathiot et al., 1988, Camicas et al., 1989, Zeller et al., 1994a, Hassenein et al., 1997, Ergönül and Whitehouse, 2007, Nalca and Whitehouse, 2007, Mohamed et al., 2008, Mild et al., 2010). These countries are listed in Table 4.1.

Table 4.1: Countries defined as 'CCHFV-endemic' (ProMED-mail, Hoogstraal, 1979, Mathiot et al., 1988, Camicas et al., 1989, Zeller et al., 1994a, Hassenein et al., 1997, Ergönül and Whitehouse, 2007, Nalca and Whitehouse, 2007, Mohamed et al., 2008, Mild et al., 2010).

Countries defined as ‘CCHFV-endemic’			
Afghanistan	Hungary	Namibia	Tanzania
Albania	India	Nigeria	Turkey
Armenia	Iran	Oman	Turkmenistan
Azerbaijan	Iraq	Pakistan	United Arab Emirates
Bulgaria	Kazakhstan	Republic of South Africa	Uganda
Burkina Faso	Kenya	Romania	Ukraine
Central African Republic	Kosovo	Russia	Uzbekistan
China	Kuwait	Saudi Arabia	Zimbabwe
Democratic Republic of Congo	Kyrgyzstan	Senegal	
Egypt	Madagascar	Somalia	
Ethiopia	Mauritania	Spain	
Georgia	Moldova	Sudan	
Greece	Morocco	Tajikistan	

The data available from TRACES included the end destination of each individual horse and this was mapped using ESRI ArcGIS 10.0. The total number of horses imported into GB from 1st April to 31st July 2011 and 1st April to 31st July 2012 was obtained, along with the end destination of each horse. This was used to calculate the density of horses imported per square kilometre for each of these time periods. Densities were calculated and smoothed using kernel density estimation (KDE) with a circular window of radius of 75 km in ERSI ArcGIS 10.0. KDE was applied to highlight areas with high densities of horse imports and removed the unrealistic assumption that horses would remain at the exact location of their end destination as recorded in TRACES. A circular window of radius 75 km was chosen as this gave the density output that best represented the original data compared to smaller or larger radii. The mean density of horses imported into GB from CCHFV-endemic countries for the period 1st April to 31st July each year (n_{horse}) was calculated for each 1 km^2 cell of GB from the data for 2011 and 2012.

Data on horse imports were also collected from the paper archives of the BIP at Heathrow Airport. The data collected from these archives ran from January 1995 to June 2010 and contained a record of every live animal import into GB that had arrived through Heathrow Airport. The archives were searched by hand for all horse importations and again, the data collected included date of importation, country of origin and the number of horses per consignment. The end destination of each horse was not provided, but the Heathrow dataset provided a longer-term study of horse importations than that available from TRACES and was used to ascertain the time of year when most horse importations occurred, to see if this coincided with the period of interest (1st April to 31st July). The data collected from Heathrow Airport from 2009 onwards would have also appeared on the TRACES database for the same period and, therefore, this was compared for accuracy and content.

4.2.3 Estimation of the number of *Hyalomma* spp. ticks present on horses in *Hyalomma* spp.-endemic countries

There are many species of *Hyalomma* that are vectors of CCHFV and also parasitize horses (Table 4.2). Spain has established populations of *Hyalomma* spp. ticks that parasitize horses, including *H. marginatum* and *H. lusitanicum* (Table 4.2), which has been found to be infected with CCHFV in Extremadura in Spain (Estrada-Peña et al., 2012b). In other CCHFV-endemic countries that export horses to GB, such as Saudi Arabia and Russia, other *Hyalomma* spp. ticks are endemic such as *Hyalomma anatolicum* and *Hyalomma asiaticum* (Table 4.2).

Table 4.2: Species of the genus, *Hyalomma*, that parasitize horses and are vectors of CCHFV (Apanaskevich, 2003, Walker et al., 2003, Apanaskevich and Horak, 2008, Apanaskevich et al., 2008, Kolonin, 2009, Apanaskevich and Horak, 2010).

Species	Distribution
H. anatolicum	Northeast Africa, southeast Europe, central and southwest Asia
H. asiaticum	Central and southwest Asia
H. detritum	Northwest Africa
H. dromedarii	North Africa and southwest Asia
H. excavatum	North Africa, southeast Europe and southwest Asia
Hyalomma impeltatum Schulze and Schlottke	North Africa and southwest Asia
H. lusitanicum	North Africa, central and eastern Mediterranean
H. marginatum	North Africa, southern Europe, east Asia
Hyalomma nitidum Schulze	Central and West Africa, Egypt and South Africa
H. rufipes	Southern Africa, Yemen and Oman
H. truncatum	Southern Africa
H. turanicum	Central and southwest Asia

A tick of the species listed in Table 4.2 could enter GB attached to a horse due to the long period of attachment for adult *Hyalomma* spp. ticks. Adult *H. marginatum* ticks have been observed to feed for 24 days on calves prior to detachment (Sahibi et al., 1997). For the purposes of this study, the prevalence of *Hyalomma* spp. ticks on horses in southern Spain was considered to be typical of a country with endemic populations of *Hyalomma* spp.. For this chapter, experimental field data on Hyalomma spp. ticks on horses in Spain were gathered. This is now described.

To estimate the number of *Hyalomma* spp. ticks per horse in a *Hyalomma* spp.-endemic area (n_{tick}), 18 horses in the provinces of Cadiz and Malaga, southern Spain were surveyed for ticks during April and May 2012 (Figure 4.2). The horses were primarily inspected under the tail and around the perineum, as well as the legs, axillae and along the mane. Of the horses surveyed, 15 were mares that were receiving routine visits from an equine veterinarian as part of an artificial insemination programme. The three remaining horses were private riding horses kept in paddocks at the same livery stables and included one gelding and two mares. All horses were healthy at the time of tick removal and any ticks found were removed with tweezers and placed immediately into tick fixative, a solution of 70% ethanol, 20% distilled water and 10% glycerol. Ticks were stored separately and labelled according to the individual horse that they were removed from. Where tick infestations were very heavy, not all ticks were removed in order to manage the stress levels of the horse and instead an estimate of the percentage of ticks removed was made. This was done by examining the whole horse for ticks and then visually estimating the area from which ticks had been removed as a percentage of the whole area where ticks were present. All collected ticks were aged and identified to species using the descriptions and keys of Estrada-Peña et al. (2004), Apanaskevich and Horak (2008) and Apanaskevich et al. (2008).

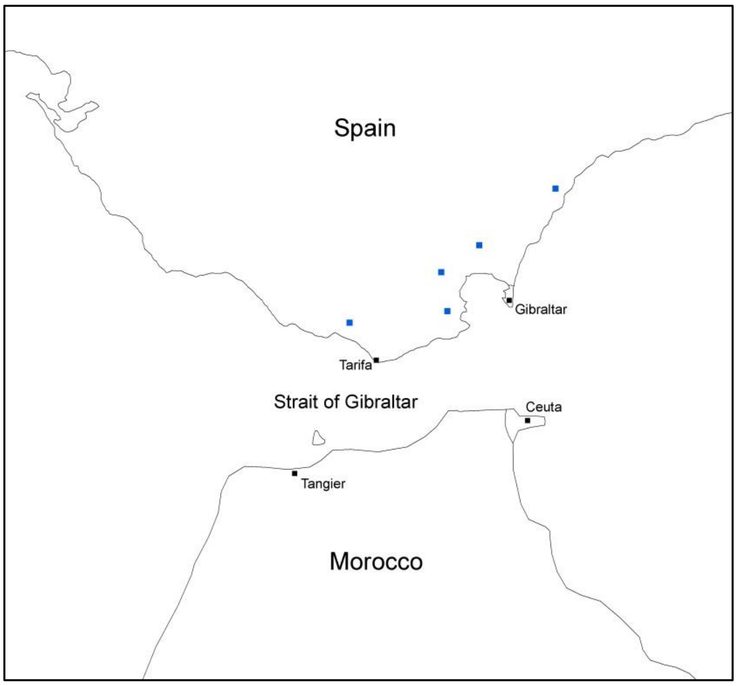


Figure 4.2: Locations in Spain where horses were surveyed for ticks in 2012, denoted by the blue symbol.

To describe the distribution of counts of *Hyalomma* spp. ticks on horses (n_{tick}), a negative binomial distribution was fitted to the data collected from Spain on *Hyalomma* spp. tick counts per horse by maximum likelihood estimation (MLE) using MATLAB R2012a. To help decide which distribution would be an appropriate fit for the data, the count of ticks per horse (from Spain) was plotted and a goodness-of-fit test was used.

4.2.4 Estimation of the prevalence of CCHFV in adult *Hyalomma* spp. ticks in a CCHFV-endemic country

Ticks found on horses in Spain were tested for CCHFV using the RT-PCR developed by Atkinson et al. (2012) using a SuperScript III (SSIII) Platinum One-step qRT-PCR kit (Invitrogen). The resultant CCHFV prevalence data were combined with published data on the CCHFV-prevalence in various known and unknown species of adult *Hyalomma* spp. ticks that were collected from 13 different studies carried out in CCHFV-endemic areas. This provided data for the prevalence of CCHFV in adult *Hyalomma* spp. ticks in a CCHFV-endemic country (p_{prev}). These values were considered to represent the possible values that p_{prev} could take and were considered to be equally likely to occur. Therefore, the data were best described by the discrete uniform distribution (an empirical distribution).

4.3 Results

4.3.1 Estimation of the number of horses imported into GB from CCHFV-endemic countries

Data on horse imports into GB between 1st April 2009 and 31st July 2012 were collected from TRACES. For 2009 and 2010, only non-EU import data were available and no data were available between January and March 2009. This was because the TRACES archive of import certificates did not go back further than April 2009. Due to time constraints, data were not collected from November 2011 to March 2012. This decision was taken as these data would not be used to estimate the final result of n_{infh}. Also, data collected from Heathrow Airport would better represent seasonal fluctuations in import numbers over many months than the data available from TRACES.

The most complete datasets available were those collected from 1st April 2011 to 31st July 2011 and from 1st April 2012 to 31st July 2012. For these periods, data were available for all horse imports from both EU and non-EU countries (combined total for both years given in Table 4.3). During these time periods a combined total of 4,464 horses were imported into GB. For 256 of those horses, data on the end destination were not available either because they had not been entered into the TRACES system or because the address was incomplete or incorrectly entered and could not be deciphered. These horses could not be mapped and were excluded from further analysis. The remaining 4,208 horses that were imported between April and July of 2011 and 2012 were mapped according to the address of their end destination point within GB (Figure 4.3). Entries for horses that had been imported from CCHFV-endemic countries were then extracted, representing 1,184 horses (highlighted in grey in Table 4.3).

Table 4.3: Total number of horses imported into GB from 1st April 2011 to 31st July 2011 and from 1st April 2012 to 31st July 2012 (TRACES). Horses from CCHFV-endemic countries are shaded in grey.

Number of horses all countries 2011 and 2012	Month				
Country of Origin	April	May	June	July	Grand Total
Argentina	265	143	18	5	431
Australia	39	51	14	8	112
Austria	1	1	14	13	29
Bahrain			8		8
Belarus				2	2
Belgium	85	204	152	206	647
Brazil		3		1	4
Bulgaria	1				1
Canada	3		15	25	43
Chile	1		3		4
Croatia				1	1
Czech Republic		8	6	1	15
Estonia			1		1
France	4	2	2	5	13
Germany	101	201	157	311	770

Hong Kong	2	6	1	2	11
Hungary		5	13	4	22
Iceland	1	1	3	4	9
Israel		1			1
Italy	6	80	7	49	142
Italy			2		2
Japan		3	6	3	12
Luxembourg				1	1
Mauritius				26	26
Netherlands	2	27	18	14	61
New Zealand	37	21	3	6	67
Norway	3	20	17	10	50
Oman	87	1			88
Poland	3		8	18	29
Portugal	3	4	15	6	28
Qatar	14	6	9		29
Russia	14		4		18
Saudi Arabia	4				4
Singapore		10	2	2	14
Slovenia	1				1
Spain	145	108	82	73	408
Switzerland	5	5	8	12	30
Thailand	6				6
Turkey				9	9
Ukraine				11	11
United Arab Emirates	339	209	26	41	615
Uruguay		22	8		30
USA	186	99	82	36	403
Grand Total	1358	1241	704	905	4208

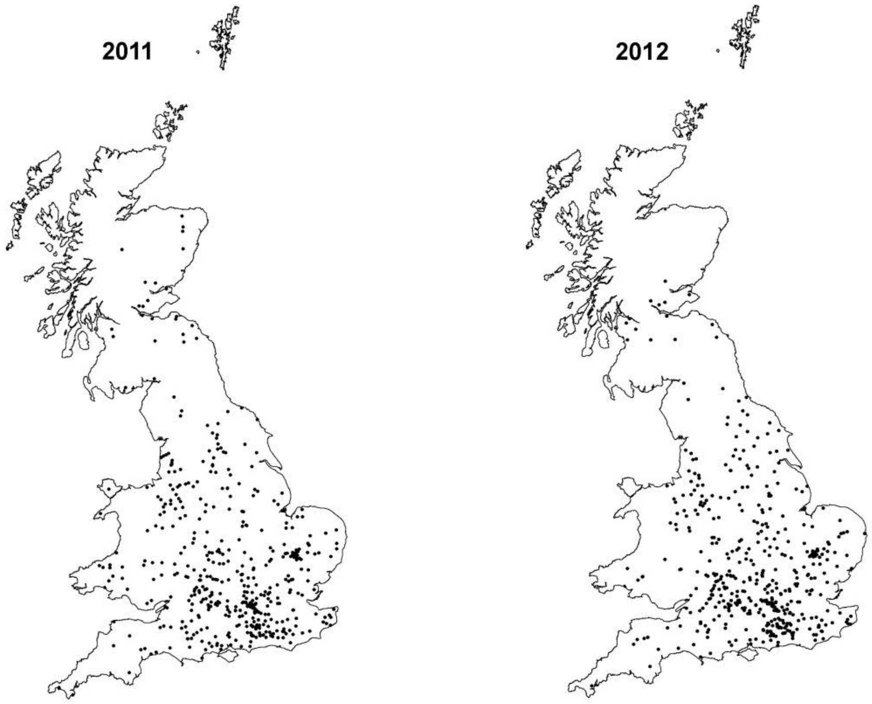


Figure 4.3: Destination of horses imported into GB from 1st April 2011 to 31st July 2011 and from 1st April 2012 to 31st July 2012.

The data collected for 2009 and 2010 are summarised by month in Table 4.4 and Table 4.5 and represent imports from non-EU countries. Table 4.6 shows the number of horses imported from all countries for 2011 by month. It is clear from the data that April was the peak month for horse importations. This pattern of imports is supported by the data collected over 14 years from Heathrow Airport which show that the spring months, particularly April and May, experience the largest numbers of horse imports to GB (Figure 4.4).

Table 4.4: Numbers of horses imported into GB from non-EU countries during 2009 by month (TRACES).

Number of horses imported from non-EU countries 2009	
Month	Total
April	410
May	158
June	145

July	73
August	69
September	96
October	383
November	108
December	171
Grand Total	1613

Table 4.5: Number of horses imported into GB from non-EU countries during 2010 by month (TRACES).

Number of horses imported from non-EU countries 2010	
Month	Total
January	104
February	39
March	280
April	470
May	308
June	96
July	73
August	46
September	66
October	321
November	386
December	243
Grand Total	2432

Table 4.6: Number of horses imported into GB from all countries during 2011 by month (TRACES).

Number of horses imported from EU and non-EU countries 2011	
Month	Total
January	374
February	202
March	632
April	671
May	629

June	307
July	431
August	670
September	178
Grand Total	4096

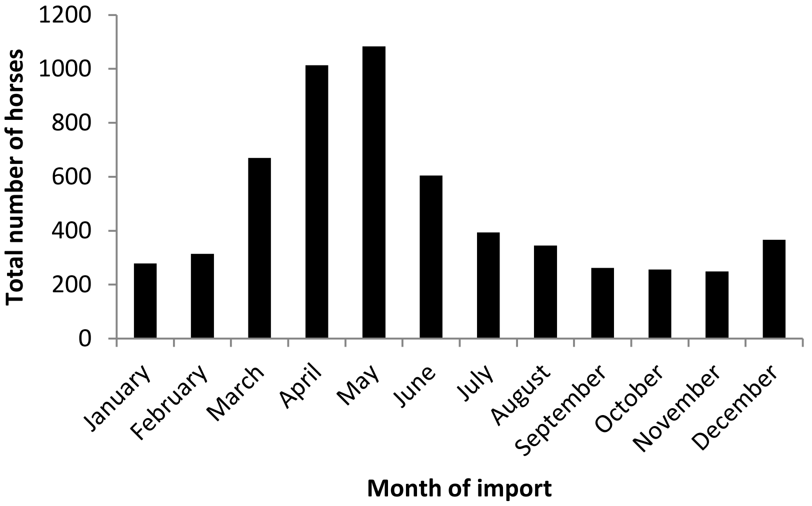


Figure 4.4: Number of horses imported into Heathrow Airport by month from all countries pooled from data collected between January 1995 and December 2009 at Heathrow Airport.

The data on number of horses imported into Heathrow Airport according to TRACES were compared with the corresponding data collected from the paper files at Heathrow Airport for the same time period. The number of horses imported into Heathrow Airport per month was summed for each data source and is shown in Table 4.7.

Table 4.7: Total number of horses imported into Heathrow Airport according to TRACES and according to paper documentation at Heathrow Airport.

Month of import	Total number of horses imported according to TRACES	Total no.of horses imported according to Heathrow Airport paper files
April 2009	37	46
May 2009	57	62
June 2009	31	36
July 2009	18	19

August 2009	1	3
September 2009	15	13
October 2009	0	0
November 2009	6	7
December 2009	22	29
January 2010	4	13
February 2010	12	11
March 2010	32	41
April 2010	39	47
May 2010	62	63
June 2010	22	26
Total	358	416

It was observed that there were differences in the data from Heathrow Airport's paper documentation and the data obtained from TRACES (Figure 4.5). The paper documentation from Heathrow Airport is believed to be an accurate account of the importations that actually occurred between April 2009 and June 2010. The data from Heathrow Airport consistently show a slightly larger number of horses being imported than the data from TRACES (with the exception of September 2009 and February 2010). TRACES holds records of cancelled, replaced and expired certificates for horse importation as well as valid certificates. The data presented here from TRACES were collected from a database of valid certificates. The certificates for some horse imports into Heathrow Airport may have subsequently expired or been replaced such that they no longer appear on the valid certificates database of TRACES. This could explain the apparently smaller number of imports into Heathrow Airport according to TRACES compared to Heathrow Airport documentation.

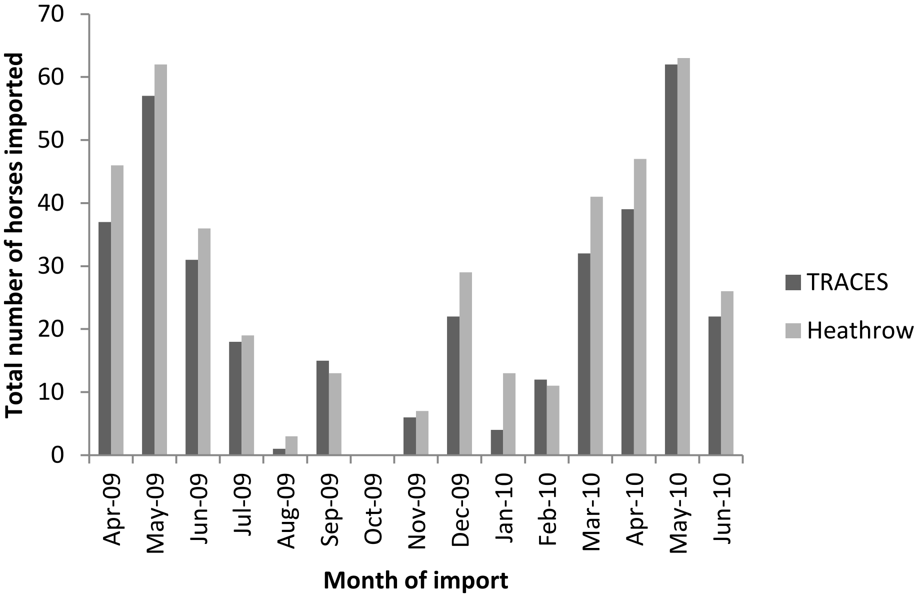


Figure 4.5: Number of horses imported into GB between April 2009 and June 2010 according to TRACES and Heathrow Airport paper documentation.

From April to July 2011 and from April to July 2012, the percentages of horses imported from CCHFV-endemic countries were 26.9% (*n*=515) and 29.1% (*n*=669), respectively. Horses that had originated from CCHFV-endemic countries between 1st April and 31st July 2011 and 1st April and 31st July 2012 were mapped according to their end destinations within GB. The horse destinations were mapped in ESRI ArcGIS 10.0 and labelled according to their country of origin (Figure 4.6). The majority of imports for both years were from the United Arab Emirates (*n* = 615) and Spain (*n* = 408) (see also Table 4.3). The mean number of horses imported between 1st April and 31st July each year from CCHFV-endemic countries (n_{horse}) was 592.

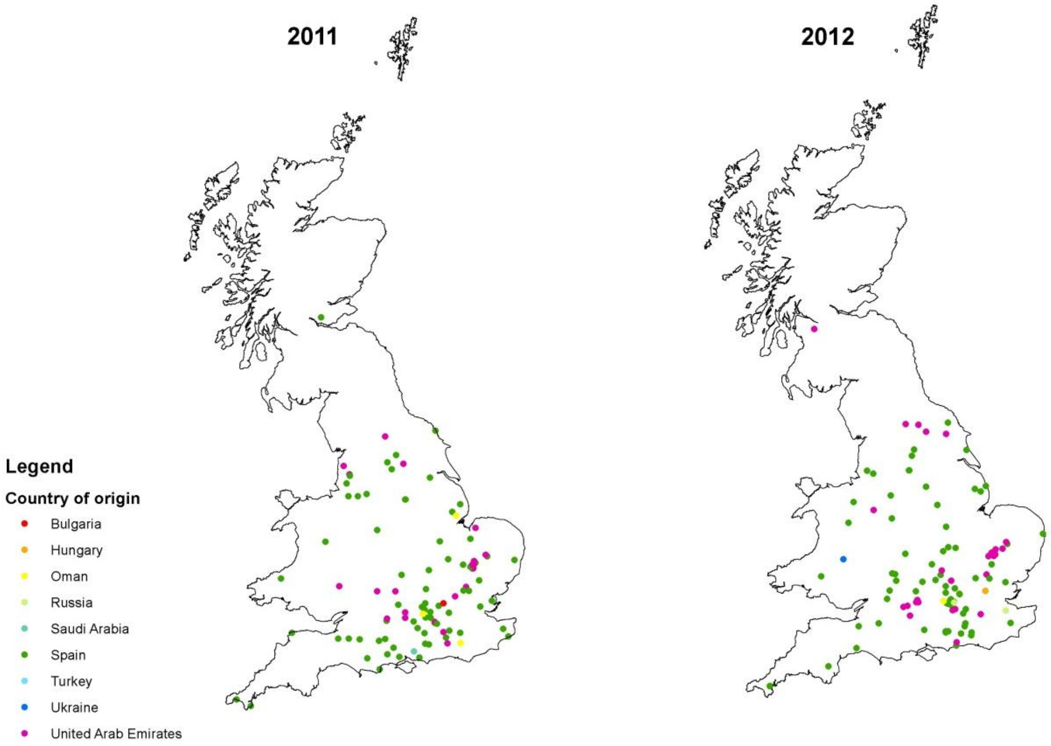


Figure 4.6: Destination of horses imported into GB from CCHFV-endemic countries from 1st April 2011 to 31st July 2011 and from 1st April 2012 to 31st July 2012.

From the maps in Figure 4.6, it can be seen that there is approximate temporal stationarity of horse imports across years. This was more clearly seen when the data were smoothed using KDE which was carried out for both April to July 2011 and April to July 2012 using a circular window of radius 75 km in ESRI ArcGIS 10.0 (Figure 4.7).

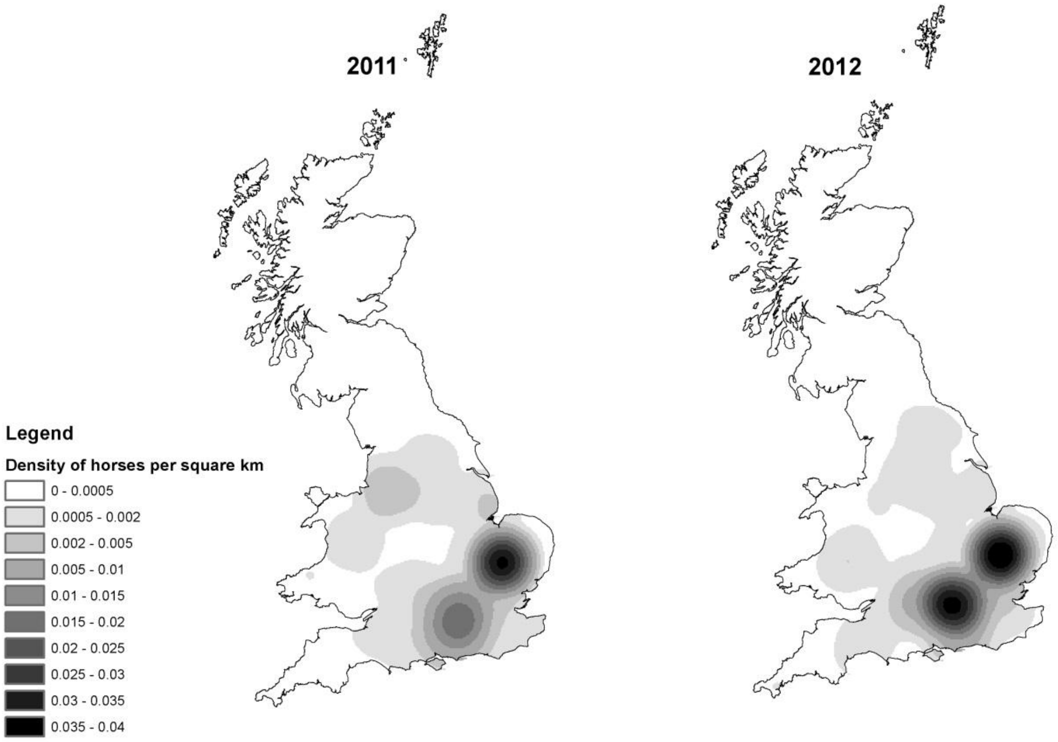


Figure 4.7: Kernel Density Estimation for the density of horses imported into Great Britain (horses per 1 km^2) from CCHFV-endemic countries during April to July 2011 and April to July 2012.

Due to the temporal stationarity of the spatial pattern, as already mentioned, the density of horses per square kilometre for 2011 and 2012 was averaged using the mean to produce a map that shows the predicted density of horses imported per km^2 from CCHFV-endemic countries between 1st April and 31st July in a typical year (Figure 4.8).

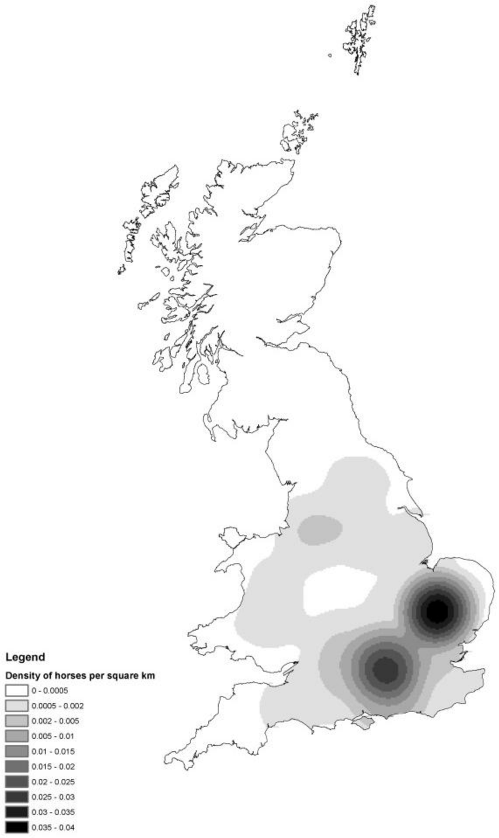


Figure 4.8: The predicted density of horses imported (number of horses per 1 km^2) between 1st April and 31st July during a typical year.

4.3.2 Estimation of the number of *Hyalomma* spp. ticks imported into GB on horses from CCHFV-endemic countries

Of the 18 horses checked for ticks in southern Spain, six were found to have ticks identified as either *Rhipicephalus bursa* Canestrini and Fanzago*, H. marginatum* or *H. lusitanicum* (Table 4.8), giving a percentage of 36.8% of horses being infested with ticks. The total number of ticks collected was 248, with *R. bursa* being the most prevalent tick (*n*=226), followed by *H. marginatum* (*n*=21) and then *H. lusitanicum* (*n*=1). The percentage of horses with *Hyalomma* spp. ticks was 33.3%. The proportion of total ticks removed from each horse varied greatly and this was related to the amount of time available to remove the ticks (the horses were waiting for veterinary procedures to be carried out), the behaviour of horses and their tolerance of the tick removal process.

Table 4.8: Number of and species of ticks found on six horses in southern Spain. When not all ticks were removed, an estimate of the total number of ticks was made.

	Horse 1	Horse 2	Horse 3	Horse 4	Horse 5	Horse 6	Total
Number of *R. bursa*	140	41	37	0	8	0	226
Number of *H. marginatum*	4	5	5	3	3	1	21
Number of *H. lusitanicum*	0	1	0	0	0	0	1
Total number of ticks removed	**144**	**47**	**42**	**3**	**11**	**1**	**248**
Estimated % of total ticks removed	66%	100%	33%	100%	100%	100%	
Estimated total number of *Hyalomma* spp.	6	6	15	3	3	1	34
Estimated total number of ticks	**216**	**47**	**126**	**3**	**11**	**1**	**416**

A total of 22 *Hyalomma* spp. ticks were removed from these six horses. However, not all ticks were removed from some of the horses (Horse 1 and Horse 3, Table 4.8). Therefore, for these horses an estimate of the total number of *Hyalomma* spp. present was made using the number of *Hyalomma* spp. ticks already removed and the estimated percentage of all ticks removed (Table 4.8). This gave a mean of 1.89 *Hyalomma* spp. ticks per horse.

A negative binomial distribution (NBD, see Chapter 2) was fitted to the number of ticks on horses using maximum likelihood estimation (MLE). The probability density function of the fitted distribution is given in Figure 4.9, along with a plot of the empirical cumulative distribution function of the data from Spain. The plots in Figure 4.9 show that an NBD provides an appropriate fit. A Poisson distribution was also fitted using MLE and the goodness-of-fit of both distributions was calculated using the negative log-likelihood (NLogL). The lower negative log-likelihood of the NBD shows that this distribution provides the more appropriate fit (Table 4.9).

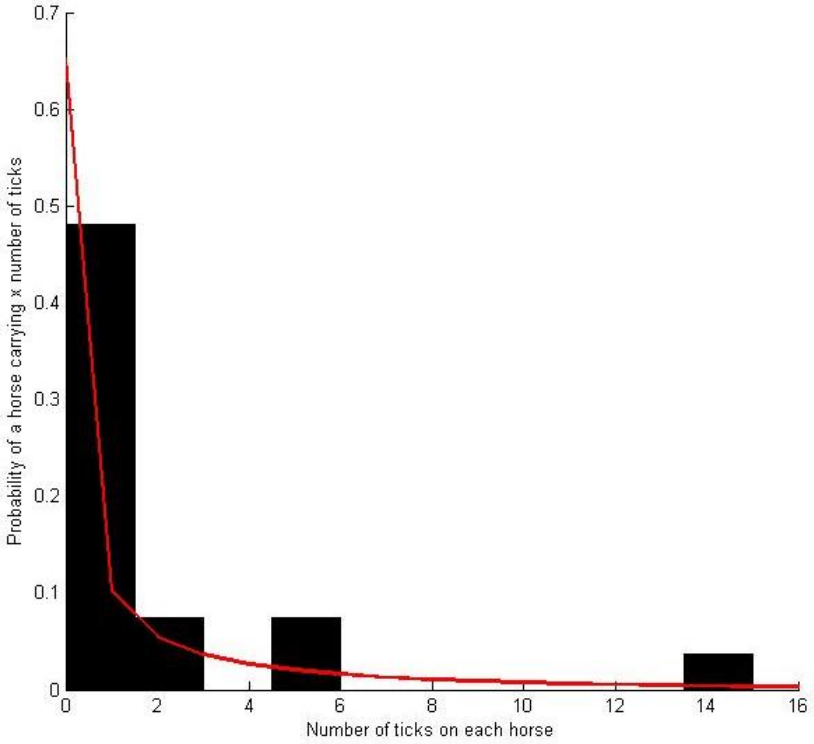


Figure 4.9: Plot of the empirical cumulative distribution function of the data collected from Spain on the number of *Hyalomma* spp. ticks per horse (black bars) and the fitted negative binomial distribution (red line).

Table 4.9: Distributions fitted to the data collected on number of *Hyalomma* spp. ticks on horses in Spain 2012.

Fitted Distribution	MLE parameter values		NLogL
Negative binomial	r	0.1715	27.9360
	p	0.0832	
	n	592	
Poisson	λ	1.8889	57.0177
	n	592	

4.3.3 Estimation of the prevalence of CCHFV in adult *Hyalomma* spp. ticks in a CCHFV-endemic country

All ticks collected on horses from Spain tested negative for CCHFV. Data on CCHFV prevalence in endemic populations of adult *Hyalomma* spp. ticks in CCHFV-endemic countries were collected from the literature and the mean CCHFV-infection prevalence was found to be 8.12% (Table 4.10). Using this figure, it is proposed that the average probability of an adult *Hyalomma* spp. tick being infected with CCHFV in a CCHFV-endemic country (p_{prev}) is 0.0812. It was considered that the largest value that p_{prev} could take was that observed in the literature (28% according to Bursali et al. (2011)) and due to the small dataset available (Table 4.10) it was not possible to fit a distribution that was considered more appropriate than the empirical distribution. Similarly, due to the lack of data for p_{prev} for each individual country, it was considered more appropriate to generalise across countries and apply this to all imports, irrespective of country of origin. Thus, an empirical distribution was fitted to the data.

Table 4.10: Data on CCHFV prevalence among populations of *Hyalomma* spp. ticks in CCHFV-endemic countries. Ticks were tested in pools of varying sizes.

Country	No. of *Hyalomma* spp. ticks tested	*Hyalomma* spp. infection rate	Reference
Spain	22	0.00%	This study
Turkey	Not specified	0.93%	Gargili et al. (2011)
Turkey	860	2.04%	Gunes et al. (2011)
Turkey	479	2.51%	Tekin et al. (2012)
Turkey	471	3.23%	Tonbak et al. (2006)
Kenya	5,104	3.30%	Sang et al. (2011)
Bulgaria	284	4.93%	Gergova et al. (2011)
Iran	63	7.94%	Fakoorziba et al. (2012)
Turkey	432	8.47%	Albayrak et al. (2010), Albayrak et al. (2010)
Oman	722	9.63%	Williams et al. (2000)
Russia	4,787	10.24%	Yashina et al. (2003)
Kosovo	272	15.80%	Avsic-Zupanc et al. (2007)
Spain	117	16.67%	Estrada-Peña et al. (2012)
Turkey	470	28.00%	Bursali et al. (2011)
Average CCHFV-prevalence		8.12%	

4.3.4 Prediction of the density of CCHFV-infected *Hyalomma* spp. ticks per 1 km^2 imported into GB on horses

The density of CCHFV-infected *Hyalomma* spp. ticks per 1 km^2 imported into GB on horses was predicted (n_{infh}). Using the mean density of horses imported from CCHFV-endemic countries, a Monte Carlo simulation of 5,000 realisations of n_{infh} was run for each 1 km^2 grid cell of GB. The 25th percentile and the 50th percentile (the median) of the model outputs were estimated for each cell and in both cases n_{infh} was found to be 0 for the whole of GB. The 68th percentile was the smallest percentile that produced values of CCHFV-infected tick density greater than 0. Therefore, the 68th percentile of the model output was mapped, along with the 75th and 95th percentiles and the mean (Figure 4.10). The mean, 68th, 75th and 95th percentile values of n_{infh} were summed for the whole of GB to give the predicted number of CCHFV-infected *Hyalomma* spp. adults imported into GB on horses between 1st April and 31st July each year (Table 4.11).

Table 4.11: The predicted total number of CCHFV-infected *Hyalomma* spp. adults imported into GB on horses between 1st April and 31st July each year. The mean, 68th, 75th and 95th percentiles of the output are given.

	Total of n_{infh}
Mean	89.8
68th percentile	5.5
75th percentile	40.4
95th percentile	494.0

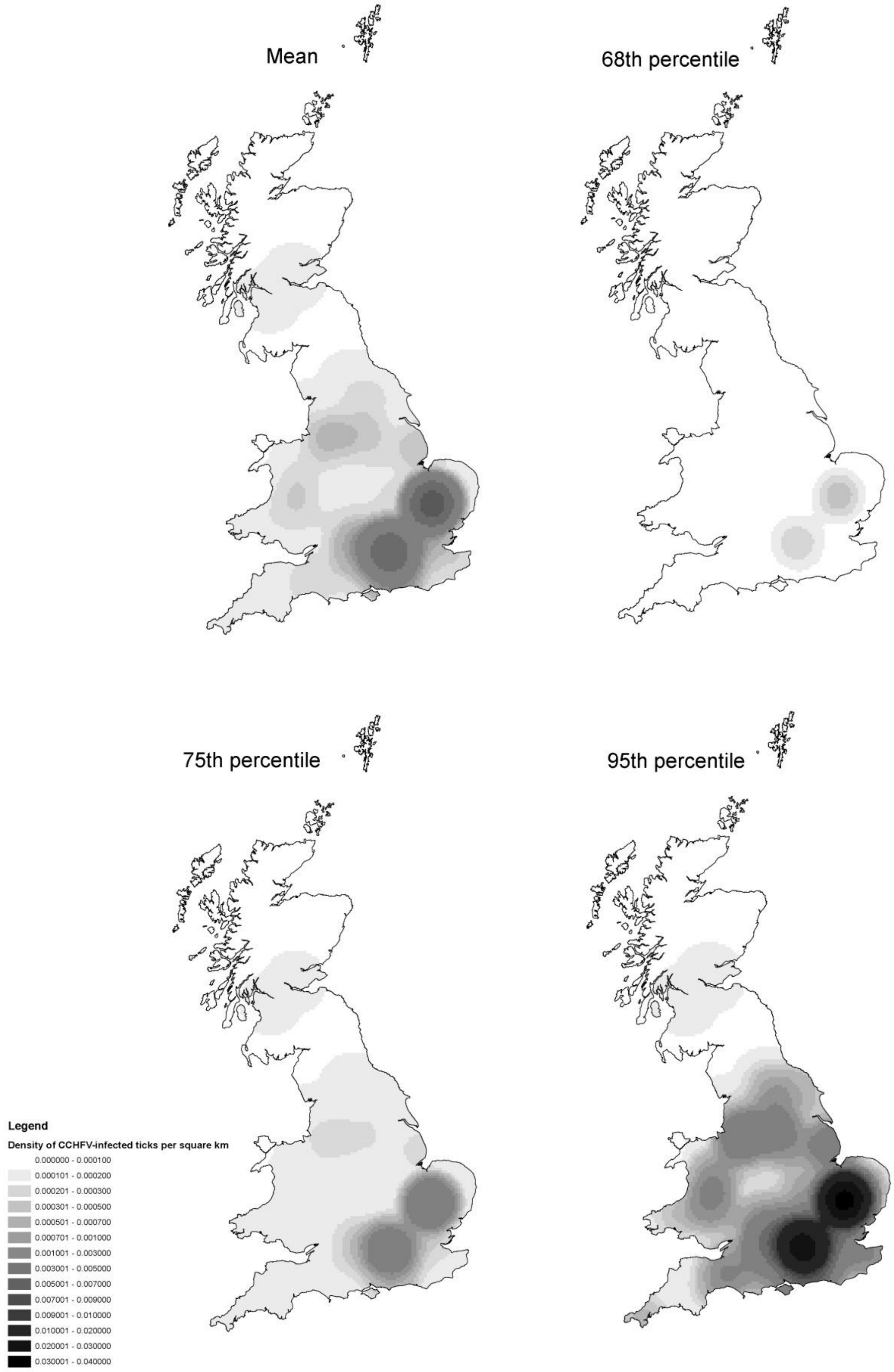


Figure 4.10: The mean, 68th, 75th and 95th percentiles of n_{infh}, the predicted density (ticks per 1 km^2) of CCHFV-infected ticks entering GB on horses between 1st April and 31st July each year.

