Revised Disease Risk Analysis for the Conservation Translocation of the Eurasian Beaver (*Castor fiber*) to England

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Foreword

This report was commissioned to inform Natural England's approach to the reintroduction of beavers in England. This Disease Risk Analysis updates a previous version published in 2020 (Donald, Common, and Sainsbury 2021) by including a new translocation pathway in which beavers held in fenced enclosures and other captive collections in Great Britain might be released in England and by assessing an additional identified 28 hazards.

Legal protection of beavers came into force on 1 October 2022 in England and brought this species one step closer to fulfil one of the ambitions of the 25-year Environment Plan to restore lost species to England. Although species reintroductions are a key conservation tool used to help restore species populations and/or ecosystem function, species translocations can facilitate the movement of parasites and risk animals encountering parasites that they normally would not be exposed to. Individual translocated specimens are a 'biological package', consisting of the host and all the associated viruses, bacteria, fungi and other parasites that the animal or plant may naturally harbour. It is this biological package that needs to be translocated as intact as possible. Translocations increase the risks the biological package. Reintroduced beavers may also act as a mechanism for the introduction of new or previously eradicated parasites or may establish new transmission routes for the infection of humans, domesticated livestock and existing wildlife.

Disease risk analysis is a qualitative risk assessment method ideally undertaken in the planning stage of a conservation intervention such as a reintroduction. During a disease risk analysis, the risk that infectious and non-infectious hazards will precipitate during or following an intervention is analysed in the absence of mitigating measures. Mitigation measures are then proposed, which in many cases will reduce the risk to an acceptable level. However, it is important to regularly review and monitor the risks of introducing new disease and pathogens when translocating beavers. Preventing the introduction of alien parasites to native populations is crucial because parasite invasions have the potential to cause catastrophic mortality outbreaks in potentially immunologically naïve populations. Disease risk analysis and the evaluation and implementation of mitigation measures is, therefore, a key step in understanding and controlling any disease risks for humans, livestock and wildlife that may arise from a reintroduction or translocation of wildlife.

Natural England commission a range of reports from external contractors to provide evidence and advice to assist us in delivering our duties. The views in this report are those of the authors and do not necessarily represent those of Natural England.

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Executive summary

In a disease risk analysis (DRA) on the conservation translocation of free-living beavers from either Norway or Great Britain, or those housed in either fenced enclosures or zoological collections in Great Britain, to England, 96 hazards (89 infectious and seven non-infectious) were evaluated and 26 received detailed analysis. This DRA was an expanded and updated version of one published in 2020 (Donald, Common, and Sainsbury 2021) and analysed an additional 28 hazards, of which four required detailed evaluation. The revision included a new translocation pathway in which beavers held in fenced enclosures, and other captive collections, in Great Britain might be released, in addition to the two pathways previously investigated. This included beavers held in naturalistic, fenced enclosures as well as those held in private and zoological collections. Contact between these beavers and with exotic mammalian species was considered in the risk analysis.

Of the 26 hazards assessed in detail, 17 were of high or medium risk of precipitating disease in beavers or sympatric mammals, including people: *Echinococcus multilocularis*; *Leptospira* spp.; *Yersinia* spp.; *Toxoplasma gondii* (as both a carrier and population hazard); *Taenia* spp.; persecution; captivity; road traffic collisions; *Eimeria* spp.; *Streptococcus castoreus; Neostichorchis subtriquetrus; Emmonsia crescens; Trichinella* spp.; gram- negative bacteria*; Brucella* spp.; and hantaviruses (Puumala-virus (PUUV) and Saaremaa-virus (SAAV)). Moreover, seven hazards were considered to be of higher risk of precipitating disease if captive beavers from enclosures are chosen for conservation translocation in preference to free-living beavers: *Brucella* spp.; *Echinococcus multilocularis*; *Trichinella* spp.; *Toxoplasma gondii* (as a carrier and population hazard); *Francisella tularensis; Taenia martis and* hantaviruses (PUUV and SAAV).

In the disease risk analysis employed in this report, which uses World Organisation for Animal Health methods, risk estimation is made prior to consideration of disease risk management, which is evaluated thereafter. Disease risk management measures are employed to reduce the risk level and therefore the risk estimations noted might be reduced when risk management is implemented.

Eleven of the 26 hazards are stressor-associated and very careful attention to translocation protocols will be required to reduce the risk from disease precipitated by them. Parasites which are stressor-associated hazards may be commensal, or beaver-specific (for example *Neostichorchis subtriquetrus*), and therefore are an important component of biodiversity. Therefore, efforts should be made to conserve these parasites following translocation, stress mitigation is the favoured method of management, and prophylactic anti-parasitic treatment must be used with care to prevent elimination of these native parasites. If the Steering Committee concludes that the benefits of translocation outweigh the costs, we recommend that a disease risk management and post-release

health surveillance protocol, which includes attention to stressor-related hazards, is drawn up.

Evidence shows that source hazards constitute the greatest risk of epidemic disease following translocation. Six parasitic source hazards were assessed within this report, all of which pose a zoonotic risk of disease in people or a risk of disease in domestic animals at the destination. These parasites, *Echinococcus multilocularis*, *Francisella tularensis*, hantaviruses (Puumala-virus (PUUV) and Saaremaa-virus (SAAV)), *Trichinella* spp., *Taenia martis*, and certain *Brucella* spp., are currently not present in England and endemic in certain areas of mainland Europe. Our analysis shows that translocations of beavers to England from endemic areas, or of beavers which originated from endemic areas, and are now in captivity, or, in some cases, beavers which have been in contact with those which originated from endemic areas, represents a greater risk from disease. Therefore, using free-living beavers from Great Britain for translocations represents a lower risk from disease than translocations from either Norway to England or through the release of beavers held in enclosures in Great Britain. Beavers held in enclosures in Great Britain have, in some cases, originated from areas, such as Bavaria (Germany) and Poland in which the six parasitic source hazards are endemic. In other cases, their history of origin is unknown, and there may have been contact with exotic rodents in captive collections including enclosures, and therefore the risk from disease from the release of these animals should be assumed to be high. Free-living beavers in Great Britain are also of uncertain origin in some cases and, if these beavers are used for translocations, we recommend that a comprehensive, methodical post-release disease surveillance plan is formulated and enacted. The risk estimations reported here can be discussed by the Steering Committee of the translocation in the context of the social, ecological and conservation dimensions of the translocation setting and views on acceptable risk may be moderated by these considerations.

The free-living beaver populations in Great Britain or Norway are a potential source of unidentified hazards and, since unknown parasites have given rise to severe epidemics as a result of translocations, this disease risk analysis should be continually updated as new information becomes available, and the literature scrutinised, and immediate efforts made to incorporate surveillance data into the DRA.

The transparent method of disease risk analysis used in this work, adapted by DRAHS at ZSL for use in free-living wildlife from the World Organisation for Animal Health Import Risk Analysis, and conforming to IUCN guidelines, allows for ready re-analysis and revised risk estimation. In conclusion, if the benefits of translocation are seen by the Steering Committee to exceed the costs, we recommend continued scrutiny and evaluation of the risks from disease and that a disease risk management and post-release health surveillance protocol is drawn up.

This disease risk analysis must be regularly reviewed, as new evidence relevant to the threat of disease to mammal populations following beaver translocation

becomes available, if it is to effectively assess and manage the risks from disease from beaver translocation.

1.0 Introduction

The Eurasian Beaver (*Castor fiber*) is believed to have become extinct in Great Britain during the 16th century as a result of human persecution, primarily hunting for fur, meat and castoreum (Nolet and Rosell 1998). Across the species' range, exploitation reduced population size in the late 1990s to approximately 1200 individuals over eight discrete locations (*ibid.*). Following greater protection, reintroductions and natural dispersal, numbers in Europe have now recovered to over one million across 32 European countries, with the addition of some non-native Canadian beavers (*Castor canadensis*) in Russia, Luxembourg and Finland (Halley, Rosell, and Saveljev 2012), with human-beaver conflict requiring careful management in some areas (Campbell-Palmer *et al.,* 2015a). Small free-living populations are currently found in Scotland and England as a result of authorised and unauthorised releases. In addition, there have been licensed imports to captive facilities in England. Interest in the beaver's potential role as a keystone species in ecosystem restoration, specifically its ability to alter landscapes to the benefit of other species and for flood mitigation (Gaywood, Batty, and Galbraith 2008), has fed enthusiasm for reintroduction of the species in Great Britain.

1.1 Beavers in Great Britain

There are currently at least five known populations of free-living beavers in Great Britain: Knapdale and in the region surrounding Tayside in Scotland (Jones and Campbell-Palmer 2014b); the River Otter in Devon, the River Tamar in Devon and the River Stour in Kent (Claire Howe, pers. comm.). Beavers in Knapdale were imported from Norway in 2008 as part of a formal trial regulated by Scottish National Heritage (Jones and Campbell-Palmer 2014b). The Tayside beavers, first sighted in 2006, are of unknown origin but genetic testing of 25 individuals indicated that they were from three distinct lineages of German, most probably Bavarian, origin with heterozygosity and allelic richness comparable to the Bavarian source population (McEwing, Senn, and Campbell-Palmer 2015). This diversity suggests that the Tayside population is derived from multiple releases. Beavers on the River Otter were first sighted in 2007 and five were trapped and found on genetic analysis to be closely related and from either Bavaria or Baden-Wurtemberg (Brazier *et al.,* 2020). The origin of the beavers on the Rivers Tamar and Stour is less certain but is believed to be Bavaria and Norway, and Poland and Bavaria respectively (Claire Howe, pers. comm.). There are less certain reports of free-living beavers in at least one site in Wales and several sites in England which are of unknown number, origin and date of release (Jones *et al.,* 2013). In addition, 67 captive beavers are currently known to be held in approximately 20 fenced sites, commonly known as 'enclosed releases', such as Ham Fen, Kent (Claire Howe, pers. comm.) and in an unknown number of zoos, wildlife parks and other captive collections. Of the beavers known to have been involved in enclosed

releases, at least one is known to have originated from each of Bavaria, Poland or Norway, and others are suspected to have originated from these areas, and many were sourced from Scotland (Claire Howe, pers. comm.). Beavers are continuing to be placed into new enclosures. Knowledge of the origin of beavers, including of their antecedents, is important because parasites which pose a risk in precipitating disease as a result of the translocation are endemic in Bavaria and Poland, such as *Echinococcus multilocularis*.

1.2 Recent developments in disease surveillance of free-living beavers in England

The precise origin of some free-living beavers and/or their antecedents in Great Britain is unknown. The release of some beavers was not subject to disease risk analysis and management, and they may harbour parasites novel to Great Britain. Since the 2020 version of the DRA was carried out, a disease surveillance programme for free-living beavers has been implemented across England which aims to improve the understanding of beaver health in England and subsequently improve conservation outcomes (Common, Gerard, and Sainsbury 2022). In 2021, four free-living beaver carcases were examined in detail providing information which has been utilised throughout this report. For example, *Streptococcus castoreus* was isolated from one beaver and two adult *Neostichorchis subtriquetrus* trematodes found in the caecum of another beaver. Three out of four beavers were considered to have been involved in road traffic collisions and, in the fourth, drowning is a differential, a previously unconsidered hazard (Common, Gerard, and Sainsbury 2022). Targeted testing for other hazards of concern has been employed on these beavers. To date, pan-hantavirus polymerase chain reaction (PCR) has not detected hantavirus DNA in the lungs and/or kidney from the four beavers, and results of SARS-CoV-2 PCR, *Francisella tularensis* PCR and culture, *Toxoplasma gondii* testing, *Leptospira* spp. testing and *Echinococcus multilocularis* serology are pending. No signs of gross lesions associated with *E. multilocularis* have been detected in the four beavers.

2.0 Assessing the risks from disease in wildlife translocations for conservation purposes

Wildlife translocations for conservation purposes (reintroduction, reinforcement, ecological replacement and assisted colonisation) have become a key conservation tool to help restore species and/or ecosystem functions (IUCN 2013). Risks from disease associated with wildlife translocations arise because individual animals moved are a biological package, consisting of the host and all of its associated parasites (Davidson and Nettles, 1992). The potential impact of infectious disease on the outcome of wildlife conservation interventions has only recently been recognised and detrimental effects may occur in the focus species or in other species within the wider destination ecosystem. The IUCN (2013) recommends health monitoring of animals involved in translocation programmes and

current scientific opinion is that a disease risk analysis (DRA) should be conducted before a translocation takes place in order to address the significant disease risks of translocation and to inform appropriate mitigation measures (Davidson and Nettles, 1992; Leighton, 2002; Miller, 2007; Sainsbury and Vaughan-Higgins, 2012).

DRA provides a structured, evidence-based process that can help decision-makers to understand the risks of disease-causing agents on translocation objectives and make decisions in light of these risks (Jacob-Hoff *et al.,* 2014). Several methods have been described. In 2012, Sainsbury and Vaughan-Higgins described a method for conducting a DRA for conservation translocations adapted from the World Organisation for Animal Health's (OIE) guidelines for DRA in domestic animal movements between countries (Murray *et al.,* 2004). The Sainsbury and Vaughan-Higgins (2012) method includes (i) hazards not known to cause harm (ii) infectious agents as hazards based solely on novelty to the source or the destination (iii) hazards based on stressor effects (iv) non-infectious hazards and (v) ignores country borders and assesses the risk from parasite hazards on the presence or absence of geographical and ecological barriers in the translocation pathway. A series of steps are completed in the DRA: (1) mapping out the translocation pathway, (2) defining geographical and ecological barriers (3) hazard identification, (4) justification of hazard status, (5) risk assessment, (6) risk management and (7) risk communication.

Sainsbury and Vaughan-Higgins' (2012) method (the ZSL method) has been used for 32 translocation and reintroduction programmes conducted over the last 20 years.

3.0 Aims of this disease risk analysis for beaver reintroduction

The purpose of this disease risk analysis (DRA) was to expand on and update the DRA published in 2020 (Donald, Common and Sainsbury 2020), for which the aim was 'to assess the risks from disease related to the conservation translocation of beavers from either Norway, or any free-living population from any area of Great Britain, to England'. There has since been interest in translocating beavers currently housed in enclosures and captive (zoological) collections in the UK, and the risks from disease in this additional translocation pathway are included here.

Our previous work has shown that the risk from disease to a conservation translocation programme is comparatively high if the animals to be translocated have been housed in zoological collections (Bobadilla Suarez *et al.,* 2017) primarily due to breach of ecological barriers and the potential for exchange of alien parasites from different ecological and geographical zones. Some beavers have been held in zoological collections in the UK and, as such, may have had direct or indirect contact with other captive, non-native species including rodents. Evidence shows that parasites have been transmitted between

species in captive collections and the prevalence of certain parasites is high in captivity, for example the prevalence of *Toxoplasma gondii* is higher in captive collections (Ippen, Kozojed, and Jira 1981; Hardgrove *et al.,* 2021). The origin of the captive beavers, including those held in fenced enclosures, may differ from the beaver origins considered in the 2020 DRA. Beavers of differing origin may harbour different parasites with ensuing effects on disease risk analysis and so we have investigated these changes in the report here.

It is important to note that if, in the future, the translocation pathway is altered again, for example, beavers housed in enclosures in countries outside the UK are included as possible source animals, a revised disease risk analysis would be required.

We have communicated the findings from this DRA to Natural England and the Steering Committee responsible for plans to reintroduce beavers to England through this report. The intention is that the Steering Committee can use this disease risk analysis in the context of other evidence, for example ecological feasibility, to make a decision on the favourability of reintroduction and on the source of beavers for that intervention.

4.0 Materials and methods

In this report we use the Sainsbury and Vaughan-Higgins' (2012) method (ZSL method) described above, as developed from previous qualitative DRA methods for wildlife (Davidson and Nettles, 1992; Leighton, 2002) and domestic animals (Murray *et al.,* 2004) and modified by Bobadilla-Suarez *et al.,* (2017) and Rideout *et al.,* (2017) to describe the translocation pathway, assess geographical and ecological barriers, identify disease hazards, assess the magnitude and probability of disease occurring, and propose methods to mitigate the risk from disease associated with the reintroduction of free-living or captive beavers to England.

4.1 Translocation Pathway(s) and geographical/ecological barrier considerations

A translocation pathway is a description of the route of the translocated animals that illustrates the points at which different types of hazards may potentially harm translocated individuals or the recipient ecosystem (Bobadilla Suarez *et al.,* 2015). A major consideration in any given translocation pathway is whether any geographical (rivers, mountain ranges, seas) or ecological barriers are to be crossed, for example by bringing species that would normally be separated by habitat or behaviour into either direct or indirect contact with each other, thereby facilitating the spread of parasites that could not occur without human intervention. If a translocation crosses geographical or ecological barriers, then there is an increased probability of translocated or recipient populations being exposed to novel infectious agents.

This assessment is crucial because empirical evidence shows that the major epidemics of disease associated with translocations have arisen from source hazards (Cunningham, 1996; Sainsbury and Vaughan-Higgins, 2012; Viggers *et al.,* 1993). Source hazards are parasites present at the source but not at the destination (until the translocation occurs). An assumption that source and destination hazards are absent or minimal in a given translocation gives the translocation manager confidence that the overall risk from disease of a given translocation is markedly reduced. If source and destination environments are not separated by barriers, and populations of the translocated species, closely-related or sympatric species and their parasites are contiguous, source and destination hazards do not require consideration and the overall risk from disease in the translocation may be reduced (Bobadilla Suarez *et al.,* 2017).

In this disease risk analysis, three potential source populations were considered: free-living beavers in Norway, free-living beavers in Great Britain and captive beavers in captive collections such as fenced enclosures, wildlife parks or zoos, or translocations in which beavers are temporarily housed in wildlife parks or zoos, or any collection which houses or has housed exotic species. Animals in some captive collections, including zoos, are considered to have crossed an ecological barrier, as described above, because their proximity to exotic species creates the potential for parasite transfer and the acquisition of non-native parasite species.

4.2 Hazard Identification

To identify hazards, we searched the scientific literature, examined unpublished data and sought experts' opinions. We used the search engines of Google Scholar, PubMed, Web of Knowledge and the ZSL library services.

We identified parasites (micro- and macro-parasites) known to be present in Rodentia, and specifically beavers, as well as multi-host parasites, using the scientific literature both in Great Britain and overseas, including a disease risk analysis undertaken for Eurasian beavers in Great Britain (Girling *et al.,* 2019a). Through consideration of (i) geographic distribution, (ii) occurrence (iii) pathogenesis and (iv) diseases associated with each parasite and (v) evidence for a negative impact on population numbers, we assigned, when possible, each hazard to an appropriate category as defined below (justification of hazard status). We included evidence for susceptibility of beavers, other rodents and other mammals to each potential hazard, or similar agents of disease, in carrying out our evaluation. We considered not only known pathogens, but also apparent commensal parasites, since the pathogenicity of many parasites of free-living wild animals is unknown. The translocation and the adaptation to a new environment could act as stressors and therefore alter the normal host-parasite dynamics resulting in disease. We also considered non-infectious agents or events, and their association with disease, and similarly assigned these to their respective hazard category.

CARRIER HAZARDS were defined as commensal parasites, or parasites which do not ordinarily cause disease in the host animal following infection, which when the host is under stress associated with translocation or is subjected to factors that affect parasite dynamics, such as alterations in host density, may cause disease in transit or at the release site.

TRANSPORT HAZARDS were defined as those hazards that may be encountered during the transport (between the source and destination sites) which may be novel to the translocated animals and/or the release environment. Translocated animals can be a potential vehicle for introduction of these hazards to the destination site. Transport hazards are also those infectious agents moved with materials such as transport boxes, equipment, food and water.

POPULATION HAZARDS were defined as those non-infectious and infectious agents present at both the source and destination sites which potentially could have a negative impact on population numbers at the destination.

SOURCE HAZARDS were defined as a hazard present at the source site which would be novel at the destination site. Conversely, **DESTINATION HAZARDS** were defined as infectious agents present at the destination but not the source.

If no geographical or ecological barriers are crossed in a translocation then it may be assumed that there are no source or destination hazards (Bobadilla Suarez *et al.,* 2015).

4.3 Disease risk assessment

We assessed the risk of disease from each hazard using the method described by Sainsbury and Vaughan-Higgins (2012), with amendments provided by Bobadilla Suarez *et al.,* (2017) and Rideout *et al.,* (2017) and using the foundation provided by the World Organisation for Animal Health (Murray 2004).

4.3.1 Release assessment

Where relevant, we determined the biological pathways that might permit a beaver from the source site to be released while infected with a parasite and the likelihood of its occurrence.

4.3.2 Exposure assessment

We described the biological pathways that might permit beavers and sympatric species at the destination to be exposed and infected with the parasite and the probability of this occurrence. We then described the processes required for the agent to disseminate

through beavers and sympatric species populations and the probability of dissemination occurring.

4.3.3 Consequence assessment

We assessed the likelihood and severity of biological, economic and environmental consequences associated with the entry, establishment and spread of the hazard.

4.3.4 Risk estimation

Using the method described in Murray *et al.,* (2004), we combined the results of the release, exposure, and consequence assessments to qualitatively assess the risk from disease associated with the hazard (negligible, very low, low, medium or high).

In our method, destination and population hazards have already “entered” the destination environment and a release assessment is not carried out for these hazards.

It is important to note that these estimates will be influenced by the information available and the risk attitudes of the specialists undertaking the DRA and therefore a reasoned, informed and transparent discussion of the risks from disease associated with each hazard is included within the DRA to justify each probability or risk estimation.

5.0 Results

5.1 Translocation Pathway

Following guidance from Natural England, three possible pathways were considered: the translocation of (i) free-living beavers from Norway, (ii) free-living beavers from Great Britain, and (iii) beavers held in captive collections, including fenced enclosures, in Great Britain, and in each case the translocation was to England. The destination sites remain unknown at this stage but were considered to be at any location in England.

5.2 Geographical and ecological barriers evaluation

The distance between source and destination site(s) is unknown as both have yet to be selected but could be as great as 2000km if considering southern Norway as a source and 500km if considering Scotland. Norway and England are separated by the North Sea. We do not know of any free-living rodents or fresh-water mammals which are contiguous between Norway and England. Many species of birds migrate seasonally between the two countries and could act as a potential route for parasite transfer. However, parasites infectious for birds may not be infectious for rodents. It therefore seems prudent to

consider that a geographic barrier exists between Norway and England for the purposes of disease risk analysis. We have additionally considered the risk associated with the proximity of Norwegian beavers to neighbouring Swedish beaver populations. Populations inhabit the areas surrounding waterways which breach the 1600km border between the two countries, such as the river Klarälven (Hartman 1995).

The origin of some beavers in Great Britain is uncertain and, as stated above, there is evidence that at least some Tayside free-living beavers originated from Bavaria. The previous reintroduction of these beavers may therefore have broken ecological and geographical barriers; no specific disease risk analysis was undertaken prior to their importation and these beavers may have brought non-native parasites into Great Britain. Beavers in the Tayside area of Scotland are now known to have extended their range as far south as the outskirts of Stirling and into the Forth catchment (Campbell-Palmer 2018). Beavers may move hundreds of kilometres when dispersing and cross watersheds in pursuit of new territories or mating opportunities (*ibid.*) and so continued natural dispersal seems likely.

Sympatric rodent and other mammalian species that are susceptible to the same parasites may be considered to increase the effective population size (Mathews *et al.,* 2006). Beavers live in close proximity to brown rats (*Rattus norvegicus*) and bank voles (*Myodes glareolus*), two ubiquitous species in Great Britain, with population numbers estimated at 7 million and 27.4 million respectively (Mathews *et al.,* 2018). There are also robust populations of other small mammals, particularly rodents, that would be expected to overlap in habitat occupation with beavers such as, but not limited to, field voles (*Microtus agrestis*), water shrews (*Neomys fodiens*) and water voles (*Arvicola amphibius*), particularly at riparian margins. It is therefore probable that sympatric mammalian species form contiguous populations for parasite transfer purposes in many areas of Great Britain.

Since non-native beavers have only recently (within decades) been translocated to Scotland, and other parts of Great Britain, it is assumed that there has been insufficient time for parasites to be transferred to all parts of England. It is therefore assumed that these free-living, recently reintroduced, beavers in Scotland, and other parts of Great Britain, will cross ecological and geographical barriers if they are translocated to England.

As a result, our analysis has included evaluation of the risks from disease posed by source and destination hazards for the translocation of free-living beavers from either Norway or Great Britain, and captive beavers (including those in fenced enclosures and zoological collections) in Great Britain, to England.

5.3 Hazard Identification

Ninety-six potential hazards were identified (89 infectious and seven non-infectious hazards). Twenty-six of these were identified as requiring full disease risk analysis in order

to determine the risk from disease that they presented as a consequence of beaver translocation. A list of the hazards receiving full disease risk analysis is provided in Table 1 and listed here by hazard category:

- Fully assessed SOURCE HAZARDS included *Francisella tularensis*, hantaviruses (Puumala-virus (PUUV) and Saaremaa-virus (SAAV)); *Echinococcus multilocularis; Trichinella* spp*.; Taenia martis* and *Brucella spp..*

- Fully assessed CARRIER HAZARDS included *Leptospira* spp.; *Yersinia* spp*.; Mycobacteria* spp.; *Emmonsia crescens;* gram-negative enteric bacteria; *Streptococcus castoreus; Neostichorchis subtriquetrus; Toxoplasma gondii*; *Giardia* spp.; *Cryptosporidium parvum* and *Eimeria* spp*.*

- Fully assessed POPULATION HAZARDS included Road Traffic Collision; Persecution; Captivity During Translocation; *Toxoplasma gondii* and SARS-CoV-2

- Fully assessed DESTINATION HAZARDS included hantaviruses, specifically Seoul orthohantavirus (SEOV) and Tatenale virus (TATV)

There may be a need to evaluate TRANSPORT HAZARDS once a transit route between the source and destination sites has been formulated.

In addition, we evaluated the risks from disease associated with three unclassified hazards: *Giardia duodenalis*, *Cryptosporidium parvum* and *Mycobacterium* spp. (risk to domestic and free-living wild animals).

Four further hazards were detected and require detailed analysis before a translocation of beavers takes place. Brief details of these hazards are described in Appendix 1.

Sixty-six potential hazards received detailed scientific review as described in Appendix 2. The scientific reviews in Appendix 2 showed that these hazards were, at least currently, of very low or negligible disease risk as a result of the translocation of beavers. These hazards should be re-evaluated with each succeeding translocation as information may become available and our understanding improves.

Table 1: Potential hazards identified for the translocation of beavers (Castor fiber) to England and for which full disease risk analysis was carried out

POTENTIAL HAZARD		Beaver susceptibility to infection and/or disease*	Other *Rodentia* susceptibility to infection and/or disease	Reference	Hazard Category
Viral	Hantaviruses – SEOV, TATV	N/K	YES	(Duggan *et al.,* 2017; Pounder 2013; Thomason *et al.,* 2017)	Destination
	Hantaviruses – PUUV, SAAV	N/K	Yes	(Vapalahti *et al.,*. 2003; Klingström *et al.,* 2002; Olsson, Leirs, and Henttonen 2010)	Source
	SARS-CoV-2	N/K	YES	(Bao *et al.,* 2020; Chan *et al.,* 2020b)	Population
Bacterial	*Leptospira* spp.	YES (I, D)	YES	(Nolet *et al.,* 1997)	Carrier
	Brucella spp.	NO	YES	(Hubálek, Scholz, and Sedlác 2007; Vershilova, Liamkin, and Malikov 1983)	Source
	Francisella tularensis	YES (I, D)	YES	(Mörner, Sandstrom, Mattsson, and Nilsson 1988; Mörner and Sandstedt 1983a; Schulze *et al.,* 2016)	Source

POTENTIAL HAZARD		Beaver susceptibility to infection and/or disease*	Other *Rodentia* susceptibility to infection and/or disease	Reference	Hazard Category
	Yersinia spp.	YES (I, D)	YES	(Nolet *et al.,* 1997)	Carrier
	Gram-negative enteric bacteria	YES (I, D)	YES	(Pratama *et al.,* 2019; Pilo *et al.,* 2015; Dollinger *et al.,* 1999)	Carrier
	Streptococcus castoreus	YES (I, D)	NO	(Lawson *et al.,* 2005; Schulze *et al.,* 2015)	Carrier
	Mycobacterium spp.	YES (I, D)	YES	(Gavier-Widén *et al.,* 2012)	Unclassified
	Mycobacterium spp.	YES (I, D)	YES	(Gavier-Widén *et al.,* 2012)	Carrier
Endopara-sites	*Neostichorchis subtriquetrus*	YES (I, D)	NO	(Demiaszkiewicz *et al.,* 2014)	Carrier
	Echinococcus multilocularis	YES (I, D)	YES	(Barlow, Gottstein and Mueller, 2011; Campbell-Palmer, Del Pozo, *et al.,* 2015)	Source
	Taenia martis	YES (I)	YES	(Campbell-Palmer, Del Pozo, *et al.,* 2015)	Source
	Trichinella spp.	YES (I)	YES	(Segliņa *et al.,* 2015;	Source

POTENTIAL HAZARD		Beaver susceptibility to infection and/or disease*	Other *Rodentia* susceptibility to infection and/or disease	Reference	Hazard Category
				Różycki *et al.,* 2020)	
Protozoa	*Toxoplasma gondii*	YES (I, D)	YES	(Herrmann *et a*l., 2013)	Carrier
	Toxoplasma gondii	YES (I, D)	YES	(Herrmann *et al.,* 2013; Hollings *et al.,* 2013)	Population
	Giardia duodenalis	YES (I)	YES	(Tsui *et al.,* 2018; Sroka *et al.,* 2015)	Unclassified
	Giardia spp.	YES (I)	YES	(Paziewska *et al.,* 2007; Cervone *et al.,* 2019)	Carrier
	Cryptosporidium parvum	YES (I)	YES	(Lv *et al.,* 2009; Paziewska *et al.,* 2007)	Carrier
	Cryptosporidium parvum	YES (I)	YES	(Paziewska *et al.,* 2007; Mackie 2014)	Unclassified
	Eimeria spp.	YES (I)	YES	(Demiaszkiewicz *et al.,* 2014; Campbell-Palmer *et al.,* 2021)	Carrier
Fungi	*Emmonsia crescens*	YES (I, D)	YES	(Mörner, Avenäs, and Mattsson 1999; Dolka *et al.,* 2017)	Carrier

POTENTIAL HAZARD		Beaver susceptibility to infection and/or disease*	Other *Rodentia* susceptibility to infection and/or disease	Reference	Hazard Category
Non-Infectious	Road traffic collisions	YES	YES	(Brazier *et al.,* 2020; Campbell-Palmer *et al.,* 2015b; Stefen 2018)	Population
	Captivity during translocation	YES	YES	(Harrington, Feber, and MacDonald 2010a; Goodman *et al.,* 2012)	Population
	Illegal persecution	YES	YES	(Campbell-Palmer *et al.,* 2015b; Stefen 2018)	Population

(*): Because of the paucity of data available on both infectious and non-infectious hazards in free living beavers, a qualitative judgement of beaver susceptibility to some hazards, based on expert opinion, was used when it could not otherwise be supported by evidence in the scientific literature. Beavers were considered to be “likely susceptible” to those parasites isolated in closely phylogenetically related species but also to those multi-host parasites known to infect many other mammalian families and orders. I = INFECTION; D = DISEASE IN SPECIES

5.4 Disease Risk Analyses

The risk from disease of 12 of 22 infectious hazards identified in the DRA published in 2020 (Donald, Common, and Sainsbury 2021) was reassessed based on the revised translocation pathway options, and four new infectious hazards were assessed in full. These re-evaluations are described in this report together with the analyses which remain largely unchanged since the 2020 DRA report.

A particular focus in this report has been to carefully reconsider the risk from source hazards given the potential new routes through which beavers in captive collections could be exposed and infected. Beavers currently held in enclosures in Great Britain are known to have originated from Germany, which is an endemic area for the source hazards *Echinococcus multilocularis*, hantaviruses (PUUV and SAAV), *Taenia martis* and

Francisella tularensis. Others are known to have originated from Poland, where the aforementioned source hazards are either known, or suspected, to be endemic. Poland is also considered to be an area at increased risk of brucellosis, raising the likelihood of beavers from this area being exposed to, and infected with, the source hazard *Brucella* spp..

Infectious carrier hazards were reassessed based on possible increased likelihood that beavers from captive collections would be exposed to, and infected with, different parasites, for example gram-negative bacteria, *Toxoplasma gondii* and *Leptospira* spp.. Four of the previously assessed infectious hazards (*Neostichorchis subtriquetrus*, hantaviruses (both as destination and source hazards) and *Streptococcus castoreus*), as well as one previously assessed non-infectious hazard (road traffic collisions) (Donald, Common, and Sainsbury 2021), were reconsidered based on new evidence gathered from disease surveillance of free-living beavers found dead in England in 2021 (Common, Gerard, and Sainsbury 2022). The population hazard SARS-CoV-2 was reassessed to consider the evidence published since the last assessment was undertaken.

Hazard identification detected a total of 96 hazards. Of these, 26 hazards were assessed in full. One of these 26 hazards was estimated to be of negligible risk: *Mycobacterium spp*. as a risk to domestic and free-living wild animals. Three hazards were estimated to be of very low risk: *Cryptosporidium parvum* as an unclassified hazard; Seoul orthohantavirus (SEOV) and Tatenale hantavirus (TATV) as a destination hazard; SARS-CoV-2 as a population hazard; and four hazards estimated to be of low risk: *Mycobacterium* spp. as a carrier hazard; *Giardia duodenalis* as an unclassified hazard; *Giardia* spp. as a carrier hazard; and *Cryptosporidium parvum* as a carrier hazard. Nine hazards were assessed as being medium risk: persecution as a population hazard; captivity as a population hazard; *Eimeria* spp. as a carrier hazard; *Streptococcus castoreus* as a carrier hazard; *Neostichorchis subtriquetrus* as a carrier hazard; *Emmonsia crescens* as a carrier hazard; *Trichinella* spp. as a source hazard; gram-negative bacteria as a carrier hazard; *Brucella* spp. as a source hazard; and six assessed as high risk: road traffic collisions as a population hazard; *Echinococcus multilocularis* as a source hazard; *Leptospira* spp. as a carrier hazard; *Yersinia* spp. as a carrier hazard. *Toxoplasma gondii*, as both a carrier and a population hazard, and *Taenia martis* as a source hazard, were both estimated to be of high risk if beavers from enclosures are chosen to be translocated, and medium risk if free-living beavers from Great Britain are chosen. The overall risk from disease associated with Puumala orthohantavirus (PUUV) and Saaremaa virus (SAAV) as novel source hazards is very low for rodents and medium for humans.

5.4.1 Disease risk analysis for the source hazard hantaviruses (Puumala orthohantavirus (PUUV) and Saaremaa virus (SAAV))

Hazard type: Source Hazard

Justification for Hazard Status

Hantaviruses are notifiable RNA viruses (Order *Bunyavirales*, Family *Hantaviridae*) found primarily in rodent, bat and insectivore reservoir hosts and identified as a significant emerging zoonotic risk in Europe (European Centre for Disease Prevention and Control 2019c). To date, 48 species of hantavirus have been identified (Forbes, Sironen, and Plyusnin 2018). However, in the host, viral species identification is difficult due to the cross-reactivity of serum antibodies with viral antigen (Vaheri, Vapalahti, and Plyusnin 2008) especially if using saliva samples (Jameson *et al.,* 2014). Four hantavirus species which circulate in Europe (Dobrava-virus (DOBV), Saaremaa-virus (SAAV), Seoul-virus (SEOV), and Puumala-virus (PUUV)) are associated with haemorrhagic fever with renal syndrome (HFRS) in humans. For the purposes of this DRA, hantaviruses of interest were identified as those present in Europe (Table 2).

Table 2: Hantavirus species identified in Europe with reservoir hosts (from Heyman et al., 2002; Klingström et al., 2002; Pounder, 2013)

Hantavirus	Reservoir host
Seoul-virus (SEOV)	*Rattus norvegicus* (brown rat) and *Rattus rattus* (black rat)
Puumala-virus (PUUV)	*Myodes glareolus* (bank vole)
Tula-virus (TULV)	*Microtus arvalis* (common vole)
Tatenale-virus (TATV)	*Microtus agrestis* (field vole)
Dobrava-virus (DOBV)	*Apodemus flavicollis* (yellow-necked mouse)
Saaremaa-virus (SAAV)	*Apodemus agrarius* (striped field mouse)
Topografov (TOPV)	*Lemmus sibericus* (Siberian lemming)

Hantavirus	Reservoir host
Khabarovsk (KBRV)	*Microtus fortis* (reed vole)

PUUV, in common with its reservoir host, the bank vole (*Myodes glareolus* syn. *Clethrionomys glareolus*), is widely distributed throughout Europe. Figure 1 shows host distribution and recorded cases of infection in humans. An average of 50 cases a year are reported in Norway and rarely in southern Sweden (Vapalahti *et al.,* 2003). The incidence of DOBV is predominantly in Eastern Europe and the Balkans (Vapalahti *et al.,* 2003), and this virus has not been detected in the UK to date.

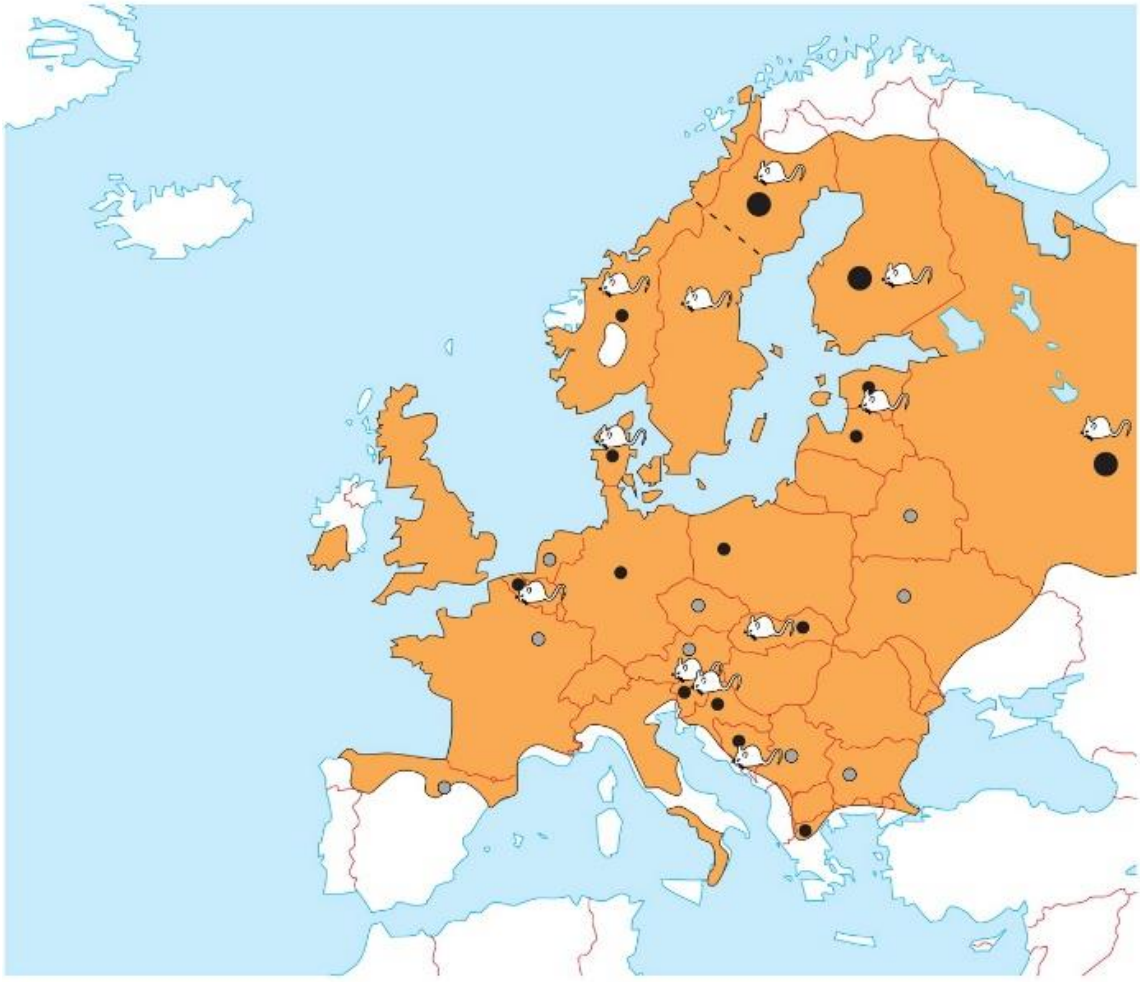

Figure 1: The distribution of Clethrionomys glareolus and human hantavirus infections. Rodent figure indicates countries where PUUV sequences are available from C. glareolus; grey dots indicate human hantavirus infections caused by PUUV; black dots indicate cases confirmed by cross-neutralisation tests or RT-PCR and sequencing. (Source Vapalahti et al., 2003)

SEOV is thought to have originated in China and has been found in captive pet and wild free-living rats (*Rattus* spp.) in the UK (Webster and Macdonald, 1995; Jameson *et al.,* 2014; Duggan *et al.,* 2017), free-living rats in Belgium and France and in pet rats in Sweden (Ling *et al.,* 2019). SEOV has not been found in rats in Germany (Hofmann *et al.,* 2018). It is not known whether SEOV is present in Norway.