The results of the simulation show that for more than 50% of the time, no CCHFV-infected *Hyalomma* spp. ticks were predicted to be imported into GB between 1st Apri and 31st July each year. However, as would be expected from the skewed distribution of ticks on hosts, there were rare occasions when a larger number of CCHFV-infected ticks could enter GB on horses. This has implications for sampling of horses for infected ticks which will be further discussed. The predicted mean number of CCHFV-infected ticks imported on horses between 1st April and 31st July for the whole of GB was found to be 89.4 ticks. This was larger than that predicted for the 68th and 75th percentile outputs (Table 4.11). For highly skewed distributions of parasites, as in this case, the median (50th percentile) (n=0 ticks) is a more appropriate measure of central tendency than the mean.

4.4 Discussion

Data on the importation of horses into GB collected from TRACES demonstrated that on average there were 592 horses imported into GB per year between 1st April and 31st July from CCHFV-endemic countries. The longitudinal data from Heathrow Airport indicated that there was an increase in the number of horse importations into GB during the spring and early summer months. This coincides with the seasonality of adult *Hyalomma* spp. ticks in the northern hemisphere (Estrada-Peña et al., 2011) and, therefore, when horses are most likely to be parasitised by adult ticks.

The data from TRACES provide a sample of the horses that are being imported into GB per year but a large number of horses move into and out of GB for which data are not recorded. Weatherbys is the only other database besides TRACES that holds information on horse importation into GB but this is limited to movements of thoroughbreds for competitions (Roberts, H., AHVLA, personal communication). Therefore, it was considered that the average import figure of 592 horses for the period 1st April to 31st July each year from CCHFV-endemic countries is a conservative figure and could greatly underestimate the number of horses that are actually being imported. A tripartite agreement exists between the Republic of Ireland, France and the UK whereby horses can move freely between these three countries. As such movements between these countries are not required to be logged on TRACES and there is no record or regulation of these movements. Horses imported into the EU from third countries (non EU-member states) must be recorded on TRACES, but onward movements of these horses within the EU are not necessarily recorded if the horse is registered. Registration of a horse means that the horse is listed in a studbook and this negates the need for the horse to be logged or checked by a veterinarian when it travels within the EU. Therefore, registered horses are entering GB without being recorded such that data on the number of registered horses imported is unavailable for risk estimation. Registered horses can enter GB from EU member states without being recorded on TRACES or checked at a BIP and it is considered (although not known) that a large number of horses enter GB on ferries in this manner. These horses will not have shown up on the TRACES database that was used in this study. Therefore, horses imported into the EU from countries such as Bahrain, Saudi Arabia and Oman can subsequently move into GB without being recorded on TRACES so long as they are registered to a studbook. Horses entering the EU from third countries have a 90 day visa within the country of import and may move to other EU member states before or when their visa expires. Ticks would detach from horses during this 90 day time period.

Horses entering GB directly from third countries are checked routinely by a veterinarian at a BIP. However, ticks are frequently found in locations on the horse’s body that are difficult to access, such as under the tail and around the genitalia. This was noted during the collection of ticks from horses in Spain during this study. As such, during a BIP check, it is unlikely that ticks would be noticed on the horse especially considering that horses can be very bad tempered after travel and detailed examination of the horse may not be possible (personal observation). Therefore, whether horses enter GB through BIPs or not, they are very unlikely to be examined thoroughly for ticks and as such there may be frequent importations of *Hyalomma* spp. ticks that go un-noticed and unreported.

The uncertainty in the number of horses entering GB from CCHFV-endemic countries (n_{horse}) means that there is a large amount of uncertainty in the model output, n_{infh}. This uncertainty was demonstrated by using two different data sources to collect the same data, TRACES and paper documentation from Heathrow Airport. Slightly larger numbers of horses were recorded by Heathrow

Airport, possibly due to subsequent cancellation or expiration of import certificates such that they were no longer held on the TRACES database. There is also spatial uncertainty in the model outputs as there may be areas of GB that are receiving large numbers of horse imports that are not currently recognised and, therefore, may be incorrectly shown as areas of low importation in this study.

The field work carried out in Spain found a mean prevalence of 1.89 *Hyalomma* spp. ticks per horse. For horses from which all ticks were removed, it was assumed that no further ticks were present that had been missed. In reality, due to the nature of the collection process, on the more heavily infested horses there may have been a few additional ticks that were missed. However, given the much greater prevalence of *Rhipicephalus* spp. ticks found on horses, missed ticks would be more likely to be *Rhipicephalus* spp.. Any missed *Hyalomma* spp. ticks would be unlikely to make any considerable difference to the final predictions of CCHFV-infected *Hyalomma* spp. ticks entering GB per year between 1st April and 31st July and would only change the results by tens rather than hundreds or thousands.

The model in this study assumes that all horses are equally susceptible to infestation by *Hyalomma* spp. ticks. Eight of the horses examined in Spain were kept in grassy paddocks and the remaining ten spent most of their time in stables. The stabled horses were those that were not infested with ticks, but they were also the horses that had been checked for ticks a week prior to examination for this study. Any ticks found at that time had been removed. Therefore, the sample of 18 horses that were checked for ticks cover two likely yet different situations from which horses might be imported – some are kept in *Hyalomma* spp. habitat with no tick removal and some are kept indoors and checked for ticks. These two situations represent two opposite ends of the spectrum and reflect the variation in husbandry practices that could exist between horses imported into GB.

None of the horses that were checked for ticks in Spain had been treated with acaricides despite the heavy tick infestations observed. Some British horse owners in Spain (whose horses were not included in those checked for ticks) used a generic insecticide on their horses on an intermittent basis and still had to remove ticks by hand (personal observation). Local Spanish horse owners did not use acaricides or insecticides on their horses as it was not deemed to be financially or practically viable. This is because during the peak tick biting season, the population of ticks and, therefore, the frequency of bites is so great that horses would have to be treated at least once a week in order to have any impact on tick infestations. It is fair to assume that a similar approach to dealing with tick infestations is common throughout Spain, and perhaps thoughout all *Hyalomma* spp.-endemic countries which support large populations of a variety of tick species. The efficacy of tick repellants is considered to be less for *Hyalomma* spp. than for other ticks such as *Ixodes* spp. (ECDC, 2012) and there are no tick repellants that have been developed or licensed specifically for use on horses in the UK (NOAH, 2010). It is considered that this is also the case in other countries (Floyd, T., AHVLA, personal communication). Overall, the use and efficacy of tick repellants or acaricides in Spain is believed to be typical of a country with endemic populations of *Hyalomma* spp. ticks. Therefore, this assumption applies to other countries with endemic populations of *Hyalomma* spp. ticks.

CCHFV was isolated from ticks in Spain in 2010 (Estrada-Peña et al., 2012b) but no human cases have occurred to date. However, the decision was taken to include Spain as a CCHFV-endemic country and, indeed, to carry out field work in Spain. Spain has endemic populations of *Hyalomma* spp. ticks and this is believed to be a prerequisite to having endemic CCHFV. It is considered that the virus is circulating in Spain and has a natural reservoir there. It may be that only some regions of Spain (or indeed only one) are CCHFV-endemic and that the levels are so low that human cases have not yet occurred. CCHFV was isolated from *H. lusitanicum* in Spain, a tick that parasitizes horses. Indeed a tick of this species was found on a horse in this study. As such, horses imported from Spain to GB have the potential to bring in CCHFV-infected *H. lusitanicum* ticks. The extent of the CCHFV-infection in Spain is not currently known and this research did not find the virus present in ticks in Cadiz and Malaga, although only a small number of *Hyalomma* spp. ticks were collected and tested for CCHFV (*n*=22 ticks). It may be that the virus is limited to certain regions within Spain and has not as yet spread further. The strain of the virus in Spain was found to be most closely related to that found in Mauritania and Senegal, which suggests an importation from a migratory bird (Estrada-Peña et al., 2012b). If this was the case, the infected tick that was imported on the bird would have been *H. rufipes* or *H. marginatum* as these are the two species that parasitize migratory birds and are present in Mauritania and Senegal. The virus may, therefore, be present in populations of *H. marginatum* in Spain as well as *H. lusitanicum* but as yet this has not been found.

The simulation results for the predicted number of CCHFV-infected adult *Hyalomma* spp. ticks entering GB each year between 1st April and 31st July (n_{infh}) reflect the highly skewed negative binomial distribution that describes *Hyalomma* spp. ticks on horses. As previously mentioned, this has implications for sampling and surveillance. If surveillance was to be carried out, sampling one in 10 horses, or even one in five horses that were imported would be insufficient to determine the number of infected ticks entering GB. A large number of horses would need to be checked to ensure that the surveillance captured the few horses that may harbour a large number of ticks.

The mean number of CCHFV-infected adult *Hyalomma* spp. ticks entering GB was predicted to be 90 between 1st April and 31st July each year. The median value was predicted to be 0. However, the actual number of horses imported into GB from CCHFV-endemic countries each year between 1st April and 31st July will be greater than that recorded in TRACES as discussed above. Therefore, the average number of CCHFV-infected *Hyalomma* spp. ticks imported into GB may be larger than that predicted here. The number of horses imported into GB is, therefore, important to studies such as this and future research should focus on finding a way to accurately estimate the uncertainty.

5 Spatial analysis of habitat suitability for *Hyalomma marginatum* in GB under current climatic conditions.

5.1 Introduction

When evaluating the overall risk of CCHF to Great Britain (GB) from the pathways of birds and horses, there are two main elements that need to be considered. The first is the probability of an infected *Hyalomma* spp. tick entering from a CCHF-endemic country and the second is the survival of the tick in GB. It is considered that established populations of *Hyalomma* spp. ticks must be present in an area for CCHF to become endemic (Ergönül, 2006) and, therefore, modelling the survival of *Hyalomma* spp. ticks in GB also gives an indication of the areas that are most suitable for population establishment, if any. The pathways for entry of *Hyalomma* spp. ticks through migratory birds and horses were addressed in Chapters 3 and 4, along with predictions of the number of infected ticks entering GB via these routes. Models for the climatic suitability of *H. marginatum* in Europe were developed by Estrada-Peña et al. (2011). This chapter, therefore, addresses the possibility of *H. marginatum* surviving after entry into GB.

A *H. marginatum* tick entering GB on wild birds or horses needs to survive to the next life stage under current climatic conditions. Generally, immature *H. marginatum* ticks feed on small animals and ground-feeding birds while adults feed on large mammals, including livestock (Hoogstraal, 1979). An immature *H. marginatum* tick entering on a migratory bird will detach as an engorged nymph and if the conditions are suitable will subsequently moult to an adult (Hillyard, 1996). An *H. marginatum* tick entering GB on a horse will detach as an engorged adult. *H. marginatum* ticks mate whilst they are on the host and, therefore, mating will occur prior to detachment from the host (Hillyard, 1996). After detachment, adult males die and females lay eggs. Therefore, mated adult females could enter GB on horses and subsequently lay eggs.

A recent study on CCHF has highlighted the need for an investigation into whether or not exotic tick species can survive and establish in new areas of suitable habitat (Estrada-Peña et al., 2012a). In this chapter, this is considered for GB using spatial analysis coupled with an environmental model to predict the areas of GB where development and survival of *H. marginatum* could occur. In particular, spatially explicit GB temperature data were used within the series of life-stage models developed for *H. marginatum* by Estrada-Peña et al. (2011). The outputs from these models were combined with land-cover data to generate maps which predict the extent of development over time, given importation of the tick. By modelling over several years, these maps give an indication of both temporal and spatial risk. The models are predicted for *H. marginatum* but the results are assumed to be applicable to all species of *Hyalomma* that would enter GB on migratory birds or horses, such as *H. rufipes* and *H. lusitanicum.*

5.2 Method

The lifecycle for a *H. marginatum* tick is shown in Figure 5.1. The process of nymph-to-adult moulting, the ability of an adult female to lay eggs and the survival of those eggs were assumed to be dependent on temperature and water vapour deficit only, as defined by the equations given by Estrada-Peña et al (2011). These processes were modelled using current climate data (2007 to 2010) and land cover data to predict habitat suitability for *H. marginatum.*

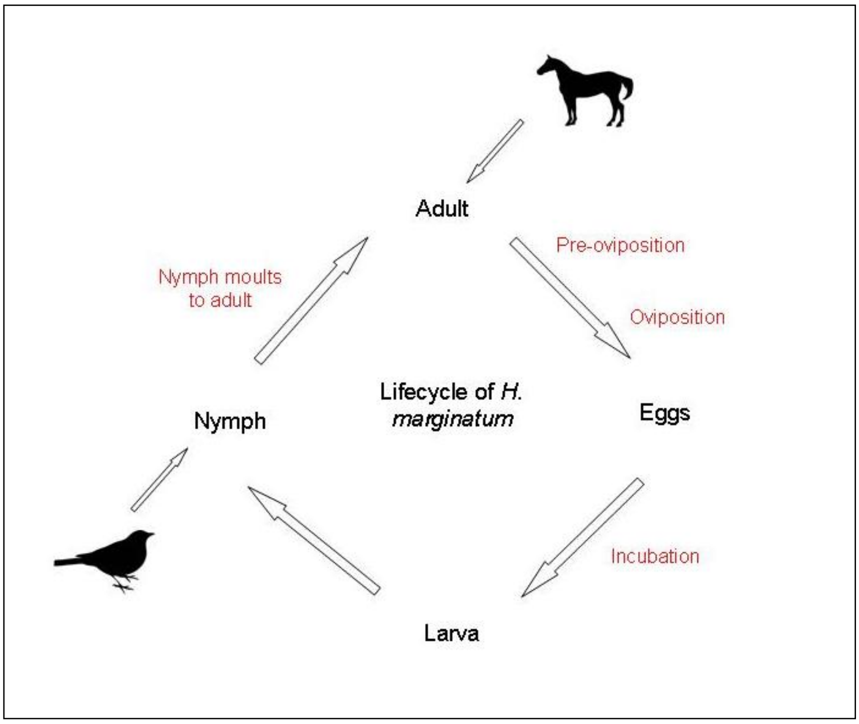


Figure 5.1: Lifecycle of a *H. marginatum* tick, showing nymphs and adults being imported by birds and horses respectively. Processes that were modelled in this study are shown in red.

5.2.1 Temperature data

Daily average temperature data from the UK Met Office for January 2007 to October 2010 had previously been obtained by AHVLA upon request. The data for each day were preformatted as a comma delimited text file with 180 columns and 290 rows producing a grid of 52,200 25 km^2 geographical units across GB in line with the British National Grid. For each 25 km^2 grid cell, the daily average temperature was given in degree Celsius (°C) measured at a height of 1.5 m above ground. Daily average temperature files were processed using the programming language, Perl, into single comma delimited text files for each month. This processed the temperature data into a more manageable format for modelling.

5.2.2 Land cover data

Land cover type was included in the model as vegetation is a basic aspect of the ecological preference of a tick species (Gray et al., 2009). Vegetation is also one of the two major factors affecting tick distribution (the other being climate) (Estrada-Peña, 2001). The Land Cover Map 2007 (LCM2007) had been previously obtained by AHVLA and was, therefore, available for use in this study. It is available at a 1 km^2 resolution and is shown in Figure 5.2. It was assumed that all potentially suitable habitat types were represented by LCM2007 and that habitats were correctly identified.

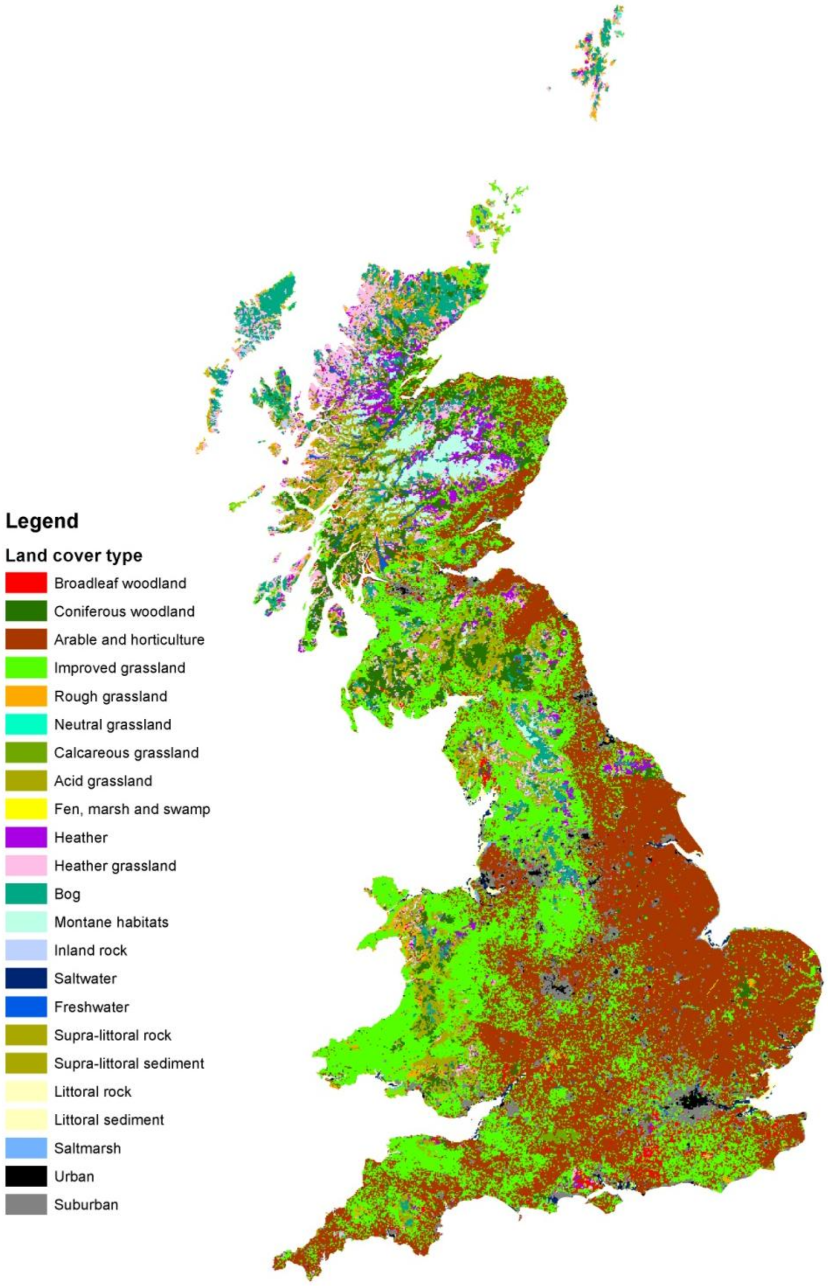


Figure 5.2: Land Cover Map 2007 at a resolution of 1 km^2.

Land cover type that was suitable for *H. marginatum* was chosen by eliciting expert opinion (Papa, A., Bouattour, A., personal communication), from published literature and from field observations from southern Spain. Opinion from Anna Papa and Ali Bouattour was elicited because they have worked extensively on CCHFV and its vectors. They were asked to describe the types of habitat where *H. marginatum* would be found. They were also given the habitat categories used in the LCM2007 and asked to assign each as suitable or unsuitable. During previous field work carried out in Spain (see Chapters 3 and 4), *H. marginatum* ticks were observed in dry grassland areas with low vegetation, but were not found in arable areas or heathlands (Figure 5.3).

Figure 5.3: Suitable habitat in southern Spain for *H. marginatum*, May 2011 (Photo taken by M. England in Spain, May 2011).

Estrada-Peña and Santos-Silva (2005) describe the preferences of *H. marginatum* as ‘hot and dry climate conditions of the Mediterranean type’ with vegetation described as ‘isolated patches of *Quercus* spp. (oak), together with natural pasture’. According to Hoogstraal (1979), *H. marginatum* is ‘characteristic of steppe, savanna and lightly wooded hill and valley biotypes, but not of deserts, semideserts, deep forests or high mountains’. Kotti et al. (2001) describe the habitat preferences of *H. marginatum* in Stavropol, Russia, as steppe and semidesert. Maltezou and Papa (2010) describe *Hyalomma* spp. ticks as being found in ‘areas of small and relatively dry vegetation’ and ‘not in forest-type vegetation’.

A study by Castellà et al. (2002) found *H. marginatum* to be present in large numbers on dairy cattle in spring on the island of Menorca. The sampled areas of Menorca were described by Castellà et al. (2002) as pastures which were interspersed with forested and bushy areas made up of oaks, pines, olive trees, pistachio shrubs, rock roses and heathers. Dry stone walls divided up the pasturelands and cattle were grazed on rotation. The elevation was below 400 m and the annual rainfall was 450 mm. There were no *Ixodes ricinus* ticks found in the survey, presumably due to the low relative humidity of the island. Some *H. marginatum* ticks were found in this survey, but twice as many *R. bursa* ticks were found (Castellà et al., 2002) suggesting that this habitat was not optimal for *H. marginatum*. However, it is important to note that this habitat is able to sustain viable populations of *H. marginatum*. There are no wild ungulates living on Menorca (no deer or wild boar) and, therefore, the dairy cattle herds may play a key role in maintenance of *H. marginatum* populations and act as the only available secondary host.

Torina et al. (2006) collected ticks from cattle on four different farms in western Sicily, an area described as ‘meso-Mediterranean’. The farms they collected from were in hilly areas between 585 m and 834 m above sea level (a.s.l.). *H. marginatum* only made up 5.3% of all the ticks they collected with the smallest number (nine) being found on the farm that was both the highest above sea level and that utilised cattle-sheds for part of the year. The farm with the largest number of *H. marginatum* was 680 m a.s.l. and the cattle were grazing on native grasses and pastures all year round. The maximum exposure of the cattle to tick bites may explain why the largest number of ticks was collected at this farm. Only 39 *H. marginatum* were found on cattle at the farm located at the lowest altitude but here cow sheds were used to house the cattle for some of the year. The two farms that yielded the largest number of *H. marginatum* were those that used a wild system of breeding and that grazed entirely on native grasslands and pastures (Torina et al., 2006). It is possible that more *H. marginatum* would have been found at the lowest altitude farm if these grazing techniques had been employed there.

From the field observations, expert opinion and literature, all types of grassland were deemed suitable, but arable and agricultural lands were considered unsuitable. Pesticides used in such areas may have detrimental effects on tick survival and irrigation may create a habitat that is unfavourably humid for *H. marginatum.* The land may also be ploughed annually destroying any suitable habitat, and crop rotation would mean that each year the habitat would change. The land cover classifications and their suitability for *H. marginatum* are shown in Table 5.1.

Table 5.1: Land cover classifications from LCM2007 and their suitability for *H. marginatum*.

Land cover classification	Suitability for *H. marginatum*
Broad-leaved/mixed woodland	Unsuitable
Coniferous woodland	Unsuitable
Arable and horticulture	Unsuitable
Improved grassland	Suitable
Rough grassland	Suitable
Neutral grass	Suitable
Calcareous grass	Suitable
Acid grass	Suitable
Fen, marsh and swamp	Unsuitable
Heather	Unsuitable
Heather grassland	Unsuitable
Bog	Unsuitable
Montane habitats	Unsuitable
Inland rock	Unsuitable
Saltwater	Unsuitable
Freshwater	Unsuitable
Supra-littoral rock	Unsuitable
Supra-littoral sediment	Unsuitable
Littoral rock	Unsuitable
Littoral sediment	Unsuitable
Saltmarsh	Unsuitable
Urban	Unsuitable
Suburban	Unsuitable

The LCM2007 provides the percentage of each type of land cover in each 1 km^2 grid cell of GB. The percentages of suitable land cover types (as defined in

Table 5.1) occurring in each cell were summed to give the total percentage of suitable land cover in each cell. In grid cells where no suitable land cover occurred, it was assumed that the tick would not survive and, therefore, moult and egg production would not complete. The percentage of suitable land cover in each cell was used to determine the proportion of suitable land cover in each cell (*LCS*). A map showing the values of *LCS* for each 1 km^2 is given in Figure 5.4.

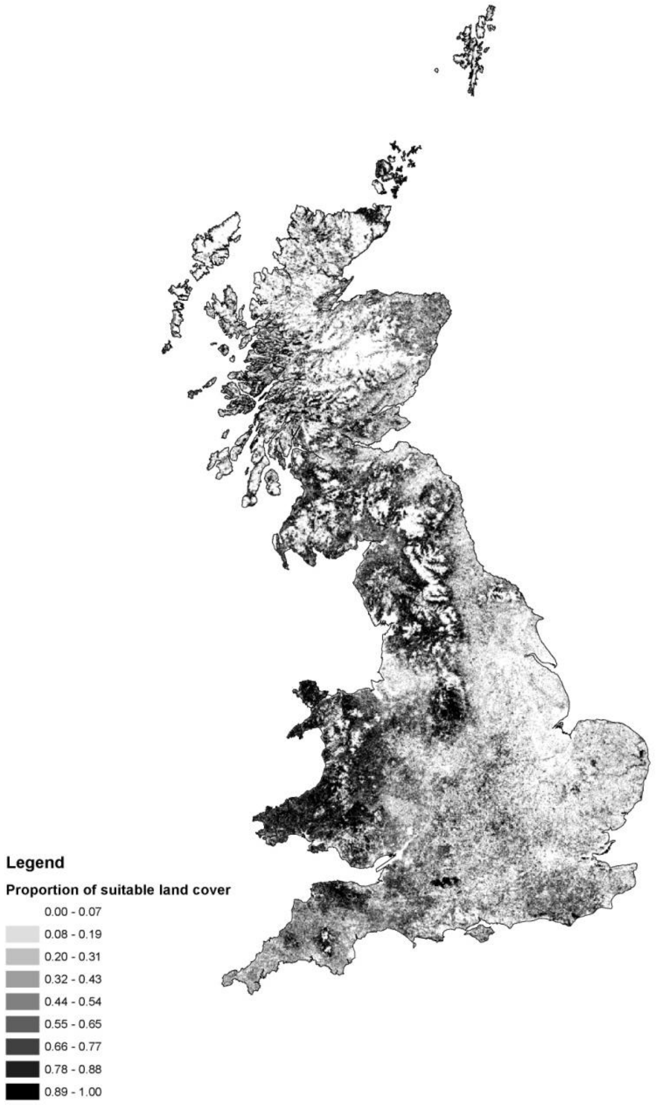


Figure 5.4: Proportion of suitable land cover, *LCS*, per 1 km^2 of GB (LCM2007).

5.2.3 Modelling moult of the nymph

The moult of an engorged *H. marginatum* nymph imported into GB on a migratory bird was modelled. Some important steps in the life cycle of the *H. marginatum* tick have to be completed for it to enter GB in the spring as an engorged nymph on a migratory bird. These steps are outlined in Figure 5.5.

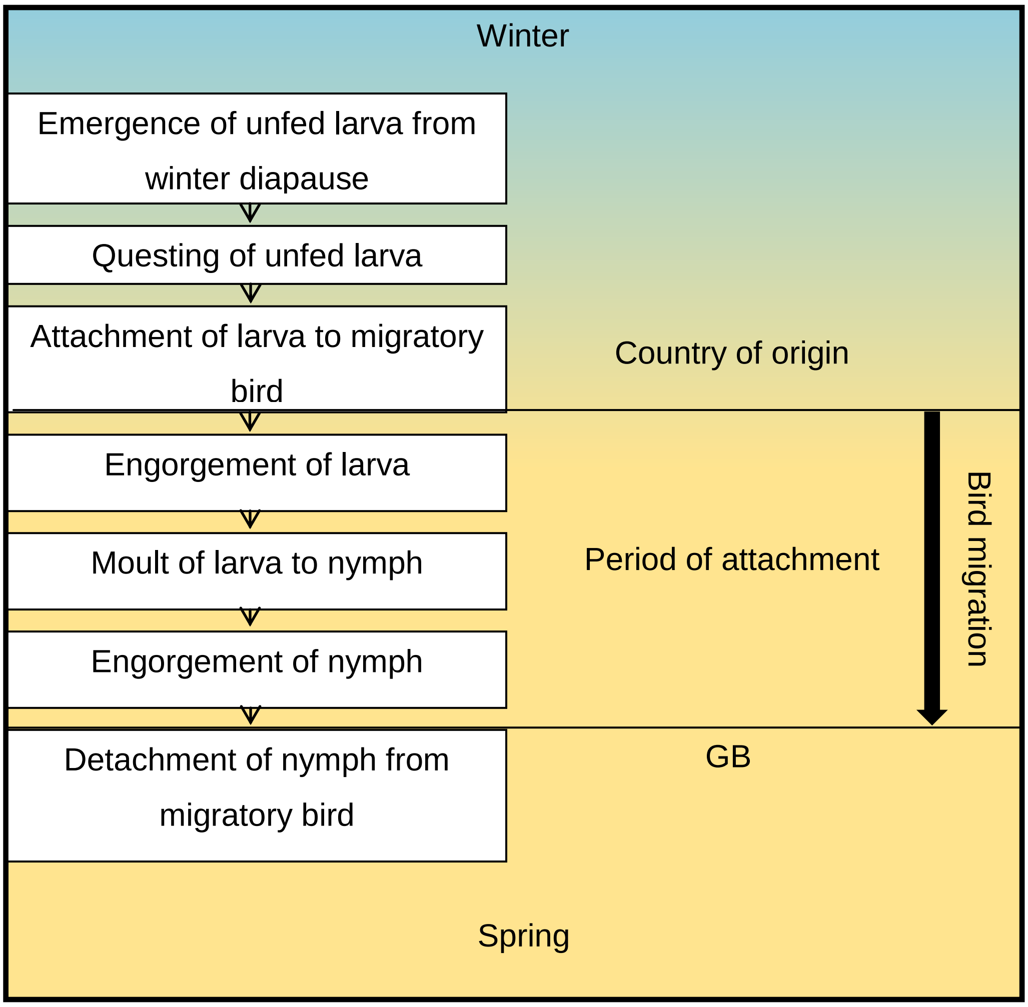


Figure 5.5: Steps in the life cycle of *H. marginatum* that occur prior to entry of an engorged nymph into GB on a migratory bird.

Given that these steps occur prior to moult commencing, it was assumed that the earliest date that an imported nymphal *H. marginatum* could begin to moult in GB was 1st April. The model developed here predicts the percentage of moult that has occurred by a specific date after the assumed start date of 1st April. It is based on a refinement of two equations which were published by Estrada-Peña et al. (2011). The refinement was based on further empirical work and details were provided by the authors of the original publication (Estrada-Peña, A., personal communication).

The first equation (Equation (5.1)) predicts the duration of the moult of an engorged nymph to an adult, T_m (days).

$$T_m = 192.2344 - 6.054T + 0.2577VD \tag{5.1}$$

Where T is daily average temperature and VD is water vapour deficit. On days when temperatures were below 7°C, moult was assumed to be suspended or put on hold (i.e. there was no development) (Estrada-Peña, A., personal communication).

The second equation (Equation (5.2)) predicts the mortality rate of engorged nymphs, M_n (%), over a 30 day period.

$$M_n = 51.4786 + 1.002524T - 0.222031VD \tag{5.2}$$

These equations were manipulated so that they could be used to predict the date that moult would be completed if a tick began moulting on 1st April, based on daily average temperature data. To investigate this, water vapour deficit was kept constant at 15 hPa and daily average temperatures were input into the model to calculate the daily moult and mortality rates. Each equation, therefore, had fixed values of VD and variable values of T. Due to the fact that the mortality equation (Equation (5.2)) was based on a 30 day period, the daily rate was divided by 30. The daily mortality M_{nd} (%) and daily moult rates T_{md} (%) are given in Equations (5.3) and (5.4).

$$M_{nd}[t] = (M_n / 100) / 30 \tag{5.3}$$

$$T_{md}[t] = 100 / T_m \tag{5.4}$$

Moult rates were compounded by mortality rates and then cumulated daily. If they reached 100%, moult was considered to be complete. The total cumulative moult rate, CT_m (%) at time t days since April 1st is given by Equation (5.5).

$$\begin{aligned} &CT_m[0] = 0 \\ &CT_m[t] = CT_m[t-1] + \left(T_{md}\left(1 - M_{nd}\right)\right) \qquad t > 0 \end{aligned} \tag{5.5}$$

Equation (5.5) was run for all grid cells with the daily average temperature data, from 1st April to 31st July of each year (2007 to 2010 inclusive) to determine the percentage of moult completed in each 25 km^2 grid cell. It was assumed that if a nymph began to moult on 1st April, the tick would expire if moult was not completed by 31st July. This assumption was a modelling decision based on empirical data of moult duration. Studies have shown great variation in moult rate from six days in *H. dromedarii* (El Ghali and Hassan, 2010b) to 36 days in *H. rufipes* (Chen et al., 2012). However, these studies were conducted in conditions with warmer average temperatures than those that would occur in GB. The moult process is affected by temperature (see Equation 5.1) (Estrada-Peña et al., 2011) and, therefore, given the lower temperatures that these ticks would be exposed to in GB, it was reasonable to allow a longer period of time for nymph-to-adult moult. According to A. Estrada-Peña (personal communication) there is no known moult duration beyond which the tick will not survive. For the purposes of risk assessment, however, it was decided that the tick would not survive beyond four months. This allowed for a reasonable extension to the moult duration observed in previous studies, whilst accepting that moult could not continue indefinitely.

Given this assumption, the 1 km^2 grid cells of GB where moult could be completed before 31st July, given that moult began on 1st April were predicted for 2007, 2008, 2009 and 2010. The probability that moult could complete by 31st July (p_m) in each 1 km^2 cell of GB was determined from the number of years in which moult was predicted to complete by 31st July. Habitat suitability for moult was defined as a function of land cover suitability and the climatic suitability for moult. Thus, for each 1 km^2 grid cell of GB, the probability of habitat suitability for nymph-to-adult moult (p_HSm) was calculated using Equation (5.6).

$$p_{\mathrm{HSm}} = LCS \times p_{\mathrm{m}} \qquad (5.6)$$

5.2.4 Modelling egg development

The process of egg development (pre-oviposition, oviposition and incubation of eggs) was modelled for an engorged *H. marginatum* adult female imported into GB on a horse. Some important steps in the life cycle of the *H. marginatum* tick have to be completed for an engorged adult female tick to enter GB in the spring on a horse and subsequently lay eggs. These steps are outlined in Figure 5.6.

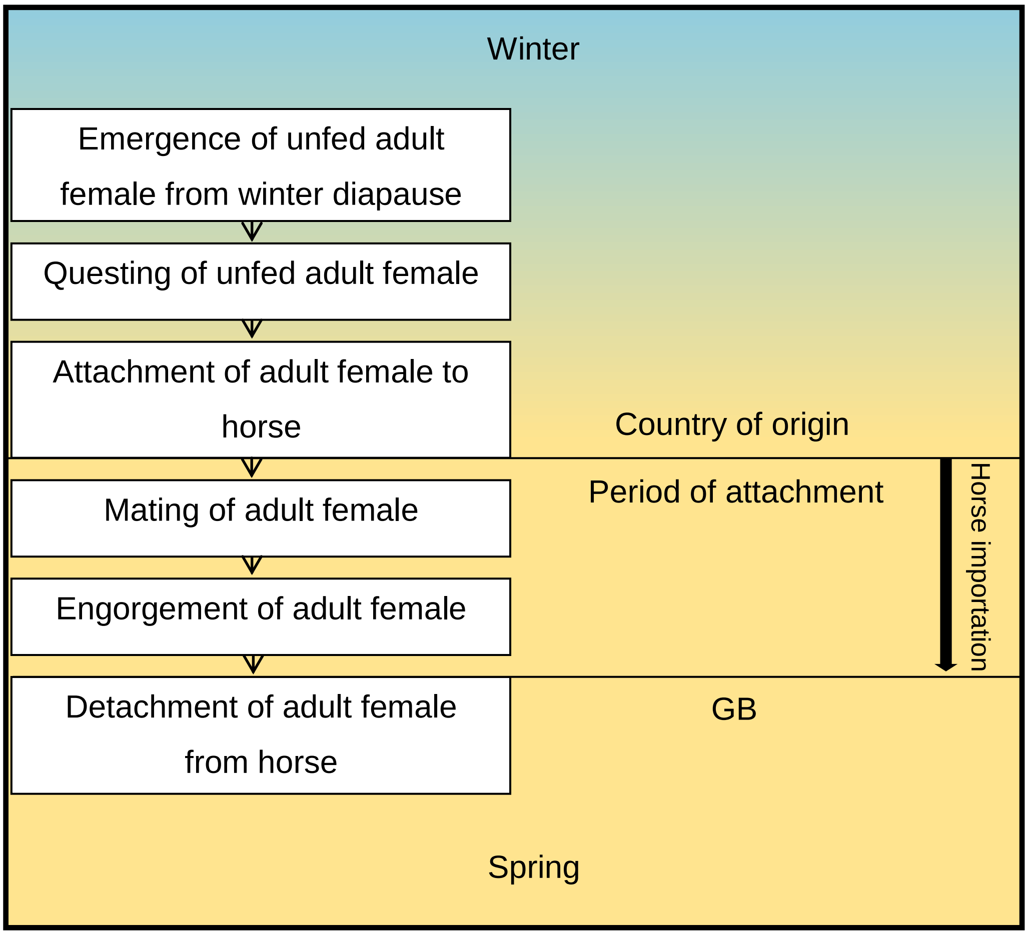


Figure 5.6: Steps in the life cycle of *H. marginatum* that occur prior to an imported adult female tick laying eggs.

The mating of the adult female tick relies on the presence of a male tick on the same host (in this case a horse) and that they find each other. For this model, it was assumed that the steps in Figure 5.6 up to the point of detachment from the horse had occurred. Given that these processes occur at the end of winter/start of spring when temperatures are starting to increase, it was considered that the earliest a mated adult female tick would enter GB and detach from its host was 1st April. The egg development model was, therefore, run with a start date of 1st April.

An adult female tick has to undergo a process called pre-oviposition prior to egg laying (oviposition) (Figure 5.1). Once the eggs are laid, they incubate (during which time the embryos develop) and subsequently hatch. The model predicts the date that pre-oviposition, oviposition and incubation would be completed after the start date of 1st April. As with the nymph-to-adult moult model, the model for egg development was based on equations published by Estrada-Peña et al. (2011).

The first equation (Equation (5.7)) predicts the duration of pre-oviposition of an adult female tick, T_p (days).

$$T_p = 50.774 - 1.0728T - 0.229VD \qquad (5.7)$$

Where T is daily average temperature and VD is water vapour deficit.