Despite the primary hosts for PUUV and DOBV, respectively the bank vole and yellow-necked mouse (*Apodemus flavicollis*), being widely present in the UK, neither virus has been detected (Duggan *et al.,* 2017). The only hantavirus species detected in the UK to date are SEOV and Tatenale-virus (TATV), a novel arvicoline virus which was identified in a field vole (*Microtus agrestis*) in northern England from samples collected between 2009 and 2011 (Pounder, 2013) and a closely related virus detected in 17% (n=8/48) of field voles examined in Kielder Forest in 2015 (Thomason *et al.,* 2017). Thomason *et al.,* (2017) concluded that the divergence in the two viruses was strongly suggestive of long-standing endemicity which may suggest that the virus is also prevalent in other areas of Britain. A number of cases of hantavirus infection have been recorded in humans in the UK, but it is not always known with certainty which species was involved as the serotype is not recorded (Bennett *et al.,* 2010) or may have been misattributed due to cross-reactivity (Duggan *et al.,* 2017) For example, SEOV cross-reacts with Hantaan-virus (HTNV) and Sin Nombre-virus (SNV) with PUUV and there may be other unidentified cross-reacting species (Jameson *et al.,* 2014). Definitive diagnosis is by reverse transcription polymerase chain reaction (RT-PCR) for viral antigen and sequencing from tissue samples. The earliest human cases noted were in Northern Ireland in the 1990s and were most probably attributable to SEOV (Clement *et al.,* 2014).

Each species of hantavirus has traditionally been regarded as host-species specific causing mostly asymptomatic and persistent (possibly lifelong) infection in its reservoir host but only transient, spillover infections in other animal species (Forbes, Sironen, and Plyusnin 2018). However, hantaviruses may have the potential to spread to new reservoir hosts; there is historic evidence of host-switching in the evolution of these viruses (Zhang 2014). Phylogenetic analysis of Hantaan-virus (HTNV), DOBV and SAAV and their reservoir hosts of the genus *Apodemus* has provided evidence of host switching in the evolution of these viruses; SAAV (host: *A. agrarius*), shared the most recent ancestory with DOBV (host: *A. flavicollis*), but not with HTNV (host: an eastern subspecies of *A. agrarius*) (Nemirov *et al.,* 2002). The authors suggested that transmission of the ancestral DOBV to the European subspecies of *A. agrarius*, leading to the evolution of ancestral SAAV (Nemirov *et al.,* 2002). Evidence of host-switching also exists for SEOV, which has been found in several rat species (Holmes and Zhang 2015), and HTNV and SEOV for which there is evidence of an expanding range in China based on identification of SEOV antigen in shrews and HTNV in house mice and brown rats (Fang *et al.,* 2015). A meta-study of all peer-reviewed reports of hantavirus infections between 1971 and 2015 found several instances of interspecies sharing, particularly in voles (*Arvicola* spp.; Milholland *et al.,* 2018). Additionally, Schmidt-Chanasit *et al.,* (2010) challenged assumptions of viral co-evolution with host species, concluding that TULV is a promiscuous hantavirus with a large

range of susceptible hosts. Klingström *et al.,* (2002) showed experimentally that wild-trapped yellow-necked mice could be infected by SAAV, suggesting that species other than *A. agrarius* may be susceptible to infection; this is pertinent as i) it suggests that other rodents can carry this virus, which is not present in the UK and poses a human health risk through HFRS, and ii) it is possible that other rodent reservoirs could be established such as the yellow necked mouse, which is present in England. Nevertheless, to the best of the authors' knowledge, there is no evidence of natural infection with SAAV of any other host.

Cases of hantavirus have also been reported in captive animals: eight primates comprising three species (*Macaca mulatta*, *M. fascicularis*, *Papio anubis*) housed in a German zoological institution were found through a serological study to have positive antibody titres for either PUUV, TULV or both viruses, with all but two animals showing long-term immunity over seven years (Mertens *et al.,* 2011). This is the first report of natural infection of primates held in outdoor enclosures and the authors hypothesised that infection occurred after transmission from rodent reservoirs gaining access to enclosures. To further investigate this hypothesis, free-living rodents from the areas surrounding the zoo were trapped and tested for hantavirus infection using polymerase chain reaction (PCR). Six of 73 bank voles and three of 19 *Microtus* spp. investigated were positive for PUUV/TULV DNA (Mertens *et al.,* 2011). This study suggests a possible route of exposure to hantaviruses through reservoir rodents gaining access to enclosures in endemic areas. Therefore, captive beavers with an unknown history, or those with a history of being housed in enclosures in hantavirus endemic areas, may have been exposed and infected with hantaviruses.

PUUV is endemic in bank voles in Scandinavia (including Norway) (Vapalahti *et al.,* 2003) and Germany (including Bavaria) (Mertens *et al.,* 2011). SAAV also circulates in North-western Europe including Germany (Olsson, Leirs and Henttonen, 2010). Neither virus is thought to be present in the UK and both can lead to human disease. Given that host-switching has been demonstrated for hantaviruses and rodents are known reservoirs, PUUV and SAAV should be considered source hazards for the translocation of beavers to England from Norway, or enclosures where the beavers have originated from continental Europe, particularly known endemic areas, or have an unknown history of origin.

Risk assessment

Release assessment

Hantaviruses may persist for some time outside the host. For example, PUUV and TULV have been shown to remain infectious for up to 11 days at room temperature and up to 18 days at 4°C (Kallio *et al.,* 2006). Cool and damp conditions may prolong viral survival (Forbes, Sironen and Plyusnin, 2018). Infection is by aerosol inhalation of viral particles or intense contact with hosts such as biting, grooming and sharing food resources (Forbes, Sironen and Plyusnin, 2018). Juvenile rodents may be protected from infection for up to 80 days by maternal antibodies and prevalence in male rodents is higher, probably due to

intra-specific aggression and dispersal distances (Kallio *et al.,* 2006). Co-infection with parasites is variably positively and negatively associated with virus infection in bank voles (Deter *et al.,* 2008; Salvador *et al.,* 2011).

Free-living bank voles and beavers in Norway and other endemic areas, for example Bavaria, Germany, are likely to be sympatric in riparian margins, and striped field mice and beavers may come into contact at riparian margins in Bavaria. Chronically infected rodent hosts will shed PUUV/SAAV in urine, faeces and saliva which may persist in the environment for up to 18 days in cool, damp conditions (Kallio *et al.,* 2006). Therefore, there is a low probability that free-living beavers in endemic areas could be exposed to viral particles when foraging on land. Moreover, the evidence of transmission to captive primates from voles in a zoological collection in Germany (Mertens *et al.,* 2011) suggests that exposure of captive beavers to hantaviruses could occur through free-living rodents accessing enclosures in endemic areas. It is not known whether beavers are susceptible to infection with hantaviruses either as reservoirs through host switching or as accidental hosts. Girling *et al.,* (2019b) found no evidence of hantaviruses in kidney tissue and urine samples from 20 free-living beavers examined between 2010 and 2015 from Knapdale and Tayside in Scotland, Telemark, Norway, and Bavaria, Germany using a pan-hantavirus nested PCR. Moreover, disease surveillance work undertaken in 2021 found no evidence of hantavirus DNA in lung and/or kidney tissue using pan-hantavirus PCR (Klempa *et al.,* 2006) in four free-living beavers found dead in England (Common, Gerard and Sainsbury, 2021). There is therefore a very low likelihood of a translocated beaver being infected with PUUV/SAAV at the time of translocation.

Exposure Assessment

Studies in laboratory rodents have shown that chronic hantavirus infection may result in occasional or no viral shedding (Forbes, Sironen and Plyusnin, 2018). However, a capture-mark-recapture investigation of naturally occurring PUUV infection in bank voles suggested that free-living host animals may be infectious for life (Voutilainen *et al.,* 2015), and shed virus in urine, faeces and saliva (Voutilainen *et al.,* 2015). Gastrointestinal transmission has also been demonstrated experimentally (Witkowski *et al.,* 2017).

Accidentally infected hosts are believed to clear infection quickly and are not considered a source of infection to other animals. The only exception to this is occasional reports of human-to-human transmission of Andes-virus (ANDV), a hantavirus species specific to South America which is believed to have unique anti-inflammatory properties that enable it to evade the host's salivary anti-viral mechanisms (Forbes, Sironen and Plyusnin, 2018). Host-switching of hantaviruses has been reported and so there is a very low likelihood that an infected beaver could act as a reservoir and shed virus into the environment through its urine, faeces or saliva or could infect con-specifics by fighting, grooming or food-sharing.

If beavers were persistently infected, there is a low likelihood that new beaver colonies at the destination could act as a reservoir of infection to sympatric species and humans. In

particular, given that the known host for PUUV, the bank vole, is native to England and likely to share habitat with released beavers in riparian margins, there is a medium likelihood that sympatric bank voles could be exposed to and infected with PUUV as a result of contact with an infected beaver. These animals could then act as a reservoir for disease transmission. There is a medium likelihood of dissemination of PUUV at the destination.

Consequence Assessment

There is a very low likelihood that one translocated beaver will become infected with PUUV or SAAV, depending on the origin of the animal. Infection of rodent reservoir hosts is believed to be asymptomatic; however, subtle histopathological changes have been recorded in infected animals in combination with a robust antibody response (Simmons and Riley, 2002). Spill-over infection to closely related sympatric species is known to occur but it is not clear whether this results in clinical disease (Simmons and Riley, 2002). Simmons and Riley (2002) reported that experimental infection of Syrian hamsters (*Mesocricetus auratus*) with PUUV, SEOV and DOBV resulted in asymptomatic serological conversion. Klingström *et al.,* (2002) further suggested that accidental spill-over infections of non-reservoir hosts result in rapid clearing of the virus. However, experimental infection of immunocompromised mice with SEOV resulted in chronic wasting disease (Golden *et al.,* 2015). There is a very low likelihood of a disease outbreak in beavers or sympatric rodents at the destination.

PUUV and SAAV are both known to cause disease in humans. In 2017, the last year for which data is available, Germany recorded the highest number of cases of human hantavirus infection of any country in Europe at 1717 cases compared to 26 in Norway, 158 in Sweden (mostly from northern Sweden) and 0 in the UK (European Centre for Disease Prevention and Control, 2019c). Baden-Wurttemberg, in south-west Germany, and Bavaria account for the majority of cases in humans in Germany (European Centre for Disease Prevention and Control, 2014). Two clinically significant syndromes have been recognised in humans (gov.uk, 2021): HFRS and Hantavirus pulmonary syndrome (HPS). Of these, only HFRS is known in Europe, usually causing a milder form of disease known as nephropathica epidemica (NE) (Klingström *et al.,* 2002). In rare cases, infection may lead to chronic conditions such as Guillain-Barre syndrome (European Centre for Disease Prevention and Control, 2019c). There is a medium likelihood of disease in humans in contact with infected beavers during the translocation.

Risk Estimation

The likelihood of a beaver from Norway being exposed to PUUV or SAAV at the source is low and the probability of infection is very low. The likelihood of dissemination to conspecifics and sympatric species is medium. There is a very low likelihood of a disease outbreak in rodents and a medium likelihood of disease in humans. The overall risk from disease associated with PUUV and SAAV as novel source hazards is very low for rodents and medium for humans.

As hantaviruses have been shown to cause morbidity in immunocompromised mice, if beavers are subsequently found to be susceptible to infection, this DRA require updating to consider the risks to beavers of hantaviruses as a carrier hazard.

Risk Management

Risk Evaluation

The level of risk associated with hantaviruses as either a source or destination hazard for beavers and other rodents is very low; the risk for humans is medium. Preventative measures for the risk management of hantaviruses as a destination and source hazard should be employed.

Risk options

Hantavirus-associated disease should be considered as a differential in any sick beaver or other rodent examined during reintroduction. Detailed pathological examination should be carried out of beavers found dead during and after translocation, and lung and kidney samples collected for diagnosis of hantaviral disease through pan-hantavirus PCR testing (Klempa *et al.,* 2006). Retrospective PCR testing of stored beaver tissue samples for hantavirus antigen or a pooled microarray for viral RNA as well as convenience blood sampling for serological conversion would be valuable to improve our understanding of hantavirus prevalence in beavers. Serological tests are currently not validated in beavers but could still provide useful information on past exposure. It is also recommended that DRM-PRHS protocols include surveillance for hantaviruses, for example screening of lung and kidney tissue from beaver carcases using pan-hantavirus PCR (Klempa *et al.,* 2006).

As hantaviruses can cause morbidity and mortality in humans, staff and volunteers working with beavers during reintroduction or post-release health surveillance should be reminded of the zoonotic risks and of the need to deploy good hygiene practices. Specifically, the wearing of masks to reduce the risk of aerosol inhalation when handling beavers is recommended.

5.4.2 Disease risk analysis for the destination hazard hantaviruses (Seoul orthohantavirus (SEOV) and Tatenale hantavirus (TATV))

Destination Hazard

Justification for Hazard Status

As data on the distribution of hantaviruses in rodent reservoirs in the UK and Europe is scant, beavers imported from Norway, or that have previously been imported from Germany (and currently free-living or in enclosures in Great Britain), may be naïve to

Seoul orthohantavirus (SEOV) and Tatenale hantaviruses (TATV) which may be present at the destination site(s). Hantaviruses should therefore be considered as a destination hazard for the translocation of beavers.

Risk assessment

Exposure assessment

Prevalence of the newly identified TATV found in field voles is not known but this virus is believed to be of long-standing endemicity in the UK so may be widely distributed throughout the country. It has not been reported outside the UK. Hantaviruses may have the potential to host-switch but, to date, there has been no evidence of TATV exposure or infection in other rodent species. Chronically infected rodents will shed the virus in urine, faeces and saliva. As beavers forage in woodland and scrub on riparian margins there is a low likelihood of a beaver being exposed to TATV and a very low probability of at least one beaver being infected. The only other hantavirus known to be present in wildlife in the UK is SEOV, identified in brown rats. Although there has been limited host switching from rats to other murines and shrews in China, SEOV has not, to date, been found in other species in Europe and there is no recorded infection of beavers. Disease surveillance work undertaken in 2021 found no evidence of hantavirus DNA in lung and/or kidney tissue of four free-living beavers found dead in England using pan-hantavirus PCR (Klempa *et al.,* 2006; Common, Gerard and Sainsbury, 2021). Nevertheless, as rats and beavers may occupy similar habitat, there is a medium likelihood of contact and exposure to SEOV through viral shedding via faeces, urine and saliva but a low likelihood of infection of beavers.

Consequence assessment

There is a very low likelihood of one beaver being infected with TATV and a low probability of one beaver being infected with SEOV. As no cases of disease have been recorded in beavers and it appears that accidental rodent spill-over hosts do not usually experience clinical disease, the likelihood of disease associated with hantaviruses in translocated beavers and failure of the reintroduction is very low.

Risk Estimation

There is a low likelihood of exposure of beavers to TATV and a very low likelihood of infection. There is a medium likelihood of exposure to SEOV and a low likelihood of infection with SEOV. The risk of morbidity and/or mortality is very low. The overall risk is very low.

Risk Management

Risk Evaluation

The level of risk associated with hantaviruses as either a source or destination hazard for beavers and other rodents is very low; the risk for humans is medium. Preventative measures for the risk management of hantaviruses as a destination and source hazard should be employed.

Risk options

Hantavirus-associated disease should be considered as a differential in any sick beaver or other rodent examined. Detailed pathological examination should be carried out of beavers found dead during and after translocation and samples collected for diagnosis of hantaviral disease. Retrospective PCR testing of stored beaver tissue samples for hantavirus antigen or a pooled microarray for viral RNA as well as convenience blood sampling for serological conversion would be valuable to improve our understanding of hantavirus prevalence in beavers, although serological tests are currently not validated for beavers. It is also recommended that DRM-PRHS protocols include surveillance for hantaviruses.

As hantaviruses can cause morbidity and mortality in humans, staff and volunteers working with beavers during reintroduction or post-release health surveillance should be reminded of the zoonotic risks and of the need to deploy good hygiene practices. Specifically, the wearing of masks to reduce the risk of aerosol inhalation when handling beavers is recommended.

5.4.3 Disease risk analysis for the population hazard SARS-CoV-2

Population Hazard

Justification for Hazard Status

SARS-CoV-2 is the name given to the newly evolved coronavirus which at the time of writing is responsible for a global pandemic of severe acute respiratory syndrome (SARS), known as Covid-19, in humans (Gorbalenya *et al.,* 2020). The virus belongs to the *Betacoronavirus* genus within the *Coronaviridae* family (Masters 2006; de Groot *et al.,* 2012). Coronaviruses are enveloped RNA viruses which cause numerous diseases across mammalian and avian species and have the largest genomes among all RNA viruses (Masters 2006; de Groot *et al.,* 2012). SARS-CoV-2 is a close relative of the human and bat severe acute respiratory syndrome coronaviruses (SARS-CoVs) which have given rise to several outbreaks of disease in people over the past 20 years (Lu *et al.,* 2020; Wassenaar and Zou 2020; Gorbalenya *et al.,* 2020).

Although some coronaviruses are host specific, others are found in a range of hosts (Drexler, Corman, and Drosten 2014). It appears that SARS-CoV-2 is likely to infect and replicate in numerous mammalian species other than humans and there is growing evidence to support its role as an anthropozoonosis, which we review here. Closely related coronaviruses to SARS-CoV-2 have been found to replicate in several free-living wild animal species. SARS-CoV-like viruses have been isolated from Himalayan palm civets (*Paradoxurus hermaphroditus)* which have been shown experimentally to be susceptible to disease from two separate virus isolates (Wu *et al.,* 2005; Guan *et al.,* 2003; Shi and Hu 2008). Evidence of infection with SARS-CoV has also been detected in raccoon dogs (*Nyctereutes procyonoides*) and numerous bat species (*Rhinolophus spp.*) although clinical disease was not reported (Cheng *et al.,* 2007; Wendong Li *et al.,* 2005; Guan *et al.,* 2003; Wassenaar and Zou 2020). These studies provide evidence that free-living wild animal species could be infected with the closely related SARS-CoV-2 and may be at risk of clinical disease as a result.

Preliminary reports have described the ability of SARS-CoV-2 to infect a number of non-human mammalian hosts. This includes companion animals, which have naturally acquired infection from owners, (domestic cats (*Felis catus*), domestic dogs (*Canis familiaris*)); members of the Musteloidea superfamily which have acquired infection naturally, such as American mink (*Neovison vison*) in fur farms across Europe (Oreshkova *et al.,* 2020) and zoo animals including Asian short clawed otters (*Aonyx cinereus*) (USDA Animal and Plant Health Inspection Service 2021d) and coatimundi (*Nasua nasua*) (USDA Animal and Plant Health Inspection Service 2021b) in the USA as well as experimentally-infected domestic ferrets (*Mustela putorius furo*); Carnivora, including numerous naturally infected zoo animals (Malayan tigers (*Panthera tigris jacksoni*), Amur tigers (*Panthera tigris altaica*), African lions (*Panthera leo*), snow leopards (*Panthera unicia*), pumas (*Puma concolor*), fishing cats (*Prionailurus viverrinus*), binturong (*Arctictis binturong*), racoon dogs and spotted hyenas (*Crocuta crocuta*) (USDA Animal and Plant Health Inspection Service 2021a; McAloose *et al.,* 2020; Wang *et al.,* 2020; Bartlett *et al.,* 2020; USDA Animal and Plant Health Inspection Service 2021c; Freuling *et al.,* 2020); non-human primates both naturally and experimentally infected including rhesus macaques (*Macaca mulatta*), long tailed macaques (*Macaca fascicularis*), African green monkeys (*Chlorocebus aethiops*) and common marmosets (*Callithrix jacchus*) (Deng *et al.,* 2020; S. Lu *et al.,* 2020); experimentally infected Egyptian fruit bats (*Rousettus aegyptiacus*); free-living, naturally-infected white-tailed deer (*Odocoileus virginianus*) (Chandler *et al.,* 2021) and experimentally-infected tree shrews (*Tupaia belangeris*) (Schlottau *et al.,* 2020). Several experimental studies which show the ability of SARS-CoV-2 to infect and cause disease in rodents also exist, including transgenic house mice, North American deer mice (*Peromyscus maniculatus*) (Griffin 2020) and Syrian hamsters (Griffin 2020; Chan *et al.,* 2020a; Sia *et al.,* 2020).

The virus has been shown to replicate effectively in the upper respiratory tract of ferrets (*Mustela* spp.) (Shi *et al.,* 2020). Two ferrets in the study developed fever and loss of appetite 10 to 12 days after experimental inoculation with the virus. Post-mortem

examination of these animals showed evidence of lymphoplasmacytic perivasculitis and vasculitis, increased numbers of type II pneumocytes, macrophages and neutrophils in the alveolar septa and alveolar lumen, and mild peribronchitis in the lungs, suggesting that ferrets are susceptible to the clinical disease associated with SARS-CoV-2. An outbreak of respiratory disease at two American mink farms in the Netherlands was thought to be associated with SARS-CoV-2 after clinically unwell animals at both farms tested positive for the virus (exact numbers not known) (ProMed International Society for Infectious Diseases 2020a). This suggests that other members of the Mustelidae family may be susceptible to the disease.

Findings by Shi and colleagues (Shi *et al.,* 2020) are supported by results of an experimental study by (Schlottau *et al.,* 2020), who reported that pigs and chickens were not susceptible to intranasal infection with SARS-CoV-2. However, the virus could replicate efficiently in ferrets and high viral RNA yields were detected in nasal washes from ferrets two to eight days post infection. Furthermore, 100% (n=3) of non-inoculated ferrets which were kept in contact with experimentally infected ferrets also became infected and viral RNA was present, detected in nasal washing fluids starting at 12 days post-contact. SARS-CoV-2 reactive antibodies were detected from day eight in the inoculated ferrets and in one contact ferret on day 21 (Schlottau *et al.,* 2020).

Schlottau *et al.,* (2020) also experimentally inoculated nine fruit bats intranasally with SARS-CoV-2, which resulted in transient respiratory tract infection. Virus replication was detectable in the nasal epithelium, trachea, lung and lung-associated lymphatic tissue, and infectious virus was isolated from the nasal epithelium and trachea of one animal after four days. Viral DNA was also detected in the nasal epithelium of one out of three in-contact bats after 21 days post-contact, suggesting that transmission is possible within this species (Schlottau *et al.,* 2020).

There is evidence to suggest that domestic cats are susceptible to Covid-19 infection and disease. Shi and colleagues (2020) showed that the virus replicates effectively in cats and can transmit between them via respiratory droplets. Moreover, two juvenile cats in the same study which were experimentally inoculated with SARS-CoV-2 were found to have severe lesions in the nasal and tracheal mucosal epithelia and lungs, highlighting their susceptibility to the disease (Shi *et al.,* 2020). This finding is supported by results of a preliminary study into populations of domestic cats in Wuhan, China. 102 serum samples were collected from domestic cats after the outbreak of Covid-19 in humans, and 14.7% (n=15) were positive for the receptor-binding domain (RBD) of SARS-CoV-2 by indirect enzyme-linked immunosorbent assay (ELISA), suggesting that SARS-CoV-2 infected the cat population in Wuhan during the outbreak (Zhang *et al.,* 2020). There are also several case reports of owned domestic cats testing positive for SARS-CoV-2, for example a case in Belgium, a case in Hong Kong, and two cases in the USA (ProMed International Society for Infectious Diseases 2020b; news.gov.hk 2020; USDA Animal and Plant Health Inspection Service 2020).

These feline cases are of further concern when considered alongside the numerous big cats infected naturally in zoos such as a captive Malayan tiger and African lion from which duplicate nasal and oropharyngeal swabs tested positive on qPCR for SARS-CoV-2 in the USA (McAloose *et al.,* 2020). The animals had shown mild respiratory disease signs after contact with an infected keeper along with one other Malayan tiger, two Amur tigers, and two other African lions (McAloose *et al.,* 2020).

Since the Covid-19 outbreak was first reported, several domestic dogs have tested positive for SARS-CoV-2, and all had been in contact with an infected owner. None of the dogs showed signs of clinical disease and, although one dog died during the infection period, it was 17 years old and had multiple underlying diseases which were attributed as the cause of death rather than Covid-19 (Goumenou, Spandidos, and Tsatsakis 2020). Over 3500 dogs, cats and horses *(Equus caballus)* showing respiratory disease (species numbers not reported) were screened for SARS-Cov-2 by IDEXX laboratories in South Korea in February and March 2020 and none were found to be positive (IDEXX 2020). This suggests that, even if it is possible for them to become infected, occurrences are likely to be rare given the 7,755 human patients with confirmed COVID-19 in Korea as of the 13th March 2020 (Covid-19 National Emergency Response Center 2020).

It has been shown that entry of SARS-CoV-2 to host cells requires binding of the viral spike protein (S) to the SARS-CoV receptor human angiotensin-converting enzyme 2 (hACE2) (Hoffmann *et al.,* 2020), as is the case for SARS-CoV (Kuba *et al.,* 2005; Li *et al.,* 2003). hACE2 transgenic mice have been used as a disease model and compared to wild-type mice (Bao *et al.,* 2020). When intranasally inoculated with SARS-CoV-2, hACE2 transgenic mice show clinical signs of weight loss along with multiple histopathological changes including interstitial pneumonia. Viral RNA was detected in the lungs of transgenic mice by quantitative PCR at one, three, five and seven days after inoculation but never in controls or wild-type mice. Infectious SARS-CoV-2 was isolated from inoculated transgenic mice, but never from wild-type mice or controls (Bao *et al.,* 2020). This study highlights the importance of the hACE2 enzyme for entry of SARS-CoV-2 into host cells, leading to infection.

A study by Chan *et al.,* (2020a) investigated the genetic components of several mammalian species with the aim to identify an appropriate animal disease model for SARS-CoV-2. They found that that rhesus macaque ACE2 is 100% identical to human ACE2 at the interface region. Syrian hamster and common marmoset ACE2 proteins were also found to be highly similar to human ACE2, each differing by only 3-4 mutations. Syrian hamsters were therefore identified as a possible disease model. In the experimental section of the study by Chan *et al.,* (2020a), Syrian hamsters were consistently infected with SARS-CoV-2 after nasal inoculation. Infected animals displayed a range of clinical signs including rapid breathing and weight loss. Histopathological changes two days after experimental inoculation included diffuse alveolar destruction and protein-rich fluid exudate, mononuclear cell infiltration, and alveolar collapse with haemorrhage. Bronchiolar lumens were filled with cell debris and epithelial cell swelling

and focal cilia loss, and mononuclear cell infiltration into the epithelium and lamina propria was noted in the trachea. Histopathological respiratory tract changes appeared to peak around seven days post-inoculation, with an increase in pulmonary cellularity and lung consolidation. After 14 days, only mild pulmonary congestion and inflammation were still detectable and gas exchange structures were restored to normal. Moreover, experimentally-infected hamsters consistently infected naïve hamsters housed within the same cage, resulting in similar clinical signs (Chan *et al.,* 2020a). This study provides evidence that hamster ACE2 can bind with SARS- CoV-2 S receptors enabling cell entry and infection.

It is likely that species susceptibility to SARS-CoV-2 is intrinsically linked to the similarity of their ACE2 gene to that of human ACE2. Although this has not been investigated in Eurasian beavers, it is feasible that they may be susceptible to SARS-CoV-2 given that other rodent species, namely Syrian hamsters, have an ACE2 gene similar enough to human ACE2 to allow infection. ACE2 has been sequenced in Ords kangaroo rat (*Dipodomys ordii*), a closely related species to the beaver (Doronina *et al.,* 2017), but not in any members of the Castoridae family (National Centre for Biotechnological Information 2020). The relatedness of the kangaroo rat ACE2 to human ACE2 has also not been analysed. Therefore, it is not possible at this stage to determine whether beavers are susceptible to SARS-CoV-2. As far as we are aware, to date there have been no coronaviruses isolated from or detected in beavers, nor have there been any coronavirus serological studies showing positive results in beavers. Guan *et al.,* (2003) tested numerous species from a wet market in China for coronavirus using PCR during the SARS-CoV-2 outbreak, including three beavers (*Castor fiber*), none of which were positive despite several other animals from different species from the same market testing positive. Nevertheless, the limited available research means that we cannot rule out the possibility that beavers are susceptible to SARS-CoV-2. SARS-CoV-2 is present both at the source and destination and therefore may represent a population hazard to reintroduced beavers.

Risk Assessment

Human Exposure Assessment

Human exposure is likely to occur through direct contact with other humans, aerosol droplets in the air spread by coughing or sneezing from an infected individual, or contact with contaminated surfaces (Kampf *et al.,* 2020; Rothan and Byrareddy 2020) as is the case for other members of the Coronaviridae family (de Groot *et al.,* 2012). The probability of human exposure to SARS-CoV-2 is medium. Human infection is thought to occur through contact of viral particles with exposed mucous membranes including the eyes, nose and mouth (Lu *et al.,* 2020; Zheng, 2020). Faecal-oral transmission may also be possible (Yeo, Kaushal, and Yeo 2020; Xiao *et al.,* 2020; Zheng 2020). The probability of infection after exposure is high.

Viral RNA has been detected in nasal washes of ferrets inoculated with the virus, as well as in several upper respiratory tract structures of inoculated and exposed cats (Shi *et al.,* 2020). SARS-CoV-2 has also been detected in faeces of humans, a Malayan tiger and an African lion and is thought to be present in the faeces of bats (Wassenaar and Zou 2020; Holshue *et al.,* 2020; Calle 2020), therefore faecal-oral transmission may also be possible, as for other closely related coronaviruses (Yeo, Kaushal, and Yeo 2020). Rectal swabs taken from experimentally inoculated ferrets tested positive for viral RNA, though at lower levels than nasal washes. Infectious virus was not detected in any rectal swabs. In the same study, rectal swabs from experimentally-inoculated beagles also tested positive for viral RNA (Shi *et al.,* 2020). The probability of dissemination through the human population following infection is estimated to be high.

Beaver Exposure Assessment

There is evidence to show exposure of free-living white-tailed deer across North America to SARS-CoV-2 through a surveillance scheme (Chandler *et al.,* 2021) and several studies suggest and evaluate the possible risk of spillback into wildlife populations from humans through either modelling or disease risk assessments (Franklin and Bevins 2020; Delahay *et al.,* 2020; Common *et al.,* 2021). This suggests the possibility that free-living beavers may have been exposed to SARS-CoV-2 before translocation and that they may be exposed after release when free-living in Great Britain. There is a very low probability that free-living beavers in Norway or Great Britain will have been exposed to SARS-CoV-2 before translocation if they are chosen for translocations. If captive beavers are chosen for translocation, there is a medium likelihood of exposure to SARS-CoV-2 because there is evidence to show natural infection of captive animals from humans across the world (USDA Animal and Plant Health Inspection Service 2021a, 2021d, 2021c; McAloose *et al.,* 2020).

Coronaviruses have been shown to persist on inanimate surfaces for up to nine days and, at low temperatures, persistence can be as long as 28 days (Ijaz *et al.,* 1985; Kampf *et al.,* 2020). Exposure through contact with infected surfaces could occur in beavers, as can occur for humans (Kampf *et al.,* 2020). During translocation of beavers, there are several opportunities during which the beavers could be exposed to SARS-CoV-2, mainly through direct contact with infected humans, or contacting surfaces contaminated by infected humans. Beavers could be exposed at capture, during the quarantine period in captivity, during transport and at release. There is a medium probability that beavers will be exposed to SARS-Cov-2 during the translocation process. There is a very low probability that beavers will be exposed to SARS-CoV-2 in the wild in Great Britain after translocation.

There is no evidence to suggest that, if beavers are exposed, they will become infected, but three other rodent species have been infected after experimental intranasal inoculation (Sia *et al.,* 2020; Chan *et al.,* 2020a; Griffin 2020), and the lack of research in this area means the eventuality of beavers becoming infected cannot be ruled out. There is a medium likelihood that beavers will become infected with SARS-CoV-2 if exposed. The

probability of the virus being disseminated amongst the reintroduced beaver population is medium since rodent to rodent transmission has been shown for other rodents experimentally (Chan *et al.,* 2020a). Animal to animal transmission has also been shown for domestic cats and ferrets under experimental conditions and naturally across zoological collections in the USA (USDA Animal and Plant Health Inspection Service 2021a; J. Shi *et al.,* 2020)

Consequence Assessment

The pathogenesis of SARS-CoV-2 in other rodents, particularly free-living wild rodents, is unclear although the literature so far suggests that severe disease and death is unlikely to occur after exposure. Covid-19 disease has been shown to occur in one non-transgenic species of rodent infected with SARS-CoV-2 in the laboratory, the Syrian hamster (Chan *et al.,* 2020a), although histopathological signs of disease were also demonstrated in experimentally-infected deer mice despite a lack of clinical disease (Griffin 2020). Wild-type house mice did not appear to be susceptible in a separate study (Bao *et al.,* 2020), implying that susceptibility is likely to be variable among rodent species. No coronavirus has ever been detected in a beaver.

There is a low likelihood that beavers will be susceptible to clinical disease if infected. Clinical signs in infected Syrian hamsters were considerable but did not result in mortality. Responses in other susceptible species have been variable and the limited available research suggests that severity may vary on a case-by case basis. It has been hypothesised that higher infective doses may lead to increased disease severity in humans: human patients with severe clinical signs had higher nasal viral loads than those with mild clinical signs (Liu *et al.,* 2020). However, until experimental challenge studies are undertaken for SARS-CoV-2, this will remain speculative. At this stage we estimate that there is a low probability of severe disease and mortality in beavers if they were to become infected.

The likelihood of Covid-19 disease outbreak within the translocated beaver population as a result of exposure to SARS-CoV-2 and the failure of the translocation is very low. The likelihood of severe economic and environmental consequences as a result of this failed translocation is very low.

Risk Estimation

At the time of writing, the probability of exposure of humans is medium and probability of infection after exposure is medium. There is a high probability of dissemination through the human population. There is a medium likelihood that captive beavers will be exposed to SARS-CoV-2 before translocation and during the translocation process through contact with workers at different stages of the translocation process and a very low likelihood of exposure and infection in free-living beavers before translocation or at the reintroduction site. There is a medium likelihood of dissemination within the beaver population at the

release site. The probability of an outbreak of disease in the beaver population and the failure of the translocation is very low. The overall risk is estimated to be VERY LOW.

Risk Management

Risk Evaluation

Simple preventative measures are likely to reduce the risk of SARS-CoV-2 to translocated Eurasian beavers.

Risk Options

The most important preventative management measure would involve reducing the exposure of translocated beavers to SARS-CoV-2 through direct contact. Since the majority of naturally occurring animal cases have been thought to have occurred as a result of anthropozoonosis, it is important to prevent exposure of beavers to infected humans. Simple measures such as appropriate personal protective equipment (PPE) for personnel in contact with beavers is likely to reduce the probability of exposure.

Moreover, since SARS-CoV-2 is active for long time periods on inanimate surfaces, proper disinfection of traps, captive enclosures, food bowls and any other possible fomites is essential to reduce the probability of transmission between humans and beavers. It is important that this is followed at every stage of the translocation pathway, including initial trapping, transport, captivity and release. Disinfectants containing 0.1% sodium hypochlorite or 62-71% ethanol lead to effective inactivation of the virus and so would be appropriate (Kampf *et al.,* 2020). Notwithstanding, all specific products should be analysed to ensure they are safe and licenced for use around animals.

5.4.4 Disease risk analysis for the population hazard *Brucella* spp.

Source Hazard

Justification for Hazard Status

Brucella is a genus of facultative intracellular bacteria containing nine species and multiple biovars of human concern, as well as a number of other atypical species (Zheludkov and Tsirelson 2010; Eisenberg *et al.,* 2017; K. Mühldorfer *et al.,* 2017; Eisenberg *et al.,* 2012; Jahans, Foster, and Broughton 1997). *Brucella* spp. are not host specific and have a wide geographic distribution. The associated disease, brucellosis, is responsible for considerable economic losses in livestock across the globe as a result of reproductive failure, and is a public health concern given the high susceptibility of humans to infection

and multi-organ disease (Hammerl *et al.,* 2017; Zheludkov and Tsirelson 2010). The species mainly associated with human infections are *B. melitensis* transmitted from sheep and goats, *B. abortus* from cattle and *B. suis* from pigs (Hammerl *et al.,* 2017) although other *Brucella* spp. have recently been implicated in human brucellosis cases including *B. neotomae*, associated with rodent infection, and *B. inopinata* (The Centre for Food Security and Public Health 2018).

Numerous wild animal species have been found to be susceptible to infection with *Brucella* spp. and associated disease; some have been implicated as reservoirs, being natural carriers of infection without associated disease (Vana 1980). Rementsova (1984) found that 24 wild animal species were naturally infected with *Brucella* spp. and 33 more could be experimentally infected (Rementsova (1984) cited by: Zheludkov and Tsirelson, 2010). Since then, many more wild animals have been found to be naturally infected, including rodents, and epidemiological studies have suggested that there is no difference in the pathogenicity or transmission rate of *Brucella* spp. between livestock and wild animals, highlighting the potential for transmission between these two animal groups (J Godfroid 2002).