The second equation (Equation (5.8)) predicts the duration of oviposition of an adult female tick, T_o (days).

$$T_o = 66.19 - 1.5638T - 0.179VD \qquad (5.8)$$

The third equation (Equation (5.9)) predicts the duration of incubation of the eggs, T_i (days).

$$T_i = 59.7 - 1.151T - 0.1014VD \qquad (5.9)$$

The fourth equation (Equation (5.10)) predicts the mortality rate of the adult female ticks, M_f (%), over a 30 day period.

$$M_f = 19.32 + 1.212T - 0.16VD \qquad (5.10)$$

The fifth equation (Equation (5.11)) predicts the mortality rate of the developing eggs, M_e (%) over a 30 day period.

$$M_e = 108.325 - 3.848T + 1.414VD \qquad (5.11)$$

These equations were manipulated so that they could be used to predict the dates that oviposition, pre-oviposition and incubation completed. As in the previous model for nymph-to-adult moult, water vapour deficit was kept constant at 15 hPa and daily average temperature data were input from 1st April for the years 2007, 2008, 2009 and 2010 in each grid cell. Equations (5.10) and (5.11) were based on a 30 day period and, therefore, the daily rate of mortality was divided by 30. The daily mortality rates (%) of an adult female tick (M_{fd}) and of the developing eggs (M_{ed}) are given in Equations (5.12) and (5.13).

$$M_{fd}[t] = (M_f / 100)/30 \qquad (5.12)$$

$$M_{ed}[t] = (M_e / 100)/30 \qquad (5.13)$$

The daily rate of pre-oviposition, T_{pd} (%) is given in Equation (5.14).

$$T_{pd}[t] = 100 / T_p \qquad (5.14)$$

The daily rate of pre-oviposition was compounded by the daily mortality rate of the adult female tick until it reached 100%, at which point pre-oviposition was considered to be complete. The total cumulative daily pre-oviposition rate, CT_p (%) at time *t* days since 1st April is given in Equation (5.15).

$$\begin{aligned} &CT_p[0] = 0 \\ &CT_p[t] = CT_p[t-1] + (T_{pd}(1 - M_{fd})) \qquad t > 0 \end{aligned} \qquad (5.15)$$

The daily rate of oviposition, T_{od} (%) is given in Equation (5.16).

$$T_{od}[t] = 100 / T_o \qquad (5.16)$$

The daily rate of oviposition was compounded by the daily mortality rate of the adult female tick until it reached 100%, at which point oviposition was considered to be complete. The total cumulative daily oviposition rate, CT_o (%) at time *t* days since 1st April is given in Equation (5.17).

$$\begin{aligned} &CT_o[0] = 0 \\ &CT_o[t] = CT_o[t-1] + (T_{od}(1 - M_{fd})) \qquad t > 0 \end{aligned} \qquad (5.17)$$

The daily rate of incubation, T_{id} (%) is given in Equation (5.18).

$$T_{id}\left[t\right] = 100 / T_i \qquad (5.18)$$

The daily rate of incubation was compounded by the daily mortality rate of the developing eggs until it reached 100%, at which point incubation was considered to be complete. The total cumulative daily incubation rate, CT_i (%) at time t days since 1st April is given in Equation (5.19).

$$\begin{aligned} &CT_i[0] = 0 \\ &CT_i\left[t\right] = CT_i\left[t-1\right] + \left(T_{id}\left(1 - M_{ed}\right)\right) \qquad t > 0 \end{aligned} \qquad (5.19)$$

In each grid cell, pre-oviposition, oviposition and incubation were run consecutively, so after CT_p reached 100%, CT_o would be calculated. When CT_o reached 100%, CT_i would be calculated. In this way, for each grid cell the whole process of egg laying was modelled from 1st April until 31st July.

The models were run using temperature data for the years 2007, 2008, 2009 and 2010 and the dates for completion of pre-oviposition, oviposition and incubation were predicted for each 25 km^2 grid cell of GB. The results were mapped in ESRI ArcGIS 10.0. It was assumed that if egg development was not completed by 31st July, given that pre-oviposition began on 1st April, then the adult female tick would no longer survive and/or the eggs would not hatch. This allowed a period of four months for egg development to take place, which was based on empirical data on egg development duration. Pre-oviposition was found to take as little as three days in *H. rufipes* (Chen et al., 2012) and up to 30 days in unspecified *Hyalomma* spp. ticks (Durrani et al., 2008). Oviposition was found to vary between 10 days in unspecified *Hyalomma* spp. (Durrani et al., 2008) and 49 days in *H. rufipes* (Chen et al., 2012). Incubation was found to take between 25.6 days in *H. dromedarii (El Ghali and Hassan, 2010b)* and 66 days in *H. rufipes* (Chen et al., 2012). As with nymph-to-adult moult, it was assumed that egg development could not continue indefinitely and, therefore, it was decided that egg development would not be successful beyond 31st July.

Given the above assumption, the 25 km^2 grid cells of GB where egg development could be completed before 31st July, given that pre-oviposition began on 1st April, were predicted for 2007, 2008, 2009 and 2010. For each grid cell of GB, the probability that egg development could complete by 31st July based on climatic suitability (p_e) was estimated from the number of years in which egg development was predicted to complete.

Land cover data were available at a spatial resolution of 1 km^2 and temperature data were available at a spatial resolution of 25 km^2. The climatic suitability for egg development was, therefore, fitted to a 1 km^2 grid across GB such that the detail in the land cover data was not lost. Each 1 km^2 grid cell was, therefore, assigned the value of p_e for the 25 km^2 cell within which it fell. This assumed that the average daily temperature for the 1 km^2 grid cell was the same as that of the 25 km^2 cell in which it fell.

Habitat suitability was defined as a function of land cover suitability and climatic suitability. Thus, for each 1 km^2 grid cell of GB, the probability of habitat suitability for egg development (p_{HSe}) was calculated using Equation (5.20).

$$p_{HSe} = LCS \times p_e \qquad (5.20)$$

Spatial autocorrelation was not accounted for in these models and it was assumed that the suitability of each grid cell in terms of land cover type, temperature and water vapour deficit was independent of the suitability in adjacent cells.

The models for nymph-to-adult moult and egg development were run and the results displayed using ESRI ArcGIS 10.0.

5.2.5 Modelling uncertainty in the equations of Estrada-Peña et al. (2011) for nymph-to-adult moult and for egg development

The equations that were used for modelling nymph-to-adult moult and egg development as described were validated for Europe against the known distribution and seasonality of *H. marginatum* by Estrada-Peña et al. (2011). However, Estrada-Peña et al. (2011) developed their equations using regression analysis to fit a line of best fit through the empirical data for each development stage (Estrada-Peña, A., personal communication). Due to environmental variation between tick populations and differences between individual ticks, the parameter estimates and constants would have inevitably taken a range of values. Thus, the coefficients and constants in Equations (5.1), (5.2), (5.7), (5.8), (5.9), (5.10) and (5.11) were each assumed to be the mean of a Normal distribution. The standard deviation of each of these distributions was not available (Estrada-Peña et al., 2011) and, therefore, it was assumed for each coefficient and constant that the 95% and 5% confidence intervals were equivalent to 20% above and below the mean, respectively. Using this assumption, the standard deviation of each coefficient and constant was determined using @Risk 5.5 in Microsoft Excel 2010 and normal distributions were fitted for each. The 25th and 75th percentile of each distribution were determined and used to characterise the uncertainty in the equations. The fitted normal distribution (denoted by $X \sim N(\mu, \sigma)$ where μ is the mean and σ is the standard variation) and the 25th and 75th percentile values for each of these fitted distributions are given in Table 5.2 for Equation (5.1), Table 5.3 for Equation (5.2), Table 5.4 for Equation (5.7), Table 5.5 for Equation (5.8), Table 5.6 for Equation (5.9), Table 5.7 for Equation (5.10) and Table 5.8 for Equation (5.11).

Table 5.2: Fitted normal distributions ($X \sim N$ (μ, σ) where μ is the mean and σ is the standard deviation) for the constant and coefficients of T and VD in Equation (5.1) for the duration of moult of an engorged nymph to an adult.

	$X \sim N (\mu, \sigma)$	25th Percentile	75th Percentile
Constant	$X \sim N$ (192.2344, 23.4)	176.5	208.0
Coefficient of T	$X \sim N$ (-6.054, 0.736)	-5.558	-6.557
Coefficient of VD	$X \sim N$ (0.2577, 0.035)	0.2341	0.2813

Table 5.3: Fitted normal distributions ($X \sim N$ (μ, σ) where μ is the mean and σ is the standard deviation) for the constant and coefficients of T and VD in Equation (5.2) for the mortality rate of engorged nymphs over a 30 day period.

	$X \sim N (\mu, \sigma)$	25th Percentile	75th Percentile
Constant	$X \sim N$ (51.4786, 6.26)	47.26	55.7
Coefficient of T	$X \sim N$ (1.002524, 0.122)	0.92	1.085
Coefficient of VD	$X \sim N$ (-0.222031, 0.027)	-0.2038	-0.2402

Table 5.4: Fitted normal distributions (X ~ N (μ, σ) where μ is the mean and σ is the standard deviation) for the constant and coefficients of T and VD in Equation (5.7) for the duration of pre-oviposition of an adult female tick.

	$X \sim N (\mu, \sigma)$	25th Percentile	75th Percentile
Constant	$X \sim N$ (50.774, 6.18)	46.61	54.94
Coefficient of T	$X \sim N$ (-1.0728, 0.13)	-0.985	-1.16
Coefficient of VD	$X \sim N$ (-0.229, 0.0278)	-0.2102	-0.2478

Table 5.5: Fitted normal distributions (X ~ N (μ, σ) where μ is the mean and σ is the standard deviation) for the constant and coefficients of T and VD in Equation (5.8) for the duration of oviposition of an adult female tick.

	$X \sim N (\mu, \sigma)$	25th Percentile	75th Percentile
Constant	$X \sim N$ (66.19, 8.05)	60.76	71.62
Coefficient of T	$X \sim N$ (-1.5638, 0.19)	-1.436	-1.692
Coefficient of VD	$X \sim N$ (-0.179, 0.0217)	-0.1644	-0.1936

Table 5.6: Fitted normal distributions (X ~ N (μ, σ) where μ is the mean and σ is the standard deviation) for the constant and coefficients of T and VD in Equation (5.9) for the duration of incubation of the eggs.

	$X \sim N(\mu, \sigma)$	25th Percentile	75th Percentile
Constant	$X \sim N$ (59.7, 7.26)	54.8	64.6
Coefficient of T	$X \sim N$ (-1.151, 0.14)	-1.057	-1.245
Coefficient of VD	$X \sim N$ (-0.1014, 0.0124)	-0.093	-0.1098

Table 5.7: Fitted normal distributions (X ~ N (μ, σ) where μ is the mean and σ is the standard deviation) for the constant and coefficients of T and VD in Equation (5.10) for the mortality rate of the adult female tick over a 30 day period.

	$X \sim N(\mu, \sigma)$	25th Percentile	75th Percentile
Constant	$X \sim N$ (19.32, 2.35)	17.73	20.91
Coefficient of T	$X \sim N$ (1.212, 0.148)	1.112	1.312
Coefficient of VD	$X \sim N$ (-0.16, 0.0194)	-0.1469	-0.1731

Table 5.8: Fitted normal distributions (X ~ N (μ, σ) where μ is the mean and σ is the standard deviation) for the constant and coefficients of T and VD in Equation (5.11) for the mortality rate of developing eggs over a 30 day period.

	$X \sim N(\mu, \sigma)$	25th Percentile	75th Percentile
Constant	$X \sim N$ (108.325, 13.2)	99.4	117.2
Coefficient of T	$X \sim N$ (-3.848, 0.468)	-3.532	-4.164
Coefficient of VD	$X \sim N$ (1.414, 0.172)	1.298	1.53

Firstly, the values of the 25th percentile of the constants and coefficients in Equations (5.1) and (5.2) were input into the model for p_{HSm} (Equation (5.6)) and the model was run. Secondly, the values of the 75th percentile of the constants and coefficients in Equations (5.1) and (5.2) were input into the model for p_{HSm} and the model was run. Similarly, the values of the 25th and 75th percentiles of the constants and coefficients in Equations (5.7), (5.8), (5.9), (5.10) and (5.11) were input into the model for p_{HSe} (Equation (5.20)). Using the 25th percentile for the coefficients and constants in the equations gave the 75th percentile of the output (p_{HSm} or p_{HSe}) as a decrease in the coefficient of temperature reduced the impact of temperature on moult and egg development, thus, increasing the probability of temperature-independent habitat suitability. Correspondingly, using the 75th percentile values for the coefficients and constants, gave the 25th percentile of the output (p_{HSm} or p_{HSe}).

The results were mapped in ESRI ArcGIS 10.0 as previously described and captured something of the uncertainty associated with the equations of Estrada-Peña et al. (2011) used in this study. The uncertainty in the models is not captured completely by this uncertainty analysis as the variable, *VD*, remained fixed at 15 hPa. However, a large amount of the uncertainty surrounding the parameters was addressed here; the impact of varying the value of *VD* is explored in a sensitivity analysis.

5.2.6 Parameter sensitivity in the equations of Estrada-Peña et al. (2011)

In the models for nymphal moult and egg development, the water vapour deficit, *VD*, was set at a constant of 15 hPa and temperature, *T*, was varied according to the temperature data. The effect of water vapour deficit was investigated by plotting the form of the functions for nymph-to-adult moult, pre-oviposition, oviposition and incubation over the respective temperature ranges with *VD* varying between 2 hPa and 26 hPa for all equations. The values for *T* and *VD* values were chosen as these are the range of values for which the equations were developed according to Estrada-Peña et al. (2011) and A. Estrada-Peña (personal communication). The results from the sensitivity analysis show the development rate per day (%) for a specific value of *T* and *VD*, not the cumulative effect considered in the baseline model.

5.2.7 Sensitivity of the models for nymph-to-adult moult and egg development to the start date

The models for nymph-to-adult moult (Equation (5.5)) and egg development (Equations (5.15), (5.17) and (5.19)) were run on the assumption that moult and pre-oviposition would begin on 1st April of each year. The start date of moult was varied for 2010 to see what impact this would have on the percentage of nymph-to-adult moult completed by 31st July in each 25 km^2 grid cell. The start date of pre-oviposition was varied for 2010 to see how this would affect the completion dates of pre-oviposition and oviposition and whether incubation could complete by 31st July. Start dates for moult and pre-oviposition of 1st April 2010, 1st May 2010 and 1st June 2010 were used and the models were run.

Table 5.9: Parameter definitions for the models of nymph-to-adult moult and of egg development.

Parameter	Definition
T	Average daily temperature °C
VD	Water vapour deficit (hPa)
T_m	Duration of moult of engorged nymph-to-adult
T_{md}	Daily nymph-to-adult moult rate (%)
M_n	Mortality rate of engorged nymphs (%)
M_{nd}	Daily mortality rate of engorged nymphs (%)
CT_m	Total cumulative nymph-to-adult moult rate (%)
T_p	Duration of pre-oviposition (days)
T_{pd}	Daily rate of pre-oviposition (%)
T_o	Duration of oviposition (days)
T_{od}	Daily rate of oviposition (%)
T_i	Duration of incubation of eggs (days)
T_{id}	Daily rate of incubation of eggs (%)
M_f	Mortality rate of engorged adult females over a 30
M_{fd}	Daily mortality rate of engorged adult females (%)
M_e	Mortality rate of developing eggs over a 30 day
M_{ed}	Daily mortality rate of developing eggs (%)
CT_p	Cumulative daily rate of pre-oviposition (%)
CT_o	Cumulative daily rate of oviposition (%)
CT_i	Cumulative daily rate of incubation of eggs (%)

5.3 Results

5.3.1 Predicted nymph-to-adult moult

The first model (Equation (5.5)) was run and the resultant data were fitted to a 25 km^2 grid with 9,832 cells that covered GB in ESRI ArcGIS 10.0. The maps in Figure 5.7 to Figure 5.10 show the predicted percentage of moult completed in each 25 km^2 grid cell across GB.

1st April to 30th April 2007

1st April to 31st May 2007

1st April to 30th June 2007

1st April to 31st July 2007

Figure 5.7: Predicted percentage of moult completed by the end of April, May, June and July 2007 if a tick began to moult on 1st April 2007.

Figure 5.8: Predicted percentage of moult completed by end of April, May, June and July 2008 if a tick began to moult on 1st April 2008.

Figure 5.9: Predicted percentage of moult completed by end of April, May, June and July 2009 if a tick began to moult on 1st April 2009.

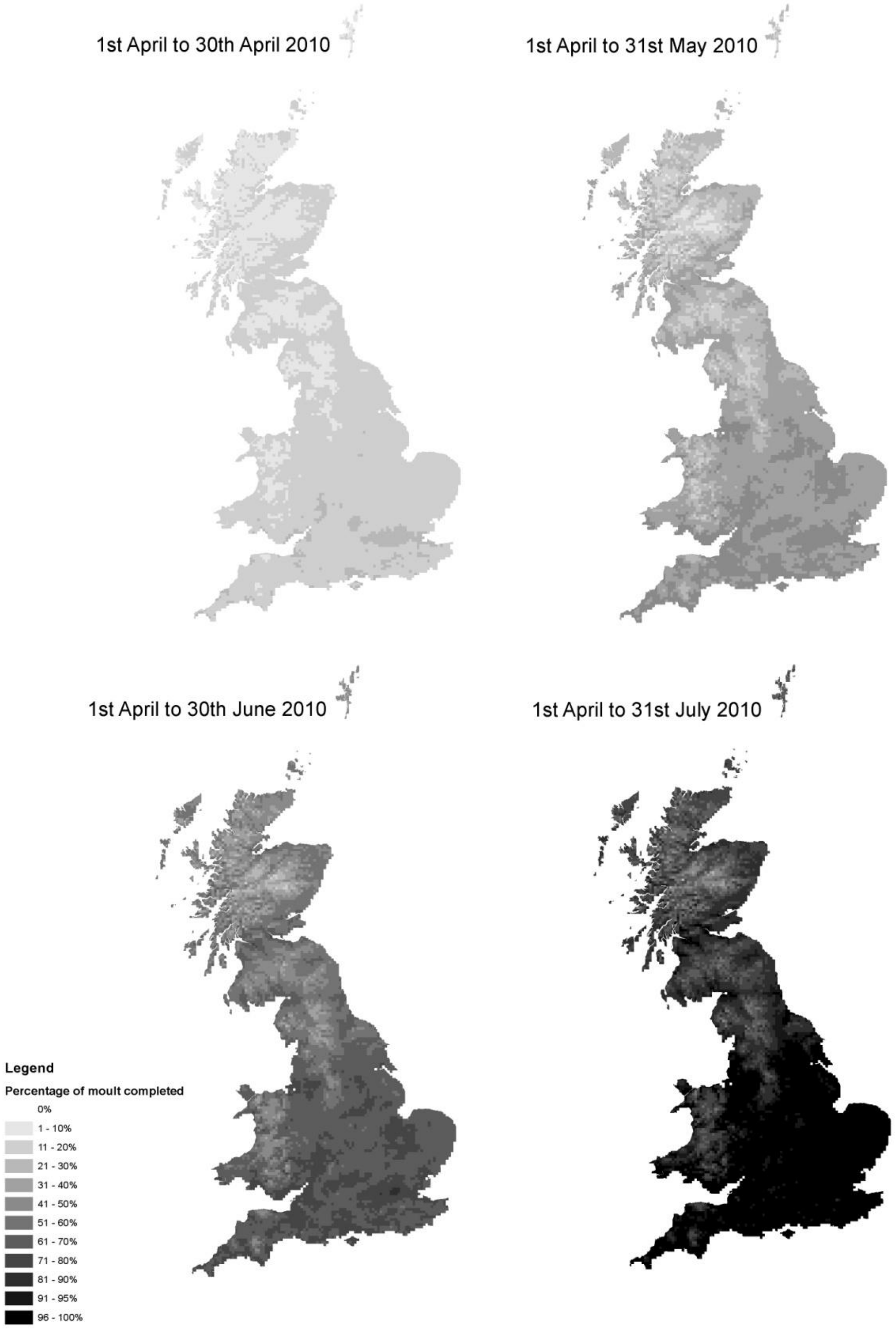


Figure 5.10: Predicted percentage of moult completed by end of April, May, June and July 2010 if a tick began to moult on 1st April 2010.

Assuming that moult began on the 1^{st} April, the earliest date that completion was predicted to have occurred was during the month of July for all years. Table 5.10 shows the predicted date of the earliest moult completion for each year of data, with the duration to earliest moult completion being longest in 2008. There was considerable between-year variation in moult rate and this was apparent when the areas where moult was completed by 31^{st} July each year were compared (Figure 5.11). Thus, the number of cells in which moult was completed by the end of July varied year-on-year. By looking at the average daily temperatures across the period of 1^{st} April to 31^{st} July for each year, it can be seen that on average temperatures were higher in 2007 and 2009 compared to the same period for 2008 and 2010 (Figure 5.12). This corroborates the fact that moult completed in a greater number of grid cells by 31^{st} July in years 2007 and 2009 compared to years 2008 and 2010. Average temperatures showed greater between-year variation during the months of April and May compared to June and July (Table 5.11). Therefore, the between-year differences in predicted moult rate suggest that April and May are critical months for the development of the tick.

Table 5.10: Earliest predicted dates of completion of nymph-to-adult moult in 2007, 2008, 2009 and 2010, assuming moult started on 1^{st} April.

Year	Earliest date of completion of nymph-to-adult moult
2007	July 13
2008	July 18
2009	July 11
2010	July 12

Figure 5.11: Areas where moult was predicted to complete by 31st July for years 2007, 2008, 2009 and 2010 if a tick began to moult on 1st April.

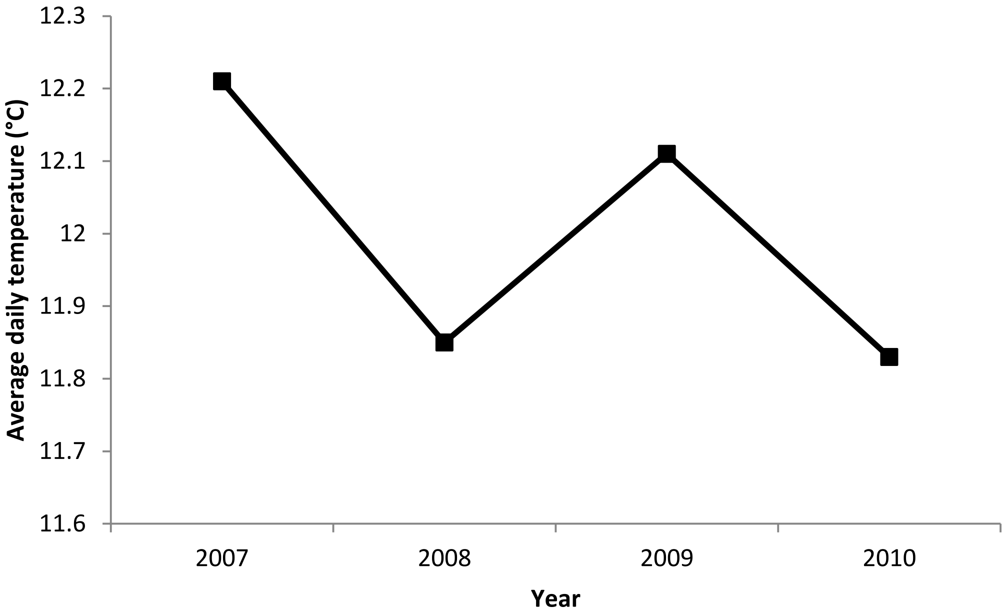


Figure 5.12: Average daily temperature between 1st April and 31st July for 2007, 2008, 2009 and 2010.

Table 5.11: Average temperature (°C) for April, May, June and July in 2007 to 2010.

Month	2010	2009	2008	2007
April	7.94	8.91	7.01	10.28
May	9.72	10.82	12.20	10.60
June	14.15	13.64	12.90	13.77
July	15.50	15.07	15.28	14.18

The probability of climate suitability for moult completion by 31st July (p_m) was determined for each 25 km^2 cell of GB and is shown in Figure 5.13.

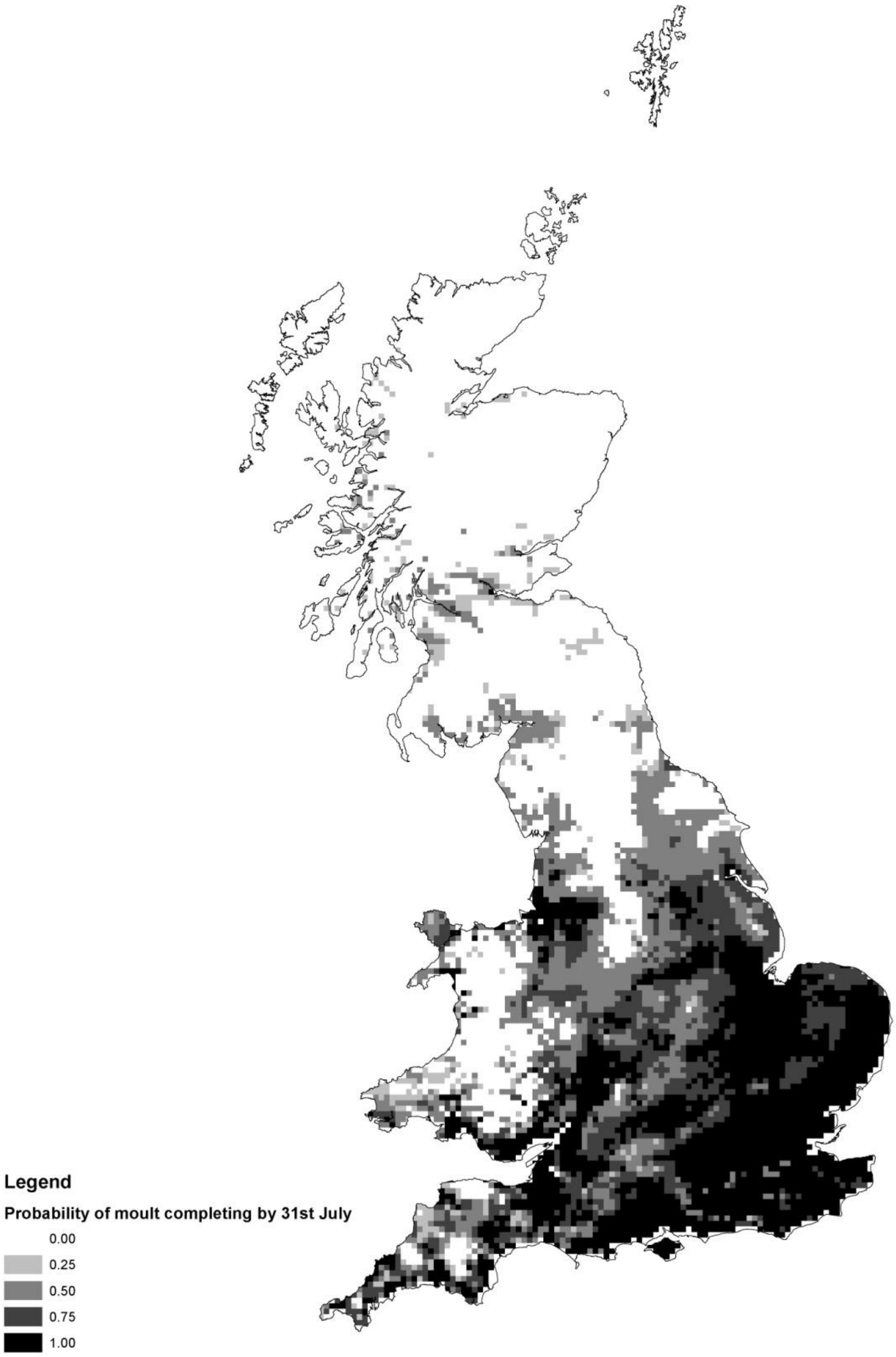


Figure 5.13: Probability of moult completing by 31st July (p_{m}) per 25 km^2, assuming that moult began on 1st April.

The predictions for p_m were transferred to the 1 km^2 land cover suitability map. The probability of habitat suitability for nymph-to-adult moult (p_{HSm}) in each grid cell was estimated using Equation (5.6) (Figure 5.14). The areas that were predicted to have the highest probability of habitat suitability for moult were around the Severn Estuary, primarily North Somerset and South Wales. The South East of England, particularly Kent and Sussex also had higher probabilities of habitat suitability and the probability of habitat suitability was greater than zero for most of the south of England and the Midlands. North of Lancashire, the probability of habitat suitability was zero in most places with the exception of a few patches including around the Firth of Forth in Scotland. As can be seen in Figure 5.11, there were areas of lowland Scotland, particularly in 2007 and 2009 where moult was predicted to complete by 31st July but only a single 25 km^2 cell had sufficient temperatures for predicted moult to complete by 31st July in all years studied (Figure 5.13).

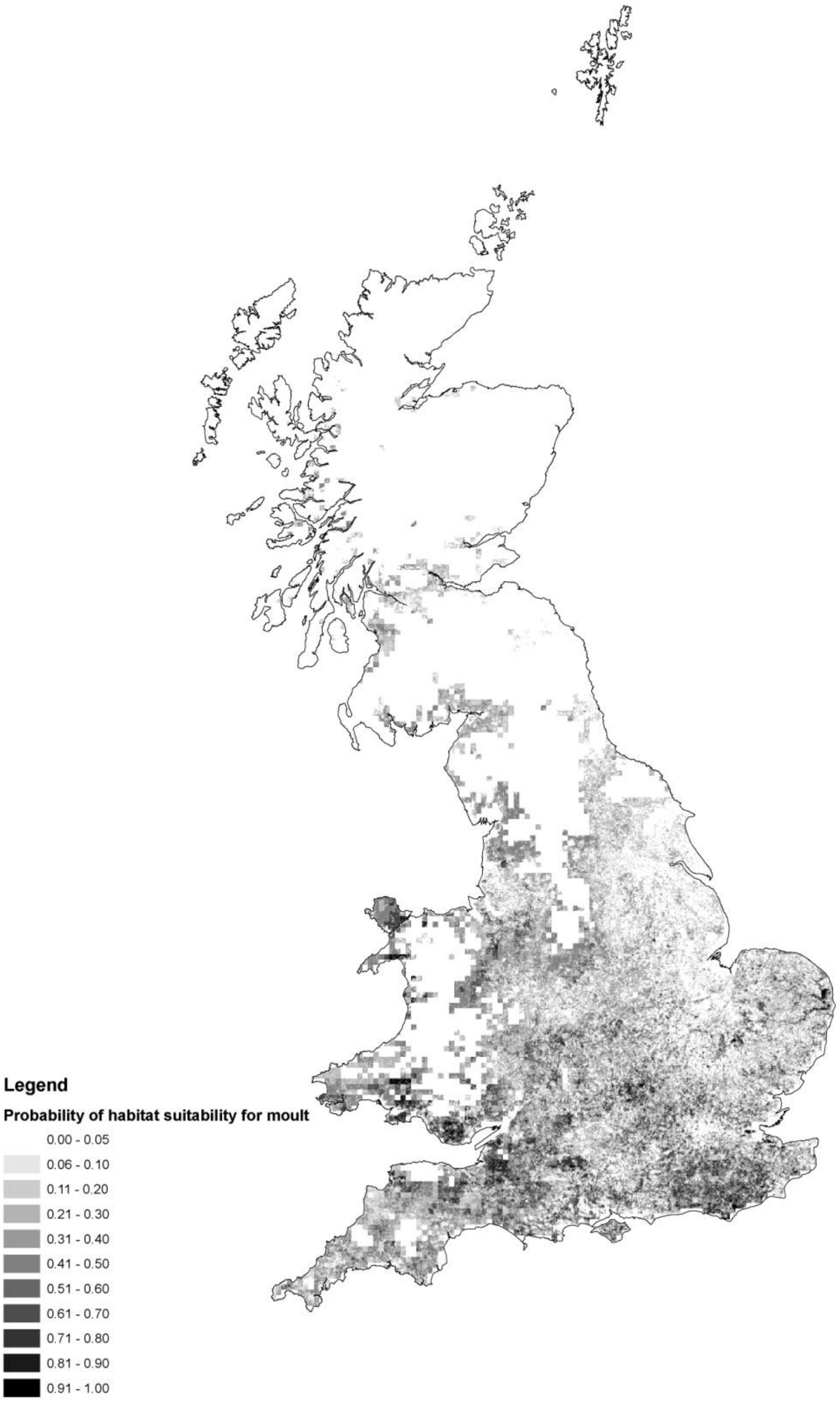


Figure 5.14: Probability of habitat suitability for nymph-to-adult moult given that a tick began to moult on 1st April.

5.3.2 Predicted egg development

The second model (Equations (5.15), (5.17) and (5.19)) was run for 2003. The predicted date for completion of pre-oviposition, oviposition and incubation for each 25 km^2 grid cell across GB was calculated for 2007, 2008, 2009 and 2010 given that an adult female tick entered GB on 1st April. The results were mapped in ESRI ArcGIS 10.0 (Figure 5.15, Figure 5.16 and Figure 5.17).

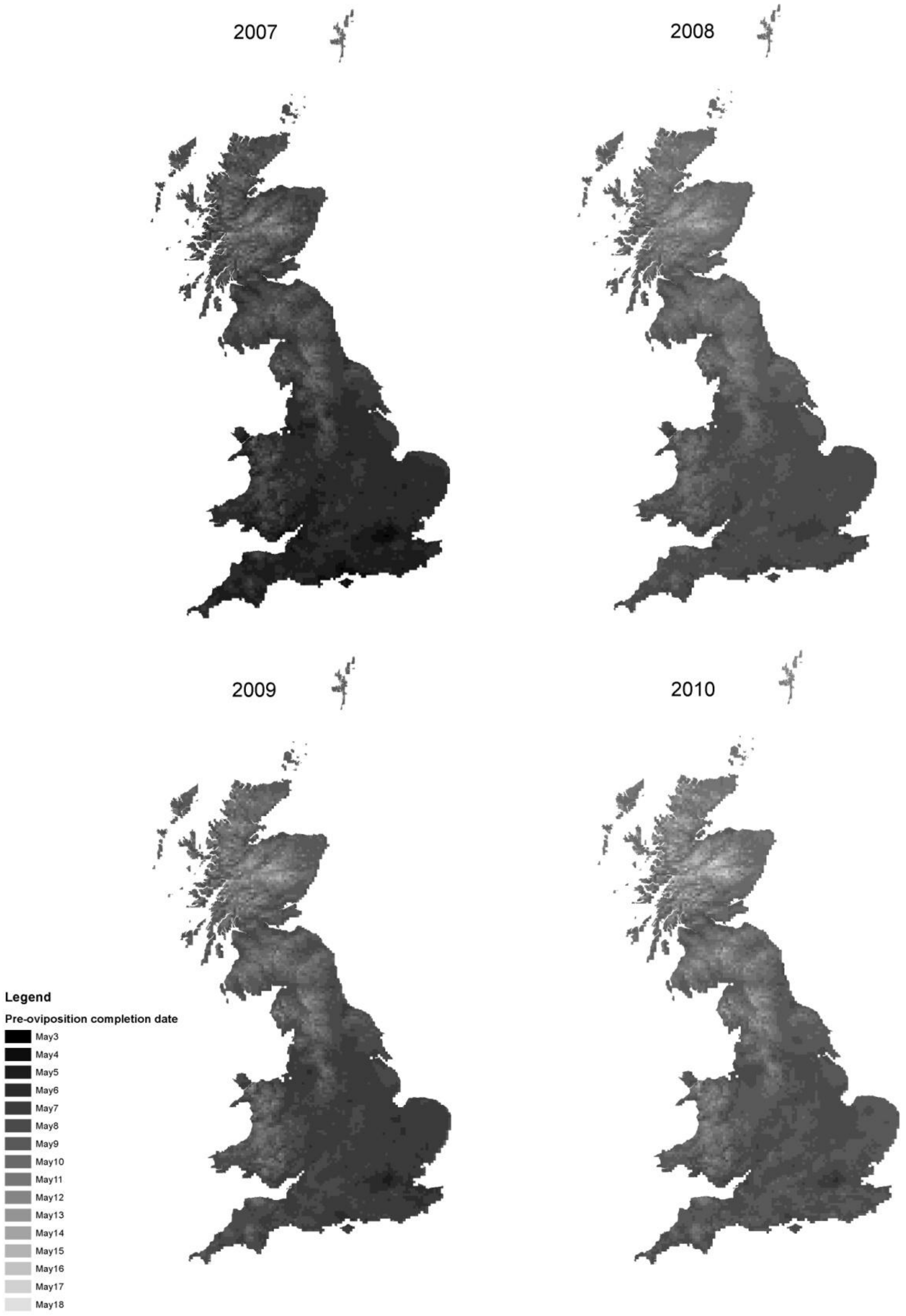


Figure 5.15: Predicted date of completion of pre-oviposition in 2007, 2008, 2009 and 2010 for each 1 km^2 of GB for a mated adult female tick starting pre-oviposition on 1st April.

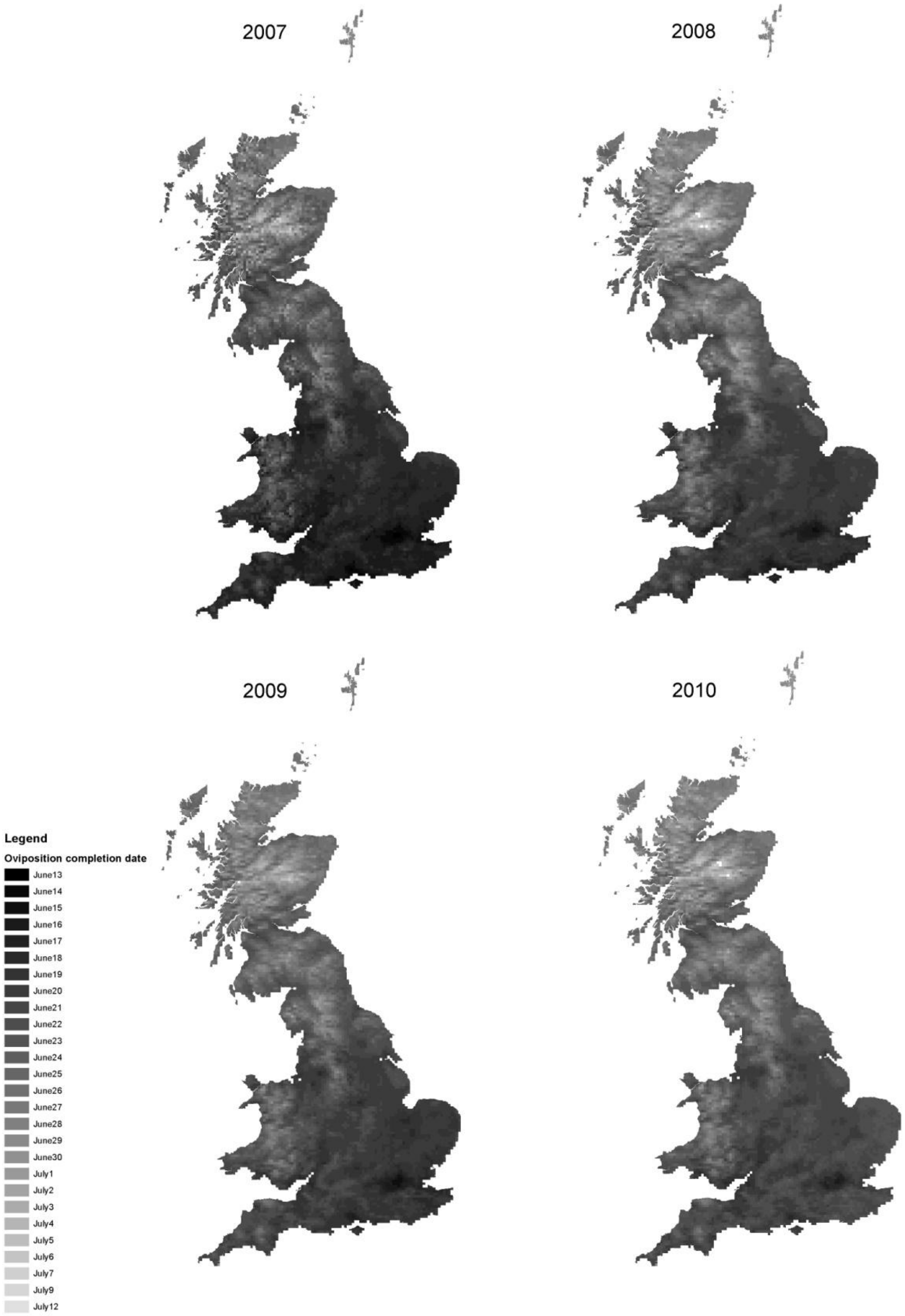


Figure 5.16: Predicted date of completion of oviposition in 2007, 2008, 2009 and 2010 for each 1 km^2 of GB for a mated adult female tick starting pre-oviposition on 1st April.