The first report of *Brucella* spp. in a rodent was published in 1957: *B. neotomae* was detected in free-living desert wood rats (*Neotoma lepida*) in North America (Stoenner and Lackman 1957). Numerous other studies have detected *Brucella* spp., or antibodies to *Brucella* spp., in free-living rodents without associated disease, highlighting their potential to act as reservoirs. A seroprevalence to *Brucella* spp. of 10.2% (7/68) was detected in free-living rodents (species not specified) with no reported clinical signs of disease in South Korea using C-ELISA (Truong *et al.,* 2011). None of the rodents in this study were positive for *Brucella* spp. on PCR or bacterial culture, suggesting past exposure rather than current infection. However, ELISA results from the study by Truong *et al.,* (2011) should be interpreted with caution as the authors highlight that false positives can occur as a result of infection with other gram-negative bacteria such as *Yersinia enterocolitica* and *Escherichia coli* (Truong *et al.,* 2011). Hammerl *et al.,* (2017) sampled 537 small, free-living mammals for *Brucella* spp. using tissue PCR; 76 animals (14.2%) were found to be positive. This included 12.3% (14/114) of voles (*Microtus* spp.), 17.1% (31/181) of bank voles and 17.1% (22/129) of mice (*Apodemus* spp.) sampled (Hammerl *et al.,* 2017). *Brucella* spp. have also been isolated from several rodent species in the former USSR without reported clinical signs, including the striped field mouse, house mouse, wood mouse (*Apodemus sylvaticus*), common vole (*Microtus arvalis*) and Eurasian harvest mouse (*Micromys minutus*) (Vershilova, Liamkin, and Malikov 1983). In Queensland, Australia, Tiller *et al.,* (2010) isolated seven new *Brucella* spp. strains from free-living, native wild rodents: four allied rats (*Rattus assimilis*), two large climbing rats (*Melomys cervinipes*) and one small climbing rat (*Melomys lutillus*) and, in Kenya, *B. suis* was isolated from free-living African grass rats (*Arvicanthus niloticus*) and Natal multimammate mice (*Mastomys natelensis*) (Heisch *et al.,* 1963). Moreover, in Venezuela numerous studies have found seropositivity for *Brucella* spp. in capybara (*Hydrochoerus hydrochaeris*) (Bello *et al.,* 1976; Plata Garcia 1973) and *B. suis* and/or *B. abortus* was

subsequently isolated from tissues of 11.4% (23/201) of free-living capybara culled around cattle ranches (Lord and Flores 1983). These studies highlight that rodents from multiple families are susceptible to infection with *Brucella* spp.. To the best of the authors' knowledge, there are no reports of *Brucella* spp. infection in beavers; one study in Alabama, North America, tested a single beaver (*C. canadensis*) as part of a surveillance study of wildlife located near cattle farms, and found it to be negative (Schnurrenberger *et al.,* 1985). However, given the broad range of susceptible rodent hosts and the lack of targeted surveillance of *Castor* spp., it is likely that beavers are susceptible to infection with *Brucella* spp..

Prevalence of *Brucella* spp. has been studied in captive collections. A serological survey in a zoo in Chile found that seven out of 158 animals tested were positive for *Brucella* spp., including a wild boar (*Sus scrofa*), a domestic goat (*Capra hircus*), two capuchin monkeys (*Cebus albifrons*), a tiger (*Panthera tigris*) and a jaguar (*Panthera onca*), indicating past infection (Olivares, Riveros, and Pinochet 1993). Prevalence of *Brucella* spp. antibodies in captive animals have also been reported from zoos in Brazil (Minervino *et al.,* 2018), and *Brucella* spp. have been isolated from wild animals in captive collections in Russia (Kulakov *et al.,* 2015) and Germany (Whatmore *et al.,* 2015; Fischer *et al.,* 2012; Eisenberg *et al.,* 2017). One study found over 20 different atypical *Brucella* spp. isolates from animals in the same zoological collection in Germany (Eisenberg *et al.,* 2020), and also noted the same isolate from a captive panther chameleon (*Furcifer pardalis*) that had previously been reported in a ribbontail ray (*Taeniura lymma*) (Eisenberg *et al.,* 2017) and numerous frog species (Eisenberg *et al.,* 2012; K. Mühldorfer *et al.,* 2017) from the same collection, although transmission routes could not be confirmed (Eisenberg *et al.,* 2020).

Brucella spp. are widely-distributed globally with the highest incidence of brucellosis in livestock reported in the Middle East, the Mediterranean region, sub-Saharan Africa, China, India, Peru, and Mexico (World Organisation for Animal Health (OIE) 2021). Several countries are considered to be free from brucellosis, including Canada, Japan, Australia and New Zealand, as well as a number of countries in Western and Northern Europe including the UK and Norway (World Organisation for Animal Health (OIE) 2021). Germany has been officially free from bovine and ovine brucellosis since 2000 although sporadic cases since then have been reported, particularly in wildlife (European Centre for Disease Prevention and Control 2019a; Al Dahouk *et al.,* 2005). Eight European Union member states reported no brucellosis cases in the last epidemiological report by the European Centre for Disease Prevention and Control (ECDC) (European Centre for Disease Prevention and Control 2019a). In the UK, brucellosis of cattle (caused by *B. abortus*) is a notifiable disease and the country has been officially free from brucellosis since 1985 excepting two contained outbreaks traced to imported cattle (Animal and Plant Health Agency 2021a).

Given the widespread infection of rodent species with *Brucella* spp., beavers are likely to be susceptible to *Brucella* spp. infection, and may be a reservoir host. Given that the UK is officially free from certain *Brucella* spp., that *Brucella* spp. are prevalent in mainland

Europe as well as in captive collections across the world, that the beavers free-living or in enclosures in Great Britain were, in some cases, known to originate from continental Europe, *Brucella* spp. should be considered to be a source hazard for the translocation of beavers.

Risk Assessment

Release Assessment

The most common transmission route of *Brucella* spp. infection in animals is through ingestion of the bacteria, although sexual transmission and transmission via wounds and through mucous membranes have also been reported (Vana 1980; Zheludkov and Tsirelson 2010). Vertical transmission is also important in the epidemiology of the bacterium and *B. abortus* has been detected in vaginal discharges of infected coyotes (*Canis latrans*) 11 days post-partum (Davis *et al.,* 1979). It has been observed that carnivores are often exposed to and infected with *Brucella* spp. through ingestion of aborted foetuses and foetal membranes in areas where brucellosis is endemic (Davis *et al.,* 1979). In humans, food-borne transmission in association with domestic livestock is the most common route of infection, through ingestion of unpasteurised dairy products and infected meat (Fischer *et al.,* 2012). Inhalation of the bacteria and infection through wounds can also occur, usually in laboratory or slaughterhouse workers, veterinarians and meat-packing employees (Centers for Disease Control and Prevention 2021a). Although there is evidence of direct transmission of infection from wildlife to humans (through ingestion of wild boar and marine mammals (Starnes *et al.,* 2004; Sohn *et al.,* 2003)), indirect transmission with domestic animals acting as an intermediate host is more likely (Zheludkov and Tsirelson 2010). In particular, rodents have been implicated in the transmission of *Brucella* spp. to domestic animals, providing a route of infection to humans (Zheludkov and Tsirelson 2010). Small rodents are most likely to be exposed through ingestion of contaminated food or water, or direct contact with other infected hosts (Hammerl *et al.,* 2017); *Brucella* spp. have been demonstrated to be able to survive in soil and water for several weeks (Scholz *et al.,* 2008). The wide distribution of infection with *Brucella* spp. in amphibians across the globe and the evidence of infection in a ray provided by Eisenberg *et al.,* (2017) do suggest that water-borne transmission is possible (Eisenberg *et al.,* 2017), which could be pertinent for aquatic rodents such as beavers. It is likely that beavers would be exposed to *Brucella* spp. in contaminated water sources or vegetation in endemic areas. Several species of bloodsucking arthropods have been identified as natural carriers of *Brucella* spp. and transmission shown under experimental conditions (Zheludkov and Tsirelson 2010; Huang *et al.,* 2020; Wang *et al.,* 2018), for example *B. abortus* from pasture ticks (*Dermacentor nuttalli* and *Hyalomma marginatum*) to guinea pigs (*Cavia porcellus*) (Pritulin 1954). Nevertheless, it has been suggested that arthropods are unlikely to play an important role in the epidemiology of *Brucella* spp. (The Centre for Food Security and Public Health 2018).

There is a very low likelihood that beavers free-living in Norway or Germany will have been exposed to *Brucella* spp. known to be associated with brucellosis in humans and livestock, given that these countries are considered to be free from these bacteria (World Organisation for Animal Health (OIE) 2021). There is a medium likelihood of exposure of beavers in mainland Europe to a novel *Brucella* spp. considering the report of a 14% (n=76) prevalence of novel *Brucella* spp. in small mammals in Germany published by Hammerl *et al.,* (2017). There is a medium likelihood that beavers held in enclosures in Great Britain, originating from Eastern Europe or with an unknown history, and those which have been held in zoological collections in contact with exotic species, will be exposed to either a novel *Brucella* spp. or a *Brucella* spp. known to be a causative agent of brucellosis. Poland is considered to be an area at risk of brucellosis (Centers for Disease Control and Prevention 2021b) and several beavers held in enclosures in Great Britain are known to have been sourced from Poland and therefore may have been exposed.

As previously discussed, to the best of the authors' knowledge, there are no reports of *Brucella* spp. infection in beavers, although, given the number of rodent species in which *Brucella* spp. has been detected, it is likely that beavers are susceptible to infection. No screening for *Brucella* spp. was undertaken for beavers in the Knapdale, Tayside or River Otter beaver reintroductions in Great Britain and so the infection status in these beavers is unclear.

Overall, there is a low likelihood that free-living beavers in Norway or those sourced from Germany will be infected with *Brucella* spp. at the time of translocation. There is a medium likelihood that beavers translocated from enclosures in Great Britain originating from Eastern Europe, or with an unknown history, and those which have been held in zoological collections in contact with exotic species will be infected with *Brucella* spp. at the time of translocation.

Exposure Assessment

If translocated beavers are infected with *Brucella* spp., there is a high likelihood of exposure and infection of domestic livestock with *Brucella* spp. in England. Rodents are thought to shed the bacterium in saliva, urine and faeces (Rajashekara *et al.,* 2005; Bosseray *et al.,* 1982; Grilló *et al.,* 2012) and could contaminate water sources and vegetation through these secretions, as well as possible contamination from infected carcases. This could lead to exposure of susceptible wild animals (particularly sympatric rodents) which, in turn, could expose domestic animals through establishment of a wildlife reservoir, or the beavers themselves could directly expose domestic livestock such as grazing animals in the local vicinity. Stowaway, infected arthropods translocated alongside the beavers may also transmit *Brucella* spp. through feeding on animals at the release site, although there is lack of evidence for the role of vector transmission in a natural setting. Once exposed, there is a high likelihood of infection of wild and domestic mammals at the release site, and dissemination through these populations.

There is a medium likelihood that arthropods within Great Britain will be exposed, through feeding on beavers, and infected with *Brucella* spp.. If one infected translocated beaver is bacteraemic when released, arthropod vectors residing at the destination site could be exposed through feeding on this animal.

Consequence Assessment

There is a low or medium likelihood that a translocated beaver will be infected with *Brucella* spp..

Although *Brucella* spp. have largely been reported to be associated with disease-free infection in rodents, there are contrasting reports. Hubálek, Scholz and Sedlác (2007) identified *Brucella* spp. as associated with systemic multi-organ disease in four free-living common voles captured and euthanised in the Czech Republic. The animals had oedematous extremities, abscessation of the skin and lymph nodes and other non-specific disease signs (Hubálek, Scholz, and Sedlác 2007), and the isolated bacteria lead to disease in experimentally inoculated captive laboratory mice (*Mus musculus*). This contrasts with other reports of disease-free infection in the same species (Vershilova, Liamkin, and Malikov 1983; Hammerl *et al.,* 2017), which could reflect differences in the pathogenicity between species of *Brucella* (the species isolated from sick voles was not reported), or differences in host immune responses to infection since voles in the study by Hubálek *et al.,* (2007) were part of a capture and release scheme, three out of four of the diseased animals were known to have been captured at least once previously during the same year, and it is possible that the stress of capture could have led to compromised immune function and disease. *B. suis* has also been isolated from a brown rat caught in Germany with thoracic abscesses (Stoll and Manz 1971). Disease following infection with *Brucella* spp. cannot be ruled out in translocated beavers, particularly given that the translocation is likely to lead to stress and subsequent immunocompromise in these animals (Dickens, Delehanty, and Michael Romero 2010; Dickens, Delehanty, and Romero 2009).

If *B. abortus*, *B. melitensi* or *B. suis* are released, there are likely to be sizeable detrimental consequences. Given that the UK holds ‘free-from brucellosis’ status (Animal and Plant Health Agency 2021a), the economic costs of the introduction of one of these agents will be substantial as a result of livestock losses through culling, disease and poor reproductive performance. Moreover, exposure of livestock to *Brucella* spp. would generate an important human health risk; brucellosis in humans can lead to multi-organ disease with long-term effects on health, and rarely death (around 2% of cases) (Centers for Disease Control and Prevention 2021a). Treatment is possible with six to eight weeks of multiple antibiotics (Centers for Disease Control and Prevention 2021a), but this presents a further cost to health authorities. There is, therefore, a high likelihood of substantial biological and economic consequences as a result of *Brucella* spp.-associated disease in people, and a medium likelihood of environmental and ecological consequences as a result of the establishment of reservoirs.

Risk Estimation

There is a very low probability that Norwegian and Great British free-living beavers will have been exposed to *Brucella* spp. and a low likelihood that these beavers will be infected at the time of translocation. There is a medium likelihood that beavers living in enclosures in Great Britain originating from Eastern Europe or with an unknown history, and those which have been held in zoological collections in contact with exotic species, have been exposed to *Brucella* spp., and a medium likelihood that they are infected.

There is a medium likelihood that arthropods within Great Britain will be exposed and infected with *Brucella* spp.. There is a high likelihood of exposure of wild and domestic mammals at the release site if an infected beaver is released. If exposed, there is a high likelihood of infection in livestock and domestic mammals at the destination site, a high likelihood of dissemination, and a low probability of resulting human infection. There is a high probability of disease in humans after infection. There is a high probability of substantial biological and economic consequences as a result of *Brucella* spp. controls in livestock and treatment of people, and a medium likelihood of environmental and ecological consequences as a result of the establishment of reservoirs. The overall risk from disease is estimated to be LOW for beavers originating from Norway or free-living in Great Britain and MEDIUM for beavers living in fenced enclosures in Great Britain originating from Eastern Europe or with an unknown history and those which have been held in zoological collections during their captivity period.

Risk Management

Risk Evaluation

Given that the risk from *Brucella* spp. is estimated to be medium, management measures should be implemented.

Risk Options

It would be preferable to use beavers already free-living in Great Britain for conservation translocations because these beavers have a lower likelihood of being exposed to and infected with *Brucella* spp..

If it is essential to release beavers housed in enclosures or those which have had contact with exotic species, then it is recommended that culture of cloacal swabs for *Brucella* spp. is performed to provide information on whether the animal is currently infected with this bacterium. Blood samples could also be taken for assessment of past exposure to *Brucella* spp. through serology. It is recommended in other species that blood is analysed using three classical *Brucella* spp. tests in order to maximise the specificity of anti-brucellae antibodies; Slow Agglutination of Wright (SAW), EDTA-modified Slow Agglutination of Wright (SAW-EDTA) and Rose Bengal Test (RB) (Tryland *et al.,* 2001;

Jacques Godfroid, Nielsen, and Saegerman 2010), and if positive results are obtained attempts should be made to isolate the bacterium (Jacques Godfroid, Nielsen, and Saegerman 2010). However, these tests are not validated or commercially available for beavers. A complement fixation test is available for cattle, sheep and pigs and is recommended by the OIE as the test of choice for assessing whether a population or individual is negative for past exposure to *Brucella* spp., although it does not differentiate between *Brucella* species (OIE 2018). This test and a serum agglutination test are available through the Animal and Plant Health Agency (APHA) in England. Neither of these tests are validated in beavers but could provide useful information on past exposure. However, positive serological tests do not necessarily indicate that a beaver is currently infected with or can be a reservoir for *Brucella* spp. and therefore should be interpreted with caution.

Routine *Brucella* spp. surveillance occurs in domestic livestock in the UK (Animal and Plant Health Agency 2021b), and the most likely exposure route of humans is through ingestion of contaminated animal products. Therefore, the risk of human exposure and infection would remain low. Nevertheless, post-release health surveillance should be undertaken for *Brucella* spp. in any beavers found dead either free-living or in enclosures in Great Britain. This can be performed through culture of swabs from visceral organs on Farrell medium. Any cultured bacteria should be further biotyped (Jacques Godfroid, Nielsen, and Saegerman 2010).

5.4.5 Disease risk analysis for the carrier hazard gram-negative bacteria

Carrier Hazard

Justification for Hazard Status

Gram-negative bacteria are a classification of bacteria which do not retain the crystal violet (gentian) stain used in the Gram staining process (Beveridge 2001). Like most bacteria, they can cause infections throughout the body. Gram-negative bacteria are enclosed in a protective capsule that helps to prevent them from being ingested by white blood cells. When disrupted, this membrane releases toxic substances, called endotoxins, which contribute to the severity of clinical signs (J. H. Kim *et al.,* 2015). Gram-negative bacteria that can colonise the gastrointestinal tract can be part of the normal flora. However, under certain circumstances, commensal gram-negative bacteria can become secondary pathogens and their relationship with the host might range from mutualism to parasitism (Kang *et al.,* 2018). Members of the gut community might express their pathogenic potential in immune-compromised animals, such as those in captive facilities (Pace *et al.,* 2019).

Gram-negative aerobic bacteria are commonly isolated from both captive and free-living rodents. For example, gram-negative infections have been reported from numerous captive rodents globally (Hardgrove *et al.,* 2021). *E. Coli* infection has been detected in crested and North American porcupines (*Hystrix cristata* and *Erethizon dorsatum* respectively) and Patagonian mara (*Dolichotis patagonum*) (Barigye *et al.,* 2007; Hardgrove *et al.,* 2021; Leotta *et al.,* 2006); *Hafnia alvei* and *Klebsiella oxytoca* in captive Balkan snow voles (*Dinaromys bogdanovi*); *Klebsiella pneumoniae* in crested porcupines; and *Enterobacta* spp. in Balkan snow voles and African rope squirrels (*Funisciurus substriatus*) (Lukac *et al.,* 2017; Craig *et al.,* 1998).

Two families are of concern: *Enterobacteriaceae* and *Epsilonproteobacteria*. Some genera such as *Yersinia* spp. (evaluated elsewhere in this report), *Salmonella* spp., *Shigella* spp. and *Escherichia coli* are considered to be important zoonoses, associated with severe morbidity and mortality (Kang *et al.,* 2018). Other genera of interest are: *Klebsiella, Enterobacter, Citrobacter, Proteus, Serratia, Campylobacter and Helicobacter* spp.. Numerous species, serotypes and serovars of varying pathogenicity and host specificity exist within each genus and *E coli* is additionally characterised by differing pathotypes expressing different virulence factors such as EPEC (enteropathogenic *E. coli*) and ETEC (enterotoxigenic *E. coli*) (Kang *et al.,* 2018), of which VTEC O157 is considered to be the most common cause of foodborne illness in humans (FSA 2021).

Disease in the host animal may occur when gram-negative bacteria either overgrow within the gastrointestinal tract or colonise a new body compartment (Bublitz *et al.,* 2014)**.** Survival of some species of gram-negative bacteria in the environment may be prolonged for several months (Kramer, Schwebke, and Kampf 2006) with direct or indirect infection of new hosts via the faecal-oral route or, occasionally, via mucous membranes (Gaffuri and Holmes 2012).