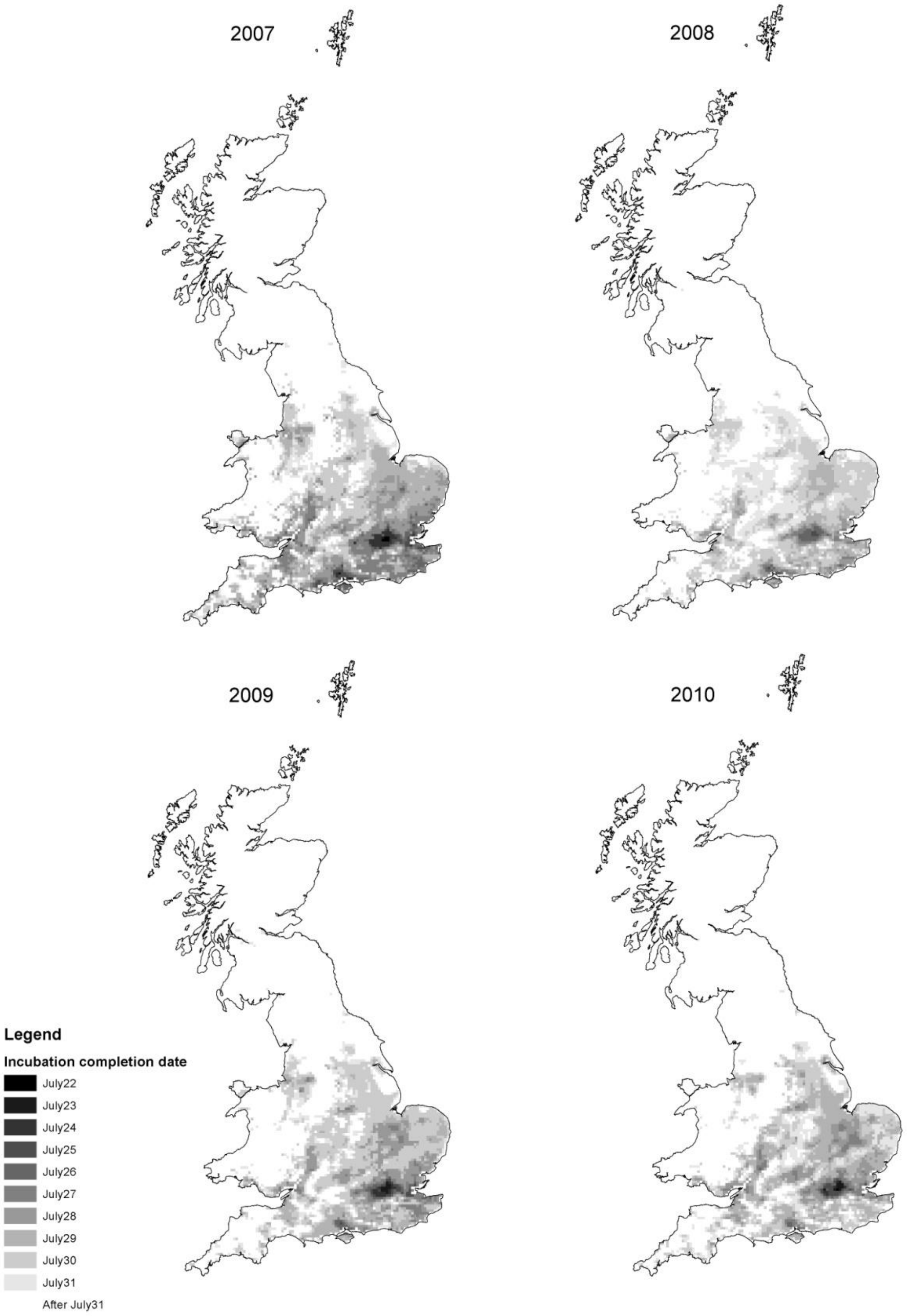


Figure 5.17: Predicted date of completion of incubation in 2007, 2008, 2009 and 2010 for each 1 km^2 of GB for a mated adult female tick starting pre-oviposition on 1st April.

The dates of completion of each stage of egg development were predicted to vary across GB, according to corresponding variation in temperature. As with nymph-to-adult moult, there was between-year variation in the predicted date that egg development completed in each cell. The earliest predicted dates of completion of egg development (i.e. first incubation completion dates) were obtained from the results and are presented in Table 5.12. For all years the earliest predicted date of completion occurred in Central London. Overall the predicted egg development completion dates did not vary greatly from year to year and as such the areas where egg development was predicted to complete by 31st July showed temporal stationarity across all years studied (Figure 5.17). For all years, there were large areas of GB where egg development did not complete by 31st July.

Table 5.12: Earliest predicted completion dates of egg development in 2007, 2008, 2009 and 2010.

Year	Earliest predicted date of completion of egg development
2007	July 22
2008	July 25
2009	July 23
2010	July 22

The predictions for egg development completion by 31st July were transferred to the 1 km^2 land cover suitability map. The probability of habitat suitability for egg development (p_{HSe}) in each grid cell was then calculated based on whether egg development could complete by 31st July for all years (2007 to 2010) and the percentage of suitable habitat within the cell (Figure 5.18). The probability of habitat suitability for egg development was found to be higher in eastern and central regions of GB compared to western regions, in particular the probability was higher around the Severn Estuary and the South East of England. The probability of habitat suitability was very low for most of Wales, northern England and Scotland.

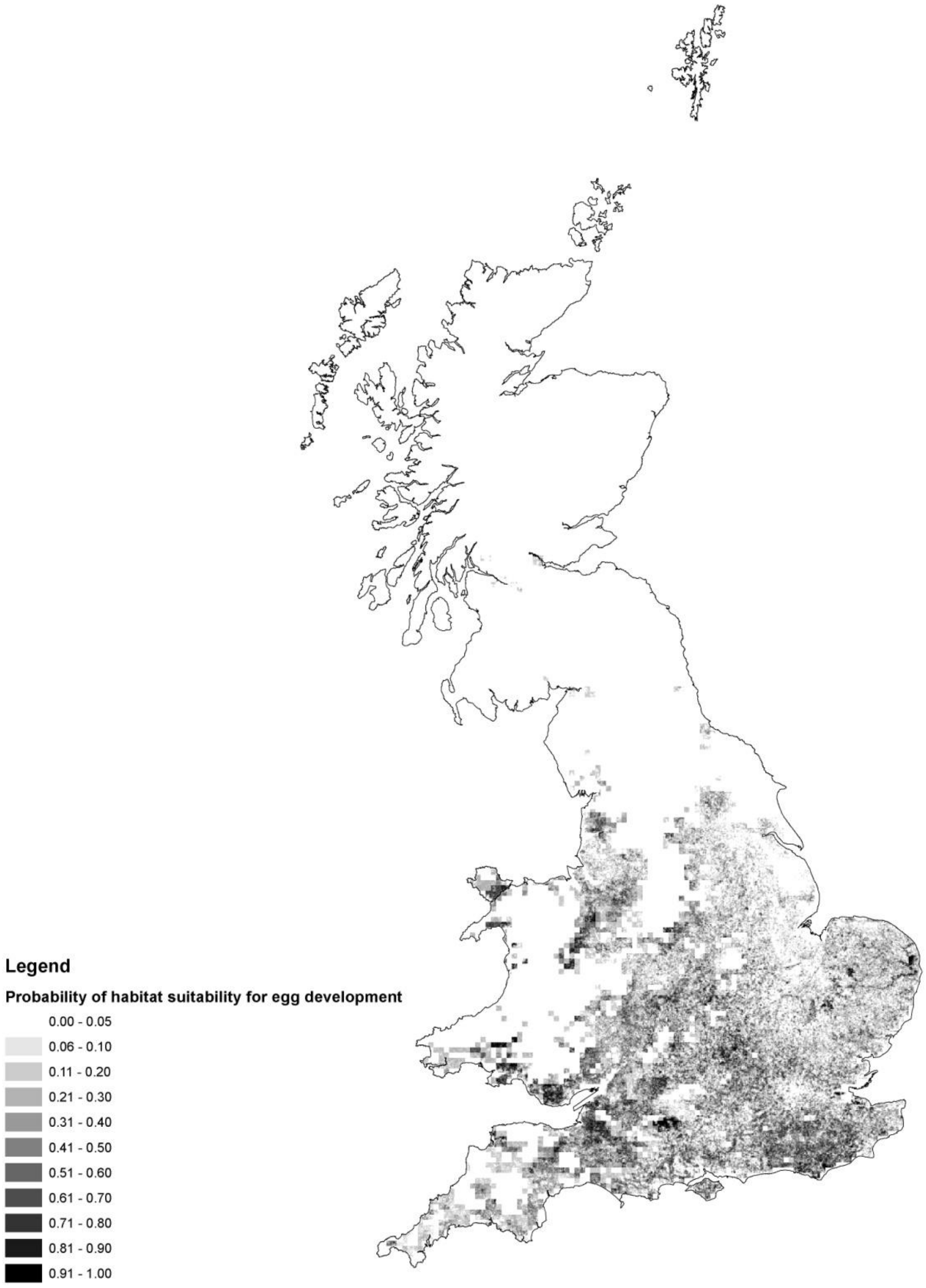


Figure 5.18: Probability of habitat suitability for egg development given that a mated adult female tick started pre-oviposition on 1st April.

5.3.3 Uncertainty in the probability of habitat suitability for nymph-to-adult moult

The uncertainty in the equations for nymph-to-adult moult of Estrada-Peña et al. (2011) was modelled and the resultant 25th and 75th percentiles of p_{HSm} were mapped (Figure 5.19). The 25th percentile map showed a probability of habitat suitability for moult of zero in nearly all areas of GB, with the exception of low probabilities throughout the South East and around the Severn Estuary. The 75th percentile map showed a moderately high probability of habitat suitability for moult throughout most lowland areas of GB.

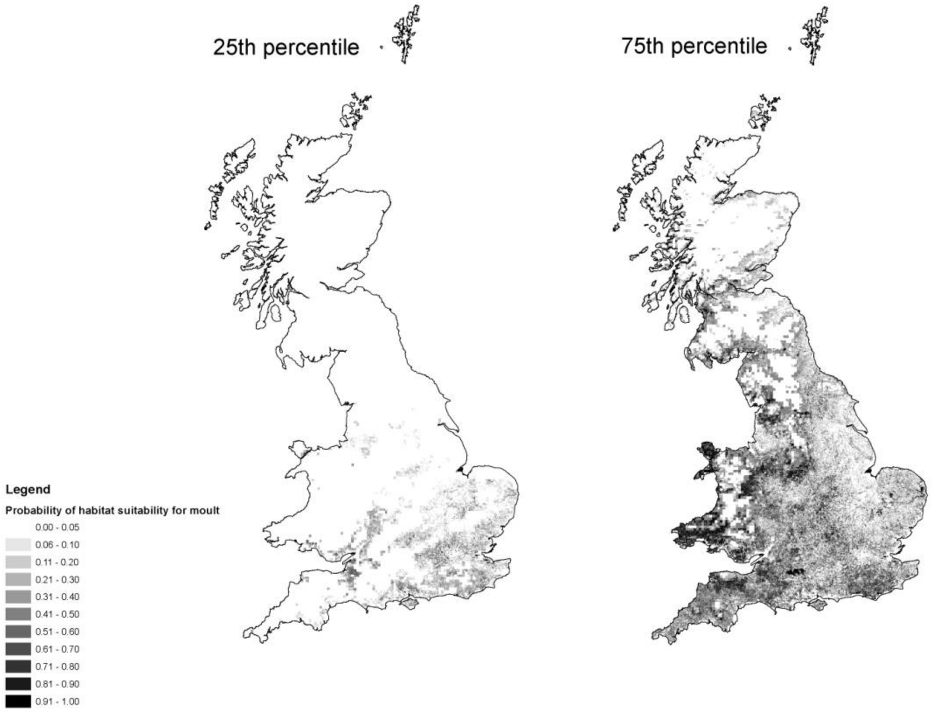


Figure 5.19: The 25th and 75th percentiles of the probability of habitat suitability for nymph-to-adult moult (p_{HSm}).

5.3.4 Uncertainty in the probability of habitat suitability for egg development

The uncertainty in the equations for egg development of Estrada-Peña et al. (2011) was modelled and the resultant 25th and 75th percentiles of p_{HSe} were mapped (Figure 5.20). There was a considerable difference between the 25th percentile output and the 75th percentile output. The 25th percentile map showed a prediction of $p_{\mathrm{HSe}}=0$ for nearly all areas of GB with the exception of a few grid cells in London. Completion of egg development by 31st July occurred in seven

1 km^2 cells in 2007, only three 1 km^2 cells in 2010, and in no cells in 2008 and 2009. The 75th percentile map showed a high probability of habitat suitability for egg development in Wales and around Derbyshire. The probability was greater in the north of England, Scotland and Wales compared to the mean.

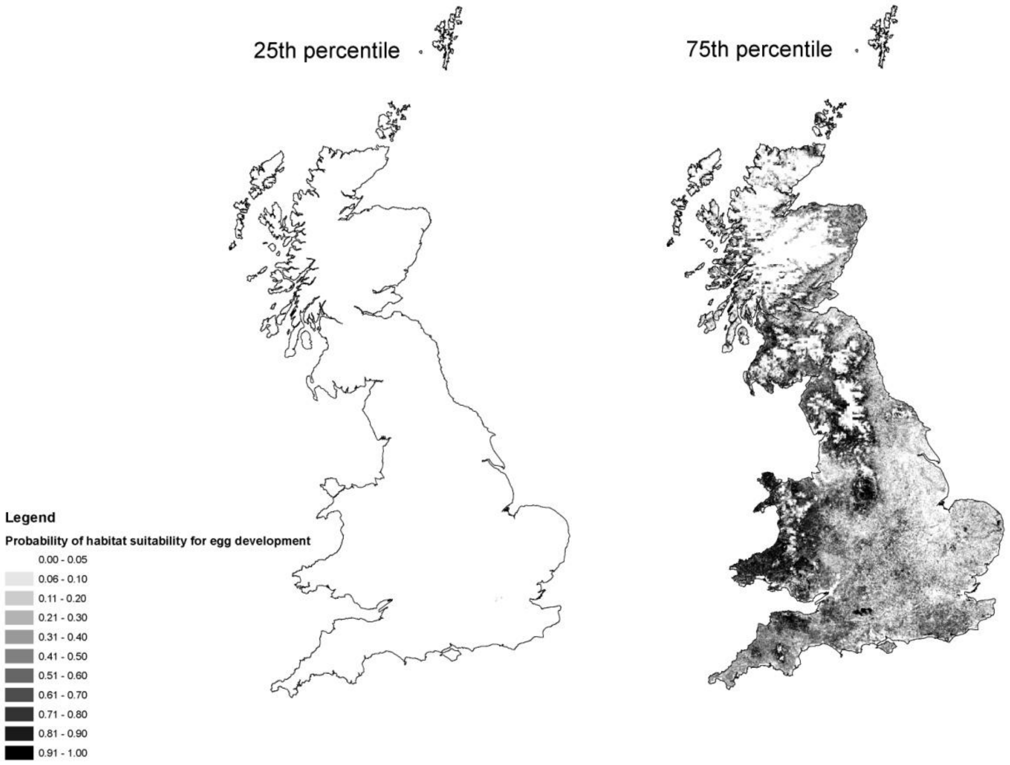


Figure 5.20: The 25th and 75th percentiles of the probability of habitat suitability for egg development (p_{HSe}).

5.3.5 Parameter sensitivity

A sensitivity analyses for the effect of water vapour deficit on nymph-to-adult moult and egg development was run and the results are shown in Figure 5.21 to Figure 5.24. The daily rate of moult was slightly higher at low values of *VD* compared to high values of *VD*. The daily rates of pre-oviposition, oviposition and incubation were slightly lower for low values of *VD* compared to high values of *VD*. When the values of *VD* were varied, the resultant development rates varied only marginally.

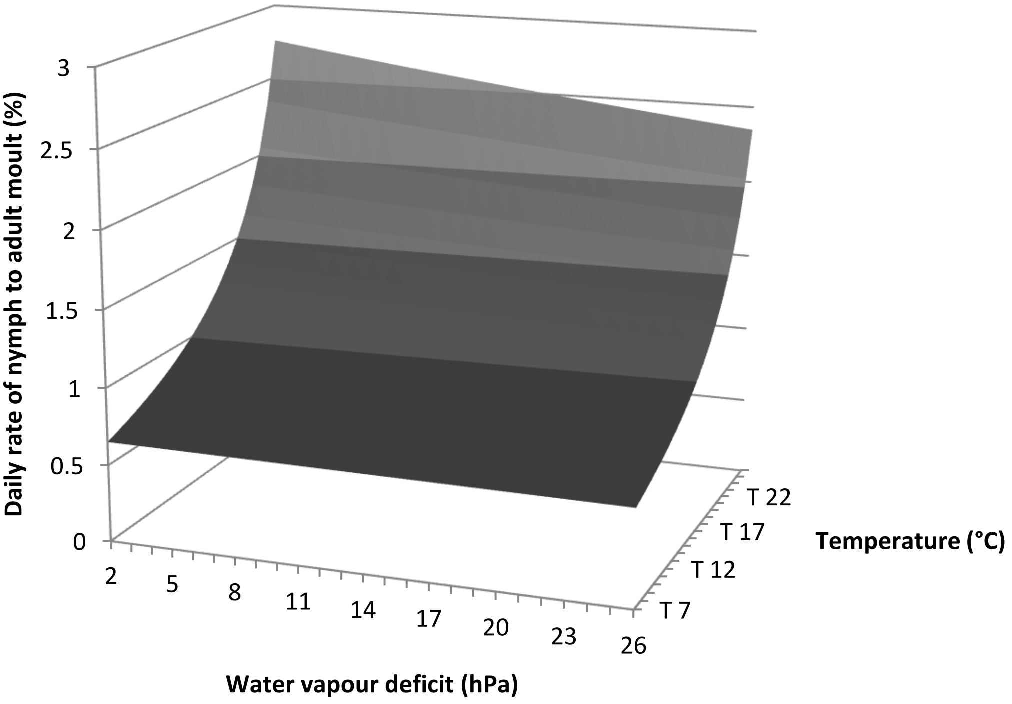


Figure 5.21: Daily rate of nymph-to-adult moult (% completed) at temperatures from 7°C to 26°C and water vapour deficit values of 2 hPa to 26 hPa.

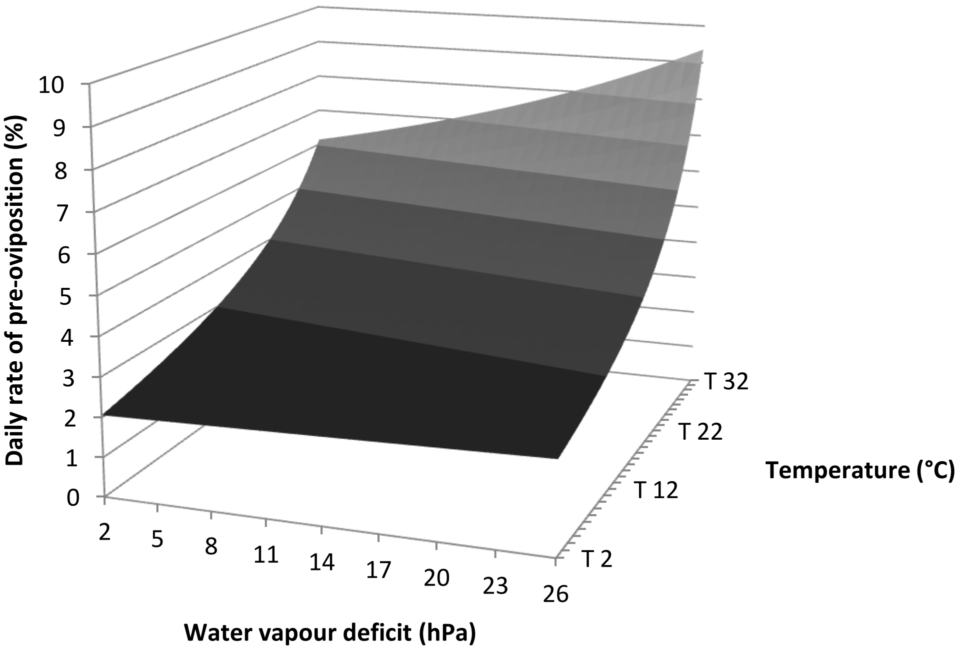


Figure 5.22: Daily rate of pre-oviposition (% completed) at temperatures from 2°C to 32°C and water vapour deficit values of 2 hPa to 26 hPa.

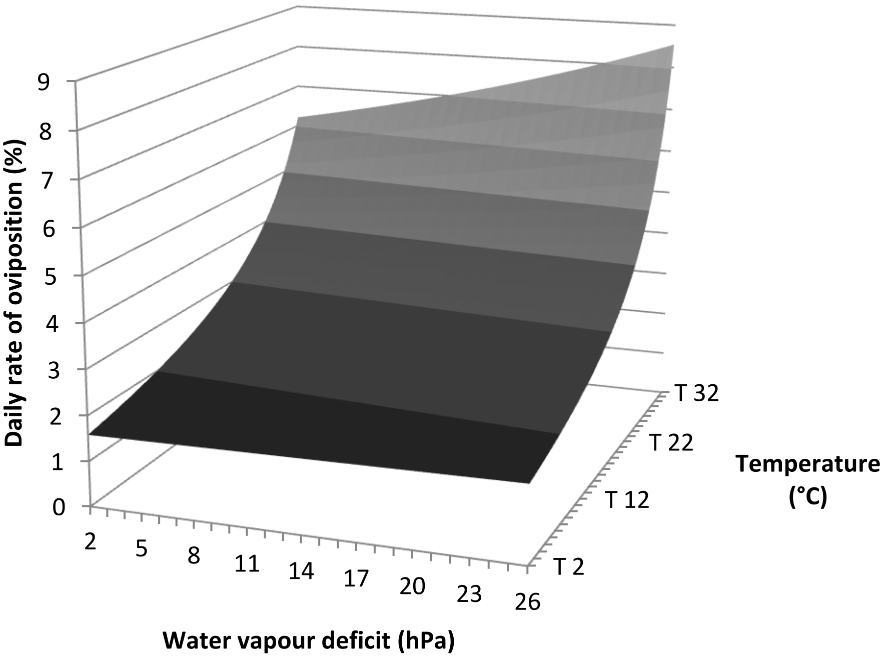


Figure 5.23: Daily rate of oviposition (% completed) at temperatures from 2°C to 32°C and water vapour deficit values of 2 hPa to 26 hPa.

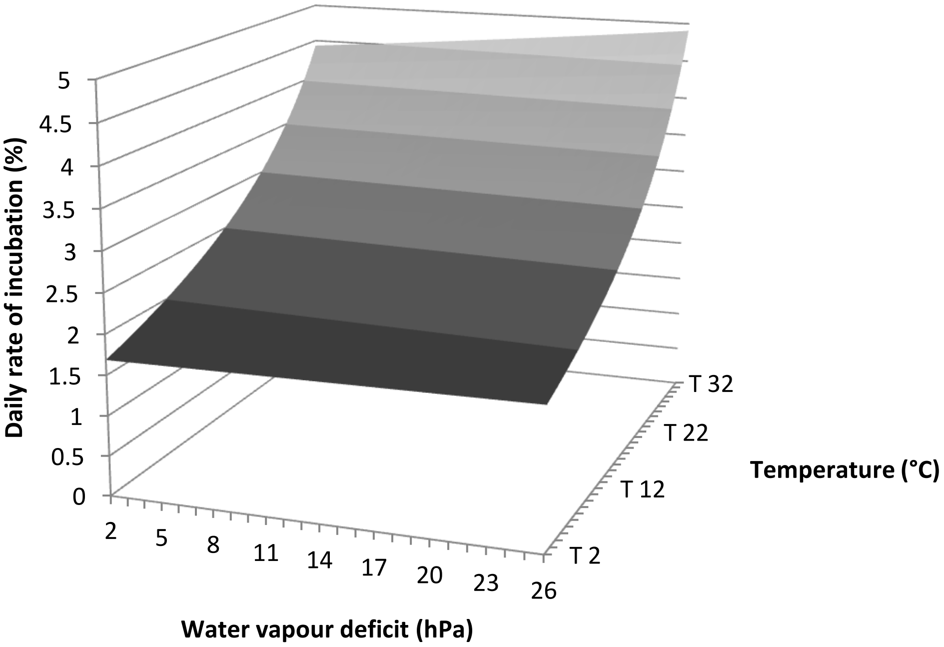


Figure 5.24: Daily rate of incubation (% completed) at temperatures from 2°C to 32°C and water vapour deficit values of 2 hPa to 26 hPa.

5.3.6 Sensitivity of the models for nymph-to-adult moult and egg development to the start date

The model for the percentage completion of nymph-to-adult moult (Equation (5.5)) was run using temperature data for 2010 with start dates of 1st April, 1st May and 1st June (Figure 5.25). Changing the start date to 1st May created a reduction in moult completion by 31st July of around 15% compared to a start date of 1st April. A start date of 1st June reduced the percentage of moult completed by 31st July by around 35% compared to a start date of 1st April.

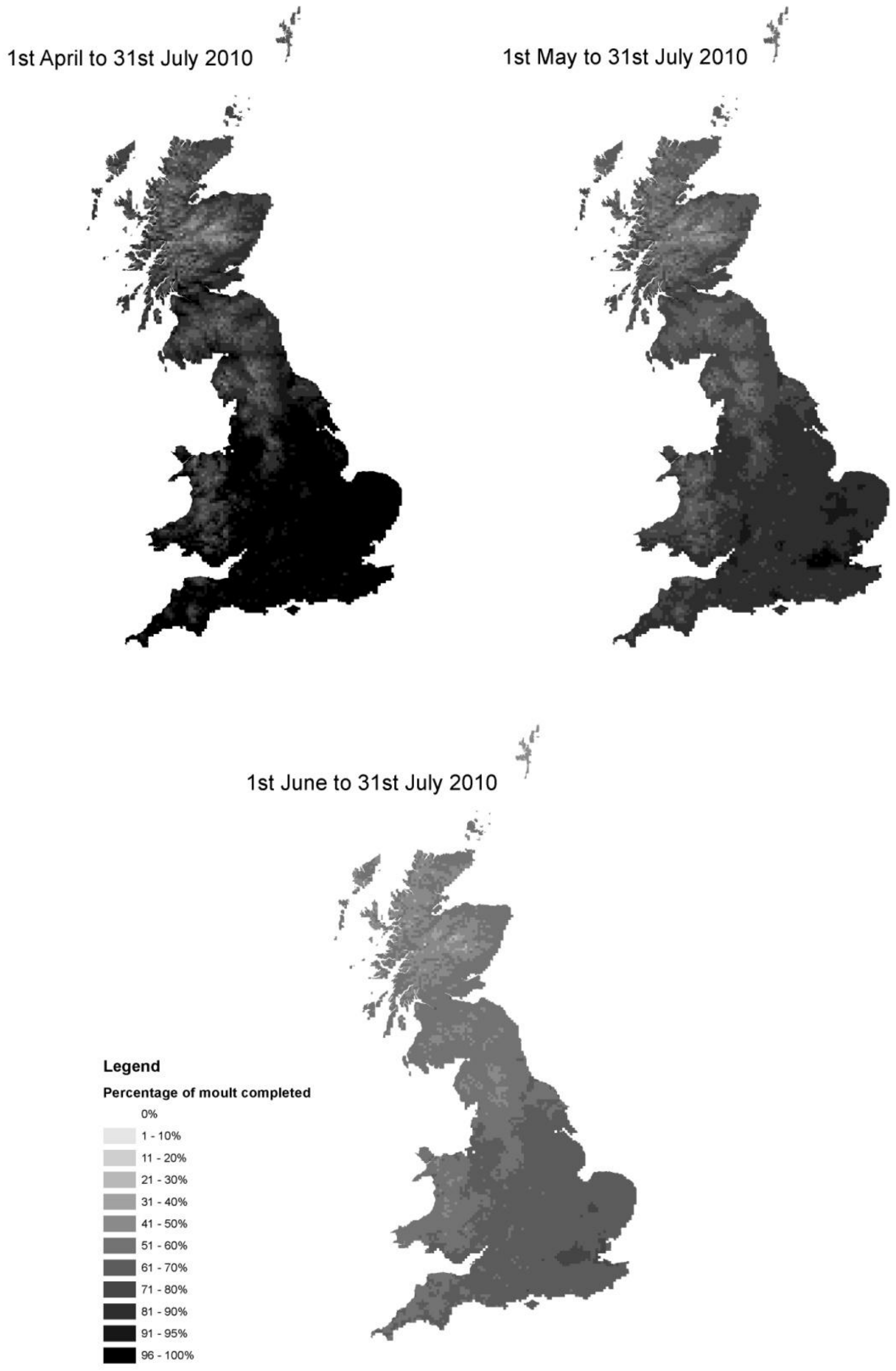
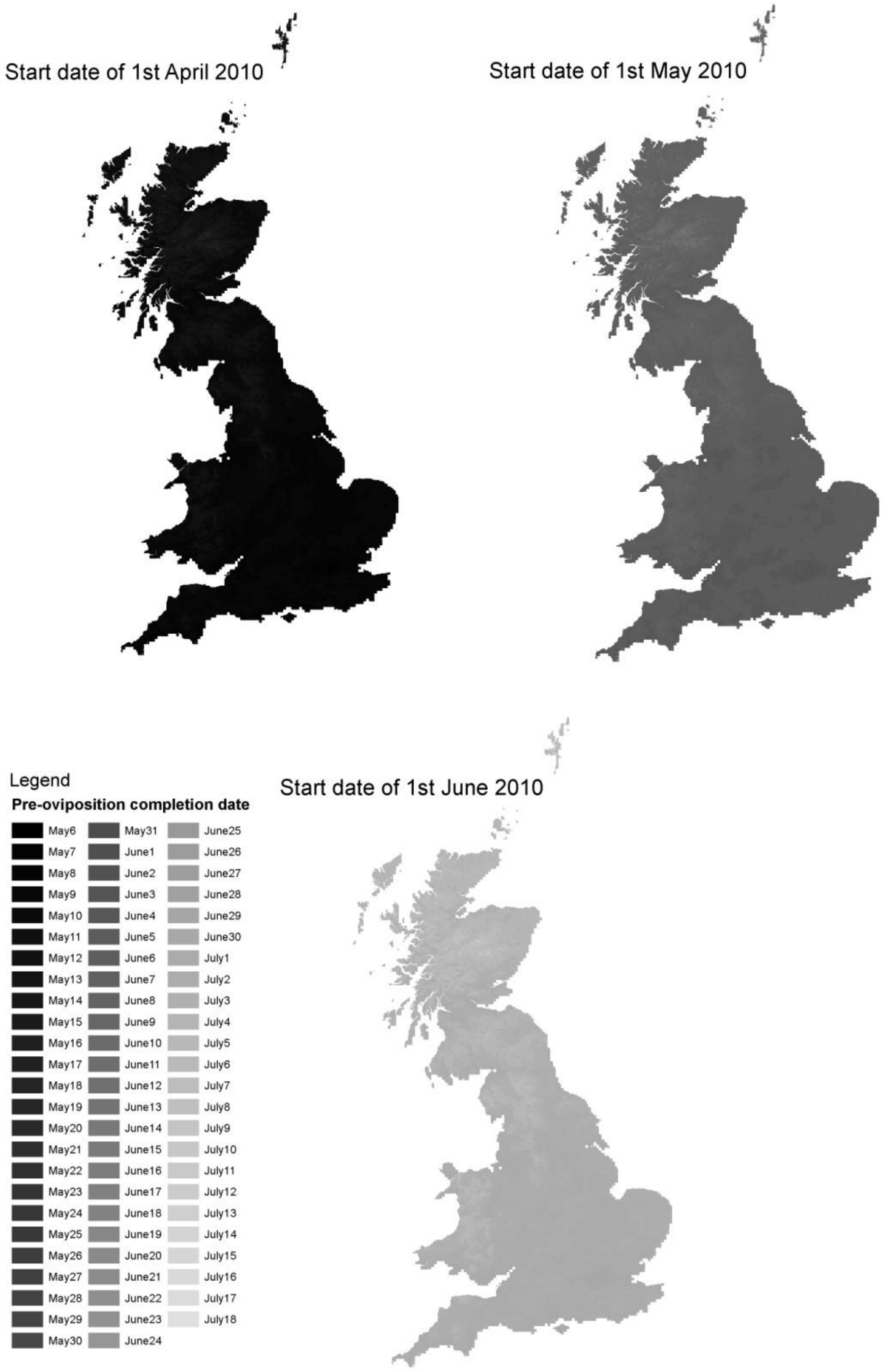


Figure 5.25: Predicted percentage of nymph-to-adult moult completed by 31st July if moult begins on 1st April, 1st May and 1st June 2010.

The predicted dates of completion of pre-oviposition were delayed by approximately one month when the start date was delayed by one month, as would be expected. The earliest dates of pre-oviposition moved from 6th May to

3rd June and then to 29th June with each delay in start date (Figure 5.26). Similarly, oviposition completion was delayed from an earliest date of 16th June when the start date was 1st April, to 8th July and 31st July with start dates of 1st May and 1st June, respectively (Figure 5.27). When the start date of pre-oviposition was 1st May or 1st June, incubation did not complete by 31st July (Figure 5.28).



Start date of 1st April 2010

Start date of 1st May 2010

Start date of 1st June 2010

Figure 5.26: Predicted date of completion of pre-oviposition if pre-oviposition starts on 1st April 2010, 1st May 2010 and 1st June 2010.

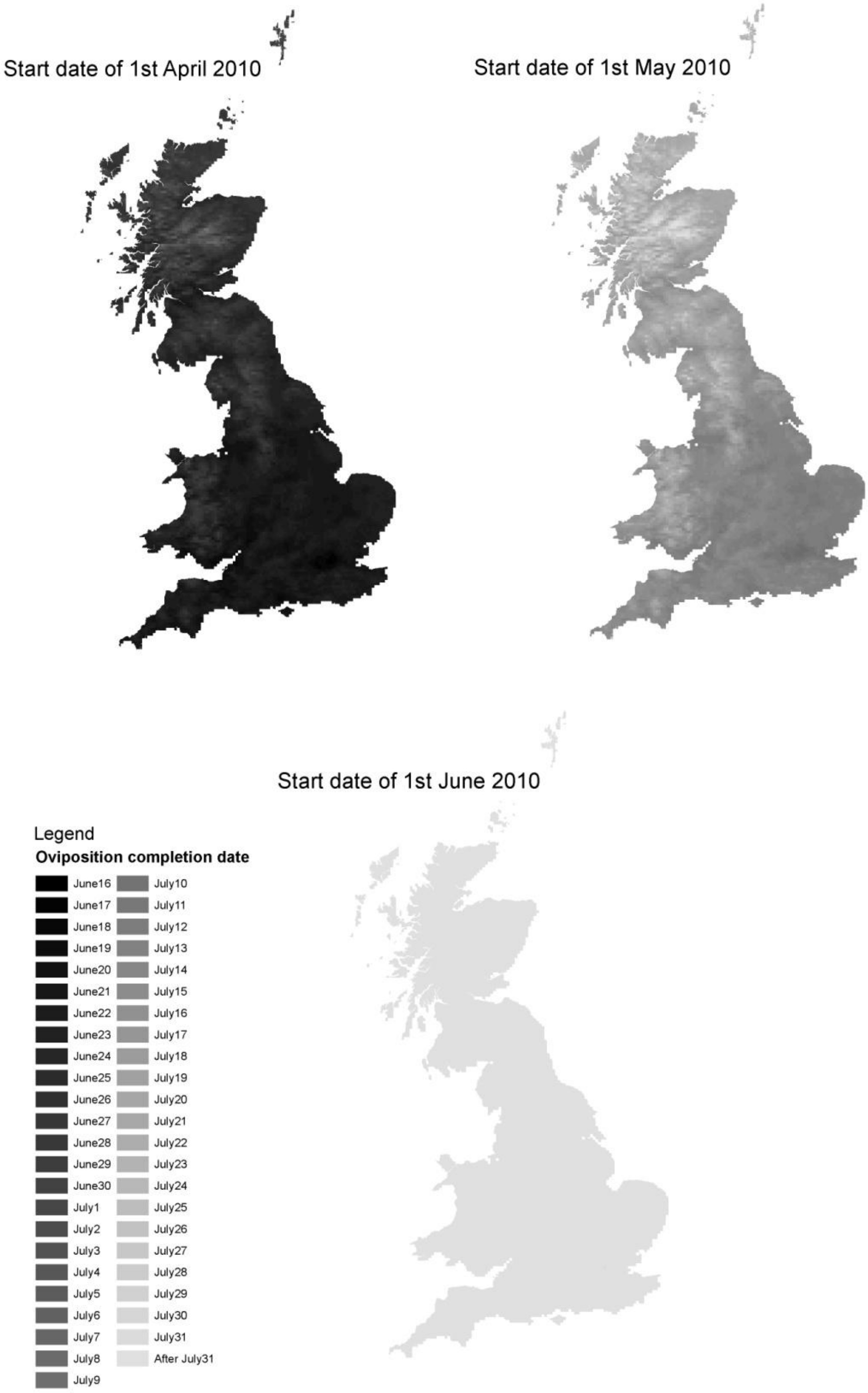


Figure 5.27: Predicted date of completion of oviposition if pre-oviposition starts on 1st April 2010, 1st May 2010 and 1st June 2010.

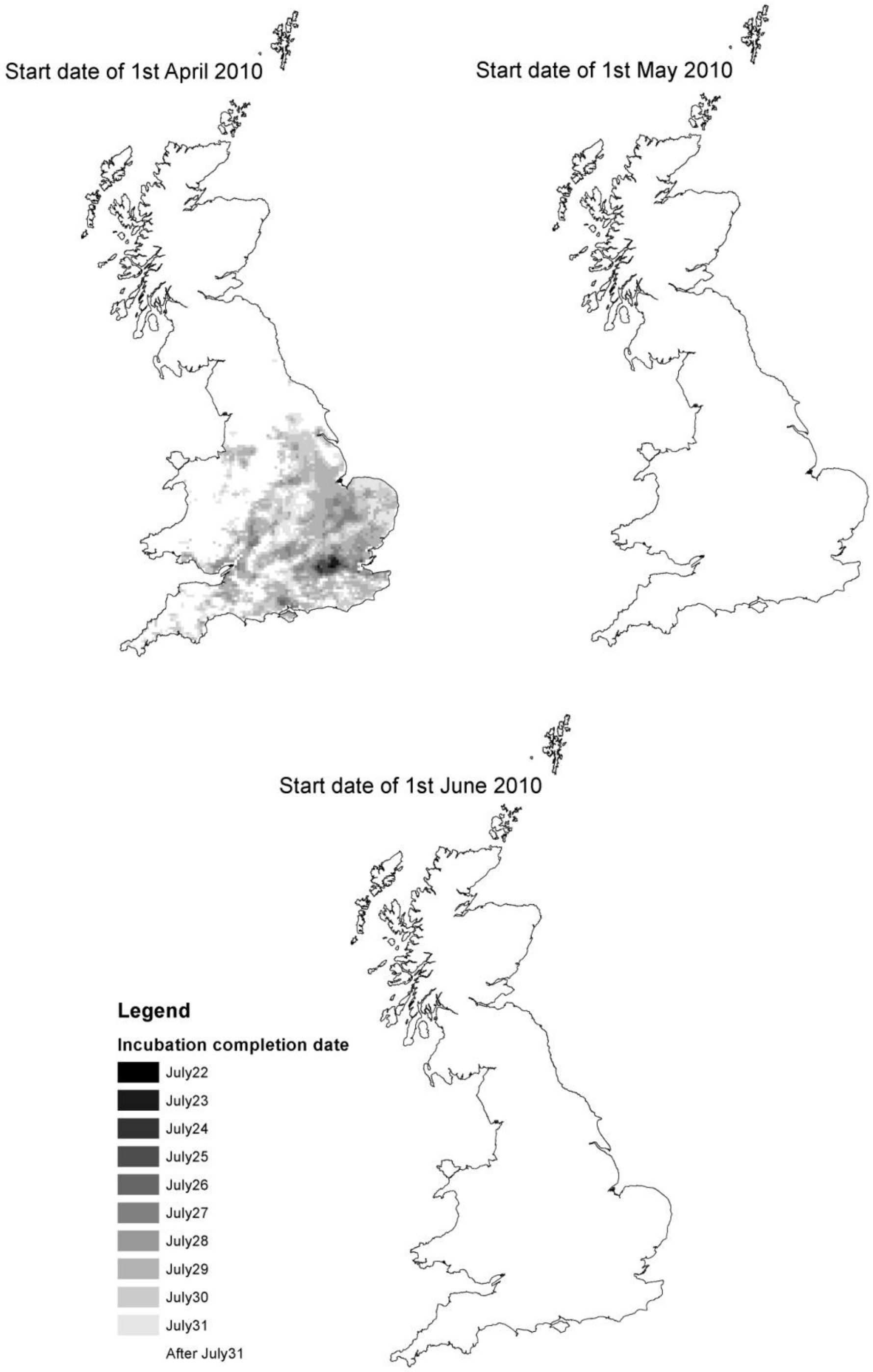


Figure 5.28: Predicted date of completion of incubation of eggs if pre-oviposition starts on 1st April, 1st May and 1st June 2010.