The role of free-living animals in maintaining reservoirs of gram-negative enteric species pathogenic to humans and livestock is unclear. There are few reports of infection and disease associated with *E. coli* in free-living wild mammals, although VTEC O157 has been isolated from rats (Nielsen *et al.,* 2004), wild boar in Sweden (Wahlström *et al.,* 2003); rabbits in Great Britain (*Oryctolagus cuniculus*) (Simpson 2008), and deer (*Cervida*e spp.) in Germany and Spain (Speck 2012a). Simpson (2008) reviewed wildlife cases of *E. coli* O157 infections in wildlife and concluded that free-living wild animals do not play a significant role in epidemiology. *E. coli* spp. are commonly found as asymptomatic infections in the small and large intestines of many mammal species, with higher prevalence levels in carnivores compared to omnivores and herbivores for reasons that are not well understood (Speck 2012a). Extra-intestinal disease in host animals usually results from translocation of normal intestinal flora rather than exogenous infection. It is thought that factors such as stress and gut dysbiosis, for example as a result of a predominantly grain-based diet, can contribute to enteric overgrowth of *E. coli* and disease in domestic livestock (Speck, 2012).

Similarly, sub-clinical carriage of *Salmonella* spp. appears to be common in free-living wild animals (Gaffuri and Holmes 2012). *Salmonella* spp., including some found in humans and/or livestock, have been reported in rodents, badgers (*Meles meles*) and red foxes (*Vulpes vulpes*) with no macroscopic or microscopic lesions consistent with salmonellosis (Millán *et al.,* 2004; Handeland *et al.,* 2008; Chiari *et al.,* 2014; Euden 1990). Prevalence estimates for wild or captive rodents are relatively scarce, variable among geographic regions, and the numbers of studies as well as the prevalence seem to have decreased over time. In general, *Salmonella* shedding rate estimates in rodents are in the range of 1 to 15% (Healing 1991; Meerburg *et al.,* 2006; Singh, Sethi, and Sharma 1980; Shimi, Keyhani, and Hedayati 1979). The majority of infections in mice and rats are asymptomatic. However, salmonellosis has been reported in laboratory rodents and several species of free-living wild mammals and is most common between November and April in Europe (Gaffuri and Holmes 2012). Healing and Greenwood (1991) found that rodents living near a poultry farm in Dorset were reservoirs of some *Campylobacter* spp. but not *Salmonella* spp. detected in poultry on the same farm and proposed that rodents were not important reservoirs for *Campylobacter* and *Salmonella* spp.. However, Meerburg and Kijlstra (2007) reviewed several studies of *Campylobacter* and *Salmonella* spp. infections of small rodents and concluded that, in agricultural environments, rodents may maintain or amplify reservoirs of *Campylobacter* and *Salmonella* spp. infection. In addition, it is reported that *Salmonella* spp. have repeatedly been isolated from wild mice and rats on farms and in food production environments (Hoelzer, Switt, and Wiedmann 2011).

Chronic infection with *Helicobacter* spp. is usually asymptomatic in immunocompetent hosts (Whary and Fox 2004) and disease occurs when host immunoregulation breaks down (Harbour and Sutton 2008). In rodents, naturally-acquired infections are common and persistent with prolonged shedding (Whary and Fox 2004). *Helicobacter* spp. infections have been reported with no association between infection and clinical signs of disease, gross or microscopic, in free-living red foxes in Sweden, Slovenia and Turkey (Mörner *et al.,* 2008) and in 60% (n=93/154) of vertebrate species studied in a captive zoological collection over 10 years (Schrenzel *et al.,* 2010).

Klebsiella pneumoniae can be found in high densities in the natural environment (Seidler, Knittel, and Brown 1975) and has clinical significance as an opportunistic pathogen of rodents; immunocompetent animals do not usually display clinical signs (Baker 1998). Intermittent and chronic stress (physical, psychological or social) is known to cause altered immunity and increased vulnerability to disease in rodents (Bailey, Engler, and Sheridan 2006; Bartolomucci 2007). Specifically, stress is known to cause altered faecal microbiota (O'Mahony *et al.,* 2009) with increased levels of *Klebsiella* spp. in the jejunum, ileum and caecum of mice (Tannock and Savage 1974). In free-living mammals, an outbreak of *Klebsiella pneumoniae* has been reported in wild hares (*Lepus sinensis*) in China, causing acute pneumonia, diarrhoea and death within one to two days (Du *et al.,* 2014). *Klebsiella pneumoniae* was also isolated in pure culture from three organs in a free-living dormouse (1730/16) in 2016 (Jaffe, Januszczak, and Sainsbury 2017). Associated haemorrhages in

the gastrointestinal tract were noted, suggesting that *Klebsiella pneumoniae* may have contributed to the death of this dormouse.

Hafnia alvei is an opportunistic pathogen of humans, having been implicated as the cause of gastrointestinal tract disease, as well as extraintestinal infections, associated with nosocomial multiple infections (Okada and Gordon 2003). *H. alvei* is found in water and is frequently isolated from dairy, meat and fish products and is classed as one of the major bacterial food contaminants (Lindberg *et al.,* 1998). Okada and Gordon (2003) reported that 158 strains of *H. alvei* were isolated from faecal or intestinal swabs of 1488 hosts representing 97 birds, 78 mammals, 54 reptiles, eight frogs, six freshwater fish and 11 arthropod species from over 190 localities throughout Australia between 1993 and 2001. This indicates the wide host range of this bacterium. The evidence for *H. alvei* being pathogenic is not conclusive but it is possible that it causes gastro-enteritis and/or septicaemia in many types of animals, including humans, secondary to stressors. Nevertheless, in most cases *H. alvei* is probably commensal (Janda and Abbott 2006).

Infectious disease is a common diagnosis in free-living beavers. Infectious disease was associated with the death of 50% (n=22) of beavers following translocation from Germany to the Netherlands between 1988 and 1994 (Nolet *et al.,* 1997) and 23.3% (n=60) beavers found dead in Germany and Austria between 1990 and 2003 (Steineck and Sieber 2003); however, there may be uncertainty as to the causative agent. Gram-negative bacteria have rarely been found in association with beaver deaths: one of the beavers examined by Steineck and Sieber (2003) was infected with an unspecified *Salmonella* spp.; *S. enteriditis* was identified in a co-infection in a Canadian beaver which died with streptococcosis at Berne Zoo (Dollinger *et al.,* 1999); a wild-caught beaver from Norway (M08K33), which died during quarantine in the UK with severe enteritis and focal hepatic necrosis, was found to have an *E. coli* bacteraemia, although histopathology was reported to be suggestive of yersiniosis (Cranwell 2009a) and Pilo *et al.,* (2015) reported the death of a free-living beaver in Switzerland in 2013 in association with *Klebsiella pneumoniae*. In addition, two of three sub-adult beavers killed in road traffic collisions in Germany were infected with unspecified *E. coli* and *Shigella* spp. (Pratama *et al.,* 2019), although it is not known whether the infections in these animals were associated with disease, and Lauková *et al.,* (2015) identified *Enterococcus* spp. with potential virulence factors in pooled faecal samples from 12 free-living beavers in Poland.

Neither *Salmonella* spp. or *Campylobacter* spp. were found on culture of faecal samples from free-living beavers (n = 65) in Great Britain screened during survey work of populations in Knapdale and Tayside, Scotland or the River Otter, Devon (Campbell-Palmer *et al.,* 2015b; Goodman 2014; Campbell-Palmer and Girling 2019). In addition, 0/235 beavers examined by faecal culture for *Salmonella* spp. in Telemark, Norway were positive (Rosell, Rosef, and Parker 2001). However, in studies in humans, the numbers of enterobacteria shed in faeces declines over time with only low numbers detected in faecal samples from chronically infected people (Ethelberg *et al.,* 2007) so it is possible that cases of infection with gram-negative enteric bacteria in beavers have been missed.

Beavers are herbivorous hindgut fermenters and are reliant for digestion on large colonies of cellulase-producing bacteria (Pratama *et al.,* 2019). In other, better-studied, hindgut fermenters such as the rabbit, gut dysbiosis as a result of an inappropriate diet or other stressors leads to changes in intestinal motility and pH precipitating enterotoxaemia and overgrowth of some bacterial species such as *E. coli* (Oglesbee and Jenkins 2012). Beavers may be susceptible to similar enteric diseases.

Given the evidence discussed above, gram-negative enterobacterial infection in beavers is probably asymptomatic in immunocompetent hosts but stressors may increase their susceptibility to the development of disease. As discussed elsewhere in this report, free-living beavers captured and translocated are known to be particularly susceptible to stressor-related disease and translocation is a known stressor (Dickens, Delehanty, and Michael Romero 2010). Gram-negative enteric bacteria should therefore be considered as a carrier hazard for the translocation of beavers.

Risk Assessment

Release Assessment

Beavers may be exposed to gram-negative enteric bacteria shed by other animals and in environmental reservoirs such as soil, water and on plant foodstuffs and infected by the oro-faecal route. In addition, they may be exposed to water-borne bacteria via mucous membranes. Survival of gram-negative enteric bacteria in the environment may be prolonged and up to several months for some species (Kramer, Schwebke, and Kampf 2006) with direct or indirect infection of new hosts via the faecal-oral route or, occasionally, via mucous membranes (Gaffuri and Holmes 2012). Since species in captive collections can harbour g-ram-negative enterobacteria, beavers held in enclosures or with an unknown history may have been exposed through direct contact with other species or indirect contact, for example through fomites. Given that beavers are known to be susceptible to infection with gram-negative bacteria, and since most species of gram-negative bacteria are ubiquitous, and commensal in numerous animal species, the likelihood of a beaver being exposed to gram-negative enteric bacteria and infected at the source site(s) and infected at the time of translocation is high.

Exposure Assessment

Translocated beavers with commensal gram-negative enteric bacterial infections may shed bacteria in their faeces and contribute to environmental reservoirs of gram-negative bacterial spp. at the destination site(s). There is a medium likelihood that other beavers, humans and sympatric mammalian species at the destination site(s) will be exposed to and infected by gram-negative enteric bacteria shed by beavers and a high likelihood that beavers and sympatric species infected at the destination site(s) will maintain and disseminate gram-negative enteric bacteria in their faeces. Since these bacteria are

harboured by many free-living wild mammals, the release of beavers is unlikely to markedly affect the dissemination of gram-negative enteric bacteria and the prevalence and intensity in mammal populations.

Consequence Assessment

There is a high likelihood of one translocated beaver being infected with gram-negative enteric bacteria.

Gram-negative enteric bacteria are usually commensal in immunocompetent mammals. However, infected beavers stressed through handling, transport, and adjustment to release environments may be more susceptible to disease. The range of diseases caused by enteric bacteria is extensive but, in addition to enteritis, includes sepsis, pneumonia, organ necrosis and wound infections. There is a low likelihood of translocated beavers suffering from stressor-precipitated disease associated with gram-negative enteric bacteria. There is a very low likelihood of failure of the reintroduction and the associated economic and biological consequences because evidence noted above suggests that cases of disease are sporadic.

We are not aware of any reports of disease in humans or other species as a result of direct or indirect contact with beavers. In immunocompetent humans, infection with gram-negative enteric bacteria usually results in self-limiting enteric disease and the probability of severe biological or economic consequences is therefore very low.

Risk Estimation

There is a high likelihood that beavers will be exposed to and infected by gram-negative enteric bacteria at the source site(s). The likelihood of exposure and infection at the destination is medium and the likelihood of dissemination is high. There is a low likelihood that the stress of translocation may precipitate disease in infected beavers and a very low likelihood of the failure of the translocation. There is a very low likelihood of biological and economic consequences as a result of disease in humans and livestock. The overall risk of disease due to gram-negative enteric bacteria in translocated beavers is MEDIUM.

Risk Management

Risk Evaluation

Mitigation measures should be implemented based on the medium risk estimation.

Risk Options

Testing asymptomatic beavers for infection with gram-negative enteric bacteria is likely to be of limited value as these agents are normal commensal organisms and infected

beavers may be healthy and not necessarily of risk to other beavers or mammals. However, post-mortem examination of any beaver found dead or electively euthanised on welfare grounds with appropriate culture and possibly sequencing of associated infectious agents is strongly recommended in order to improve our understanding of gram-negative enteric spp. harboured by beavers.

Appropriate measures to minimise stress during capture, handling and transport should be undertaken. In addition, appropriate dietary provision should be made during any period in captivity, with emphasis on the provision of suitable browse, ideally taken from the source site.

To reduce the risk of zoonotic diseases, routine hygienic precautions such as use of disposable gloves and hand washing should be employed. Gloves should be worn whenever handling animals, and during the cleaning and disinfection of all equipment and transport materials. Equipment such as transport crates should be cleaned with detergent and water and then disinfected with a suitable agent diluted according to the manufacturer's guidelines.

It may be important to conserve commensal parasites during translocation because it may be counterproductive to create a population of beavers at the release site without exposure and immunity to these parasites, should a non-immune population be subsequently exposed to them.

5.4.6 Disease risk analysis for the carrier hazard *Francisella tularensis*

Source Hazard

Justification for Hazard Status

Francisella tularensis is a small, gram-negative coccobacillus which is one of five species within the *Francisella* genus, family *Francisellaceae*. It is the aetiological agent of tularaemia, an infectious and zoonotic septicaemic disease. Tularaemia was first described in 1911 in rodents exhibiting plague-like clinical signs (McCoy 1911) and the bacteria were later identified after isolation from Californian ground squirrels (*Otospermophilus beecheyi*) (McCoy and Chapin, 1912). *F. tularensis* has since been isolated from over 250 species and is considered to have the broadest host range of all zoonotic agents (Mörner 1992; Gyuranecz 2012). Eurasian beavers have been implicated as reservoir hosts of *F. tularensis* and one case of clinical disease has been reported (Mörner *et al.,* 1988; Mörner and Sandstedt, 1983; Schulze *et al.,* 2016). Tularaemia is a complex disease and many aspects of the epidemiology are poorly understood, including transmission cycles and reservoir hosts (Hestvik *et al.,* 2015). Mammals within the orders *Lagomorpha* and *Rodentia* are thought to be particularly important within the parasite's lifecycles (Gyuranecz 2012).

Four subspecies of *F. tularensis are* currently recognised: *F. tularensis* subsp*. tularensis*, *F. tularensis* subsp*. holarctica*, *F. tularensis* subsp*. mediasiatica* and *F. tularensis* subsp*. novicida*. The moderately virulent *F. tularensis* subsp. *holarctica* is the causative agent of disease in Europe (Gyuranecz 2012). *F. tularensis* subsp*. holarctica* is associated with aquatic ecosystems. Aquatic mammals, including Eurasian beavers, have been implicated as reservoirs of the bacterium in countries where the disease is endemic (Mörner and Sandstedt, 1983). *F. tularensis* subsp. *holarctica* can also be transmitted by haematophagous arthropods, including mosquitos (*Aedes aegypti*) and ticks (*Ixodae* spp*.*). Mosquitoes become infected through the aquatic cycle during their larval stages, but are not considered to be true reservoirs as transovarial transmission has not been shown, suggesting that the infection will die with the mosquito (Petersen, Mead, and Schriefer 2009). The tick *Dermacentor reticulatus* is thought to be a true reservoir of *F. tularensis* subsp. *holarctica* and transmits the parasite between mammals in Central Europe through a separate terrestrial cycle (Keim, Johansson, and Wagner 2007).

Francisella tularensis is widespread across continental Europe and its current geographic range encompasses Czech Republic, Finland, France, Germany, Liechtenstein, Netherlands, Norway (pers. comm., Turid Vikøren, 11th February 2020), Sweden and Switzerland (World Health Organisation, 2007). It is also suspected to be present in Italy, Denmark and Russia, and has previously been reported in Austria, Belgium, Bulgaria, Hungary and Poland, although it is currently apparently absent in these areas (Maurin and Gyuranecz 2016). The bacterium is currently considered to be absent from Great Britain and therefore is considered a source hazard for the translocation of beavers to England.

Risk Assessment

Release Assessment

There are two known transmission cycles of *F. tularensis*: the aquatic and terrestrial cycles. *F. tularensis* is highly adaptable to a wide range of arthropod vectors (Petersen, Mead, and Schriefer 2009), and it is possible that an infected arthropod could be released at the destination alongside translocated beavers from Norway. Prevalence of *F. tularensis* within the European tick population has been reported as between 0 and 3% (Hubálek and Halouzka, 1997).

Hare and rodent species, such as lemmings (*Lemmus lemmus*), are important hosts and have also been implicated as reservoir species in previous outbreaks (Berdal *et al.,* 1996; Larssen *et al.,* 2011; Mörner *et al.,* 1988; Nordstoga *et al.,* 2014). The bacterium can be transmitted directly through environmental contamination with bodily discharges such as faeces and urine, leading to alimentary or aerogenous infection (Friend 2006; Gyuranecz *et al.,* 2010; Reintjes *et al.,* 2002; Gyuranecz 2012). These routes of infection are particularly important during winter when arthropod density decreases (Mörner *et al.,* 1988).

In the aquatic cycle, aquatic mammals including voles (*Microtus* spp.), muskrats (*Ondatra zibethicus*) and beavers are thought to be important hosts and contribute to environmental contamination through shedding of live bacteria in secretions (Mörner and Sandstedt, 1983; Schulze *et al.,* 2016). Contamination from carcases can also occur (Schulze *et al.,* 2016; Gyuranecz 2012). *F. tularensis* subsp. *holarctica* has been detected in water and sediment samples from areas in which tularaemia is endemic in both outbreak and non-outbreak years. Its presence in sediments and water indicates that environmental persistence may contribute to the complex epidemiology of the disease (Berdal *et al.,* 1996; Broman *et al.,* 2011).

F. tularensis has not been detected in beavers in Great Britain during testing in the River Otter Beaver Trial and monitoring of the Scottish populations at Knapdale and Tayside. PCR and ELISA were used to test 29 beavers in Knapdale, with no positive results noted (Gaywood *et al.,* 2015; Goodman, 2014). At Tayside, PCR of blood was negative *for F. tularensis* in all 17 live trapped animals as well as PCR of blood or tissue samples of six carcases submitted for post-mortem examination. Serum PCR was performed on five live-trapped animals as part of the River Otter Beaver Trial and all were negative for *F. tularensis* (Campbell-Palmer and Girling, 2019).

Cases of tularaemia in Norway have been sporadic in humans, wildlife and domestic species over the past century but showed an increase in 2019 (Agren *et al.,* 2019). 116 human cases of tularaemia were reported in Norway between 1926 and 1972 along with sporadic identification of *F. tularensis* in lemmings and *Ixodes* spp. of tick (Pearson 1975; Výrosteková 1993), while an additional 179 cases of disease in humans were reported in 2019 (Agren *et al.,* 2019). A report published in 2014 by Nordstoga *et al.,* (2014) described a case of tularaemia in a domestic dog in Norway after ingestion of an infected mountain hare (*Lepus timidus*), suggesting a further route of transmission (Nordstoga *et al.,* 2014). More recent outbreaks in humans and domestic dogs were linked to increased free-living lemming populations and subsequent contamination of drinking water. Lemmings are now widely considered to be the main reservoir in Norway (Nordstoga *et al.,* 2014; Larssen *et al.,* 2011; Berdal *et al.,* 1996). Human tularaemia outbreaks have been associated with increased population numbers of free-living rodent reservoirs (Larssen *et al.,* 2011) and with insect bites (Agren *et al.,* 2019).

There is a high likelihood that Eurasian beavers in Norway will have been exposed to *F. tularensis* through contaminated water sources during these outbreak periods. A recent report of tularaemia diagnosis in 16 hares (*Lepus* spp.) from the Eastern part of Norway in 2019 (pers. comm., Turid Vikøren, 11th February 2020) confirms that the disease has recently been occurring within the country. It is possible that free-living beavers in Norway were exposed to *F. tularensis* through environmental contamination at this time. There have been no known surveys of *F. tularensis* infection or tularaemia in free-living Eurasian beavers in Norway, and it is therefore not possible to conclude that these animals have not been exposed to and infected with *F. tularensis* over the last decade. It is also unclear if

and for how long beavers shed the bacterium after infection and whether they may become persistent shedders.