5.4 Discussion

The models in this chapter have shown for the first time where in GB it may be possible for *H. marginatum* ticks to survive following importation on migratory birds and horses. The outputs from the nymph-to-adult moult model show that, for temperatures similar to those observed over the last few years, it is not possible for ticks to complete their nymph-to-adult moult before 1st July, in any area of GB (Figure 5.7 to Figure 5.10). The earliest predicted date that nymph-to-adult moult was completed, given that a tick dropped off its host and started moulting on 1st April was 11th July and this occurred for 2009 temperature data (Table 5.10). The model was run until the end of July each year and in many areas of GB moult still did not complete. This highlights the variation in moult rates across GB and reflects the dependence that ticks have on temperature to complete their moult, as shown in laboratory studies (Estrada-Pena et al., 2011). It also shows that there are areas of GB with a much higher suitability for *H. marginatum* survival than others.

Given that in GB there are no populations of *H. marginatum* (to the best of my knowledge) it is possible that the shortest period of time to moult that has been observed in these models is too long to support survival. However, there is no known period of time beyond which moult will not occur such that the tick dies (Estrada-Peña, A., personal communication). In this model, it was assumed that each day the probability of death is an independent event. As such cumulative mortality over time was not calculated. According to A. Estrada-Peña (personal communication), it may be that if the moult period is long, the risk of mortality accumulates over time and ‘overtakes’ the rate of moult, such that there is an overwhelming probability that the tick will die before it is able to successfully moult. The actual physical process of the tick moulting and hardening off takes at most one day (Phipps P., AHVLA, personal communication). Therefore, it would appear that the period of time when the adult tick is developing within its nymphal body is long in this study. The longer this takes, the longer the tick is exposed to pressures such as predation, extreme weather events and desiccation and, thus, more likely to die.

Previous empirical studies can inform whether the time frames for moult in GB as determined in this model are realistic or not. *H. marginatum* were found to take 15 to 27 days to moult from nymph-to-adult, with an average of 20 days at 28 °C and 57% relative humidity (Hueli, 1979). There have not been many experimental studies on *H. marginatum*, but those carried out on other species of *Hyalomma* spp. ticks indicate a similar length of time. *H. rufipes* took 24 days to moult from the time that it detached from its host when kept at 27 °C and 75% relative humidity (Okorie and Fabiyi, 1980). *Hyalomma impeltatum* took 32 days to moult when kept at 26 °C and 92-96% relative humidity (Dohm et al., 1996) and *H. truncatum* took 57 days to moult at 26 °C and 92-98% relative humidity.

In experimental studies ticks are kept at conditions that are optimum for their survival, which would not necessarily occur in nature or indeed be necessary for populations to survive. However, these studies are indicative of the approximate timings that the moult should take and are, therefore, a useful comparison to the results of the model. The shortest period of time in which moult was predicted to occur according to the model of *H. marginatum* in this study was 102 days which is almost double the time for *H. truncatum* and approximately five times as long as that observed for laboratory kept *H. marginatum*. Therefore, the moult times that have been predicted from the model are considerably longer than those observed and it may be justifiable to conclude that the nymph-to-adult moult takes too long under current temperature scenarios in GB for the tick to be able to survive or for populations to establish.

There was substantial between-year variation in moult rates in GB (Figure 5.11) reflecting temperature fluctuations in each cell from year to year. Thus, in 2008 there were fewer grid cells where moult was completed by the end of July compared to other years. There are not many areas of GB where temperatures were consistently high enough across all four years for moult to be completed by the end of July (Figure 5.13). This is very interesting as it shows that new adults could be recruited into a population one year but not the next and, therefore, the population would not be sustained. It may be that during warmer years in GB, temperatures are sufficient for nymph-to-adult moult, but these temperatures are not consistent over a number of years and, thus, populations cannot establish.

The maps in Figures 14 to 16 show the predicted climatic suitability for an adult female *H. marginatum* to successfully produce eggs that are fully incubated and ready to hatch. As with the nymph-to-adult moult model, there was large variation in completion dates across GB and some between-year variation was also observed. The earliest predicted date that egg development completed over all four years was 22nd July and this occurred in 2007. Once again, it is not known if there is a length of time beyond which any of the stages of egg development will fail. By studying the empirical evidence in the literature on observed timings for these development processes it is possible to again evaluate if the model predictions are realistic.

A study on *H. marginatum* found that pre-oviposition took between one and six days with an average of 4.2 days when kept at 28 °C and 57% relative humidity (Hueli, 1979), but another study found this to take 25 to 27 days (Petrelli et al., 1973). Oviposition was observed to take between 13 and 31 days with an average of 22.4 days and incubation took 14 to 22 days in the study by Hueli (1979). Incubation was found to be much longer in a study where *H. marginatum* were kept at 22 °C (Honzáková, 1971). The pre-oviposition period for *H. rufipes* was 7.1 days and the incubation period was between 26 and 32 days, with an average of 29.3 days when ticks were kept at 26 °C and 80% relative humidity (Knight et al., 1978). *H. truncatum* ticks were found to take an average of 11.9 days for pre-oviposition, up to 20 days for oviposition and an average of 35 days for incubation at a temperature of 26°C and 92-96% relative humidity (Linthicum et al., 1991). Interestingly, one study found that high relative humidity (90% at a temperature of 25 °C) had a detrimental effect on the embryonic development of *H. marginatum*, with 22.2% of larvae failing to hatch or hatching with abnormalities (Buczek, 2000). From these previous studies, the whole process of egg development from the start of pre-oviposition to the end of incubation would take an average of 60 days. The results from the model in this study predict that it would take a minimum of 113 days from the start of pre-oviposition to the end of incubation. This is substantially longer than the experimental data suggest is normal for *Hyalomma* spp. ticks. As with the nymph-to-adult moult model, mortality rates were not cumulated daily and mortality was built into the model as a compounding factor to egg development. Therefore, again, the adult female tick and/or the eggs may die before development is completed due to accumulated mortality. Hatching takes an average of 13.6 days under suitable conditions (Hueli, 1979) and hatched larvae undergo a hardening off process, which takes about 21 days (in the case of *I. scapularis*) (Ogden et al., 2005). This is a significant amount of time in addition to the egg development that is required to take place for larvae to survive the winter. It has been suggested that the most vulnerable point of the tick life cycle is the production of larvae (Yuval and Spielman, 1990) and, therefore, the successful establishment of a population may be limited by reproductive capability. It has also been suggested for other tick species that the northern limits of the range are at the point when winter temperatures arrive too early for larval development to have completed and eggs run out of energy stores (Ogden et al., 2005). In these models winter temperatures were not considered as the establishment of viable populations was not directly considered. Only the survival of imported ticks was investigated, as these may be infected with CCHFV as addressed in Chapters 3 and 4.

The greatest between-year variation in temperature from 2007 to 2010 occurred during the months of April and May (Table 5.11). The peak migration of birds into GB occurs during these months and, therefore, it is likely that this coincides with the peak arrival time for immature ticks entering GB. The between-year temperature variation during the months of April and May is responsible for the predicted between-year variation observed in predicted nymph-to-adult moult. Thus, temperatures during these months may be critical for moult success and consequently, may be a limiting factor for northerly populations of *H. marginatum*.

Both the model for nymph-to-adult moult and the model for egg development are based on the assumption that a tick enters GB on a host and detaches on the 1st April. It was assumed that a migratory bird would be the most likely route of entry for a nymphal *H. marginatum*. The main passage of migratory birds into GB (in particular *O. oenanthe*, *S. communis* and *A. schoenobaenus*) is during April and May. Some migrants enter GB in March, but it was considered that attached ticks would not be fully engorged until 1st April. Given that the tick must be replete before drop off, it is reasonable to suggest 1st April as the earliest date that this could occur. Earlier dates of tick importation were considered unlikely as immature *H. marginatum* ticks would not be actively questing for hosts when early migrants leave their wintering grounds or stop at staging posts. It may be that the peak number of birds carrying ticks is not until May, but the model was run from the date when it was believed ticks could start to drop-off in GB. When the start date for the model was changed to 1st May and 1st June in the sensitivity analysis, the predicted percentage of moult completed by 31st July decreased, as would be expected. There were no areas of GB where nymph-to-adult moult could complete by 31st July when the start date was 1st June. Moult progressed at a faster rate during the months of June and July compared to during April and May, with corresponding higher temperatures. When the model was run from 1st June, the highest predicted percentage completion of moult observed was 77.8% and this occurred in central London. As the main passage of migrant birds into GB occurs in April and May, it was considered that only very few immature ticks would be imported into GB such that moult began on 1st June. When the model was run with a start date of 1st May, moult was predicted to complete by 31st July in only nine of the 25 km^2 cells of GB and these were in central London. The date of moult completion was delayed by approximately one month when the start date was delayed by one month (1st May and 1st June) and, therefore, the areas of GB where moult could complete within the designated time frame of four months did not change.

The model for egg development was also run from 1st April, as this allowed time for adult ticks to emerge from winter diapause and find a host in the country of origin. As with nymph-to-adult moult, delaying the start date of pre-oviposition delayed the completion of incubation by approximately one month. The areas of GB where egg development could complete within four months did not change relative to one another and, therefore, the outputs from the main model were considered to correctly represent the areas where egg development could occur, given that a four month period for completion was reasonable.

The lifecycle equations used in this study to build the models are taken from a previous laboratory study (Estrada-Peña et al., 2011). It was, therefore, assumed that these equations were correct and were the best available representation of the tick’s lifecycle. The equations have been refined since the paper was published (Estrada-Peña, A., personal communication) and some of these revisions have been used in the current study. The equation for nymph-to-adult moult (Equation 5.1) was not calculated for temperatures below 7 °C and development was assumed to be zero on days when daily average temperatures were below this level. This is a limitation of the model and may be an ecologically incorrect assumption. It is not known if other temperature constraints apply to other equations, but none are stated in Estrada-Peña et al. (2011). The standard deviation around each of the coefficient and constant values in the equations was not available and as such it was assumed that each value was the mean of a Normal distribution with 5% and 95% confidence intervals of 20% below and above the mean value, respectively (Table 5.2 to Table 5.8). This was done to attempt to capture the uncertainty in the equations in the model. The 25th and 75th percentiles of these distributions were mapped (Figure 5.19 and Figure 5.20) and showed the variation due to uncertainty in the model. It was predicted that there may in fact be no areas of habitat suitability in GB where moult or egg development could occur or indeed there could be a wide range of areas of GB where p_{HSe} could be > 0.

In both models water vapour deficit (*VD*) was set at 15 hPa. Northern populations of *H. marginatum* are believed to be constrained by temperature and southern populations by humidity and, thus, water vapour deficit (Gray et al., 2009). Estrada-Peña et al. (2011) carried out their experiments with a water vapour deficit range of 2 hPa to 26 hPa. This study looked at the habitat suitability for nymph-to-adult moult and egg development of *H. marginatum* based on temperature data and, therefore, *VD* was set at a value that would not limit the moult or egg development of *H. marginatum* in GB. Estrada-Peña et al. (2011) found that populations of *H. marginatum* died out at values of *VD* higher than 15 hPa, but that survival increased significantly up 15 hPa. Thus, 15 hPa was chosen as the constant value of *VD* in this study such that the model outputs were not constrained by VD and the effect of temperature could be predicted. When *VD* was varied in the sensitivity analyses, there was little change in either the nymph-to-adult moult rates or egg development rates (Figures 21 to 24). Therefore, different values of *VD* would have had little impact on the model outcomes and be unlikely to affect the predictive potential of the models. Nymph-to-adult moult rates decreased and egg development rates increased with increasing values of *VD* (Figure 5.21 to Figure 5.24). Therefore, different levels of water vapour deficit may affect the tick differently at different stages in its lifecycle and habitats with consistently high or low values of *VD* (e.g. deserts) may not provide suitable climatic conditions for *H. marginatum.*

Some previous models have included precipitation as a variable for tick survival (Estrada-Peña and Venzal, 2007). Precipitation was not included in the models in this study for two reasons. Firstly, it was not incorporated as a variable in the set of equations by Estrada-Peña et al. (2011) on which the models in this study were based. Secondly, relative humidity or a derivative of relative humidity (in this case water vapour deficit) was included, as well as vegetation data which are considered to be more important for tick survival than precipitation (Lindgren et al., 2000). As a result of excluding this variable from the model, any impact of flooding on ticks was also excluded. However, as there is no ecological information on the impact of flooding on *H. marginatum* populations it would have been difficult to quantify this effect in the model.

Suitable land cover type for *H. marginatum* was determined to be all types of grassland, as suggested by the relevant literature, expert opinion and field observations. It was difficult to be certain that areas were chosen correctly as suitable due to the limited knowledge about the ecology of *H. marginatum* and its habitat requirements. The geographical range of *H. marginatum* is fairly broad and, thus, the tick exhibits slightly different habitat preferences in different regions. However, grassland seems to be a consistent habitat type across most areas where *H. marginatum* is found and, thus, it was chosen as a suitable land cover type. It was difficult to exclude other land cover types, as in some cases it was not known whether *H. marginatum* could survive in certain habitats. Heather and heathlands were excluded based on observational data from Spain and woodlands are widely recognised as unsuitable. It was decided that agricultural and horticultural land would be deemed unsuitable and the reasons for this are given previously (see Method). Agricultural land is often fragmented which is favourable for hares, an important host of immature *Hyalomma* spp. ticks (Estrada-Peña et al., 2012a). Therefore, although agricultural land was considered to be unsuitable in this study, it may be that an abundance of suitable hosts reverses this outcome. However, fragmented habitat around agricultural land commonly has low mammalian diversity (Estrada-Peña et al., 2012a) and arable farming would mean a lack of domestic livestock in the area. This demonstrates the difficulty in evaluating whether a habitat type may or may not be suitable for *H. marginatum* as many factors need to be considered and balanced.

Ticks generally live in a microhabitat that is affected by microclimatic factors, such as soil moisture and leaf litter. This is especially true for ticks such as *I. ricinus* for which soil humidity, leaf litter and the microrelief of the terrain are critical to their survival (Daniel and Dusbábek, 1994). As such, to determine if an area of land is suitable for survival of *I. ricinus*, data on microclimatic variables are needed. This type of data is rarely available and requires extensive and time-consuming field work. *Hyalomma* spp. ticks, unlike *I. ricinus*, are xerophilic (they can survive and reproduce in conditions with low water availability) and do not require moist leaf litter, tending to inhabit open, dry grasslands. As such, they are exposed to macroclimatic elements and being cursorial (able to run) are able to find suitable microclimates if needed within a broader area of macroclimatic suitability. The overall habitat of an area dictates the presence or absence of a tick population, whilst microclimatic features influence the population dynamics (Daniel and Dusbábek, 1994). It is, therefore, justifiable to use macroclimatic data to study the presence or absence of *H. marginatum*. The temperature data used were measured at 1.5 m above the ground, which is a realistic measure of the temperatures that *H. marginatum* would be exposed to on the ground. Within each 1 km^2 cell, the percentage of suitable land cover was given by the LCM2007. A cursorial *H. marginatum* can reasonably be expected to find this suitable habitat based on the fact that *H. asiaticum* can migrate 400 to 500 m in a 30 day period (Daniel and Dusbábek, 1994). It is important to note that the use of macroclimatic data may not be appropriate for calculating habitat suitability for other tick species such as *I. ricinus*.

The overall conclusion of this chapter is that the climate in some areas of GB may permit nymph-to-adult moult and/or egg production of imported *H. marginatum*, although the length of time these processes are predicted to take is longer than that observed in empirical studies. It has been noted that for other tick species (*Boophilus microplus*) the length of time required for a population to establish in a new area is unknown (Estrada-Peña, 2001). If an area of GB were to become suitable under future climate scenarios (as investigated in Chapter 6), it would be difficult to predict how long it would take for a new population to establish, but the work here focuses on the immediate survival of an imported tick.

In terms of the risk of CCHFV to GB, it does not seem likely that an endemic situation could occur without the establishment of *H. marginatum* (unless *I. ricinus* were a competent vector), but the survival of imported CCHFV-infected *H. marginatum* may be possible. It is considered that for populations of

Hyalomma spp. ticks to establish there would need to be consistently warmer temperatures in GB enabling tick development rates to increase. To investigate establishment, at a minimum, host availability and winter temperatures would also need to be considered.

6 Current and future introduction of CCHFV-infected *Hyalomma* spp. ticks into Great Britain from migratory birds and imported horses

6.1 Introduction

CCHFV-infected ticks can enter GB through migratory birds and imported horses as set out in Chapters 3 and 4, respectively. The mean number of CCHFV-infected *Hyalomma* spp. ticks entering GB each year on the five bird species studied was predicted to be 72.9 (5% and 95% confidence intervals of 0 and 64.2, respectively). The mean number of CCHFV-infected *Hyalomma* spp. ticks entering GB on imported horses between 1st April and 31st July each year was predicted to be 89.9 (5% and 95% confidence intervals of 0 and 493.9, respectively). The median number of CCHFV-infected ticks entering GB for both birds and horses was 0 over this period. However, the 95% confidence intervals of these model outputs suggest that infected ticks could enter GB and, therefore, what happens to them subsequently is of interest. This chapter looked at the survival potential of CCHFV-infected *Hyalomma* spp. ticks upon arrival in GB, now (2007 to 2010) and in the future (2077 to 2080). The ability of an imported *H*. *marginatum* tick to survive to the next life stage or reproduce under current climatic and land cover scenarios was investigated in Chapter 5. In this Chapter, this was investigated under future climatic and land cover scenarios and comparisons were drawn between the current and future model outputs. By combining the outputs from the models in Chapters 3, 4 and 5, the number of CCHFV-infected unfed adult *Hyalomma* spp. ticks in GB arising from migratory birds and horses was predicted under current habitat conditions. A prediction of the number of CCHFV-infected unfed adult *Hyalomma* spp. ticks in GB arising from migratory birds and horses was also made for the future (2070s) using a future habitat scenario. Throughout this chapter, ‘habitat’ is defined as a function of climate and land cover. It was assumed that only unfed adult *Hyalomma* spp. ticks (not nymphs or larvae) could bite a human.

As set out in Chapter 3, *Hyalomma* spp. ticks enter GB on birds as engorging immatures. The moult from larva to nymph occurs on the bird host and it has been observed that *Hyalomma* spp. ticks enter GB on birds as nymphs

(Jameson et al., 2012a). The results of the tick collection presented in Chapter 3, showed that 13 out of 14 *Hyalomma* spp. ticks removed from migratory birds were nymphs. It was, thus, assumed that only the nymph-to-adult moult of the tick needed to be modelled after entry into GB. As unfed adults, *Hyalomma* spp. ticks parasitize a wide range of hosts, which include humans (Hoogstraal, 1979, Bursali et al., 2011, Karaer et al., 2011). Therefore, imported CCHFV-infected nymphs have the potential to infect a human with CCHFV after they have moulted to an adult, given human exposure to the tick.

Horses are parasitized by adult *Hyalomma* spp. ticks (Jameson and Medlock, 2009) and, therefore, CCHFV-infected *Hyalomma* spp. ticks can enter GB on horses as engorging adults as explored in Chapter 4. *Hyalomma* spp. ticks only feed once per life-stage (Hillyard, 1996), so fed adults ticks imported on horses pose no direct transmission risk to humans through biting. However, as described in Chapter 4, mated adult female *Hyalomma* spp. ticks could detach from an imported horse in GB and lay eggs which hatch and survive to become unfed adults, which could then bite a human. The CCHF virus is able to persist in the *Hyalomma* spp. tick throughout its lifecycle through trans-stadial transmission (Wilson et al., 1991, Gonzalez et al., 1992, Zeller et al., 1994b) and, therefore, the progeny of a CCHFV-infected tick imported on a horse may be able to infect a human with CCHFV, given exposure through tick bite.

The aim of this chapter was to predict the number of CCHFV-infected *Hyalomma* spp. ticks imported on horses and on birds that can survive to the adult stage, whilst still being infected with CCHFV and where in GB this might occur both now and in the future. CCHFV-infected unfed adult *Hyalomma* spp. ticks have the potential to bite and infect a human. The pathways for entry of CCHFV-infected ticks on migrant birds and horses were presented in Chapter 5 and subsequent habitat suitability for moult and egg production was modelled. The pathway showing the process that could occur in GB from the point of detachment from the bird is shown in Figure 6.1. The relevant parameters are also shown, some of which were predicted in previous chapters. The white area shows the processes that were modelled in this chapter under current and future habitat conditions.

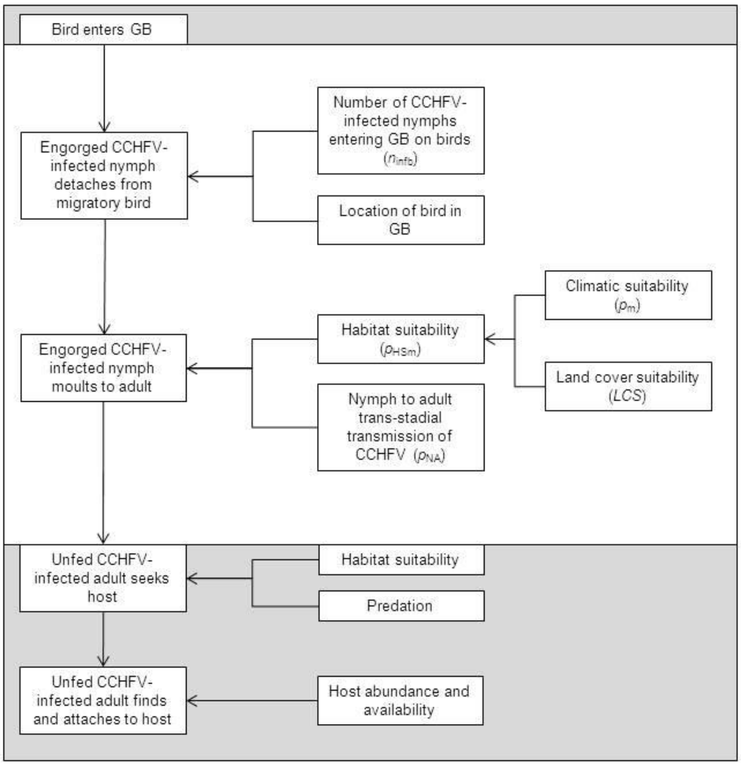


Figure 6.1: Pathway for a CCHFV-infected *Hyalomma* spp. tick entering GB on a bird, showing the relevant parameters. The processes shown in the white section are those that were modelled in this chapter.

The pathway showing the process that could occur in GB from the point of detachment from a horse is shown in Figure 6.2. As with the pathway shown in Figure 6.1, the relevant parameters are shown, with the white area showing the processes that were modelled in this chapter under current and future habitat conditions.

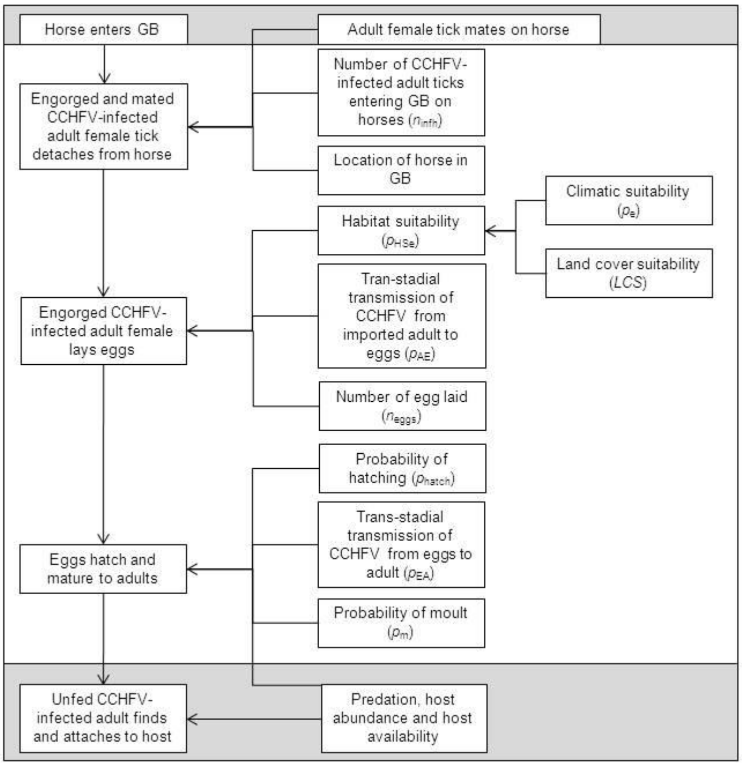


Figure 6.2: Pathway for a CCHFV-infected *Hyalomma* spp. tick after entry into GB on a horse, showing the relevant parameters. The processes in the white area are those that were modelled in this chapter.

6.2 Method

6.2.1 Predicted density per 1 km^2 of CCHFV-infected unfed adult *Hyalomma* spp. ticks from nymphs imported by migratory birds into GB under a current habitat scenario

The number of adult CCHFV-infected *Hyalomma* spp. ticks that moulted from nymphs imported on migratory birds was predicted. *Hyalomma marginatum* and *Hyalomma rufipes* are the two species of *Hyalomma* that have previously been found on migratory birds (Hasle et al., 2009a, Foley-Fisher et al., 2012, Jameson et al., 2012a). Hereafter, when discussing *Hyalomma* spp. ticks entering GB on migratory birds, it is these two species which are being referred to. For each of the five bird species selected (as in Chapter 3), the resultant number of CCHFV-infected adult *Hyalomma* spp. ticks ($n_{infadultb}$) was predicted for each 1 km^2 of GB using Equation (6.1), where the parameters are described in Table 6.1.

$$n_{\mathrm{infadultb}} = D_{\mathrm{Tb}} \times p_{\mathrm{HSm}} \times p_{\mathrm{NA}} \qquad (6.1)$$

For each bird species a Monte Carlo simulation of 10,000 realisations of $n_{\mathrm{infadultb}}$ was run using MATLAB R2012a and the mean, 95th and 99th percentiles were mapped. Finally, the total mean density of CCHFV-infected unfed adult *Hyalomma* spp. ticks in each 1 km^2 was predicted by summing the mean values of $n_{\mathrm{infadultb}}$ for each species. The following sections describe how estimates were made for each parameter.

Table 6.1: Summary of parameters for prediction of the number of *Hyalomma* spp. ticks able to infect a human with CCHFV in GB per 1 km^2 as a result of tick importation on migratory birds ($n_{\mathrm{infadultb}}$) according to Equation (6.1).

Parameter	Description
D_{Tb}	Predicted density of CCHFV-infected *Hyalomma* spp. nymphs entering GB on birds per 1 km^2
p_{HSm}	Probability of habitat suitability for the imported *Hyalomma* spp. tick
p_{NA}	Trans-stadial transmission of CCHFV during nymph-to-adult moult in the imported *Hyalomma* spp. tick

6.2.1.1 *Predicted density of CCHFV-infected* Hyalomma *spp. nymphs entering GB on migratory birds per 1 km²*

The number of CCHFV-infected *Hyalomma* spp. nymphs entering GB in spring each year was predicted in Chapter 3. This was based on the numbers of ticks entering on five species of bird that were considered to be the most likely to carry *Hyalomma* spp. ticks – *Acrocephalus schoenobaenus* (sedge warbler), *Oenanthe oenanthe* (northern wheatear), *Phoenicurus phoenicurus* (common redstart)*, Phylloscopus trochilus* (willow warbler) and *Sylvia communis* (common whitethroat). Data on the ranges of these birds within GB were used to predict where CCHFV-infected ticks might be deposited by these five bird species. Data were obtained at a spatial resolution of 10 km^2 across GB for each bird species from the British Trust for Ornithology (BTO). The data were taken from bird ringing and volunteer survey reports from 1988 to 1991 which had been collated by the BTO (Gibbons et al., 1993). In each 10 km^2 cell across GB, each species was defined as 'breeding', 'seen' or 'not present'. For the purposes of this study, the range of each species included both 'breeding' and 'seen', as the presence of the species is enough to indicate the possible presence of the tick. The area where each species was 'present' was mapped in ESRI ArcGIS 10.0. A grid of cell size 1 km^2 was created (as in Chapter 5) and, for each bird species, 1 km^2 cells that fell within the range of the bird species were assigned a value of one. Those outside of the range were assigned a value of zero.

For each bird species, the total number of 1 km^2 cells that fell within the range of the bird species (and therefore took a value of one) were summed (n_{cells}). The value of n_{cells} for each bird species range is given in Table 6.2. For each species, the number of CCHFV-infected *Hyalomma* spp. ticks imported (n_{infb}) was predicted in Chapter 3. The predicted density of CCHFV-infected ticks per 1 km^2 (D_{Tb}) imported by each bird species could, therefore, be calculated across the range of each bird species by Equation (6.2):

$$D_{\text{Tb}} = \frac{n_{\text{infb}}}{n_{\text{cells}}} \qquad (6.2)$$

Table 6.2: The number of 1 km^2 cells within the range of each of the five bird species.

Species of bird	Number of 1 km^2 cells in bird range, n_{cells}
Acrocephalus schoenobaenus	157,809
Oenanthe oenanthe	131,736
Phoenicurus phoenicurus	122,240
Phylloscopus trochilus	219,550
Sylvia communis	183,570

In Chapter 3, a simulation of 10,000 realisations of n_{infb} for each bird species was run in MATLAB R2012a and this was used to generate 10,000 values of D_{Tb} for input into Equation (6.1). It was assumed that birds were evenly distributed across their species range and that outside the range of these five bird species *Hyalomma* spp. ticks would not be imported by birds.

6.2.1.2 Probability of habitat suitability for the imported CCHFV-infected **Hyalomma** *spp. ticks*

Once the nymphal tick has detached from the bird, it needs to be in a suitable habitat for it to survive such that it can moult to the next life stage. The habitat suitability for *H. marginatum* under current climatic conditions (2007 to 2010) and using current land cover data was predicted in Chapter 5 for each 1 km^2 of GB. This was assumed to represent the habitat suitability for all *Hyalomma* spp. ticks imported by migratory birds. The mean output from Chapter 5 (p_{HSm}) was input into Equation (6.1) for each cell.

6.2.1.3 Trans-stadial transmission of CCHFV during nymph-to-adult moult in the imported **Hyalomma** *spp. tick*

For the moulted *Hyalomma* spp. adult to be able to infect a human, the CCHF virus has to persist within the tick throughout its lifecycle. The probability of trans-stadial transmission (TST) of CCHFV from nymphs to adults (p_{NA}) was determined from the literature for various *Hyalomma* spp. (Table 6.3). The resultant dataset was described by a beta distribution, X ~ Beta(360,36).

Table 6.3: Trans-stadial transmission rates of CCHFV from nymph-to-adult *Hyalomma* spp. ticks.

Reference	Species of tick	Nymph-to-adult TST rate
Okorie and Fabiyi (1980)	*H. rufipes*	100%
Logan et al. (1989)	*H. truncatum*	10%
Shepherd et al. (1991)	*H. rufipes*	41%, 5.88%
Shepherd et al. (1991)	*H. truncatum*	40%

6.2.2 Predicted density per 1 km^2 of CCHFV-infected unfed adult *Hyalomma* spp. ticks from ticks imported by horses into GB under a current habitat scenario

The processes outlined in Figure 6.2 were modelled to predict the density of unfed CCHFV-infected adult *Hyalomma* spp. ticks that could occur in GB per 1 km^2 as a result of horse importation between 1st April and 31st July each year ($n_{\mathrm{infadulth}}$). Several species of *Hyalomma* in different countries parasitize both horses and humans (see Chapter 4) and, hereafter, when referring to ticks imported on horses, '*Hyalomma* spp.' refers to all of these. For each 1 km^2 cell of GB $n_{\text{infadulth}}$ was predicted using Equation (6.3):

$$n_{\text{infadulth}} = \frac{n_{\text{infh}}}{3} \, x \, p_{\text{HSe}} \, x \, n_{\text{eggs}} \, x \, p_{\text{hatch}} \, x \, p_{\text{m}} \, x \, p_{\text{AE}} \, x \, p_{\text{EA}} \quad (6.3)$$

The parameters in the above equation are described in Table 6.4. A Monte Carlo simulation of 5,000 realisations of $n_{\text{infadulth}}$ was run using MATLAB R2012a and the mean, 95th and 99th percentiles were mapped. The number of 5,000 realisations of the simulation was chosen as after this number the results converged. The following sections describe how values were predicted for each parameter.

Table 6.4: Summary of parameters for prediction of the density of *Hyalomma* spp. ticks able to infect a human with CCHFV in GB per 1 km^2 via the horse pathway ($n_{\text{infadulth}}$).

Parameter	Description
n_{infh}	Predicted density of CCHFV-infected *Hyalomma* spp. adult ticks entering GB on horses per 1 km^2
p_{HSe}	Probability of habitat suitability for egg development in each 1 km^2 cell of GB
n_{eggs}	Number of eggs laid by an adult female *Hyalomma* spp. tick
p_{hatch}	Probability that eggs laid by an adult female *Hyalomma* spp. tick will hatch
p_{m}	Climatic probability of nymph-to-adult moult of a *Hyalomma* spp. tick
p_{AE}	Probability of trans-ovarial transmission of CCHFV from infected *Hyalomma* spp. adult female to eggs
p_{EA}	Probability of trans-stadial transmission of CCHFV from infected eggs to unfed adults

6.2.2.1 *Predicted density of CCHFV-infected* **Hyalomma** *spp. adults entering GB on horses per 1 km^2*

The density of CCHFV-infected *Hyalomma* spp. adults per 1 km^2 imported from CCHFV-endemic countries between 1st April and 31st July (n_{infh}) was predicted in Chapter 4. The mean values of n_{infh} for each 1 km^2 cell as predicted in Chapter 4 were used in Equation (6.3). The density of CCHFV-infected ticks entering GB each year was divided by three, as only females would go on to lay eggs. *Hyalomma* spp. ticks had been collected from horses in Spain (see Chapter 4) and the ratio of male-to-female ticks was found to be 2:1. Therefore, using the results from Chapter 4, the mean number of CCHFV-infected female *Hyalomma* spp. ticks was predicted to be 30 ticks.

6.2.2.2 Probability of habitat suitability for egg development in each 1 km^2 cell of GB

The imported adult female tick detaches from the horse after it is fully engorged and then lays eggs. Therefore, the habitat where the tick detaches has to be suitable for egg production and development. The habitat suitability for *H. marginatum* under current climatic conditions (2007 to 2010) and using current land cover data was predicted in Chapter 5 for each 1 km^2 of GB. This was assumed to represent the habitat suitability for all *Hyalomma* spp. ticks imported on horses. The mean output from Chapter 5 (p_{HSe}) was input into Equation (6.3) for each cell.

6.2.2.3 Number of eggs laid by adult female **Hyalomma** *spp. tick*

The number of eggs laid by an adult female *Hyalomma* spp. tick (n_{eggs}) was determined from published experimental studies on various different *Hyalomma* spp. (Table 6.5). The data were described by a discrete uniform distribution (an empirical distribution) as there were too few values to confidently fit any other mathematical distribution.

Table 6.5: Published data on the average number of eggs laid per *Hyalomma* spp. female.

Reference	Species	Average no. of eggs laid per female
Estrada-Peña et al. (2011)	*H. marginatum*	6500
Ammah-Attoh (1966)	*H. rufipes*	4899
Arthur (1970)	*H. anatolicum*	3353.5
Durrani et al. (2008)	*Hyalomma* spp.	3045
Ouhelli and Pandey (1984)	*H. lusitanicum*	6320
Široký et al. (2012)	*H. aeyptium*	6900
Shoukry et al. (2000)	*H. schulzei*	12596
Sweatman (1968)	*H. aeyptium*	5198

6.2.2.4 Probability that eggs laid by an adult female **Hyalomma** *spp. tick will hatch*

Given that the adult female tick lays eggs, the probability that the eggs hatch (p_{hatch}) was determined. There is a single study by Chen et al. (2012) that has looked at the hatching success of *Hyalomma* spp. eggs. Laboratory experiments showed that 68% of *H. rufipes* eggs hatched. This can be described by a beta distribution, X ~ Beta(69,33).

6.2.2.5 Climatic suitability for development of larvae to adults

The newly emerged larvae are able to overwinter in an unfed state but need to find a host and feed the following spring. It was assumed that the *Hyalomma* spp. larvae would be able to overwinter in GB and subsequently find a small mammal or bird host the following spring. This was a reasonable assumption as *H. marginatum* ticks are established in countries such as Kosovo, Ukraine and Bulgaria where winter temperatures can drop below those experienced in GB (www.weatheronline.co.uk). The following spring, the larvae require sufficient temperatures to moult to nymphs and then moult again to adults. According to the equations for moult produced by Estrada-Peña et al. (2011), the larva-to-nymph moult is less dependent on temperature than the nymph-to-adult moult. Moulting of larva to nymph also occurs on the host such that the ticks are, to a certain extent, protected from external climatic influences. Therefore, in the areas of GB where nymph-to-adult moult could occur, larva-to-nymph moult would also be able to occur. Thus, the probability of nymph-to-adult moult completing between 1st April and 31st July (p_{m}) as determined in Chapter 5 was used as a proxy for the probability of larva-to-adult moult. The mean value of p_{m} for each 1 km^2 cell of GB was used in the simulation model for $n_{\mathrm{infadulth}}$ (Equation (6.3)).

6.2.2.6 Probability of trans-ovarial transmission of CCHFV from imported **Hyalomma** *spp. adult female to eggs*

As with the bird pathway, the TST of CCHFV between life stages had to be taken into account. This initially involved trans-ovarial transmission (TOT) from the adult female to the eggs (p_{AE}) and this was previously found to be described by X ~ Beta(5,13) in Chapter 3.

6.2.2.7 Probability of trans-stadial transmission of CCHFV from eggs to unfed adult **Hyalomma** *spp. ticks*

The trans-stadial transmission rate from eggs to adults (p_{EA}) was given by Equation (6.4):

$$p_{EA} = p_{EL} \text{ x } p_{LN} \text{ x } p_{NA} \qquad (6.4)$$

where the parameters and their fitted beta distributions are described in Table 6.6. The TST rates for p_{EL} and p_{LN} were previously obtained from the literature and described by beta distributions in Chapter 3. The fitted beta distribution describing p_{NA} was also previously determined for the bird pathway in this chapter.

Table 6.6: Definition and description of parameters in Equation (6.4).

Parameter	Description	Fitted distribution
p_{EL}	Trans-stadial transmission of CCHFV from eggs to larvae	$X \sim$ Beta(2,6)
p_{LN}	Trans-stadial transmission of CCHFV from larvae to nymphs	$X \sim$ Beta(2,22)
p_{NA}	Trans-stadial transmission of CCHFV from nymphs to adults	$X \sim$ Beta(360,36)

6.2.3 Predicted density per 1 km^2 of CCHFV-infected unfed adult *Hyalomma* spp. ticks from nymphs imported by migratory birds into GB under a future habitat scenario

The density per 1 km^2 of CCHFV-infected unfed adult *Hyalomma* spp. ticks in GB arising from nymphs imported by migratory birds in the future was investigated using future predictions for the parameter values in Equation (6.1) as given in Equation (6.5):

$$n_{\text{infadultbf}} = D_{\text{Tbf}} \times p_{\text{HSmf}} \times p_{\text{NA}} \qquad (6.5)$$

The parameter definitions are given in Table 6.7 (and Table 6.1 for p_{NA}) and each was estimated using predicted data for the future. It was assumed that there was no change in trans-stadial transmission of CCHFV from nymph-to-adult between current and future scenarios. Therefore, p_{NA} was described by a beta distribution, $X \sim$ Beta(360,36) as in the current model (Equation (6.1)). The ‘future’ period of interest in this chapter refers to 2077 to 2080 and hereafter all future predictions made will be for this period. These predicted data included predicted daily temperatures for GB (2077 to 2080), predicted future land cover and predicted future bird ranges. For each bird species a Monte Carlo simulation of 10,000 realisations of $n_{\text{infadultbf}}$ was run using MATLAB R2012a and the mean, 95th and 99th percentiles were mapped. Each of the future data sources will now be described along with how each parameter was estimated.