In neighbouring Sweden, tularaemia has been considered to be endemic in wildlife for the past decade and widely prevalent in domestic animal populations before this time (World Organisation for Animal Health (OIE) 2020). The number of cases in humans in Sweden showed a marked increase in 2019 (Agren *et al.,* 2019). Furthermore, exposure to the bacterium has been detected in free-living Eurasian beavers in Sweden using serological studies. Positive antibody titres were found in 21% (n= 23/110) of investigated beavers in one study (Mörner and Sandstedt, 1982). The beaver is likely to be important in the epidemiology of tularaemia in Scandinavia, and could act as a reservoir of *F. tularensis*, although the bacterium has never been isolated from this species in Sweden (Mörner *et al.,* 1988; Mörner and Sandstedt, 1983; Tärnvik *et al.,* 1996). In Sweden, several beaver populations are distributed close to the Norwegian border. Populations inhabit the areas surrounding waterways which breach this border, such as the river Klarälven (Hartman 1995). There is a risk that Norwegian beavers have been exposed to *F. tularensis* through contact with Swedish beaver populations in these areas. While it is known that beavers in parts of Europe, including Sweden, have been exposed to *F. tularensis,* there is a lack of evidence on the proportion infected and the persistence of infection. In other rodent species, infection rates appear to be low. In one study, 547 small rodents were trapped in Finland and multiple samples tested using PCR. *F. tularensis* DNA was unequivocally detected in liver samples of only five field voles.

Beavers released from enclosures in England may have an unknown history of origin, or may have originated from continental Europe, and therefore may have been exposed to *F. tularensis* when either captive or free-living, for example through direct or indirect contact with susceptible species. There have been several examples of tularaemia outbreaks in captive collections among numerous species. One such outbreak occurred at a wildlife safari park in North America leading to mortality of a bushbaby (*Galago* spp.) and a cottontop tamarin (*Saguinus oedipus*), and non-fatal disease in another cottontop tamarin (Beest *et al.,* 2017). It was hypothesised that the outbreak occurred after transmission from free-living California ground squirrels which were able to gain access to the enclosures, although prevalence in these squirrels was found to be only 2% (n= 1/45) in a concurrent serological study (Beest *et al.,* 2017). Another example of an outbreak occurred in a Canadian collection which lead to mortality in three black and red tamarins (*Sanguinus nigricollis*) and one talapoin (*Cercopithecus talapoin*), as well as non-fatal disease in a second talapoin (Nayar, Crawshaw, and Neufeld 1979). The source of the disease was again identified as wild ground squirrels and the causative organism was recovered from the liver and spleen of one squirrel and from fleas found on it (Nayar, Crawshaw, and Neufeld 1979). These studies provide evidence of *F. tularensis* transmission in zoological collections, particularly involving rodents. Beavers currently in enclosures in England are known, in some cases, to have originated from Germany (n = 32/67; 47.8%), an area where *F. tularensis* is endemic, and therefore these beavers may

harbour the bacterium. For this reason, the unknown history of enclosure beavers in Great Britain is of particular concern for their possible *F. tularensis* exposure.

There is a medium likelihood that, at the time of translocation, beavers either from Norway or from enclosures in Great Britain with an unknown history of origin,or known to have originated from endemic areas in continental Europe, will be infected with *F. tularensis.* There is a low likelihood that free-living beavers in Great Britain are infected with *F. tularensis* because some beavers from these populations originated from geographic areas in which the parasite occurs but *F. tularensis* has not to date been detected from free-living populations of beavers in either Scotland or England.

Exposure Assessment

There is a medium likelihood of exposure of mammals at the release site to *F. tularensis*. Eurasian beavers carrying the bacteria when translocated could lead to contamination of water sources and exposure of susceptible species via this route. Alternatively, direct transmission through aerosol, gastrointestinal secretions or urine could lead to infection of susceptible rodents and lagomorphs at the destination. Stowaway infected arthropods translocated with the beavers may also transmit *F. tularensis* through feeding on susceptible animals at the release site. Once exposed, there is a high likelihood of infection of mammals at the release site and dissemination through these mammal populations.

There is a medium likelihood that arthropods within Great Britain will be exposed and infected with *F. tularensis.* If one infected translocated beaver is bacteraemic when released, arthropod vectors residing at the destination site could be exposed through feeding on this animal.

There is a low likelihood of human exposure to *F. tularensis* at the destination through contamination of water sources. Human to human transmission does not occur (TARNVIC, WHO) meaning that dissemination amongst the human population in the face of an outbreak would not occur. Once the source of infection is identified the outbreak would be self-limiting.

Consequence Assessment

In humans, clinical signs of tularaemia are variable and can be non-specific and so, without appropriate testing, it is not possible to distinguish tularaemia from other septicaemic diseases (Nordstoga *et al.,* 2014; Tärnvik *et al.,* 1996). When infection is associated with contaminated water sources, symptoms are commonly fever and pharyngitis (considered the ‘oropharyngeal form’). An ulceroglandular form can also occur as a result of insect bites. In general, disease as a result of *F. tularensis* subsp. *holarctica* in Europe is generally less severe than disease caused by *F. tularensis* subsp. *tularensis* in North America (Larssen *et al.,* 2011). The disease course is thought to be dose-

dependent, with individuals exposed to higher doses more likely to die acutely than to become chronic shedders (Ellis *et al.,* 2002; Frederick and Stewart, 1975; Staples *et al.,* 2006; World Health Organisation, 2007). To our knowledge, no cases of tularaemia have been reported in humans working with beaver translocations. Several outbreaks of tularaemia have occurred in Europe, including Norway, but appear to be sporadic and are associated with contaminated water sources as a result of increased populations of lemming reservoirs (Larssen *et al.,* 2011). The likelihood of a tularaemia outbreak in humans living downstream of beaver release sites is low. The likelihood of negative consequences to humans as a result of a disease outbreak, including severe clinical signs, is high.

Clinical signs of tularaemia vary between mammal species. Mountain hares in Sweden appear to die of acute disease with non-specific clinical signs. Post-mortem examination findings have included pinpoint necrotic foci throughout abdominal organs (Mörner *et al.,* 1988). A more chronic course has been reported in brown hares (*Lepus europaeus*) in central Europe, although post-mortem examination findings are comparable to those in mountain hares (Gyuranecz *et al.,* 2010). One case of tularaemia in a Eurasian beaver has been reported in Germany, demonstrating the possibility of disease occurring in this species; findings post-mortem were comparable to those in other free-living species (Schulze *et al.,* 2016).

The probability that one translocated beaver is infected is medium if from Norway or from enclosures, and low if free-living in Great Britain. Eurasian beavers are susceptible to tularaemia, but the disease appears to be rare and only a single case has been reported, as noted above. Those beavers exposed to *F. tularensis* and infected are not likely to show clinical signs and instead will act as reservoirs (Mörner *et al.,* 1988; Mörner and Sandstedt, 1983). There is a very low likelihood of systemic disease leading to death in an infected beaver and of an outbreak in the translocated beaver population and of biological and economic consequences through failure of the reintroduction.

There is a low likelihood of cases of disease in humans in contact with contaminated water sources. Cases of tularaemia in humans would be limited by the fact that human to human transmission is not thought to occur (Tärnvik *et al.,* 1996; World Health Organisation, 2007). There is a very low likelihood of economic consequences as a result of the increased resource requirement of trained staff including vets, doctors and government agency workers to manage cases of the disease (Tärnvik, Sandström and Sjöstedt, 1996; World Health Organisation, 2007).

As far as we are aware, no autochthonous cases of tularaemia have been diagnosed in Great Britain and the differing epidemiological risk factors between continental Europe and Great Britain underlying the absence of disease in Great Britain are uncertain. There is a low likelihood of disease outbreaks in exposed susceptible mammalian species, particularly from the orders *Rodentia* and *Lagomorpha,* including several endangered

species including the already endangered hazel dormouse (*Muscardinus avellanarius*), water vole and red squirrel (*Sciurus vulgaris*).

Risk Estimation

There is a medium likelihood that Eurasian beavers translocated from Norway or from enclosures, and a low likelihood that beavers originating from free-living population in Great Britain, will be infected with *F. tularensis*. There is a low likelihood that an infected arthropod vector will be translocated alongside the beavers. There is a medium probability of exposure and a high probability of infection of mammals at the destination and dissemination through mammal populations. There is a medium probability that arthropods at the destination will be exposed to and infected with *F. tularensis* if an infected beaver is released. There is low likelihood of exposure of people and negligible likelihood of dissemination through the human population. There is a very low likelihood of a disease outbreak in the translocated beaver population and a low likelihood of a disease outbreak in other susceptible mammalian species. There is a low likelihood of sporadic disease in people. The overall risk is LOW.

Risk Management

Risk Evaluation

Measures should be undertaken to reduce further the risk of *F. tularensis* as a source hazard.

Risk options

There is an advantage in translocating free-living beavers in Great Britain to England in preference to those in fenced enclosures in England with unknown origin, or from areas in which *F. tularensis* is endemic, because limited testing of free-living beavers in Great Britain, as reported above, has failed to detect *F. tularensis*.

The following serological tests are available for *F. tularensis*: microagglutination, indirect immunofluorescent assay or ELISA-type western blot assay (Hepburn and Simpson, 2008; Maurin and Gyuranecz, 2016; Tärnvik and Chu, 2007; World Health Organisation, 2007). PCR testing of secretions to detect active shedding is also available and culture can be undertaken on body fluid (Sting *et al.,* 2013). Both serological and PCR tests would be valuable for research purposes if possible and to modify the disease risk analysis in future years. It is recommended that, if beavers are of unknown origin or originate from endemic areas for tularaemia, PCR of blood samples is undertaken to diagnose active infection ahead of release. This testing is available through Public Health England.

Treatment of all beavers with anti-parasitic agents prior to transport should be considered to avoid co-transport of arthropod vectors infected with *F. tularensis* to the destination site

if moved directly from an area in which *F. tularensis* is endemic. If Norway is chosen as the source, investigations into the conservation status of native arthropods should be undertaken and consideration given to conserving these species.

5.4.7 Disease risk analysis for the carrier hazard *Leptospira* spp.

Carrier Hazard

Justification for Hazard Status

Leptospires are globally distributed gram-negative, spirochete bacteria belonging to the genus *Leptospira* that currently comprises about 20 species of varying pathogenicity and as many as 300 recognised serovars (Adler, 2015). Nomenclature is complex, comprising species, serogroup, serovar and strain (Levett 2001).

Different *Leptospira* species and serovars have evolved to exploit different mammal species as reservoir hosts and it has been shown that almost every mammal species can serve as a carrier (Adler and de la Peña Moctezuma 2010). Leptospires do not survive well in acid conditions so animals producing alkaline urine such as herbivores are more prolific shedders (Adler and de la Peña Moctezuma 2010). Rodents, in particular, rats, are considered among the most important reservoirs of some *Leptospira* spp., including zoonotic serovars. Other mammals in environments where rats are believed to be the main reservoir tend to harbour the same *Leptospira* serovar but it is not known whether they also play a reservoir role or are accidental (incidental) hosts (Adler and de la Peña Moctezuma 2010). Aquatic rodents, including the muskrat, coypu (*Myocastor coypus*) and water vole have been shown to harbour leptospires (Meyer-Scholl *et al.,* 2012; Aviat *et al.,* 2009; Gelling *et al.,* 2015). It is recognised that an animal can be a reservoir host for one serovar but susceptible to infection and disease as an accidental host from another (Levett 2001).

Reservoir hosts are usually asymptomatically and chronically infected and may shed bacteria for extended periods (Adler and de la Peña Moctezuma 2010). However, chronic disease in reservoir hosts causing interstitial nephritis, renal fibrosis and failure has been reported in wild rats and experimentally induced in rats inoculated with *L. interrogans* serovar Copenhageni (Monahan, Callanan, and Nally 2009). Additionally, severe disease has been experimentally induced in immunocompromised mice inoculated with *L. interrogans* serovar Icterohaemorrhagiae (Evangelista and Coburn 2010). As a result, it would appear that animals within reservoir host groups may under certain circumstances experience either chronic or acute leptospirosis following infection with *Leptospira* serovars that do not normally cause disease in the host species.

There have been over 20 reported cases of leptospiral infection associated with mortality in Eurasian beavers in mainland Europe (Nolet *et al.,* 1997; Woll *et al.,* 2012; Marreros *et al.,* 2018; Giovannini *et al.,* 2012). The serovar was not reported in every case but has included five associated with infection with *L. interrogans* serovar Icterohaemorrhagiae and five with *L. interrogans* serovar Copenhageni (Marreros *et al.,* 2018; Nolet *et al.,* 1997). However, leptospiral infection, including of *L. interrogans* serovar Icterohaemorrhagiae, has been found on serology in Eurasian beavers without clinical signs (Goodman *et al.,* 2017; Girling *et al.,* 2019c). Girling *et al.,* (2019c) concluded that previously reported mortalities associated with leptospires may have been associated with other factors such as concurrent infection with other parasites. It is possible that the pathogenicity of leptospiral infection in beavers is influenced by stressors, which affect the immunocompetence, and change host-parasite dynamics leading to disease. Acute leptospirosis associated with the stress of translocation has been previously observed in beavers (Nolet *et al.,* 1997). Of 58 beavers translocated from Germany to the Netherlands, Nolet *et al.,* (1997) reported that three beavers were found dead in association with leptospiral infection between 24 and 31 days post-release.

Leptospira spp. have been detected in many exotic rodent species globally, including those housed in captive collections. In a review study, *Leptospira* was identified as the most common pathogenic genus detected in rodents in zoological institutions (Hardgrove *et al.,* 2021). Moreover, Cueva *et al.,* (2010) found that *Leptospira* spp. infection was endemic in capybara in a zoological collection in Peru (Cueva *et al.,* 2010) and, similarly, serological testing of Malayan porcupines (*Hystrix brachyura*) in a captive collection in Malaysia found that 18% (9/50) of animals had antibodies to *Leptospira* spp. (Siti-Nurdyana *et al.,* 2016). Siti-Nurdyana *et al.,* (2016) concluded that the Malayan porcupines could possibly be a source of leptospires for other zoo animals. An outbreak of leptospirosis in seals (*Phoca vitulina*) in a zoo in The Netherlands was attributed to transmission from a reservoir in coypu which were found to be positive for *Leptospira* spp. and housed in the same water system (Kik *et al.,* 2006), which suggests that leptospires can be transmitted in captive collections, particularly in aquatic species. Other rodents including Patagonia mara (*Dolichotis patagonum*), Californian ground squirrels, chestnut white-bellied rats (*Niviventer fulvescens*) and plantain squirrels (*Callosciurus notatus*) have also been found to be infected with *Leptospira* spp. (Nadia *et al.,* 2019; Hanichen *et al.,* 1992; Beest *et al.,* 2017). These reports suggest that beavers held in captive collections in close proximity to other rodents may be exposed to *Leptospira* spp..

Leptospira spp. are ubiquitous in both potential source and destination sites. As translocation is a known stressor (Dickens, Delehanty, and Michael Romero 2010), beavers, either as accidental or reservoir hosts, may be susceptible to disease when immunocompromised by stressors. Given that mortalities have occurred in beavers across Europe associated with *Leptospira* spp., *Leptospira* spp. should be considered as a carrier hazard for the translocation of beavers.

Risk Assessment

Release Assessment

Beavers at the source site(s) may be exposed to and infected with *Leptospira* spp. in the environment via mucous membranes or skin abrasions as leptospires can survive in water for several months and shedding by infected reservoir hosts is prolonged. Infected mammals may shed leptospires in their urine with warmth and moisture favouring leptospire persistence in the environment (Birtles 2012). Leptospires have been shown experimentally to survive for up to several months in water at room temperature and for up to 7 weeks in soil (Levett 2001). Cases of leptospirosis reportedly peak in summer following periods of hot, dry weather (Levett 2001).Infection is from contaminated watercourses via mucus membranes or skin lesions or, less commonly, by direct contact with infected animals' urine (Evangelista and Coburn 2010).

There is scant evidence for *Leptospira* spp. in Norway. Akerstedt *et al.,* (2010) reported a prevalence of 9.9% in red foxes tested by micro-agglutination test (MAT) serology for *L. interrogans* serovar Icterohaemorrhagiae between 1994 and 2005 (n=20/202). However, 0/52 Norwegian beavers tested by PCR of kidney tissue (Girling *et al.,* 2019c) were positive for leptospiral DNA and we are not aware of any other studies finding evidence of leptospiral infection in beavers in Norway. 9/30 beavers trapped in Norway for release in Scotland as part of the Knapdale trial tested positive on MAT (Girling *et al.,* 2019c) but this was towards the end of their 6 months rabies quarantine in the UK and so infection in the UK cannot be ruled out as none of the serovars identified was novel to the UK (Goodman *et al.,* 2012). Of these beavers, four were positive for *L. interrogans* serovar Icterohaemorrhagiae and nine for *L. interrogans* serovar Copenhageni. On retrapping, one beaver remained seropositive to *L. interrogans* serovar Icterohaemorrhagiae.

0/25 beavers trapped in the Tayside region of Scotland (origin unknown) tested positive for leptospires on MAT serology or urine or kidney PCR (Girling *et al.,* 2019c). Additionally, *Leptospira* spp. were not isolated from any of the beavers examined post-mortem in the UK to date that have been reported to us or the 12 beavers examined by ZSL. 3/6 beavers trapped in Devon as part of the River Otter trial (origin unknown, presumed Bavaria) were positive on MAT but the serovars were all known to be present in the UK (Girling *et al.,* 2019c). Similarly, 2/9 Bavarian beavers (wild-caught or captive-bred) were positive by kidney PCR or MAT but to serovars already present in the UK (Girling *et al.,* 2019c). None of these beavers was positive for *L. interrogans* serovar Icterohaemorrhagiae or *L. interrogans* serovar Copenhageni.

As previously discussed, numerous reports exist which document *Leptospira* spp. infection in exotic rodents in captive collections; a report of transmission between coypu and other species through a shared water source indicates the possibility of exposure of beavers in captive settings (Kik *et al.,* 2006). As animals infected with leptospires have been found in potential source sites and *Leptospira* spp. are considered to be ubiquitous, beavers at the

source site(s) are highly likely to be exposed to *Leptospira* spp. before translocation. Given that beavers are susceptible to infection with *Leptospira* spp. the likelihood of infection after exposure is estimated to be high. There is, therefore, a high likelihood of an infected beaver being translocated and released.

Exposure Assessment

As infected beavers may shed leptospires for prolonged periods and leptospires are able to survive for prolonged periods in the environment, there is a high probability of beavers and other mammals, including people, being exposed to *Leptospira* spp. at the destination site(s). Many mammal species are susceptible to infection and those that are already or become infected have the potential to become long term carriers and to contribute to the maintenance of the agent at the destination site(s) by shedding leptospires in their urine into water and adjacent habitat. There is therefore a high likelihood that mammals at the destination site(s) will disseminate *Leptospira* spp. to other mammals.

Consequence Assessment

In humans, leptospirosis is an important emerging zoonotic disease of which the most severe form involves multi-system organ complications, known commonly as Weil's Disease or Syndrome (Evangelista and Coburn 2010). Susceptibility and severity of disease is believed to vary with infective dose, serovar, strain, host species and individual MHC variation (Monahan, Callanan, and Nally 2009). Infection of humans can result in a range of symptoms from mild flu-like illness to jaundice, pulmonary haemorrhage and kidney failure with occasional reports of aseptic meningitis and myocarditis (Schreiber *et al.,* 2015). Histopathological examination of beavers, infected with pathogenic strains of *Leptospira* spp. found dead, recorded lung haemorrhage as the most common lesion, consistent with fatal cases in humans (Marreros *et al.,* 2018). There is a medium likelihood of disease in people associated with the translocation programme and in contact with beavers.