Table 6.7: Summary of parameters for prediction of the number of CCHFV-infected unfed adult *Hyalomma* spp. ticks in GB per 1 km^2 as a result of tick importations on migratory birds in 2077 to 2080 ($n_{\text{infadultbf}}$) according to Equation (6.5).

Parameter	Description
D_{Tbf}	Predicted future density of CCHFV-infected *Hyalomma* spp. nymphs entering GB on birds per 1 km^2
p_{HSmf}	Future probability of habitat suitability for the imported *Hyalomma* spp. tick

6.2.3.1 Future temperature predictions for 2077 to 2080

Daily predictions for air temperature at noon at 1.5 m above ground level were previously obtained by AHVLA from the British Atmospheric Data Centre (BADC). These predictions were made using the HadRM3 model under a medium emissions scenario (SRESA1B) at the UK Met Office Hadley Centre. The spatial resolution for these data was 25 km x 25 km and they were available on a rotated pole grid of 2,080 cells covering the UK. Daily noon temperature files were processed using the programming language Perl into single comma delimited text files for each month, as in Chapter 5. The predicted data were based on a 30 day month and, therefore, all future scenario outputs in this chapter were also based on 30 day months.

6.2.3.2 Future land cover predictions

The LCM2007 was used in Chapter 5 to describe the areas of GB where land cover was suitable for *H. marginatum* based on their habitat preferences. A future land cover map for GB does not exist and, therefore, for the purposes of this risk assessment, a land cover scenario for GB in the future was created. This was done by reviewing the literature on land cover and land use change and then changing the LCM2007 to match the findings. This is now described in detail.

Review of future land cover and land use change in GB

For the next 40 years it is considered that socio-economic drivers will have a greater impact on land use change than climate change (Rounsevell and Reay, 2009). Most future land use scenarios agree that there will be an increase in urban areas, although the spatial patterns of urban growth and road-based transport differ. According to Rounsevell and Reay (2009), urban development is ‘increasingly dispersed through urban sprawl’ whilst Bibby (2009) believes that urban sprawl is over-exaggerated and that it is the density of urban areas that is increasing.

Residential property currently takes up 5.4% of England’s land area (Bibby, 2009). An increase in urbanisation is not just related to housing and if all infrastructure is included (transport networks, commercial buildings, industry etc.), the land take for all development could increase by 41% by 2060. The total land area that could be developed by 2060 would, therefore, be 12% (Foresight Land Use Futures, 2010). In many land use scenarios, technological development was cited as one of the most important drivers of change, although probably the least well understood and, therefore, highly uncertain. However, this technological advancement is related to scenarios where agriculture becomes more intensive with increased productivity and more efficient farming methods. Agricultural land has been shrinking with a fall of 14% between 1961 and 2005. Arable land decreased by 20% from 1961 to 2007, whilst there was increase in the use of chemical inputs and machinery (Rounsevell and Reay, 2009).

Land that was previously used to grow crops is expected to be used for bioenergy production. The UK government has said that the biofuel industry must not replace agricultural land that would otherwise be used for food production, but in reality the demand for biofuels is likely to displace food crops (Foresight Land Use Futures, 2010). This may be countered, however, by the intensification of food production. Agricultural land is also expected to give way for reforestation and afforestation. There has been an increase in the planting of commercial conifer species such as the Sitka spruce (Rounsevell and Reay, 2009). Woodland cover in the UK has increased dramatically over the last 90 years with woodland covering 5% of the UK in 1924, 9% of the UK in 1980 and 11.7% of the UK in 2008 (Foresight Land Use Futures, 2010). It is difficult to predict where the reforestation and afforestation will occur, but it is embedded in rural development policy (Rounsevell and Reay, 2009) and, therefore, it is highly likely that the rapid increase in woodland cover that has occurred in recent history will continue into the future.

Beyond the land changes affected by urbanisation and human activity, the composition of the landscape may also alter due to climate change. There is anticipated to be water stress particularly in the south of England (Rounsevell and Reay, 2009) and this may change the types of crops that are grown. Although water shortages may lower production in some cases, it is likely that there will be a transition towards more drought resistant crop species. Water shortages may be countered by restoring or creating wetlands by planned flooding (Foresight Land Use Futures, 2010). The warmer temperatures that are predicted to occur, especially in the south of England may enhance the growth of grasses (Rounsevell and Reay, 2009), the favoured habitat type of *Hyalomma* spp. ticks. Warmer, drier summers may increase the suitability of current grassland for *H. marginatum* which has a preference for dry grassland within its current range (see Chapter 5).

The predicted land use changes as outlined above will have an impact on the suitability of the landscape for *Hyalomma* spp. ticks. Here, it is considered that the main impact would be the expansion of urban areas into grassland. It is expected that a large proportion of urban development will take place in rural areas, as well as the expansion of cities and towns. This is due in part to the current trend of people moving to areas of rural character that are close to their cities of work (Rounsevell and Reay, 2009). There is also a trend for people moving out of the cities into rural areas and a quarter of new housing is built in small rural developments (Foresight Land Use Futures, 2010). As in Chapter 5, ‘sub-urban’ and ‘urban’ are considered unsuitable land cover types for the survival of *Hyalomma* spp. ticks.

It is also considered that there will be an increase in wetland habitats in the future (Foresight Land Use Futures, 2010). Historically, there were extensive areas of wetland in GB and plans have been drawn up to restore some of these wetland areas and create new wetland habitats through controlled flooding

(Wetland Vision). Wetland Vision is a collaborative project between Natural England, RSPB, English Heritage, Environment Agency, the Wildlife Trusts and the Wildfowl and Wetlands Trust that ‘sets out a 50-year vision for England’s freshwater wetlands’ (Wetland Vision). Similar future wetland visions are being considered for Scotland (Scottish Borders Council) and Wales (Countryside Council for Wales) but are not as yet available. Sea level rise as a result of climate change and the resultant development of coastal flood plains may also contribute to land cover change by 2080. Sea levels around GB are predicted to rise between 18.6 cm and 43.3 cm by 2080 (compared to 1990) (Millin, 2010), although this may actually be an underestimate (Rahmstorf et al., 2012). Sea level rise may have an impact upon land availability particularly for agriculture. It is believed that after urbanisation, the creation and restoration of wetlands is the most important land use change affecting the habitat suitability of GB for *Hyalomma* spp. ticks.

Development of a future land cover map

The urbanisation of GB in the future was predicted from the baseline land cover map for 2007 (LCM2007, as used in Chapter 5). Future urbanisation is believed to involve an increase in the density of urban areas and/or urban sprawl, particularly in rural areas as described above. An urbanisation scenario based on these assumptions was created. For each 1 km^2 cell of GB, if the percentage of land cover type was more than 50% ‘urban’ or ‘sub-urban’ according to LCM2007, the future proportion of suitable land cover for *Hyalomma* spp. ticks (LCS_f) was given a value of zero. For 1 km^2 cells that were adjacent to cells with more than 50% cover of ‘urban’ or ‘sub-urban’, if the current proportion of land cover suitability (LCS) was more than 50%, the future proportion of land cover suitability (LCS_f) was given a value of 0.5. In the same adjacent cells, if LCS was less than or equal to 50%, LCS_f was given a value of zero. These changes were applied and mapped in ESRI ArcGIS 10.0 to create a future urbanisation scenario.

A map of England showing areas where wetlands could be restored or created over the next 50 years was available from reports on the Wetland Vision website as an image file (Wetland Vision) and is shown in Figure 6.3.

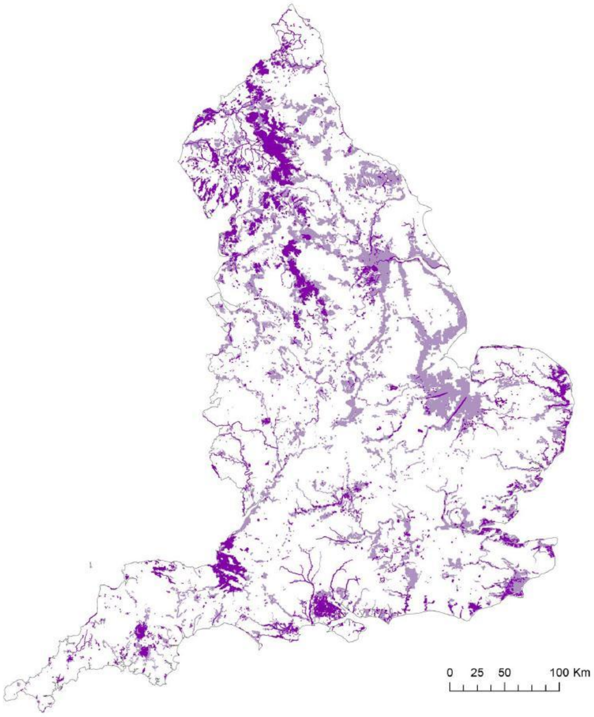


Figure 6.3: Wetland Vision map of future wetland potential (Wetland Vision). Dark purple areas show current extent of wetlands and pale purple areas show areas where wetlands could be created or restored over the next 50 years.

This map was combined with the future urbanisation scenario map in ESRI ArcGIS 10.0 using georeferencing tools. Areas where there were current and planned wetlands were considered to have a value of LCS_f of zero. Woodland and agricultural land are not considered to be suitable land cover types for *Hyalomma* spp. ticks as determined in Chapter 5. It is not known where in GB afforestation and reforestation will take place in the future, although it is believed that a proportion of agricultural land will be given over to woodland and forests. Given that both these land cover types are unsuitable for *Hyalomma* spp. ticks, it is not believed that there will be any change in habitat suitability as a result of agricultural to woodland transition. For this reason and due to the uncertainty surrounding the location of future afforestation, for the purposes of risk assessment it was assumed that forest and agricultural land cover would not change in GB between 2007 and 2080 beyond that already accounted for in the future urbanisation and wetland scenarios described above. However, whilst existing grasslands may increase in suitability for *Hyalomma* spp. ticks as a result of warmer, drier summers in 2077 to 2080, some areas of grassland may be lost from urbanisation and wetland expansion.

The future land cover suitability for *Hyalomma* spp. ticks was given as a proportion of each 1 km^2 cell of GB and is shown in Figure 6.4.

Figure 6.4: Proportion of suitable land cover in the future, LCS_{f}, per 1 km^2 of GB. See text for details.

6.2.3.3 Future bird range predictions

The future bird ranges across Europe of European breeding birds have been predicted for the late 21st century (2079 to 2099) using a future climate scenario derived from the HadCM3 simulation (Huntley et al., 2007). The predicted data on the future ranges of the five bird species of interest in this thesis were provided by Prof. Brian Huntley and Dr. Yvonne Collingham of Durham University. The data were available at a resolution of 50 km x 50 km and each bird species was defined as ‘present’ or ‘absent’ in each 50 km x 50 km grid cell. The data provided also included simulated ranges for the current climate

(1961 to 1990). Due to the coarse spatial resolution of the data when considering only GB, it was decided that changes between the simulated current range data and simulated future range data would be used to map expansion or contraction of the current BTO range data for each species. In this way, areas of no change would retain the 10 km^2 resolution of the BTO data. Therefore, for each of the five bird species modelled in this thesis, a future range map was produced using the baseline range of the current BTO data with an increase or decrease in range according to the difference between the current and future range simulations of Huntley et al. (2007). The future range maps are shown in Figure 6.5 and were used as future bird range predictions for 2077 to 2080.

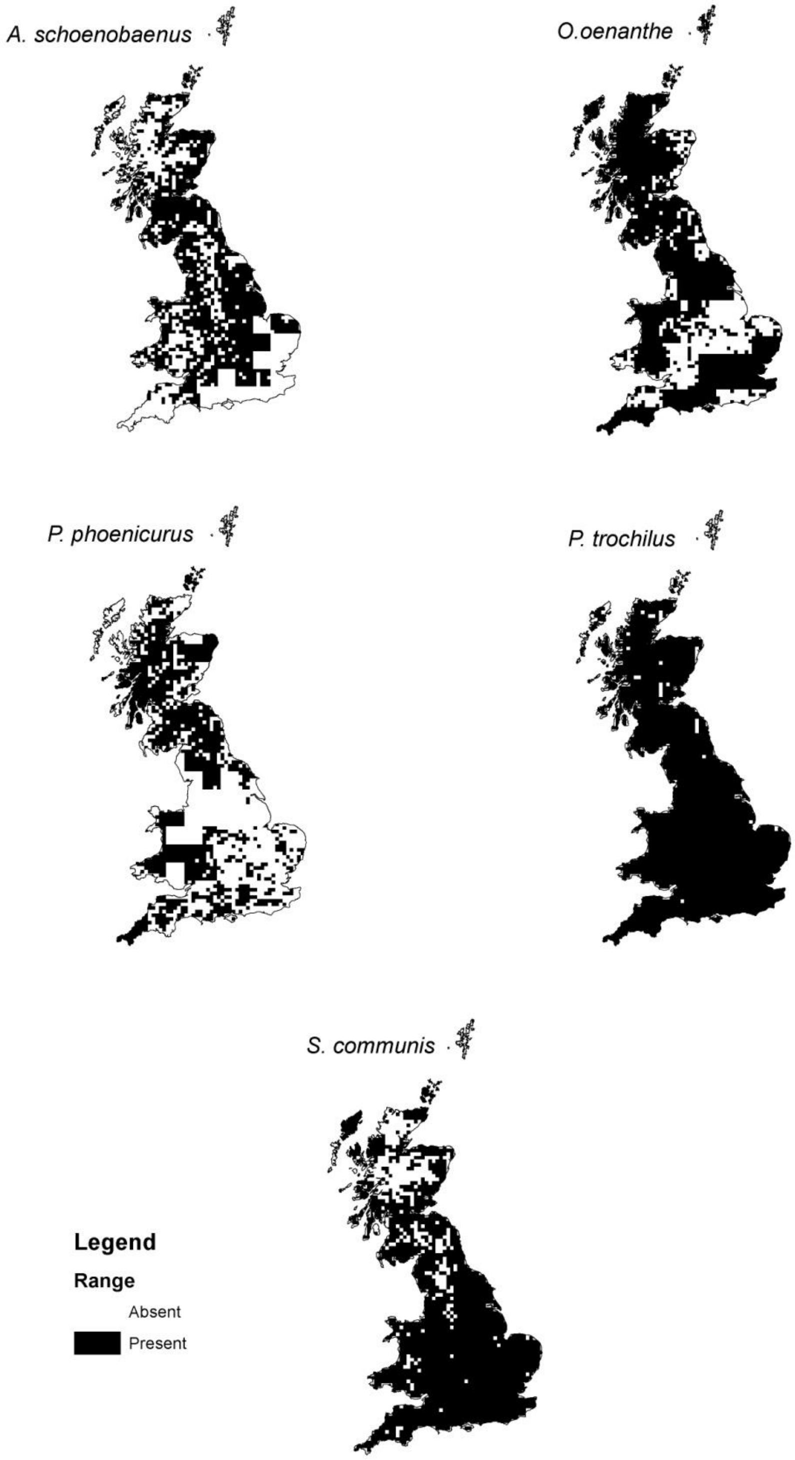


Figure 6.5: Predicted future range of each bird species.

6.2.3.4 *Future predicted density of CCHFV-infected* **Hyalomma** *spp. nymphs entering GB on migratory birds per 1 km^2*

The number of ticks entering GB on each of the five selected bird species was predicted for the future. It was assumed that the number of *Hyalomma* spp. ticks on each bird remained the same as current estimates (see Chapter 3). However, the total number of *Hyalomma* spp. nymphs entering GB in the spring on each bird species was adjusted for predicted future changes to the population size of each species in GB. Data on the future population sizes of bird species do not exist and, therefore, had to be estimated. It was assumed that the change in population size of each bird was proportional to the predicted change in range. Therefore, the percentage change between current and future bird range size was applied to the current estimates for bird population. The percentage change in range for each species was calculated from the difference between the number of 1 km^2 in the current range (n_{cells}) and the number of 1 km^2 cells in the future range (n_{cellsf}, Table 6.8). The future GB population estimates for each bird species are given in Table 6.8.

Table 6.8: Future GB population estimates for each bird species. Data on the current population size were taken from Robinson (2005).

Bird species	Current population size	Number of 1 km^2 cells in future bird range, n_{cellsf}	Change in GB range (%)	Predicted future population size
A. schoenobaenus	594,000	131,929	16.4% decrease	496,587
O.oenanthe	540,000	169,989	29.0% increase	696,803
P.phoenicurus	202,000	112,425	8.03% decrease	185,781
P. trochilus	4,000,000	221,477	0.88% increase	4,035,108
S. communis	1,862,000	191,647	4.39% increase	1,943,927

For each species, the predicted number of CCHFV-infected *Hyalomma* spp. ticks entering GB each year in the future (n_{infbf}) was estimated using Equation (3.1) in Chapter 3, with future estimates for the number of CCHFV-infected ticks entering GB on each bird species each spring based on the future population size predictions. For the purposes of risk assessment, it was assumed that trans-stadial transmission rates of CCHFV and CCHFV-prevalence for adult ticks on the East Atlantic flyway would remain the same. The predicted density of CCHFV-infected ticks per 1 km^2 imported by each bird species in the future (D_{Tbf}) could, therefore, be calculated across the future range of each bird species by Equation (6.6):

$$D_{\mathrm{Tbf}} = \frac{n_{\mathrm{infbf}}}{n_{\mathrm{cellsf}}} \qquad (6.6)$$

A Monte Carlo simulation of 10,000 realisations of n_{infbf} for each bird species was run in MATLAB R2012a and this was used to generate 10,000 values of D_{Tbf} for input into Equation (6.5). It was again assumed that birds were evenly distributed across their species range and that outside the range of these five bird species *Hyalomma* spp. ticks would not be imported by birds.

6.2.3.5 Future probability of habitat suitability for the imported CCHFV-infected Hyalomma spp. ticks

The future probability of habitat suitability for *Hyalomma* spp. ticks (p_{HSmf}) was predicted for each 1 km^2 of GB using future climate data (2077 to 2080) and the future land cover suitability map (Figure 6.4). The probability that nymph-to-adult moult could be completed by 31st July if moult began on 1st April (p_{mf}), was estimated using Equation (5.5) in Chapter 5. The value of water vapour deficit (*VD*) was kept constant at 15 hPa as in the current habitat suitability models (see Chapter 5) and daily noon temperatures were input into Equation (5.5) of Chapter 5 using the future temperature predictions for 2077 to 2080. The values of p_{mf} were transferred onto a 1 km^2 grid that covered GB in ESRI ArcGIS 10.0 and were input into Equation (6.7):

$$p_{\mathrm{HSmf}} = LCS_{\mathrm{f}} \times p_{\mathrm{mf}} \qquad (6.7)$$

The future probability of habitat suitability for the imported CCHFV-infected *Hyalomma* spp. ticks was mapped in ESRI ArcGIS 10.0 and is shown in Figure 6.6.

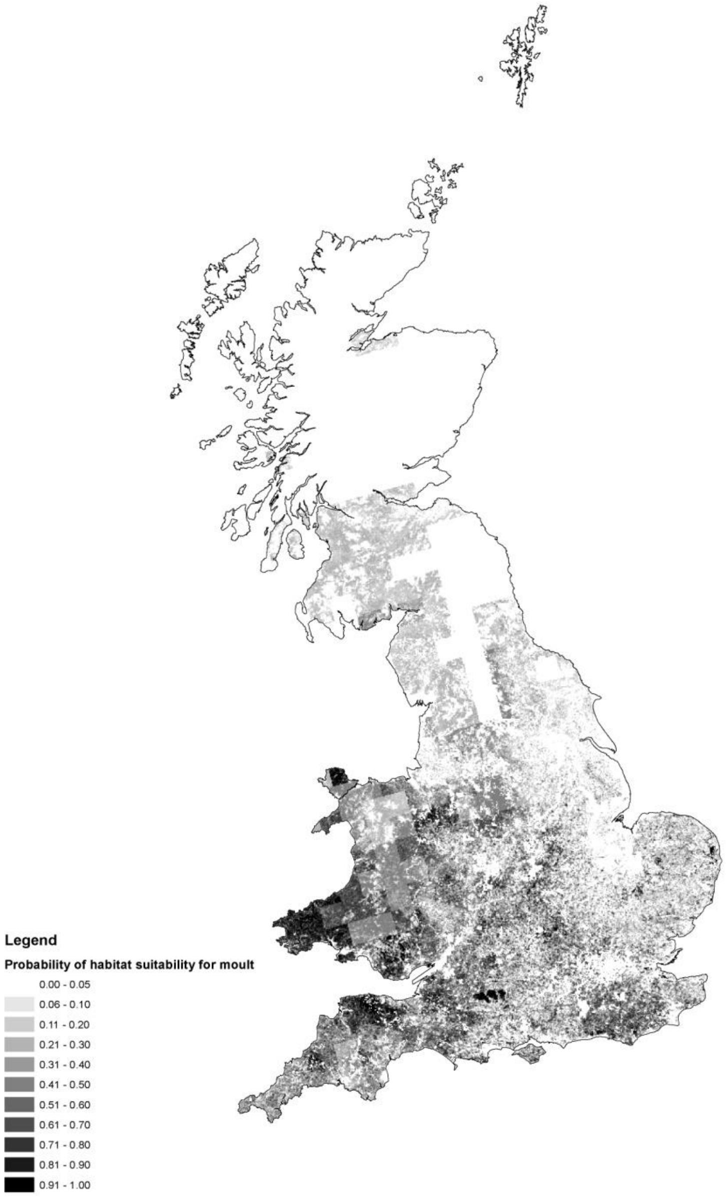


Figure 6.6: Future probability of habitat suitability for nymph-to-adult moult given that a tick began to moult on 1st April.

6.2.4 Predicted density per 1 km^2 of CCHFV-infected unfed adult *Hyalomma* spp. ticks from ticks imported by horses into GB under a future habitat scenario

The future density per 1 km^2 of unfed CCHFV-infected adult *Hyalomma* spp. ticks that could occur in GB as a result of horse importation between 1st April and 31st July each year ($n_{\text{infadulthf}}$) was predicted. For each 1 km^2 cell of GB $n_{\text{infadulthf}}$ was predicted using Equation (6.8):

$$n_{\text{infadulthf}} = \frac{n_{\text{infh}}}{3}\; x\; p_{\text{HSef}}\; x\; n_{\text{eggs}}\; x\; p_{\text{hatch}}\; x\; p_{\text{mf}}\; x\; p_{\text{AE}}\; x\; p_{\text{EA}} \qquad (6.8)$$

where the parameter values are defined in Table 6.4 and Table 6.9. A Monte Carlo simulation of 5,000 realisations of $n_{\text{infadulthf}}$ was run using MATLAB R2012a and the mean, 95th and 99th percentiles were mapped.

Table 6.9: Summary of parameters for future prediction of the density of *Hyalomma* spp. ticks able to infect a human with CCHFV in GB per 1 km^2 via the horse pathway ($n_{\text{infadulthf}}$).

Parameter	Description
p_{HSef}	Future probability of habitat suitability for egg development in each 1 km^2 cell of GB
p_{mf}	Future climatic probability of nymph-to-adult moult of a *Hyalomma* spp. tick

It was assumed that the future number of CCHFV-infected *Hyalomma* spp. ticks imported on horses (n_{infh}) would not change from current predictions. The number of horses being imported into GB each year from CCHFV-endemic countries was considered to remain unchanged and this is supported by the data collected over 15 years from Heathrow Airport in Chapter 4 which showed little long-term change in the number of horses imported each year. The parameters, n_{eggs}, p_{hatch}, p_{AE} and p_{EA} were also kept the same as in the current scenario. The parameters, p_{HSef} and p_{mf} (Table 6.9) were predicted under a future habitat scenario. The future probability of nymph-to-adult moult (p_{mf}) was previously estimated for the prediction of p_{HSmf} (Equation 6.7). As with the current habitat scenario model in Equation (6.3), the areas where nymph-to-adult moult was predicted to complete by 31st July were also indicative of the areas where larva-to-nymph moult would complete. The assumptions of survival of over-wintering larvae and successful host questing were also made in this model, as in the current scenario model. The method of estimation of p_{HSef} is now described.

6.2.4.1 Future probability of habitat suitability for egg production in each 1 km^2 cell of GB

The future probability of habitat suitability for *Hyalomma* spp. egg production (p_{HSef}) was predicted for each 1 km^2 of GB using future climate data (2077 to

2080) and the future land cover suitability map (Figure 6.4). The probability that egg development could be completed by 31st July if pre-oviposition began on 1st April (p_{ef}), was estimated using Equation (5.19) in Chapter 5. The value of water vapour deficit (VD) was kept constant at 15 hPa as in the current habitat suitability models (see Chapter 5) and daily noon temperatures were input into Equation (5.19) of Chapter 5 using the future temperature predictions for 2077 to 2080. The values of p_{ef} were transferred onto a 1 km^2 grid that covered GB in ESRI ArcGIS 10.0 and were input into Equation (6.9):

$$p_{\mathrm{HSef}} = LCS_{\mathrm{f}} \times p_{\mathrm{ef}} \qquad (6.9)$$

The future probability of habitat suitability for egg development was mapped in ESRI ArcGIS 10.0 and is shown in Figure 6.7.

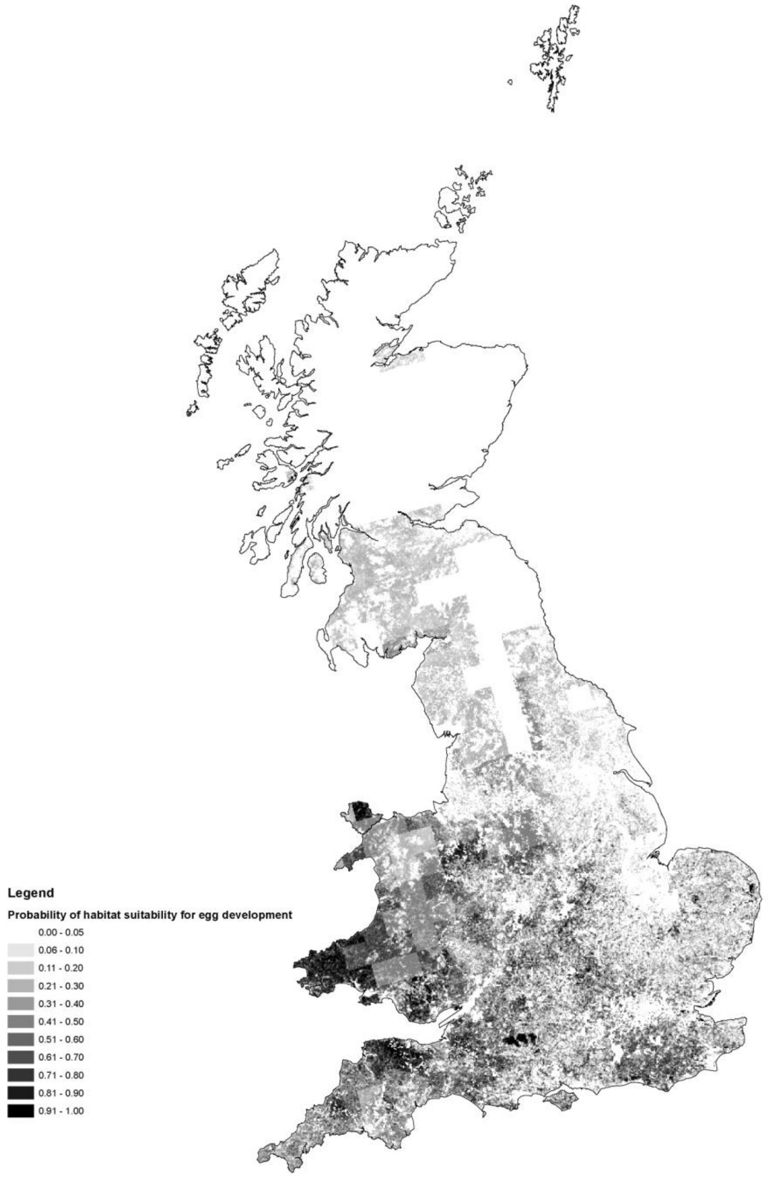


Figure 6.7: Future probability of habitat suitability for egg development given that a mated adult female tick started pre-oviposition on 1st April.

6.3 Results

6.3.1 Predicted density per 1 km^2 of CCHFV-infected adult *Hyalomma* spp. ticks from nymphs imported by migratory birds into GB under a current habitat scenario

A Monte Carlo simulation using Equation (6.1) with 10,000 realisations was run for each of the five bird species and maps of GB were produced to show the predicted density per 1 km^2 of CCHFV-infected unfed adult *Hyalomma* spp.

ticks moulted from nymphs imported by birds each year ($n_{\text{infadultb}}$) for the mean, 95th and 99th percentiles of the output for each species. In some cases the 95th percentile in all areas of GB was zero ticks and so for these bird species, only maps of the mean and 99th percentile output are shown. The maps for each species are given in Figure 6.8 to Figure 6.12. The predicted density of CCHFV-infected unfed adult *Hyalomma* spp. ticks in each cell from all five bird species was mapped (Figure 6.13). The total number of ticks imported for each bird species for the whole of GB is given in Table 6.10.

Table 6.10: The predicted number of CCHFV-infected unfed adult ticks moulted from nymphs imported each spring by each bird species ($n_{\text{infadultb}}$). The mean, 95th and 99th percentile results are presented for the total number of ticks imported into GB.

Species	Mean	95th percentile	99th percentile
A. schoenobaenus	4.03	9.23	86.4
O. oenanthe	2.45	0	39.6
P. phoenicurus	0.19	0	4.31
P. trochilus	0.11	0	2.41
S. communis	4.23	0.72	90.3

The British breeding population of *S. communis* was predicted to be responsible for the largest number of CCHFV-infected unfed adult *Hyalomma* spp. ticks in GB and *P. trochilus* was responsible for the fewest. For 95% of the time, *O. oenanthe*, *P. phoenicurus* and *P. trochilus* were predicted to not import any CCHFV-infected ticks that could survive to the next life stage. *P. trochilus* had the largest British breeding population and the largest range in GB (Figure 6.11) but had the lowest infestation rates of *Hyalomma* spp. ticks of all the bird species studied (see Chapter 3). The predicted 95th percentile value of $n_{\text{infadultb}}$ for *A. schoenobaenus* was larger than the corresponding value for *S. communis*. This was because despite having a smaller GB population and range within GB, the infestation rate of *Hyalomma* spp. ticks on *A. schoenobaenus* was higher (see Table 3.8 in Chapter 3).

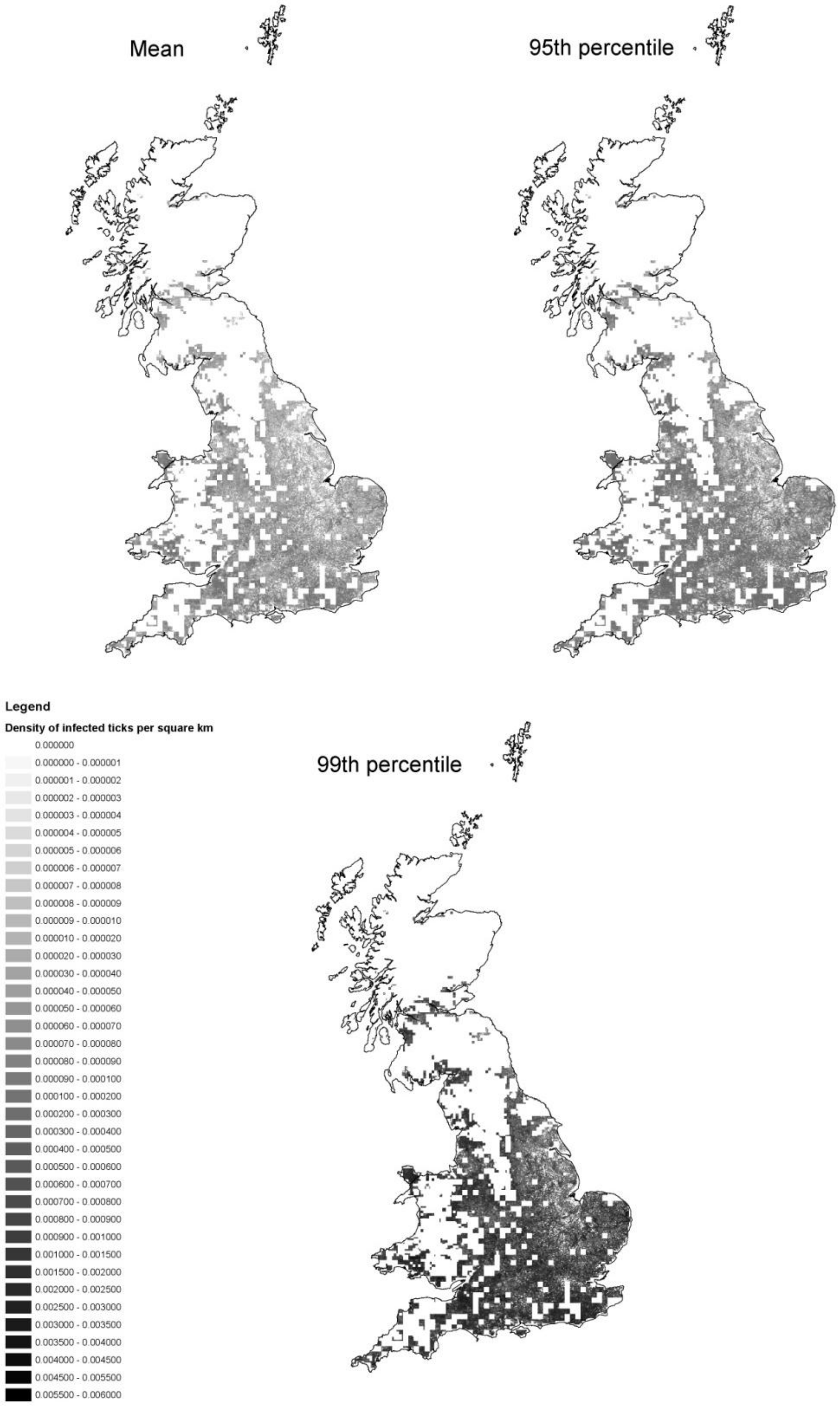


Figure 6.8: The density of CCHFV-infected unfed adult *Hyalomma* spp. ticks per 1 km^2 of GB from nymphs imported on the British breeding population of *A. schoenaenus*.

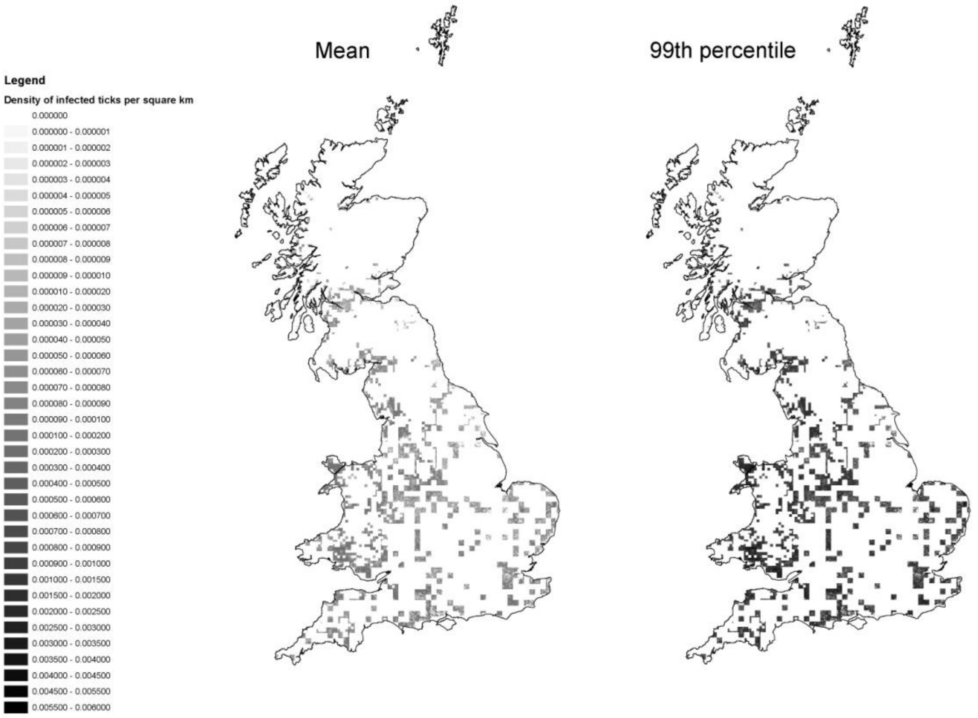


Figure 6.9: The density of CCHFV-infected unfed adult *Hyalomma* spp. ticks per 1 km^2 of GB from nymphs imported on the British breeding population of *O. oenanthe*.

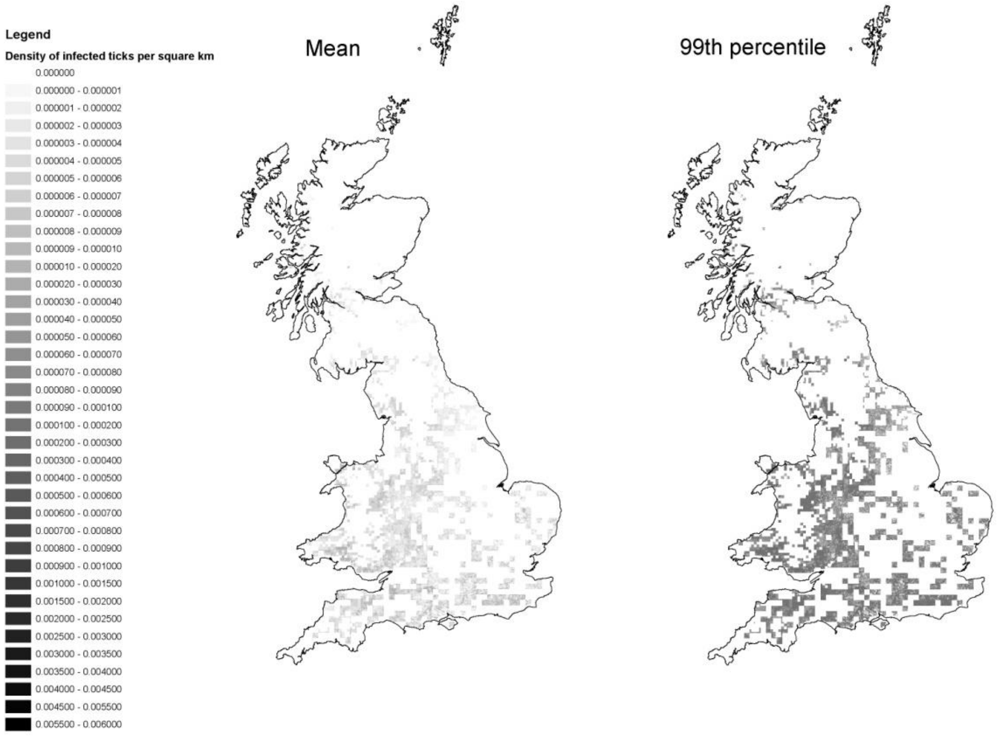


Figure 6.10: The density of CCHFV-infected unfed adult *Hyalomma* spp. ticks per 1 km^2 of GB from nymphs imported on the British breeding population of *P. phoenicurus*.

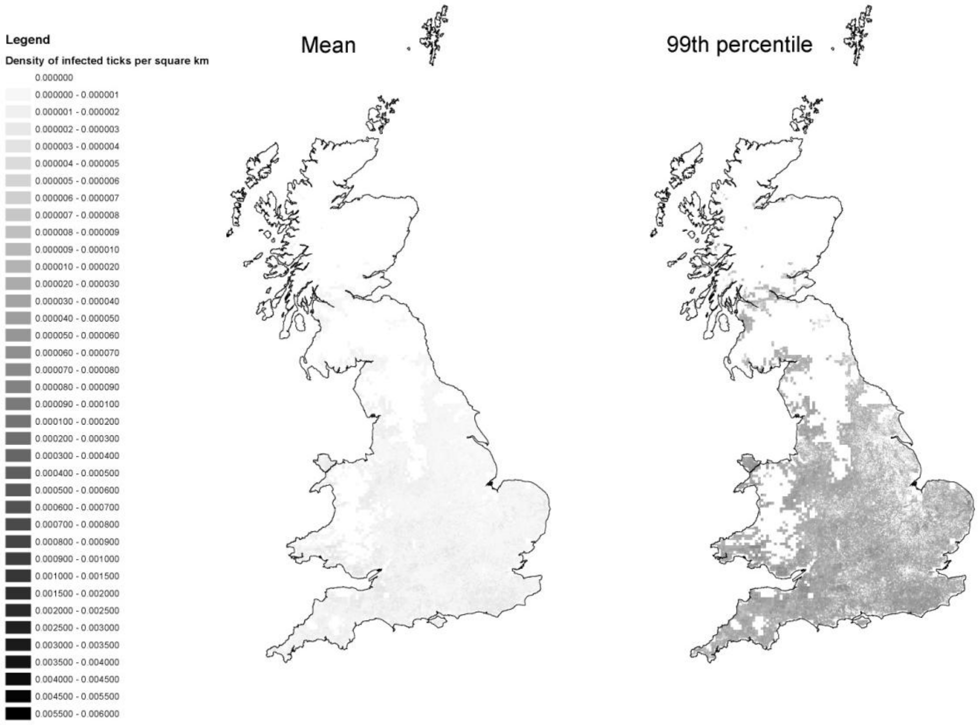


Figure 6.11: The density of CCHFV-infected unfed adult *Hyalomma* spp. ticks per 1 km^2 of GB from nymphs imported on the British breeding population of *P. trochilus*.

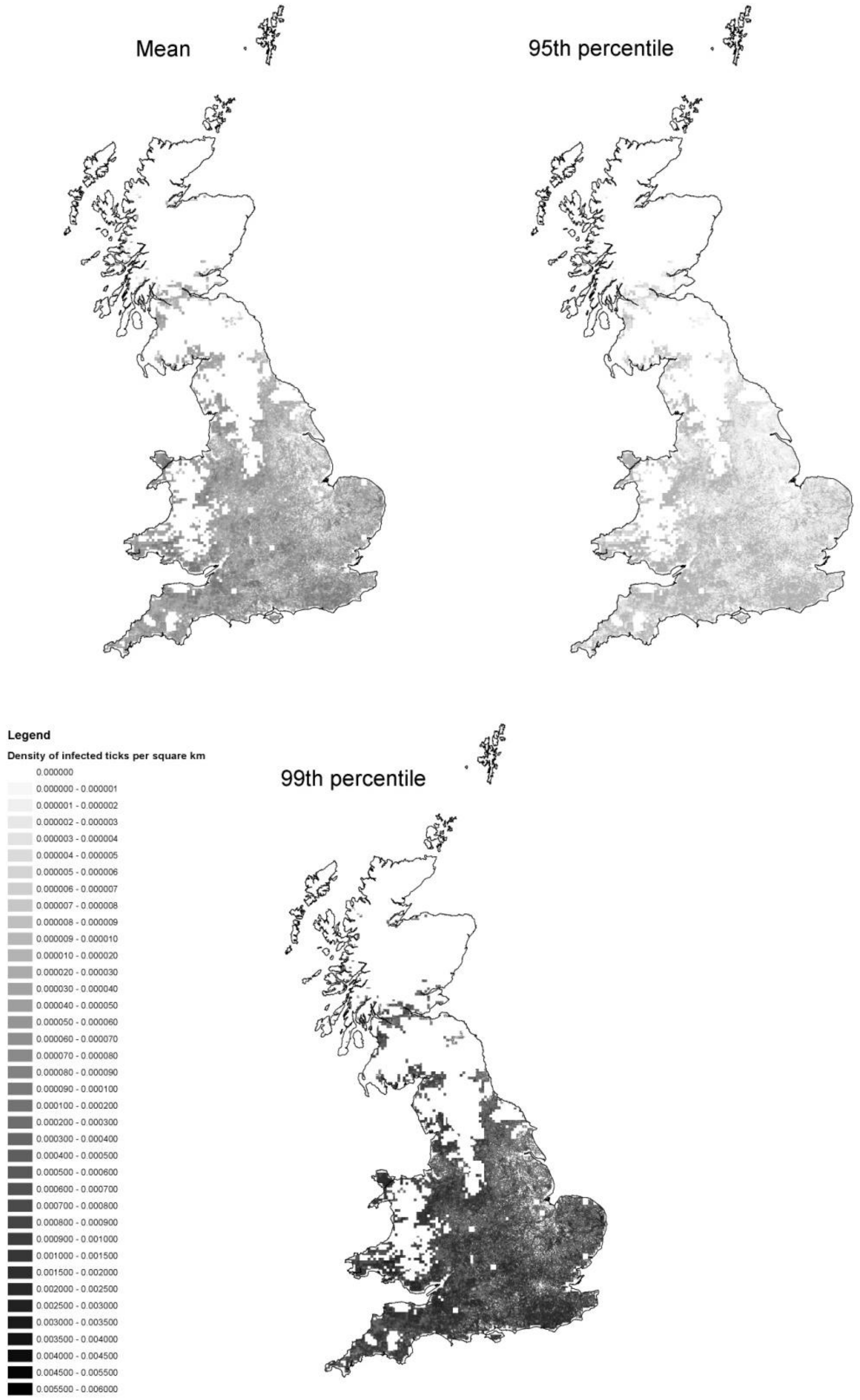


Figure 6.12: The density of CCHFV-infected unfed adult *Hyalomma* spp. ticks per 1 km^2 of GB from nymphs imported on the British breeding population of *S. communis*.

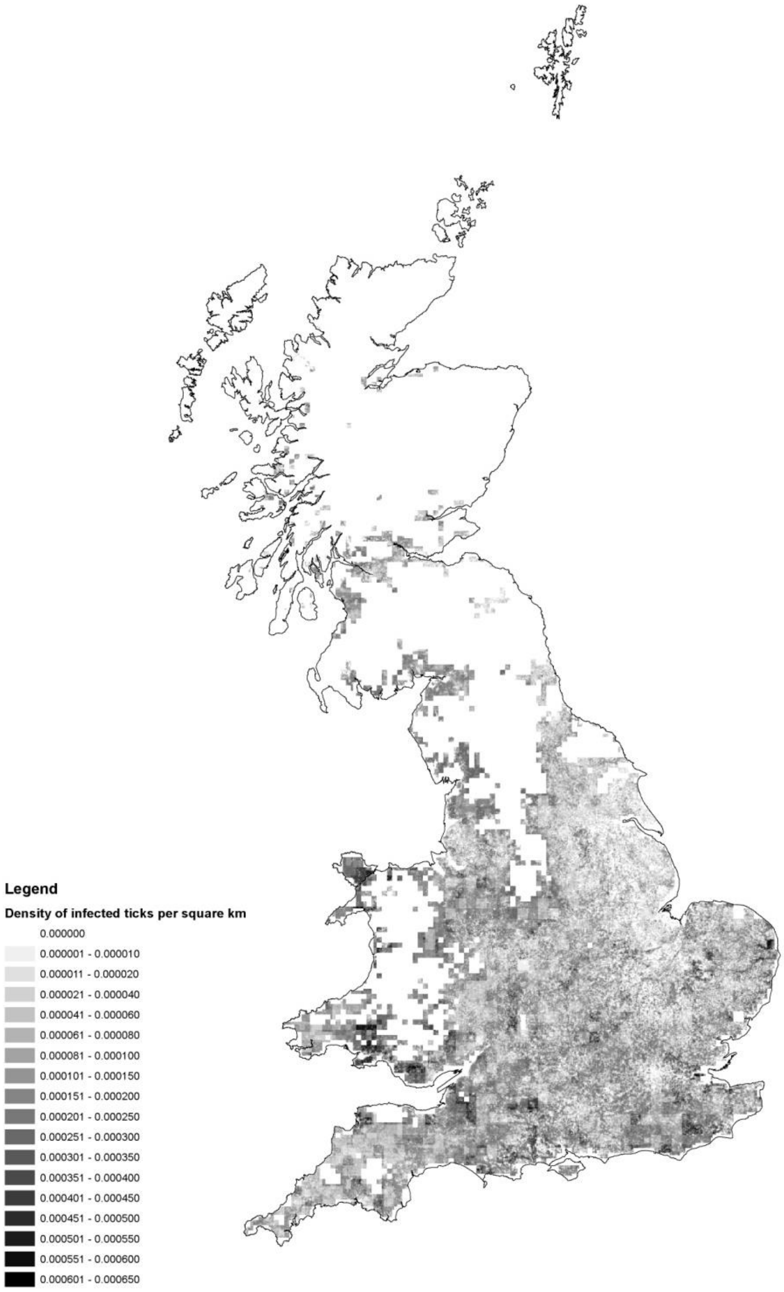


Figure 6.13: The density of CCHFV-infected unfed adult *Hyalomma* spp. ticks per 1 km^2 of GB from nymphs imported on the British breeding population of all five bird species.

Imported nymphs were unable to moult to adults within the defined time frame of 1st April to 31st July in upland areas of Wales, the north of England and Scotland, as determined in Chapter 5. When the five bird species were considered together, the greatest density of infected ticks per 1 km^2 was found in lowland parts of Wales, Kent and north Somerset (Figure 6.13). For all birds the total mean number of infected ticks, $n_{\text{infadultb}}$, was predicted to be 11 ticks per year.

6.3.2 Predicted density per 1 km^2 of CCHFV-infected adult *Hyalomma* spp. ticks from ticks imported by horses into GB under a current habitat scenario

A Monte Carlo simulation using Equation (6.3) with 5,000 realisations was run and the density per 1 km^2 of CCHFV-infected adult *Hyalomma* spp. ticks produced from ticks imported by horses between 1st April and 31st July each year ($n_{\text{infadulth}}$) was predicted. The mean, 95th percentile and 99th percentile of the simulation results were mapped in ESRI ArcGIS 10.0 (Figure 6.14). The predicted numbers of CCHFV-infected adult *Hyalomma* spp. ticks for the whole of GB are given in Table 6.11.

Table 6.11: The total predicted number of CCHFV-infected unfed adult *Hyalomma* spp. ticks arising from adults imported by horses between 1st April and 31st July each year. The mean, 95th and 99th percentile results are presented.

Species	Predicted total number of infected ticks
Mean	143.3
95th percentile	643.1
99th percentile	2724.0

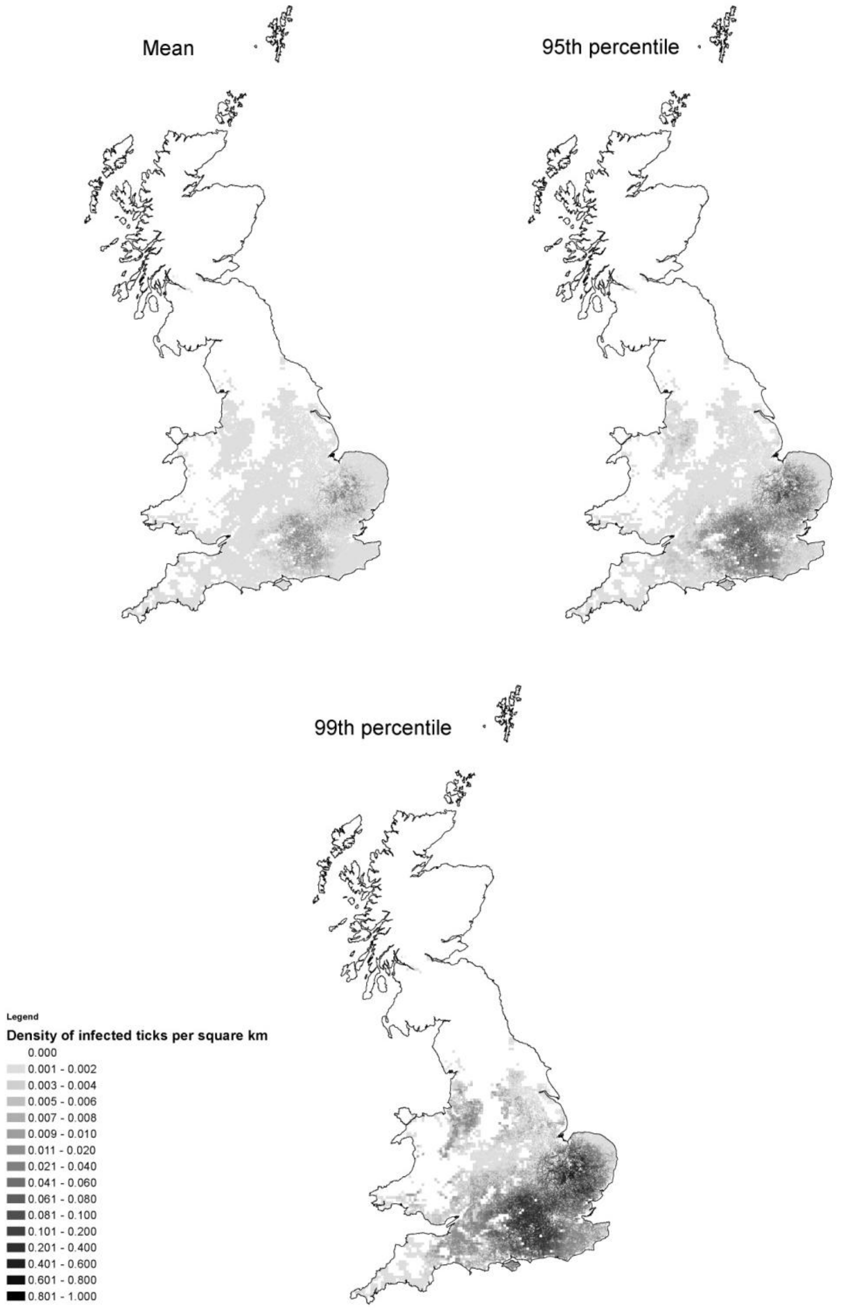


Figure 6.14: Predicted density per 1 km^2 of CCHFV-infected adult *Hyalomma* spp. ticks from ticks imported by horses into GB under a current habitat scenario.

Between 1st April and 31st July each year, under current climatic and land cover conditions, a mean number of 143.3 CCHFV-infected adult *Hyalomma* spp. ticks were predicted to be present in GB as a result of tick importations on horses from CCHFV-endemic countries. The highest density of infected ticks was predicted to be in the south-east of England, particularly around Oxfordshire, Berkshire, Surrey and East Anglia. This is where the largest number of horse importations occurred (see Chapter 4) and where the probability of habitat suitability for *Hyalomma* spp. ticks was highest (see Chapter 5). The greatest predicted mean density of infected ticks ($n_{\text{infadulth}}$) was 0.049 ticks per 1 km^2 (5th and 95th percentiles of 0 and 0.218, respectively) and this occurred near Newmarket, Suffolk, where the highest number of horse importations occurred. Most of Wales, the south-west of England, northern England and Scotland were predicted to have no CCHFV-infected *Hyalomma* spp. ticks.

6.3.3 Predicted density per 1 km^2 of CCHFV-infected adult *Hyalomma* spp. ticks from nymphs imported by migratory birds into GB under a future habitat scenario

A Monte Carlo simulation using Equation (6.5) was run with 10,000 realisations for each of the five bird species and the density per 1 km^2 of CCHFV-infected unfed adult *Hyalomma* spp. ticks moulted from nymphs imported by birds each year ($n_{\text{infadultbf}}$) was predicted. The mean, 95th and 99th percentiles of the simulation results were mapped. The mean output for each species is presented along with the 95th and 99th percentiles, if they were greater than zero. The maps for each species are given in Figure 6.15 to Figure 6.19. The total of $n_{\text{infadultbf}}$ for each bird species for the whole of GB is given in Table 6.12.

Table 6.12: The predicted number of CCHFV-infected unfed *Hyalomma* spp. adult ticks moulted from nymphs imported each year by each bird species under a future scenario. The mean, 95th and 99th percentile results are presented for the total number of ticks in GB for each bird species.

Species	Mean	95th percentile	99th percentile
A. schoenobaenus	4.66	12.8	105
O. oenanthe	2.55	0	50.4
P. phoenicurus	0.24	0	4.94
P. trochilus	0.13	0	0
S. communis	5.33	0	133

The total mean number of CCHFV-infected adult ticks for all bird species was 12.9 ticks for the whole of GB. This was slightly larger than that predicted under a current habitat scenario. The mean density of CCHFV-infected ticks from importation on all five bird species is shown in Figure 6.20.

Figure 6.15: The density of CCHFV-infected unfed adult *Hyalomma* spp. ticks per 1 km^2 of GB from nymphs imported on the British breeding population of *A. schoenaenus* in the future (2077 to 2080).

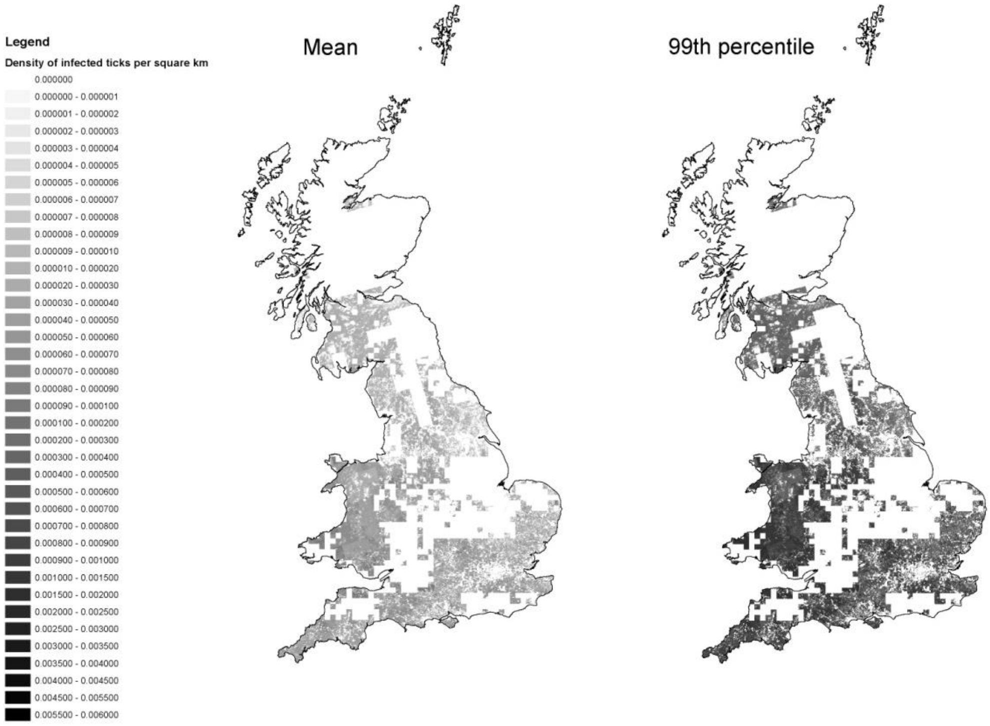


Figure 6.16: The density of CCHFV-infected unfed adult *Hyalomma* spp. ticks per 1 km^2 of GB from nymphs imported on the British breeding population of *O. oenanthe* in the future (2077 to 2080).

Figure 6.17: The density of CCHFV-infected unfed adult *Hyalomma* spp. ticks per 1 km^2 of GB from nymphs imported on the British breeding population of *P. phoenicurus* in the future (2077 to 2080).

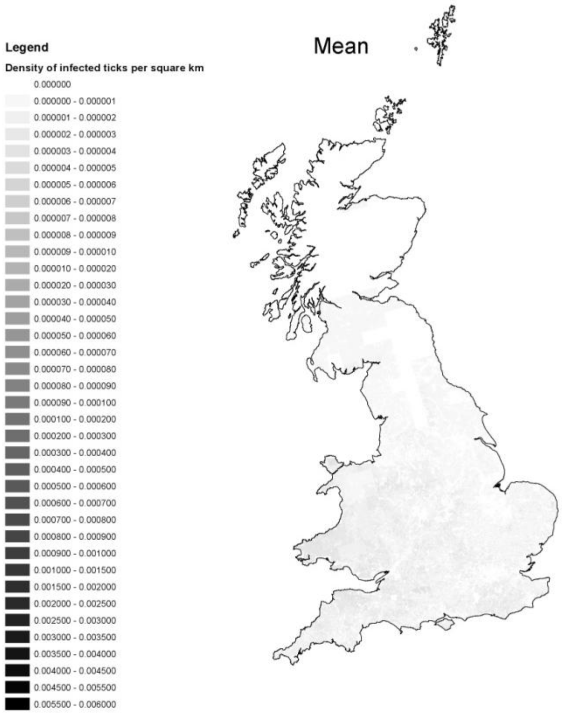


Figure 6.18: The density of CCHFV-infected unfed adult *Hyalomma* spp. ticks per 1 km^2 of GB from nymphs imported on the British breeding population of *P. trochilus* in the future (2077 to 2080).

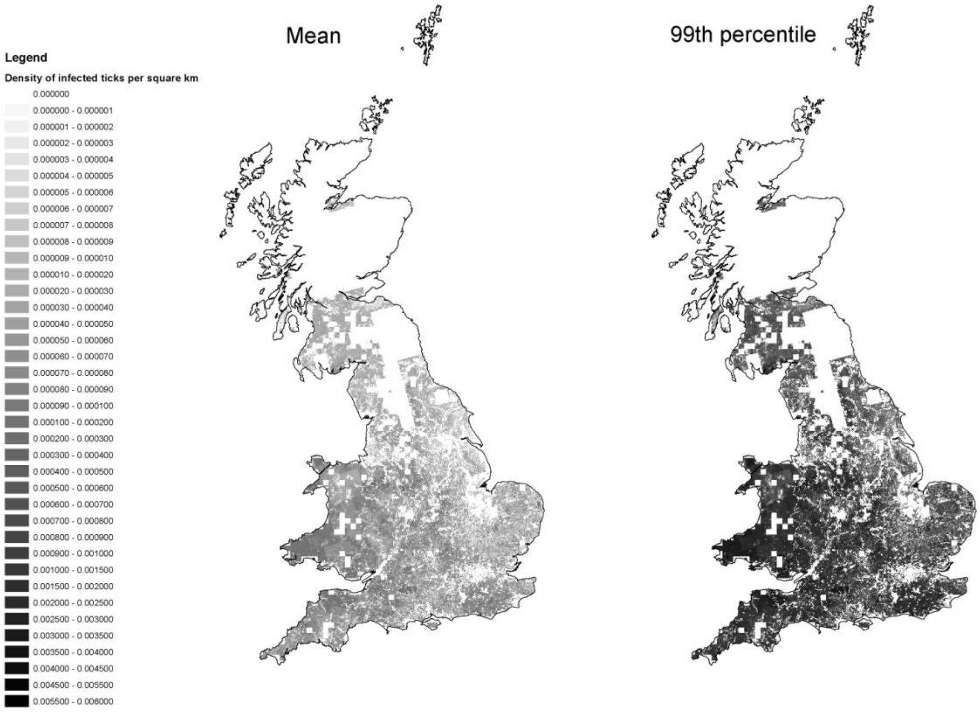


Figure 6.19: The density of CCHFV-infected unfed adult *Hyalomma* spp. ticks per 1 km^2 of GB from nymphs imported on the British breeding population of *S. communis* in the future (2077 to 2080).

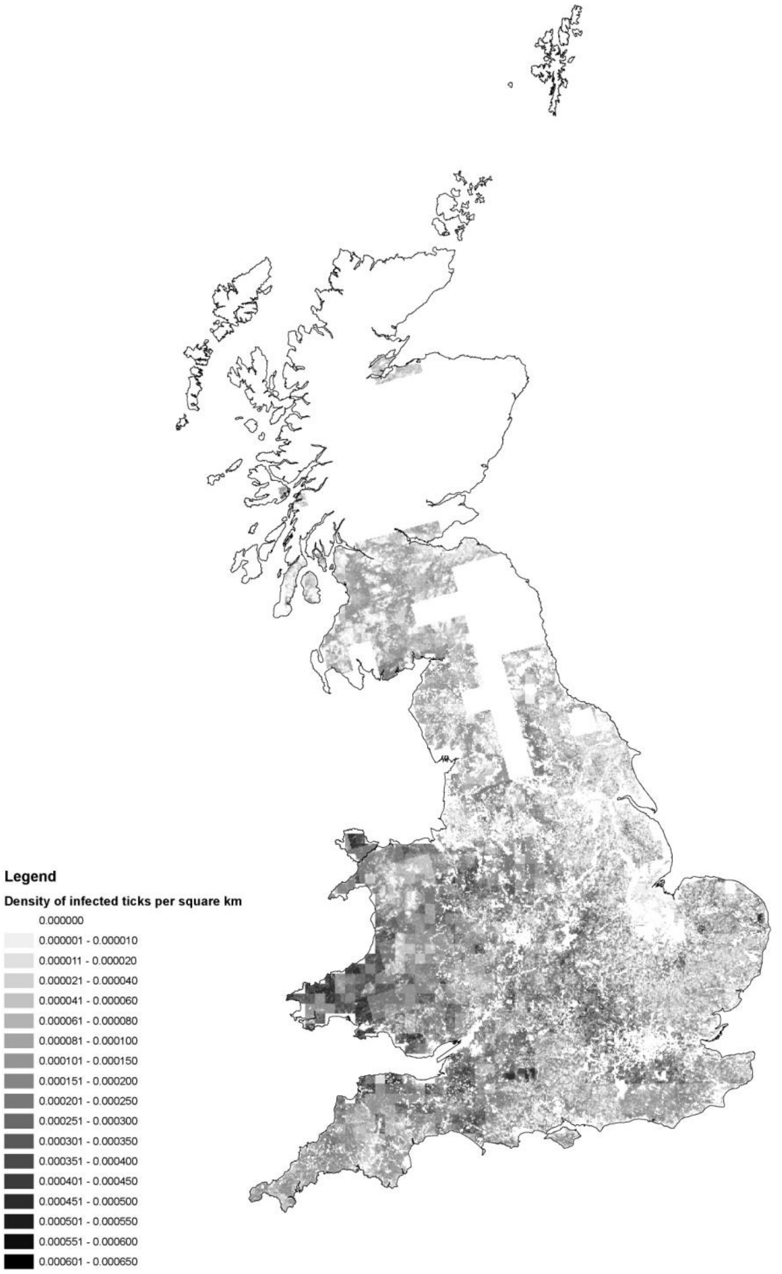


Figure 6.20: The density of CCHFV-infected unfed adult *Hyalomma* spp. ticks per 1 km^2 of GB from nymphs imported on the British breeding population of all five bird species under a future habitat scenario.

There was predicted to be a reduction in the density of CCHFV-infected unfed adult *Hyalomma* spp. ticks per 1 km^2 arising from migratory birds in the east of England due to urbanization around London and the creation of wetland areas particularly around The Wash. Areas of Wales, the south-west of England and lowland parts of northern England and southern Scotland were predicted to have higher densities of CCHFV-infected *Hyalomma* spp. ticks compared to the current scenario. This is because of the increase from current air temperatures to future predicted air temperatures as used in this model. The range of all bird species was predicted to increase from 814,905 km^2 in the current model to 827,467 km^2 in the future model, contributing to the increase in the mean total number of infected ticks in GB predicted under the future habitat scenario.

6.3.4 Predicted density per 1 km^2 of CCHFV-infected adult *Hyalomma* spp. ticks from ticks imported by horses into GB under a future habitat scenario

A Monte Carlo simulation using Equation (6.8) was run with 5,000 realisations and the density per 1 km^2 of CCHFV-infected adult *Hyalomma* spp. ticks produced from ticks imported by horses between 1st April and 31st July each year ($n_{\text{infadulthf}}$) was predicted. The mean, 95th percentile and 99th percentile of the simulation results were mapped in ESRI ArcGIS 10.0 (Figure 6.21). The predicted numbers of CCHFV-infected adult *Hyalomma* spp. ticks for the whole of GB are given in Table 6.13.

Table 6.13: The total predicted number of CCHFV-infected unfed adult *Hyalomma* spp. ticks in GB arising from adults imported by horses between 1st April and 31st July each year under a future habitat scenario. The mean, 95th and 99th percentile results are presented.

Species	Predicted total number of infected ticks
Mean	121.8
95th percentile	539.0
99th percentile	2505.0

The predicted number of CCHFV-infected ticks in GB as a result of tick importations along the horse pathway under a future habitat scenario was lower than under a current habitat scenario (Table 6.11). This is likely to be due to the decrease in land cover suitability for *Hyalomma* spp. ticks in the areas that were predicted to receive the largest numbers of imported ticks. In Norfolk, the future expansion of wetland areas was predicted to encroach on grassland in areas that currently have large numbers of horse imports. The urbanization of outer London and the ‘home counties’ is also in an area of greater horse importation. However, some areas of GB had larger predicted values of $n_{\text{infadulthf}}$ under a future scenario than under a current scenario and this was most likely to be due to increasing temperatures aiding the survival of *Hyalomma* spp. ticks. These areas included Wales, the south-west of England, the north of England and southern Scotland.

Figure 6.21: Predicted density per 1 km^2 of CCHFV-infected adult *Hyalomma* spp. ticks from ticks imported by horses between 1^{st} April and 31^{st} July each year into GB under a future habitat scenario.

6.4 Discussion

Migratory birds and horses as routes of introduction of CCHFV-infected *Hyalomma* spp. ticks into GB were investigated under both current and future habitat scenarios. The two pathways present the most likely entry routes for an infected tick into GB as both horses and migratory birds have been found in GB with *Hyalomma* spp. ticks attached (Jameson and Medlock, 2009, Jameson et al., 2012a). There are a large number of *Hyalomma* spp. ticks predicted to be entering GB each year on migratory birds (*n*=506,223 ticks (5th and 95th percentiles of 0 and 3,050,000 ticks), see Chapter 3) but the mean number of these ticks predicted to be infected with CCHFV was 72.9 (5th and 95th percentiles of 0 and 64.2 ticks). In this study, five bird species were selected for modelling but there are likely to be other bird species that carry *Hyalomma* spp. ticks into GB. For example, *H. marginatum* ticks have been recorded at least twice on *S. rubetra* (whinchat) in GB (Martyn, 1988). These birds frequent open grassland and meadow (Robinson, 2005), the preferred habitat of the *Hyalomma* spp. tick (see Chapter 4) and migrate along the East Atlantic flyway each spring between Europe and Africa. The British breeding population of *S. rubetra* is smaller at just 42,000 individuals (Robinson, 2005) compared to that of the birds included in this study (e.g. 202,000 individuals of *P. phoenicurus* and 4,000,000 individuals of *P. trochilus*; (Robinson, 2005). Thus, species of bird other than the five considered here may make a small contribution to the number of CCHFV-infected *Hyalomma* spp. ticks in GB.

In Chapter 3, the number of CCHFV-infected ticks imported on the British breeding population of the five selected bird species was predicted. In this chapter, the models were run from 1st April to 31st July and, thus, an assumption was made that the breeding population of the five bird species would enter GB during this time period. Data over five years (2007 to 2012) available from Portland bird observatory showed that 92% of migrants (of the five species studied) arrived at Portland on the south coast of England after 1st April. Data from Bardsey bird observatory available for 2007 to 2012, showed that 97% of migrants (of the five species studied) arrived at Bardsey Island (off the northwest coast of Wales) after 1st April. Finally, data were available from Fair Isle bird observatory from 2007 to 2011 showed that 100% of migrants (of the five species studied) arrive at Fair Isle in the Shetland Islands after 1st April. Therefore, a small number of early migrants were not accounted for by running the model from 1st April. The model could, therefore, be improved by setting a slightly earlier start date in the south of GB compared to more northerly areas to accommodate the difference in bird arrival times across GB. However, it is not known how long after arrival into GB ticks may detach from birds and it may be that ticks on migratory birds that arrive earlier are not yet fully engorged and ready to detach immediately after arrival in GB. Birds that leave their wintering grounds early may in fact leave before ticks emerge from winter diapause and, thus, may be less likely to be carrying ticks than later migrants.

The number of horses imported into GB each year (n=592 horses) was not of the same magnitude as the number of migratory birds entering GB each year (n=7,198,000), but in Chapters 3 and 4 horses were predicted to be importing a greater number of CCHFV-infected *Hyalomma* spp. ticks (n_{infh}=89.8 ticks , 5th and 95th percentiles of 0 and 494 ticks) than migratory birds (n_{infb}=72.9 ticks, 5th and 95th percentiles of 0 and 64.2 ticks). In this chapter the number of CCHFV-infected unfed adult *Hyalomma* spp. ticks in GB arising from imported horses (mean=143.3 ticks for current habitat suitability and 121.8 ticks for future habitat suitability) was greater than the number arising from migratory birds (mean=11 ticks for current habitat suitability and 12.9 ticks for future habitat suitability). This was because ticks imported on horses are able to breed soon after arrival in GB and lay an average of 6,101 eggs per individual female (Table 6.5) that in some areas of GB are able to develop into unfed adult ticks.

The future scenario presented in this chapter predicted conflicting results for the two pathways considered. For the bird pathway, the number of CCHFV-infected ticks in GB that could bite and infect a human was larger in the future scenario compared to the current scenario (Table 6.14).

Table 6.14: Mean number of CCHFV-infected unfed adult Hyalomma spp. ticks in GB arising from ticks imported on migratory birds between 1st April and 31st July each year, under current habitat conditions and a future habitat scenario.

	Current habitat conditions	Future habitat scenario
Mean number of CCHFV-infected unfed adult ticks	11	12.9

The future prediction for the nymph-to-adult moult showed an increase in the probability of habitat suitability. This was particularly noticeable in Wales, northern England and southern Scotland, where current temperatures were too low for moult completion. The future increase in the area of GB where the five species were predicted to be present and, therefore, a predicted increase in the number of individual birds, meant a larger number of CCFHV-infected ticks being imported. In the case of the bird pathway, these factors outweighed the decrease in the proportion of suitable land cover for *Hyalomma* spp. ticks predicted for the future.

The future scenario for the horse pathway produced a decrease in the number of CCHFV-infected ticks in GB (Table 6.15).

Table 6.15: Mean number of CCHFV-infected unfed adult Hyalomma spp. ticks in GB arising from ticks imported on horses between 1st April and 31st July each year, under current habitat conditions and a future habitat scenario.

	Current habitat conditions	Future habitat scenario
Mean number of CCHFV-infected unfed adult ticks	143.3	121.8

It was assumed that there would be no increase in the number of horses imported into GB in the future, unlike the increase of 2.23% predicted for migratory birds. Therefore, the number of CCHFV-infected ticks imported into GB on horses remained the same as under the current scenario. Horse importations are currently difficult to quantify and the data available are most likely a sample of the total imports into GB (see Chapter 4). It may be that in the future, tighter regulations are imposed (possibly as a result of introduced equine diseases such as African Horse Sickness) that result in fewer horse importations into GB particularly from non-EU member states. It is unlikely that horse import numbers would increase dramatically in the future, although an improved recording system of all horse movements may give this impression in the future. For this risk assessment it was reasonable to assume that horse importations would remain roughly the same, with an average of 592 horses being imported into GB from 1st April to 31st July each year.

The probability of egg development increased in areas of suitable land cover under future climatic conditions. Thus, within areas of suitable land cover, there was predicted to be an increase in the survival potential for *Hyalomma* spp. ticks. However, the areas of GB where land cover suitability was predicted to decrease through wetland expansion and urbanisation, were the main areas where imported horses were previously predicted to be introducing *Hyalomma* spp. ticks. This was the main reason for the decrease in the number of CCHFV-infected ticks from the horse pathway under a future scenario. It may be that as these areas are so important for the racehorse industry, they are to a certain extent ‘protected’ from the predicted land cover changes. It is very difficult to predict whether these areas will be indeed be affected by urbanisation and/or wetland development, as the future demand for housing and freshwater may take priority over leisure activities and the horse racing industry.

There were a number of assumptions made in the future models presented here for the bird and horse risk pathway. The number of eggs oviposited by the female and trans-ovarial and trans-stadial transmission rates were assumed to remain the same as under the current scenario. Studies on the effect of temperature on trans-ovarial and trans-stadial transmission of CCHFV have not been conducted, but temperature was found to have no effect on the number of eggs oviposited by *Amblyomma cajennense* Fabricius or on Venezuelan equine encephalitis viral replication rate in ticks held at various different temperatures (Dohm and Linthicum, 1993). It was, therefore, considered reasonable that transmission rates and the fecundity of ticks would not change under a future climatic scenario.

The future temperature predictions used in the models were predicted daily noon temperatures whereas the current temperature data consisted of observed average daily temperatures. Noon temperatures are likely to be higher than the average daily temperatures and although noon is not the hottest part of the day (Radin et al., 1994) the future predictions for moult rate and egg development rate may be slight overestimates. As such, the predicted differences between the current and future scenarios may also be slightly overestimated. The predicted noon future temperatures are based on a medium emissions scenario and it may be that temperatures over the next 50 years actually follow a high emissions scenario. Thus, the future noon temperatures used in this model may underestimate the average temperatures that could occur in 2080. With this uncertainty in mind, it was considered that these future temperature data represented a possible future scenario and were, therefore, appropriate for use in predicting future moult and egg development.

The current and future models in this chapter have predicted the number of CCHFV-infected unfed adult *Hyalomma* spp. ticks in GB arising from imported horses and migratory birds between 1st April and 31st July each year. Due to the greater number of infected unfed adult ticks arising from imported horses, it is concluded that horses are a more important factor in the spread of CCHFV into GB than migratory birds. Indeed, the small number of infected ticks imported in GB by migratory birds supports the finding of Gale et al. (2012) that that the risk of incursion of CCHFV in livestock through immature ticks carried on migratory birds is very low. Different climate change scenarios may produce different results and a more comprehensive future land cover prediction is needed to increase the accuracy of the predictions for future risk assessment.

7 Conclusions and Discussion

7.1 Introduction

The aim of this thesis was to understand the risks and factors associated with the introduction of CCHFV into GB. The CCHF virus can enter GB in infected *Hyalomma* spp. ticks and the probability that these ticks can subsequently infect a human is dependent on their capacity to survive in GB. The number of CCHFV-infected unfed adult *Hyalomma* spp. ticks present in GB is, thus, related to the CCHFV risk to humans. These ticks can bite and infect a human with CCHFV, given human exposure to the tick. The two routes of entry of CCHFV-infected ticks into GB under investigation were migratory birds and horses. Migratory birds have previously been considered as a factor in the spread of CCHFV (Leblebicioglu, 2010, Maltezou and Papa, 2010, Bosch et al., 2012, Gale et al., 2012) and indeed the risks to livestock in Europe have been assessed broadly for four migrant bird species (Gale et al., 2012). The role of migrant birds and, for the first time, the role of horses in the spread of CCHFV into GB were assessed. The research presented here predicts, quantitatively, the number of CCHFV-infected ticks entering GB on birds and horses. The habitat suitability within GB for imported *Hyalomma* spp. ticks was assessed based on current temperature and land cover data. Establishment of *Hyalomma* spp. ticks (and of the CCHF virus) in GB was not addressed here, although the probability of completion of part of the life cycle of *H. marginatum* ticks within GB was predicted under current habitat conditions and under a future habitat scenario for the late 2070s. Quantitative models were fitted to predict the number of CCHFV-infected ticks that could survive and, thus, potentially transmit the virus to humans following importation on migratory birds and horses for both current and future habitat scenarios. The main conclusions drawn from these models are now presented together in this chapter. The construction and fitting of quantitative models requires assumptions to be made. The key assumptions made in this thesis are now outlined with discussion of their impact on predictions and an indication of future research that could increase the appropriateness or reduce dependence on some of these assumptions. The findings presented in this thesis provide the basis for future research which could inform a full assessment of the risk to humans from CCHFV-infected ticks entering GB on migratory birds and horses.

7.2 Key findings

7.2.1 Introduction of CCHFV-infected ticks into GB by migratory birds

Fieldwork was conducted in Spain in the spring of 2011 and 2012, where birds were trapped in mist nets and checked for ticks (Chapter 3). It was found that the distribution of *Hyalomma* spp. ticks on birds was highly skewed, with most birds carrying no ticks and a few birds carrying several ticks. This skewed distribution of ectoparasites on hosts has previously been found in other studies (Shaw and Dobson, 1995, Jameson et al., 2012a). The fieldwork carried out in 2011 along the East Atlantic flyway found a *Hyalomma* spp. infestation rate of 0.5% for migratory birds which contrasted with the *Hyalomma* spp. infestation rate of 2.55% found in Molin et al. (2011) along the Central and Eastern Mediterranean flyway (Foley-Fisher et al., 2012). Initially, therefore, it seemed that more ticks were being carried on migratory birds into Europe along the Central and Eastern Mediterranean flyways than along the East Atlantic flyway (Foley-Fisher et al., 2012). The fieldwork carried out in 2012 along the East Atlantic flyway showed this not to be the case, as the *Hyalomma* spp. infestation rate for 2012 was found to 2.19%. This did, however, serve to highlight between-year variation and, thus, the importance of long-term sampling.

The *Hyalomma* spp. infestation rate varied between the bird species studied. The mean number of *Hyalomma* spp. ticks per bird was greatest for *A. schoenobaenus* (*n*=0.3 ticks per bird) with just 0.0018 *Hyalomma* spp. ticks per bird on *P. trochilus*. This meant that, despite *P. trochilus* having the largest British breeding population of the five birds studied (*n*=4,000,000 individuals), the species was predicted to carry very few CCHFV-infected *Hyalomma* spp. ticks into GB each year (mean=0.8 ticks). A combination of a large population of British breeding birds and a relatively high infestation rate meant that *S. communis* was predicted to import the most CCHFV-infected *Hyalomma* spp. ticks into GB each year (mean=33.9 ticks) out of the five bird species studied.

The *Hyalomma* spp. ticks found on birds during fieldwork in southern Spain in 2011 and 2012 tested negative for CCHFV. CCHFV was isolated recently from

H. lusitanicum in Spain (Estrada-Peña et al., 2012b). In conclusion, it was considered likely that species of *Hyalomma* ticks in Spain other than *H. lusitanicum* are infected with CCHFV despite the negative findings of this study. The similarity between the strain of CCHFV found in Spain to that found in Mauritania and Senegal suggests that the virus may have been brought in by a migratory bird carrying an infected tick (Estrada-Peña et al., 2012b) and, thus, species of tick that parasitize birds such as *H. marginatum* may carry CCHFV in Spain. The CCHF virus has recently been found in *H. marginatum* on migratory birds in Morocco (Palomar et al., 2012), including on *I. opaca*. The study carried out in Spain in Chapter 3 also found *H. marginatum* ticks on *I. opaca*. This confirms the circulation of the virus in North West Africa, where many migrants which breed in GB stop-over to feed prior to making the crossing to Europe. CCHFV is, therefore, circulating in regions along the East Atlantic flyway (Morocco and Spain) where the vast majority of sub-Saharan migrants that breed in GB pass through and/or stop-over. It is, therefore, recommended that studies testing ticks in Spain for CCHFV should continue to inform the risk to humans of CCHFV, both in Spain and GB.

In Chapter 3, the importation of CCHFV-infected ticks into GB on migratory birds was addressed. The mean number of CCHFV-infected *Hyalomma* spp. nymphs imported into GB between 1st April and 31st July each year on the five migratory birds studied was predicted to be 72.9 ticks. The mean number of *Hyalomma* spp. ticks imported into GB on migratory birds for the same time period was predicted to be 506,223 ticks.

7.2.2 Introduction of CCHFV-infected ticks into GB by imported horses

Fieldwork was carried out in southern Spain in 2012 to sample horses for ticks (see Chapter 4). The number of *Hyalomma* spp. ticks found on horses was highly skewed as seen for birds in Chapter 3 and was described by a negative binomial distribution, with a mean of 1.89 *Hyalomma* spp. ticks per horse. In Chapter 4, the importation of CCHFV-infected ticks into GB on horses was addressed. The mean number of CCHFV-infected *Hyalomma* spp. adult ticks imported into GB between 1st April and 31st July each year on horses was predicted to be 89.8 ticks. This was based on the mean number of horses entering GB from CCHFV-endemic countries between 1st April and 31st July each year (*n*=592 horses). The uncertainty around this number due to lack of data was discussed in Chapter 4 and it was concluded that the mean of 592 horses represented a sample of the total number of horses imported into GB from CCHFV-endemic countries in the defined time period. It was, therefore, concluded that the prediction of 89.9 CCHFV-infected *Hyalomma* spp. adult ticks entering GB on horses was probably an underestimate.

7.2.3 Spatial analysis of the habitat suitability for *Hyalomma marginatum* in GB under current climatic conditions

As discussed above, the number of CCHFV-infected *Hyalomma* spp. ticks imported into GB on migratory birds and horses was predicted in Chapter 3 and 4, respectively. In Chapter 5, the survival potential of these imported ticks was modelled by predicting the current habitat suitability for nymph-to-adult moult and egg production of *H. marginatum* in each 1 km^2 cell of GB. Thus, the models in Chapter 5 predicted the locations within GB where it may be possible for *H. marginatum* ticks to survive following importation on migratory birds and horses. The survival of nymphs imported on migratory birds was dependent on the probability of nymph-to-adult moult following detachment. The survival of adult ticks imported on horses was evaluated by the probability of production of fully incubated eggs. By modelling the habitat suitability for moult and egg development using temperature and land cover data, it was predicted that the probability of moult and egg development (given that moult/pre-oviposition started on 1st April) was largest around the Severn Estuary, Kent and Sussex. These were lowland areas and, therefore, experienced warmer temperatures than areas of higher altitude allowing moult and egg development to complete at a faster rate (and, thus, by 31st July). The land cover type of these areas was grassland, the preferred land cover type of *H. marginatum*. Although there were extensive areas of grassland in Wales (Figure 5.2 of Chapter 5), these were in the Brecon Beacons and the Cambrian Mountains where cooler temperatures resulted in slow moult completion and egg development. Similarly, the Pennines, the Cumbrian Mountains and Exmoor had large areas of suitable land cover type, but cooler temperatures due to higher altitudes meant that moult and egg development could not complete by 31st July. Conversely, temperatures around The Wash allowed for moult and egg development to

complete by 31st July but the land cover in that area is predominantly arable farming and horticulture, making it unsuitable for *H. marginatum* survival.
In Chapter 5, it was concluded that long-term establishment of *H. marginatum* would be unlikely under the current habitat conditions of GB. However, it was predicted that in some years, survival would be possible in certain areas of GB, as highlighted above. Therefore, it may be possible for an imported CCHFV-infected tick to bite and infect a human, given human exposure to the imported tick, but endemic cycles of CCHFV in GB were not believed to be currently possible. This was due to annual fluctuations in temperature resulting in *Hyalomma* spp. tick populations being unsustainable over a number of years. Whilst some ticks may survive to form a population in one year, in subsequent years it may not be possible for reproduction and maturation to occur making population establishment impossible.

7.2.4 Current and future introduction of CCHFV-infected *Hyalomma* spp. ticks into Great Britain from migratory birds and imported horses

The number of CCHFV-infected ticks entering GB on birds and horses that could survive to bite a human was predicted in Chapter 6 under current habitat conditions and under a future habitat scenario. Under current habitat conditions, it was predicted that from migratory birds entering GB between 1st April and 31st July each year, there would be a mean of 11 CCHFV-infected unfed adult *Hyalomma* spp. ticks surviving in GB. It was predicted that *S. communis* imported the most CCHFV-infected nymphs from 1st April to 31st July (mean=33.9) and *P. phoenicurus* imported the fewest (mean=0.8). The difference in the number of ticks imported by different bird species was discussed above. The density of CCHFV-infected unfed adult ticks per 1 km^2 arising from migratory birds each year was predicted to be greatest in lowland areas of Wales, north Somerset and Kent, which corresponded to areas of highest habitat suitability for nymph-to-adult moult. The predicted density of infected ticks per square kilometre was low with the highest value being 0.00065 ticks per 1 km^2. Most areas of south England and the Midlands were predicted to have a density of CCHFV-infected adult *Hyalomma* spp. ticks greater than zero ticks per 1 km^2. Most areas of north England, Scotland and upland areas of Wales and the southwest of England were predicted to have densities of zero ticks per 1 km^2. This was due to nymphs being unable to moult to adults after importation on birds as a result of low temperatures rather than because ticks were not imported into these areas by birds.

Under the future habitat scenario for the late 2070s, the mean number of CCHFV-infected unfed adult *Hyalomma* spp. ticks present in GB from tick importations by migratory birds between 1st April and 31st July each year was predicted to be 12.9 ticks. This was an increase of 17.3% from the result of 11 ticks under current habitat conditions. CCHFV-infected adult ticks were distributed over a larger area of GB under a future habitat scenario (52.2% of GB) than under current climatic conditions (48.6% of GB). Areas of Wales, the southwest of England, northern England and Scotland had a predicted density of ticks greater than zero where previously no imported ticks were predicted to survive. This was due to a combination of higher predicted temperatures, a predicted increase in the total number of birds entering GB (of the five species studied) and an expansion in the total range of these bird species. Interestingly, the future increase predicted in the range of these bird species within GB is in contrast to the range contraction that is predicted for Europe as a whole. In GB, *A. schoenobaenus* and *P. phoenicurus* were predicted in future to decrease in range by 16.4% and 8.03%, respectively. *O. oenanthe, P. trochilus* and *S. communis* were predicted to increase in range of 29%, 0.88% and 4.39%, respectively in GB. According to Huntley et al. (2007), bird ranges in Europe are predicted to be reduced on average by a fifth in the future and there is a northward shift in the range of most species. This predicted increase in some breeding bird populations in GB may be due to these birds being ‘compressed’ into the northern limits of their habitat range within Europe. Changes in temperatures and land cover across Europe may also have an impact upon bird migration, whereby birds over-winter in more northerly areas than where they currently spend the winter. The exposure of these birds to CCHFV-infected ticks may, therefore, change although the current observed northward expansion in the range of CCHFV and *Hyalomma* spp. ticks may match the northward transition of bird ranges. Therefore, in the future, the origin of CCHFV-infected ticks entering GB may be predominantly from southern Europe rather than from Africa.

The number of CCHFV-infected unfed adults *Hyalomma* spp. ticks that could survive to bite a human between 1st April and 31st July each year as a result of tick importations on horses was predicted to be 143.3 ticks under current habitat conditions. The density of these ticks was highest around the south east of England (particularly Oxfordshire, Berkshire, Surrey and East Anglia) with northern England, Scotland and most of Wales predicted to have a density of zero ticks per 1 km^2. The areas with the highest densities of CCHFV-infected unfed adult *Hyalomma* spp. ticks coincided with the areas that had the highest densities of imported horses. From Chapter 5, it was predicted that Surrey, in particular, had relatively high habitat suitability for *H. marginatum* and, thus, ticks imported into this area on horses were predicted to have a larger probability of reproduction and development as required for a tick to bite a human.

The number of CCHFV-infected unfed adult *Hyalomma* spp. ticks that could survive to bite a human between 1st April and 31st July each year as a result of tick importations on horses was predicted to be 121.8 ticks under a future habitat scenario. This amounted to a 15% decrease when compared with the result from the current habitat scenario. The current mean number of horses imported into GB each year from 1st April to 31st July was assumed to remain the same in the future. The future land cover scenario map in Chapter 6 used predictions from the literature of increased urban and suburban areas and wetlands. These predictions were then translated into a future land cover map using published ‘visions’ for wetland expansion available online (Wetland Vision) and an algorithm for urbanisation, as described in Chapter 6. Due to an increase in predicted temperatures in the future, the probability of habitat suitability for moult and egg development increased overall for GB. However, due to a predicted reduction in the proportion of land cover suitable for *H. marginatum* (as a result of urbanisation and wetland expansion) some areas with high densities of imported horses had unsuitable land cover types for tick survival in the future. Urban sprawl around London was predicted to spread into Surrey and Berkshire where imported horse densities were relatively high. Wetland expansion around The Wash into East Anglia also encroached into areas with relatively high densities of imported horses from CCHF-endemic countries. Land cover type was not expected to change a great deal in Wales in the future and, with predicted increasing temperatures, Wales was predicted to have higher densities of CCHFV-infected unfed adult *Hyalomma* spp. ticks per 1 km^2 as a result of tick importations from horses from CCHFV-endemic areas in the future compared to under current climatic conditions. The decrease in the total predicted number of CCHFV-infected unfed adult *Hyalomma* spp. ticks in the future from 143.3 ticks under current habitat conditions to 121.8 ticks under a future habitat scenario was, therefore, thought to be as a result of a reduction of suitable land cover type in areas with high densities of horse importations.

To date, there have been no maps produced (or at least made available to the research community) that show possible future land cover scenarios for GB. The map created in Chapter 6 is considered to be one possible scenario for future land cover and represents the most consensual scenario as determined from the available literature. Thus, the predicted decrease in the proportion of suitable land cover for imported *Hyalomma* spp. ticks in areas of high horse import density is considered to be the most likely outcome as result of future land cover change. There is, of course, uncertainty in this prediction which could have been captured by creating a range of future land cover maps using different scenarios of urbanisation and wetland expansion. This was beyond the scope of this project but clearly there exists a need for the production of future land cover maps using a range of scenario-based algorithms. These would be useful for future risk assessment of CCHF and other tick-borne diseases in GB such as Lyme Disease.

As previously mentioned, birds are considered to be a factor in the spread of CCHFV (Leblebicioglu, 2010, Maltezou and Papa, 2010, Bosch et al., 2012, Gale et al., 2012). However, in Chapters 3 and 4, it was predicted that a slightly larger number of CCHFV-infected *Hyalomma* spp. ticks would be imported into GB on horses between 1st April and 31st July each year (mean=89.9) compared to the number imported for the same period by the five migratory bird species studied (mean=72.9). This, therefore, places horses alongside migratory birds as an important route for the importation of CCHFV-infected ticks and, thus, horses may be a previously unrecognised factor in the spread of CCHFV into, and across, Europe. In addition to this it was proposed, from the results of Chapters 3 and 4, that horse movements have a more significant role in the spread of CCHFV into GB (and possibly other European countries) than migratory birds. The predicted mean numbers of CCHFV-infected unfed adult *Hyalomma* spp. ticks present in GB that could bite a human as a result of importations on the five chosen migratory bird species and horses (for the period 1st April to 31st July reach year), both now and in the future, are presented in Table 7.1.

Table 7.1: The predicted mean number of CCHFV-infected unfed adult *Hyalomma* spp. ticks in GB from importations by the five chosen migratory bird species and horses under current and future habitat conditions between 1st April and 31st July each year.

Route of importation	Current mean no. of birds/horses entering GB each year	Future mean no. of birds/horses entering GB each year	Current no. of ticks	Future no. of ticks
Migratory birds	7,198,000	7,358,206	11.0	12.9
Horses	592	592	143.3	121.8

The predicted number of CCHFV-infected adult *Hyalomma* spp. ticks in GB arising from horse importations was 13 times larger than that from migratory birds under current habitat conditions, and nine times larger under a future habitat scenario. Although only five bird species were included in this study, these were considered to be the most likely species to carry *Hyalomma* spp. ticks into GB (as described in Chapter 3). However, other species may also carry CCHFV-infected ticks into GB and, therefore, the number of CCHFV-infected unfed adult *Hyalomma* spp. in GB from migratory birds could be larger than that predicted in Chapter 6. However, as previously discussed in Chapter 4, the number of CCHFV-infected unfed adult *Hyalomma* spp. ticks in GB from horse importations could also be much larger due to the large number of unrecorded horse imports into GB for which there are no data. Thus, it was considered that the predictions for the two pathways as given in Table 7.1 could be compared. The greater number of CCHFV-infected unfed adult ticks from the horse pathway was a result of the large number of eggs predicted to be laid by imported adult female ticks after entry into GB, as discussed in Chapter 6. Nymphs imported on migratory birds that subsequently moulted to adults were considered to be very unlikely to find a mate in GB and, hence, give rise to eggs. The infestation rate of *Hyalomma* spp. ticks on horses was found to be larger than that of the migratory birds studied (a mean of 1.89 ticks per horse compared to 0.0018 ticks per bird for *P. trochilus* to 0.3 ticks per birds for *A. schoenobaenus*). Despite considerably fewer horses being imported each year between 1st April and 31st July compared to migratory birds under both current and future habitat scenarios (Table 7.1), the greater infestation rate of *Hyalomma* spp. ticks on horses combined with the ability of adult female ticks to reproduce upon entry into GB led to the prediction of a greater number of CCHFV-infected unfed adult ticks in GB from imported horses than from migratory birds.

The level of exposure of humans to imported ticks and/or their progeny may vary depending on the route of introduction of the ticks. Ticks imported on the five migratory birds studied in this thesis are likely to be deposited in the areas and habitats of GB that are preferred by their host bird species. These include rural areas of Somerset and Sussex, and in the future, also Wales as predicted in Chapter 6. By contrast, ticks from horses are likely to be deposited in areas around Newmarket in Suffolk, Surrey, Oxfordshire and Berkshire (as predicted in Chapter 6) and, thus, in closer proximity to humans. Domestic horses are closely associated with humans and human dwellings, whilst the wild birds do not have this close association and the species considered here are more likely to frequent areas of undisturbed habitat away from human populations. The majority of common garden birds are not migratory or only partial migrants (only migrate over short distances and unlikely to cross international boundaries), with the exception of some species such as *H. rustica* (barn swallow). Thus, ticks detaching from horses after import into GB and, subsequently, reproducing are likely to do so in close proximity to humans and human dwellings. Humans could then act as an available host for the unfed adult progeny. Nymphs imported on migratory birds that subsequently moult to adults may be more likely to then feed on wild herbivores such as deer or rural livestock such as cattle. Therefore, the exposure of humans to CCHFV-infected ticks and their progeny from horse importations may be greater than the exposure of humans to CCHFV-imported ticks from migratory birds. A complete risk assessment incorporating exposure of humans to imported ticks would be needed to assess fully the risk to humans from CCHFV-infected ticks imported on horses and migratory birds. However, the hypothetically greater exposure of humans to ticks imported on horses compared to birds could support the important conclusion of Chapter 6 that horses are a factor in the spread of CCHFV, with a potentially greater role in disease spread than migratory birds.

7.3 Discussion of assumptions used in this thesis

7.3.1 CCHFV and the *Hyalomma* spp. tick vector

For this study, it was assumed that CCHFV would occur only in areas where *Hyalomma* spp. ticks were present. This is supported by numerous studies that show CCHFV is present within the range of *Hyalomma* spp. ticks. Within Europe, human cases of the virus, isolation of the virus from ticks and positive serological evidence from livestock have occurred only within the range of the tick, *H. marginatum* (see Chapter 1). There is, however, one known exception in Madagascar. Here, the virus was isolated from *Rhipicephalus* (*Boophilus*) *microplus* Canestrini collected from cattle at a slaughterhouse in Antananarivo, the capital of Madagascar, in 1985 (Mathiot et al., 1988). Subsequently, serological surveys of humans showed low seroprevalences of 0.5% (Mathiot et al., 1989) and 0.8% (Andriamandimby et al., 2011). There are no species of *Hyalomma* present in Madagascar and, thus, it is believed that the CCHF virus circulates in *R.* (*Boophilus*) *microplus* and their main host, cattle (Mathiot et al., 1989). This tick is very rarely found on humans, and, therefore, the human seroprevalence observed in Madagascar may represent ‘accidental’ parasitization by *R.* (*Boophilus*) *microplus* (Mathiot et al., 1989) or occasional infection from the blood of virus-positive cattle. The majority of humans tested in these studies were considered to be from ‘at-risk’ groups (slaughterhouse workers and people in cattle breeding areas) and, therefore, the seroprevalence for the general population is likely to be much lower. The occurrence of CCHFV in Madagascar is believed to be the result of cattle importation from Asia (as the Madagascar virus strain is closely related to strains from Pakistan and the United Arab Emirates) (Burt and Swanepoel, 2005).

Human cases of CCHF have not been reported from Madagascar and this is likely to be because the virus circulates at very low levels in ticks that do not commonly parasitize humans. However, a virus introduction could occur in a country with the virus subsequently circulating in a vector species that does parasitize humans as has happened in areas where *Hyalomma* spp. ticks are endemic. As demonstrated in Madagascar, irrespective of the mode of virus introduction into a new area, there exists the possibility of local ticks to act as efficient reservoirs and vectors. When considering GB, *I. ricinus* is a candidate species that could act as a vector, but in this thesis it was assumed that this was not possible (as discussed below). Within Greece, the tick *Rhipicephalus bursa* is the vector for a non-virulent strain of CCHFV, AP-92 (Papa et al., 2011b) and, thus, the future establishment of this tick in GB could also be considered. An assessment of the tick species present in Senegal that act as vectors for CCHFV has been carried out and their respective roles in virus transmission have been assessed and weighted against each other (Camicas et al., 1990). A similar approach could be carried out for GB, whereby the endemic tick fauna are analysed by their ecological characteristics to determine which species are the most likely candidates for CCHFV transmission.

7.3.2 *Ixodes ricinus* as a vector for CCHFV

The importance of *Hyalomma* spp. ticks in the maintenance and spread of CCHFV has long been acknowledged (Hoogstraal, 1979, Ergönül, 2006) but the role of other tick species, such as the GB tick, *I. ricinus*, is poorly understood. An assumption in this thesis was that a human case of CCHFV in GB could arise only from a CCHFV-infected *Hyalomma* spp. tick and, thus, the survival of imported *Hyalomma* spp. ticks was considered. It was been assumed that *I. ricinus* is not a competent vector of CCHFV. CCHFV has been isolated from *I. ricinus* but this does not indicate vector competence. However, there is some evidence to suggest that *I. ricinus* may at least serve as a reservoir for CCHFV. A study in Turkey found that 12.06% of *I. ricinus* collected from mammals were positive for CCHFV whilst only 8.49% of *H. marginatum* were positive for CCHFV (Albayrak et al., 2010a). CCHFV was also isolated from *I. ricinus* in Moldavia and the Crimea in the 1970s (Hoogstraal, 1979). In Bulgaria, a CCHFV-endemic country, it is thought that *I. ricinus* plays a role in enzootic transmission cycles of CCHFV in some areas (Avsic-Zupanc, 2007). However, *I. ricinus* is a common tick throughout most of Europe and, yet, it does not appear to be a significant player in the maintenance or spread of CCHFV. Indeed, if any, its role may be only of local significance (Ergönül and Whitehouse, 2007). It is very common in many countries, such as GB and in Scandanavia, where CCHFV does not occur. This is important as according to a report by Hoek et al. (2012), ‘*Hyalomma* ticks, including CCHF virus, are probably introduced into Western Europe by migrating birds every year, but the tick species and virus have apparently not persisted. If local tick species are competent vectors, it is not understood why establishment of CCHFV in Western Europe hasn’t occurred yet’. (The introduction of CCHFV through ticks on migrating birds and horses is supported by modelling studies for GB undertaken here). This supports the assumption that *I. ricinus* is not a competent vector of CCHFV, and if it were capable of playing a role in the endemicity of CCHFV in GB, it would likely have done so by now. A vector competency study of *I. ricinus* for CCHFV has recently commenced at Public Health England (PHE) and this will help to determine whether *I. ricinus* is a competent vector of CCHFV (Leech, S., personal communication). Of course, future adaptation of CCHFV to new tick vectors cannot be ruled out and a strain of CCHFV transmitted by *Rhipicephalus sanguineus* in Iran showed greater genetic variation than those in *Hyalomma* spp. ticks (Tahmasebi et al., 2010). Apart from *I. ricinus*, two other species of tick are endemic to GB that could act as vectors for CCHFV. *Rhipicephalus sanguineus* Latreille most commonly parasitizes dogs in GB (Martyn, 1988) and CCHFV has been isolated from this species in Bulgaria, Turkmenistan, the Crimea (Hoogstraal, 1979) and Iran (Tahmasebi et al., 2010). *Haemaphysalis punctata* Canestrini and Fanzago is found in warmer regions of GB, where it exists at the northern-most part of its range (Martyn, 1988). CCHFV has been isolated from *H. punctata* in Moldavia and the Crimea (Hoogstraal, 1979). *I. ricinus* is much more common in GB than either of these species and parasitizes a much wider range of species (Martyn, 1988). For this reason, it is the British species of greatest concern for CCHFV incursion into GB. The results of the vector competency study at PHE will shed more light on the relationship between *I. ricinus* and CCHFV. H. Albayrak (personal communication) believes that *I. ricinus* is a ‘mechanical vector’ of CCHFV having detected both CCHFV RNA and antigens from *I. ricinus*. A. Papa (personal communication) says that although no human cases have been associated with *I. ricinus*, we should ‘expect the unexpected – we cannot exclude any option’. If *I. ricinus* could be a natural reservoir for the CCHF virus, it would be important to consider the possibility of viraemic horses and, possibly birds, entering GB and being bitten by an *I. ricinus* tick such that the virus enters the GB population of *I. ricinus*. A separate quantitative assessment of this route of introduction of CCHFV into GB could, therefore, be undertaken, but in this project it was assumed that there was no risk from viraemic animals entering GB and this assumption is now discussed.

7.3.3 CCHFV-infected migratory birds and horses entering GB

It was assumed that the risk of introduction of CCHFV to GB from migratory birds and imported horses is from the ticks that they may be carrying. The direct risk from a CCHFV-infected bird or horse was not considered. The most likely method of onward transmission of the virus after entry into GB, would be parasitization of the bird or horse by a British tick species. *I. ricinus* parasitizes both horses and migratory birds, having been found on all the bird species considered in this risk assessment (Martyn, 1988). However, the onward transmission of CCHFV by *I. ricinus* is considered to be unlikely, as discussed above.

Generally, birds are considered to be refractory to CCHFV infection (Hoogstraal, 1979) although this does not necessarily mean that the virus cannot be transmitted (Estrada-Peña et al., 2012a). There are some species of bird that do have detectable viraemia and are able to transmit the virus to ticks. Zeller et al. (1994b) were able to elicit a significant antibody response from *Tockus erythrorhynchus* Temminck (red-beaked hornbills) and *Lamprotornis caudatus* Statius Muller (glossy starlings). Despite no detectable viraemia, these birds were able to transmit the virus to *H. rufipes* ticks that were feeding on them. Chickens, however, had no antibody response and were unable to transmit the virus to ticks (Zeller et al., 1994b). Therefore, it may be incorrect to generalise across all bird species about their ability to amplify and transmit the CCHF virus. Studies on the East Atlantic flyway, or the Black Sea flyway, testing a range of migratory birds for CCHFV antibodies would help to determine which bird species, if any, are involved in virus circulation and, potentially, transmission.

Horses have been found to develop low levels of viraemia when experimentally infected with CCHFV (Nalca and Whitehouse, 2007). Antibodies to CCHFV have been detected in horses in Iraq (Nalca and Whitehouse, 2007), Bulgaria (Avsic-Zupanc, 2007) and Russia (Hoogstraal, 1979). As it is considered that at present there are no competent tick vectors for CCHFV present in GB, the consequences of CCHFV-infected birds and horses entering GB have not been addressed here. A study to test for CCHFV antibodies on horses imported into GB (particularly those imported from CCHFV-endemic countries) would help to quantify the risk from this specific pathway, should it be that *I. ricinus* is a competent vector, or that *H. marginatum* establishes in GB in the future.

7.3.4 Winter temperatures and the availability of hosts for *Hyalomma* spp. ticks in GB

In Chapter 5, the survival of CCHFV-infected *Hyalomma* spp. ticks in GB was considered, under current and future climatic and land cover scenarios. Adult *Hyalomma* spp. ticks imported into GB on horses were predicted to lay eggs that hatched to larvae. These larvae were assumed to be able to survive the winter in GB and find a host the following spring. There are records of *H. marginatum* ticks in Romania and Bulgaria (Estrada-Peña, A., personal communication), where average daily temperatures in winter can be as low as those experienced in GB. According to the Met Office, daily average temperatures in Bucharest in January are around 1°C (Met Office). In Astrakhan where *H. marginatum* ticks are endemic, temperatures in winter ‘rarely drop to -20 °C’ (Hoogstraal, 1979) and there were far fewer *H. marginatum* ticks per cow after a severe winter when temperatures of -20°C to -30°C continued for two months (Hoogstraal, 1979). These temperatures are far lower than those experienced in GB during the winter and yet Astrakhan has established populations of *H. marginatum*. It is, therefore, considered that winter temperatures in GB are not a limiting factor for the survival of imported *H. marginatum* ticks and the assumption made in this risk assessment is justified. The assumption that hosts are available for the *Hyalomma* spp. larvae, following emergence for winter diapause is also believed to be justified. Hoek et al. (2012) state that ‘there is no shortage of suitable hosts for *Hyalomma* spp. ticks in (Western) Europe. Any CCHFV *Hyalomma* tick introduced is, therefore, likely to find and bite a suitable host’. Many *Hyalomma* spp. ticks are able to parasitize a wide range of hosts, with immatures parasitizing hedgehogs, rodents, rabbits, hares and birds (Kolonin, 2009). In particular, *H. marginatum* utilises a wide range of hosts (Apanaskevich and Horak, 2008), many of which are present in GB.

In the models in this thesis, it was considered that the inclusion of some host species, such as hares (which are considered to be important hosts of *H. marginatum* and are amplifying hosts of CCHFV (Estrada-Peña et al., 2012a)) and the exclusion of other species, such as rabbits (whose role in CCHFV epidemiology is unknown) may lead to some areas of GB being falsely predicted to have low densities of infected *Hyalomma* spp. ticks, due to the absence of the ‘included’ host species. Therefore, the assumption was made that in areas where habitat was suitable for *Hyalomma* spp. ticks, there would also be available hosts for the ticks to feed on. This approach was believed to be more realistic than including a few, important host species such as hares, considering the fact that *H. marginatum* are able to utilise a wide range of hosts.

7.3.5 *Hyalomma* spp. larvae and nymphs feeding on humans

Immature *Hyalomma* spp. ticks, particularly *H. marginatum*, usually feed on small mammals or birds, whilst the adults feed on larger hosts, such as livestock and humans (Hillyard, 1996, Apanaskevich and Horak, 2008). Adult *Hyalomma* spp. ticks have been found on humans in Turkey (Gargili et al., 2010, Gunes et al., 2011) and in Russia (Kotti et al., 2001). A study in Greece removed 39 *Hyalomma* spp. ticks from humans, all of which were adults (Papa et al., 2011a). It was assumed that CCHFV could be transmitted to humans from the bite of an adult *Hyalomma* spp. tick. A case of CCHF in humans from the bird pathway was considered to arise from an imported CCHFV-infected nymph having moulted to an adult and subsequently biting a human. For the horse pathway, it was assumed that imported CCHFV-infected adult *Hyalomma* spp. would need to reproduce and the progeny mature to adults before direct transmission of the virus to a human could occur by tick bite.

A study in Anatolia, Turkey, collected 2,957 *Hyalomma* spp. ticks from humans, of which 1,512 were nymphs and 12 were larvae (Karaer et al., 2011). Of the nymphs collected, 20 were kept alive and they moulted to *Hyalomma aegyptium*. On this basis, and by morphological identification, all other nymphs and larvae were believed to be *H. aegyptium*. The high nymph biting rate was observed in cities and was, therefore, attributed to opportunistic biting of an abundant source of hosts (humans) rather than host preference (Karaer et al., 2011). Similarly, large numbers of *H. aegyptium* nymphs were found on humans in Thrace Province, Turkey (Gargili et al., 2010).

The range of *Hyalomma* spp. ticks imported on horses into GB may be quite broad as there at least 12 species of *Hyalomma* that parasitize horses and are vectors of CCHFV (see Table 4.2, Chapter 4). For those species that are also known to bite humans (*H. anatolicum, H. detritum, H.excavatum*, *H. marginatum* and *H. rufipes*), only adults have been found on humans (Gargili et al., 2010, Gunes et al., 2011, Karaer et al., 2011, Papa et al., 2011a). As with *H. aegyptium*, it may be that some species of *Hyalomma* imported on horses could bite humans if the preferred host is not available. However, as previously mentioned, for the models in this thesis, suitable hosts for immature *Hyalomma* spp. ticks were justifiably assumed to be available. It can, therefore, be safely assumed that imported *Hyalomma* spp. ticks on horses would need to produce adult progeny for direct virus transmission to humans.

7.4 Future research

In Chapter 5, it was predicted that some areas of GB had a higher probability of habitat suitability for the survival of *H. marginatum* ticks. These areas were, therefore, considered to be the most likely areas in GB where imported CCHFV-infected *Hyalomma* spp. ticks could survive and, thus, where human cases of CCHF may arise. This geographical output could be used to direct future tick surveillance within GB. It allows for targeted, cost-effective surveys in areas that are predicted to have the highest density of imported *Hyalomma* spp. ticks. Therefore, research that builds on this PhD could involve tick surveillance on livestock farms in Kent, Suffolk and northern Somerset. In the future, tick surveys could extend into lowland areas of Wales, an area that was predicted to show a relatively large increase in the climatic suitability for ticks in the future.

There are several areas of study that are currently lacking in available data which could increase the predictive power of models in this thesis and future risk assessments. Although there is much discussion and speculation on the changing landscape of GB in the future, definitive maps for different climatic and economic scenarios have not yet been produced. Whilst a future land cover scenario has been developed in this thesis, there is still a need for future land cover predictions for GB based on a more rigorous approach than that possible within the scope of this project. The number of horses imported into GB each year is another area where data are limited. Without data on the total number of annual imports into GB and from which countries, quantification of this risk pathway will continue to represent a 'snapshot' of the complete picture. Due to EU legislation (as discussed in Chapter 4) and the tripartite agreement between the UK, the Republic of Ireland and France, the full data on horse movements across country borders do not currently exist. To obtain them would be time consuming and possibly require legislative changes, but as emerging infectious diseases increasingly pose a threat to GB, the economic importance of fully understanding the risk from all possible pathways also increases.

By the 2070s, it is possible that populations of *H. marginatum* may have become established in GB. The risk pathways would, therefore, widen to incorporate the risk from CCHFV-infected migrant birds and imported horses, which could be viraemic and could, therefore, infect endemic populations of *H. marginatum*. Horses experimentally infected with CCHFV have developed little or no viraemia, with donkeys developing low levels of viraemia (Nalca and Whitehouse, 2007). Horses do develop high levels of antibodies and indeed antibodies to CCHFV have been found in horses in Bulgaria, Iraq and Russia (Hoogstraal, 1979, Nalca and Whitehouse, 2007), but it is not considered likely that viraemic horses are entering GB.

Some bird species have been shown to develop viraemia when experimentally infected with CCHFV including *Numidia meleagris* (blue-helmeted guinea fowl) (Shepherd et al., 1987). Zeller et al. (1994b) inoculated *Tockus erythrorhynchus* (red-beaked hornbills) and *Lamprotornis spp.* (glossy starlings) with CCHFV and allowed immature *H. rufipes* to feed on them. The virus was detectable in the ticks following drop off and the birds developed an antibody response. In South Africa in 1984, there was a case of CCHF in a worker from an ostrich abattoir. When a serological survey of ostriches was conducted in the area, it was found that 23.9% of ostriches were positive for antibodies to CCHFV (Shepherd et al., 1987) and, thus, it was believed that the worker contracted the virus after contact with the blood of a viraemic ostrich (Burt et al., 2007). *Corvus frugilegus* Linnaeus (rooks), *Columba livia* Gmelin (rock doves) and domestic chickens do not develop viraemia when infected with CCHFV (Hoogstraal, 1979, Shepherd et al., 1987). Studies to determine whether GB migratory birds develop viraemia when infected with CCHFV have not been carried out and, therefore, the possibility of viraemic migrants entering GB cannot be ruled out. CCHFV infection in migratory birds could be investigated further (such as by taking blood samples from migratory birds) as the current literature suggests that although birds are generally considered to be refractory to CCHFV infection, some species do develop viraemia and, thus, may have a role in virus transmission.

Randolph and Ergönül (2008) argued against birds introducing CCHFV into Turkey as a cause of the current outbreak that began in 2002. They suggest that CCHFV was previously circulating in enzootic transmission cycles in Turkey and that changes in a combination of environmental and socio-political factors may have caused the virus to become zoonotic. This occurred in the Crimea in 1945 during World War II where hares (which develop viraemia when infected with CCHFV and are hosts are *H. marginatum*) became abundant due to a disruption in agricultural practices and abandonment of hare shooting. When humans returned to these areas, there was an outbreak of CCHF. The causative factors of CCHF outbreaks have been postulated for Kosovo and Turkey. In Kosovo, the rise in cases of CCHF was believed to be attributable in part to a decline in agriculture, expansion of grassland for grazing, imbalanced predator-prey cycles and the introduction of naïve cattle breeds (Jameson et al., 2012b). Terrorist activity in the Anatolian region of Turkey led to fields being abandoned which were previously hunting and pasturing areas in 1995 to 2001, resulting in an increase in hosts for *H. marginatum*. Therefore, it was suggested by Randolph and Ergönül (2008) that these factors may have (at least partly) caused the outbreak of CCHF in 2002 when humans returned to these areas. According to Estrada-Peña et al. (2010), case incidence of CCHF in Turkey was positively correlated with highly fragmented habitats within the range of the tick vector.

The status of CCHFV in south-west Europe is considered to be of great importance for northern Europe. Although the origin of CCHFV in Spain is not clear and it may have been circulating for some time prior to being found, the similarity of the Spanish strain to that of Mauritania and Senegal suggests ticks on migratory birds are a medium of virus dispersion (Estrada-Peña et al., 2012b). As the climate in northern Europe becomes increasingly favourable for *Hyalomma* spp. ticks, as predicted in this thesis, the continued northward spread of CCHFV is anticipated. CCHFV may be endemic in Spain in populations of *Hyalomma* spp. ticks that parasitize migratory birds, such as *H. marginatum* and in this thesis it was assumed that this is the case. It seems highly likely that this is true as CCHFV was found in *H. lusitanicum* on horses (Estrada-Peña et al., 2012b), which are also parasitized by *H. marginatum* (see Chapter 4). The prevalence of CCHFV may be low and/or localised in *H. marginatum* populations in Spain and hence, current surveillance has so far not isolated CCHFV from this species. Low levels of circulating virus may also explain the lack of human cases of CCHF in Spain. As suggested by Randolph and Ergönül (2008), a combination of factors may converge such that a human outbreak arises, but for now this has not occurred. Randolph and Ergönül (2008) also refer to endemic areas with human cases of CCHF as the ‘tip of the zoonotic iceberg’ with enzootic cycles being far more widespread than is apparent from human cases. If this is the case, the extent of the ‘submerged bulk’ of CCHFV-infection within Europe is of great importance to GB when determining risk factors associated with CCHFV introduction, such that CCHFV infection may be geographically much closer to GB than is apparent.

There is historical serological evidence of CCHFV in France and Portugal. In France, CCHFV antibodies were detected in bats close to the border with Spain (Hoogstraal, 1979). In Portugal, antibodies to CCHFV were found in six humans in southern Portugal (Filipe et al., 1985). There are established populations of *H. marginatum* in southern France and in Portugal (Kolonin, 2009) and, thus, surveillance is required to see if CCHFV is endemic in these areas. CCHFV-infected ticks could be imported on both horses and birds from these areas, and the models presented in this thesis do not include Portugal and France as CCHFV-endemic countries. Even if CCHFV is only circulating in low levels in localised foci in Portugal and France, the geographical proximity of the virus to GB is of interest when assessing the risk of introduction.

The current and future situation of CCHFV in these areas is, therefore, of great importance for the risk of CCHFV introduction into GB, and other north European countries. Further research into the extent of CCHFV in Spain, as well as surveillance in Portugal and France is needed to better understand the risk to GB of CCHFV. Monitoring of the situation in countries like Spain where CCHFV has recently been found is needed to anticipate and prepare for an outbreak of human cases, like that seen in Turkey. It is likely that in countries such as Italy and Portugal where *H. marginatum* is endemic, CCHFV is present in enzootic cycles as in Spain. Until *H. marginatum* becomes endemic in GB, it is likely that any human outbreaks of CCHF in GB will be sporadic, localised and short-lived. If in the future, populations of *Hyalomma* spp. ticks do become established in GB (which is considered to be likely given the predicted increase in habitat suitability in some areas), CCHFV may become enzootic. An outbreak on the scale of that seen in Turkey is unlikely to occur in GB if the socio-economic situation of GB remains reasonably stable. With the current economic recession affecting some parts of southern Europe at present, unanticipated localised outbreaks cannot be ruled out.

8 References

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