Marreros *et al.,* (2018) reviewed the histopathology of lung and kidney tissue and serology from 13 free-living beavers found dead in Switzerland between 2010 and 2014. The authors noted multifocal haemorrhages with variable levels of associated inflammation on histopathology of lung samples from all 13 beavers and interstitial fibrosis in renal tissue from two thirds (n=8/12) of the beavers. PCR testing confirmed the presence of leptospiral antigen in nine of the 11 beavers tested with five beavers PCR-positive in both lung and kidney tissue. Sequencing identified genotypes of leptospiral strains in the *L. interrogans* serovar Icterohaemorrhagiae and *L. interrogans* serovar Copenhageni serovars (serogroup *Icterohaemorrhagiae*). Ten of the 11 beavers for which blood samples were available were positive on MAT (titre => 1/100) for leptospiral antibodies with the highest titres (1/3200) to serovars Icterohaemorrhagiae, Copenhageni and Verdun (serogroup *Icterohaemorrhagiae*). All but one of the beavers was in poor body condition and

leptospirosis was cited by Marreros *et al.,* (2018) as the cause of morbidity and mortality in all cases.

The histopathology samples from beaver lung and kidney tissues examined by Marreros *et al.,* (2018) exhibited features associated with both acute and chronic leptospiral infection. Low levels of inflammatory infiltrate in lung tissue, seen in accidental hosts such as humans or dogs experiencing acute leptospirosis (Marreros *et al.,* 2018), were noted in some sections while interstitial renal fibrosis, associated with chronic rather than acute leptospirosis (Monahan, Callanan, and Nally 2009), were noted in sections from other beavers. Marreros *et al.,* (2018) therefore concluded that beavers are capable of being both acutely and chronically infected i.e. can act as both accidental and reservoir hosts of pathogenic leptospires. As both forms of infection, acute and chronic, have been variably observed following infection with *L. interrogans* serovar Icterohaemorrhagiae in beavers, it seems probable that immunocompetence to leptospiral infection is similarly variable in the species.

Immunocompetent beavers infected with pathogenic *Leptospira* spp. would be expected to mount a humoral antibody-mediated response to infection and recover quickly without experiencing clinical disease. However, the observation of signs of chronic infection such as bacterial colonisation of renal tubules and interstitial renal fibrosis in beavers suggests that some individuals may become chronically infected with the potential to become reservoir hosts.

Translocated beavers will be under stress and there is therefore a high likelihood that infected beavers will experience clinical disease, leptospirosis, leading to the failure of the translocation. Of the 58 beavers translocated from Germany to the Netherlands and reported by Nolet *et al.,* (1997), three were found dead in association with leptospiral disease, 57 were released in the autumn, and 43 had undergone general anaesthesia shortly prior to release for the intra-peritoneal implementation of radio-transmitters. The stress of trapping, handling and captivity could therefore have increased the susceptibility of beavers to disease and increased the likelihood of morbidity and mortality from leptospirosis.

Risk Estimation

There is a high probability of beavers being exposed to *Leptospira* spp. at either the source or destination site and a high likelihood of infection. The risk of dissemination to other animals at the destination site(s) is high. There is a high probability that the stress of translocation may precipitate acute disease in infected beavers and result in the failure of the translocation. The overall risk from disease caused by *Leptospira* spp. is HIGH. There is a medium likelihood of disease in people in contact with beavers involved in translocation.

Risk Management

Risk Evaluation

Based on the risk assessment above, preventative measures should be employed to reduce the risks from *Leptospira* spp. as a carrier hazard.

Risk Options

Diagnosis of exposure is usually by MAT serology, identifying host antibodies to specific leptospiral serovars or serogroups. Where antibodies are detectable on MAT, a minimum titre of 1/100 is usually regarded as indicative of infection although, given the specificity of the MAT, lower levels may be interpreted as confirming exposure (World Organisation for Animal Health (OIE) 2018). A titre of over 1/400, consistent with a four-fold increase, is regarded as indicative of current or recent infection (Girling *et al.,* 2019c).

However, it may be up to three to four weeks before a positive test is returned following infection (Schreiber *et al.,* 2015) so acute infection may be missed on serology. Additionally, host-adapted strains appear to trigger only minimal serological response in reservoir (carrier) hosts compared to accidental hosts (Shearer *et al.,* 2014) and bacteraemia may be transient (World Organisation for Animal Health (OIE) 2018) so serology is not a reliable means of identifying whether a host is actively shedding leptospires and so potentially infectious (Aviat *et al.,* 2009). Serology is therefore likely to be of limited value in identifying infected beavers and infected beavers may be healthy and not necessarily of risk to other beavers or mammals.

Isolation of bacteria by urine culture or PCR of urine is a preferred method of identifying carriers but leptospires are fastidious and incubation is lengthy, potentially up to 30 weeks (Birtles 2012) and leptospire shedding may be intermittent, so carriers may be missed on testing (Birtles 2012). If pathological findings are suggestive of leptospirosis, PCR testing of kidney tissue for leptospiral nucleic acid at post-mortem, followed by sequencing, in conjunction with histopathology, is currently regarded as the gold-standard method of identifying leptospiral-associated disease and should be considered as part of routine post-mortem examination of all beavers found dead or euthanised on welfare grounds if signs suggest leptospirosis is a differential.

Given that *Leptospira ‘’* spp. are considered to be a carrier hazard for beavers, and are already widely present throughout the UK, testing is not recommended ahead of beaver translocations/release as the risks associated with the stress of sampling and prolonged captivity while awaiting results are likely to outweigh any benefits. Instead, management should focus on stress mitigation and measures should be undertaken to reduce stress in beavers undergoing translocation. Specifically, handling, invasive testing, journey times and human presence, and scent, at capture and release sites should all be kept to the lowest practical level. General anaesthesia for clinical examination or implantation of

tracking devices is not recommended due to the associated stress of additional handling and confinement.

Infection of people associated with beaver translocation can be prevented through use of standard hygienic measures such as wearing of gloves, aseptic technique in procedures and hand hygiene.

5.4.8 Disease risk analysis for *Mycobacterium* spp. as a hazard for domestic and free-living mammals in England

Hazard for Domestic and Free-living Mammals in England

Justification for Hazard Status

Mycobacteria are rod-shaped, non-spore-forming acid-fast bacilli. About 200 species have been identified to date, many of which can infect a wide range of hosts, including humans, causing a range of clinical outcomes from latent and asymptomatic infection to active infection with severe disease (Larsen *et al.,* 2020). Reactivation of latent infection may be more likely with increasing age and reduced immunocompetence (Gavier-Widén *et al.,* 2012). Most mycobacterial spp. are environmental, opportunistic pathogens, existing as saprophytes in soil and water (Percival and Williams 2014). Two mycobacterial complexes are of particular interest: *Mycobacterium tuberculosis* (MTBC) and *Mycobacterium avium* (MAC). MTBC includes *M. bovis*, the most common cause of tuberculosis in domestic livestock and wildlife in the UK; *M. tuberculosis*, mainly found in humans; and *M. microti.* The principal species of interest in MAC are *M. avium* subsp. *avium* (MAA) and *M. avium* subsp. *paratuberculosis* (MAP), the causative agent of Johne's Disease in livestock (Percival and Williams 2014).

Mycobacterium bovis

The primary host for *M. bovis* in the UK is cattle with uncertainty regarding the role of wildlife species, notably the European badger and deer, in maintaining the cycle of transmission (Gavier-Widén *et al.,* 2012). Estimates of *M. bovis* prevalence in the European badger in the UK vary but may be as high as 24.2% (Allen, Skuce, and Byrne 2018). *M. bovis* has also been reported in a wide range of free-living wildlife hosts including rodents which are considered to be relatively resistant to disease following infection (Gavier-Widén *et al.,* 2012). Delahay *et al.,* (2007) cultured and spoligotyped 4,715 tissue samples from 32 wildlife species trapped or culled in south-west England in areas with high prevalence of *M. bovis* infection in cattle. Low levels of prevalence were found in 12 species tested (Table 3). These results were compared to gross pathological findings. No gross lesions were observed in culture-positive small mammals, grey squirrels (*Sciurus carolinensis*) and polecats (*Mustela putorius*). Delahay *et al.,* (2007) concluded

that species other than deer and badgers were therefore probably not a high risk to livestock. Comparison of *M. bovis* strains in a national park in Spain has similarly indicated that spill-back events from most species of wildlife to livestock are probably rare (Gortazar *et al.,* 2011). These results show that the prevalence of *M bovis* in rodents, and therefore their susceptibility to infection, is probably very low.

Table 3: Prevalence of M. bovis infection in mammals, south-west England. (From Delahay et al., (2007))

Species name	Prevalence (%)	Number tested positive
Red fox (*Vulpes vulpes*)	3.17	24/756
Stoat (*Mustela erminea*)	3.85	3/78
Polecat (*Mustela putorius*)	4.17	1/24
Common shrew (*Sorex araneus)*	2.44	1/141
Yellow-necked mouse (*Apodemus flavicol*	2.78	1/36
Wood mouse (*Apodemus sylvaticus)*	0.006	2/333
Field vole (*Microtus agrestis*)	1.49	1/67
Grey squirrel (*Sciurus carolinensis*)	0.44	2/450
Roe deer (*Capreolus capreolus*)	1.02	9/885
Red deer (*Cervus elaphus*)	1.02	2/196
Fallow deer (*Dama dama*)	4.37	22/504
Muntjac deer (*Muntiacus reevesi*)	5.17	3/58

M. avium* subsp. *paratuberculosis

M. avium subsp. *paratuberculosis* (MAP) is predominantly associated with ruminant species but has been found in non-ruminants, in particular lagomorphs which probably serve as a reservoir of infection (Gavier-Widén *et al.,* 2012). Annual surveillance of domestic livestock in Norway has found no new cases of MAP infection since 2014 (Kampen *et al.,* 2021). However, MAP is reported by Tryland *et al.,* (2004) to have been endemic in goat (*Capra* spp.) herds in western Norway prior to implementation of a vaccination programme from 1967 with prevalence in 1997 and 1998 in these areas of 12.2% in roe deer (*Capreolus capreolus*) (n=6/49) and 3.8% in red deer (*Cervus elaphus*) (n=14/371) suggesting historic spill-over into wildlife hosts. MAP is considered ubiquitous in Great Britain (APHA 2020). A study of 591 animals from 18 non-ruminant wildlife

species in Scotland (Beard *et al.,* 2001) isolated MAP by culture and PCR from 10 species (Table 4).

Table 4: Diagnosis of M. avium subsp. paratuberculosis in wildlife, Scotland. (From Beard et al., (2001))

Species	Tissue culture +/ve	Faeces culture +/ve	Histopathology +/ve
Red fox (*Vulpes vulpes*)	23/27	3/27	12/26
Stoat (*Mustela erminea)*	17/37	1/6	1/13
Weasel (*Mustela nivalis*)	2/4	N/A	2/4
Hare (*Lepus europaeus*)	1/6	0/3	0/4
Badger (*Meles meles*)	½	NA	0/1
Rat (*Rattus norvegicus*)	3/35	0/7	0/23
Wood mouse (*Apodemus sylvaticus*)	3/88	2/2	1/88
Carrion crow (*Corvus corone*)	36/60	4/12	1/60
Rook (*Corvus corax*)	3/53	1/1	0/53
Jackdaw (Corvus monedula)	1/38	NA	0/38

Where a positive diagnosis of MAP infection was made, histopathological signs were subtle or absent. Rats and mice, in particular, had minimal lesions. However, MAP was cultured from the faeces of wood mice, suggesting rodents' potential to act as a source of transmission of MAP to other species, either through predation/scavenging, or through faecal contamination of food sources (Beard *et al.,* 2001).

M. avium* subsp. *avium

M. avium subsp. *avium* (MAA) is the recognised cause of avian tuberculosis, which is particularly prevalent in water-fowl, and detected in a wide range of captive and free-living mammals (Gavier-Widén *et al.,* 2012). MAA has been isolated from brown rats and grey squirrels without visible lesions and is of low virulence in field voles and coypu (Grange, Yates, and Boughton 1990). Humans are considered resistant to disease following infection unless immunocompromised and this may be true for other species where stress-induced morbidity has been reported in captive animals (Grange, Yates, and Boughton

1990). The main route of infection is faeco-oral, via the environment, and direct transmission between mammals is probably very rare (Thorel, Huchzermeyer, and Michel 2001).

Other *Mycobacteria* spp.

M. microti is considered to be endemic in the UK with mice and voles the main reservoir hosts (Gavier-Widen *et al.,* 2012). 21% (n=38/180) of field voles in Kielder were found to have grossly visible cutaneous or abdominal lesions on post-mortem examination (Cavanagh *et al.,* 2002). *M. microti* spoligotypes were confirmed in 12/13 cutaneous lesions and 5/7 abdominal lesions but no confirmed cases were positive on urine or faecal spoligotyping, suggesting that shedding of *M. microti* bacilli is intermittent (*ibid.*). Cavanagh *et al.,* (2002) also isolated *M. microti* from three bank voles and two wood mice. Cats (*Felis catus*) that hunt small rodents are recognised as frequent spillover hosts but infection has also been occasionally reported in other species such as the badger, Eurasian otter (*Lutra lutra*) and grey squirrel (Michelet *et al.,* 2015).

Mycobacterium lepromatosis, M. leprae and *M. lepraemurium* are the cause of lepromatous leprosy in many species including red squirrels and humans (Meredith *et al.,* 2014) and rats, mice and cats (Rojas-Espinosa and Løvik 2001).

Hazard for Domestic and Free-Living Mammals in England - Justification of Hazard Status

Mycobacterial infections, in particular *M. bovis* and MAP, are a major cause of morbidity and economic loss in many species, particularly dairy cattle (*Bos taurus*). Large areas of Europe, including Norway, Sweden, Germany and Scotland, are considered free from *M. bovis* and stringent measures are underway in all European Union (EU) countries to eradicate reservoirs of infection (Visavet 2020). However, the UK continues to be the most severely affected of European member states, accounting for more than half of the *M. bovis* test-positive dairy herds in the EU in 2018 (n=10,334/18,801) with prevalence over 10% (EFSA and ECDC 2019). If beavers infected with mycobacterial species currently the subject of a control programme in the UK are translocated, their translocation may affect control goals in England and therefore these mycobacteria are evaluated as a hazard, with an emphasis on *Mycobacterium bovis*.

Risk Assessment

Release Assessment

Scotland and Norway, as outlined above, are considered free from *M. bovis*. The origin of most free-living beavers in Scotland is not known with certainty but includes Germany, also considered free from *M. bovis.* It is possible that historic, unauthorised releases of beavers in Scotland, England and Wales could have included beavers from captive collections or geographic regions which were exposed to *M. bovis* and with the potential to transmit *M. bovis* to conspecifics and offspring. Free-living beavers in England and Wales

may have been exposed to *M. bovis* from free-living wildlife or domestic cattle reservoirs since they were released. In England and Wales exposure will be more likely in areas with known infection in wildlife / cattle, being highest for beavers inhabiting areas in close proximity to dairy cattle or badgers.

Transmission of MTBC species is primarily aerogenous, and faeco-oral for MAC species, but a wide range of transmission routes, including bite-wounds, is possible for all species with the environment a key source of exposure due to the potential for prolonged survival of bacilli in water and soil. The environment, in particular water, is probably the main reservoir of MAA (Percival and Williams 2014). By contrast, animal hosts are probably the primary reservoirs for the other *Mycobacteria* species of interest. Animals that do not develop granulomas following infection may, therefore, have low infection potential but Gavier-Widén *et al.,* (2009) report that microscopic lesions are frequently detectable by histopathology in animals without visible granulomas and that these animals may still present a risk to other animals if predated, scavenged or inadvertently ingested via contaminated foodstuffs.

Beavers may be exposed to *Mycobacteria* spp. in water and soil and on plant materials. In addition, MAA and MAP probably replicate in soil and water, increasing the environmental reservoir of infectious bacilli (Percival and Williams 2014). *Mycobacteria* spp. are capable of prolonged survival in the environment due to their hydrophobic, lipid-rich cell walls which enable them to withstand desiccation and ultra-violet light (Gavier-Widén *et al.,* 2012).

Prevalence of infection with *M. bovis* in rodents is very low as indicated above, and rodents appear to be less susceptible than other mammals. Therefore, the likelihood of *M. bovis* infection in a translocated beaver is very low.

Beavers could be exposed to other *Mycobacteria* spp. such as *M. microti* and MAC species through accidental ingestion of contaminated plant material or water. MAA and *M. microti* are ubiquitous and MAP is widely distributed in Great Britain and may be present in wildlife reservoirs in Norway. Prevalence of *M. microti,* and probably MAC, is higher than *M. bovis* in rodents and, therefore, there is a medium probability that translocated beavers are infected with *M. microti* and MAC.

Exposure Assessment

An infected beaver could shed *Mycobacteria* bacilli in saliva, urine or faeces, depending on the location of lesions, which could be either inhaled by other animals or ingested from the environment in contaminated soil, water or food items. In addition, animals could become infected by predating or scavenging an infected beaver or through bite wounds from an infected beaver. The likelihood of transmission to conspecifics depends on host density, distribution and behaviour (Gavier-Widén *et al.,* 2012). For example, badgers tend to aggregate in underground setts, use communal latrines, move between family groups

and fight frequently, increasing their risk from all routes of transmission: aerogenous, environmental, ingestion and bite-wounds (*ibid.*).

The likelihood of conspecific transmission among beavers is unknown but is likely to be low as beavers live in small family groups at low density (Gurnell *et al.,* 2009) and rodents rarely experience extensive granuloma formation. As beavers inhabit aquatic environments there is potential for widespread dissemination of infectious bacilli within watercourses and in riparian margins to sympatric species. However as rodent species do not appear to be susceptible to severe disease following infection, shedding of bacilli is likely to be low and beavers are unlikely to act as a major source of mycobacteria, and increase the mycobacterial load, in the destination environment. There is a low likelihood that mammals at the destination will be exposed and infected with mycobacteria.

Many different mammalian species have been shown to be susceptible to infection with *Mycobacteria* spp. and bacilli are extremely persistent in the environment and so there is a high probability of dissemination.

Consequence Assessment

There is a low likelihood of one translocated beaver being infected with *Mycobacteria* spp..

Following infection with *Mycobacteria* spp., a cell-mediated immune response may result in the formation of granulomas in organs and lymphatic tissue. Lympho-haematogenous dissemination and granuloma rupture facilitate the spread of infectious bacilli within the host and shedding, for example through nasal secretions, urine or faeces (Gavier-Widén *et al.,* 2012). As a result, shedding is intermittent and may be related to the size and location of granulomas (*ibid.*). The location of mycobacteria lesions is thought to relate to the route of infection: aerogenous infection causing predominantly pulmonary lesions, ingestion causing primarily alimentary lesions, and bites causing cutaneous lesions. However, as disease progresses, bacilli may spread by haematogenous distribution to multiple organs. (*ibid*.). Haematogenous dissemination of large numbers of mycobacterial bacilli simultaneously may result in miliary tuberculosis, a fast developing spread of numerous, small, white foci of infection. More typically, disease progress is slow, with growth and coalescence of large granulomas ultimately resulting in organ failure and death (*ibid*.). MAP infection of ruminants causes chronic enteritis and progressive weight loss (Beard *et al.,* 2001) and has been associated with Crohn’s Disease in humans (Percival and Williams 2014).

Infected animals and humans are variably susceptible to disease following infection with *Mycobacteria* spp. and even individuals from species normally resistant to disease may, under some circumstances, develop severe lesions (Gavier-Widén *et al.,* 2012). However, in general, domestic mammals and humans are relatively resistant to MAA infection unless immunocompromised (*ibid*.) which may result in pulmonary lesions and/or lymphadenitis (Percival and Williams 2014).

There are severe biological and economic costs as a result of mycobacterial disease in livestock and sympatric species, and humans, following infection. However, the biological and economic consequences attributable to beaver translocation are likely to be negligible since *Mycobacteria* spp. are widely distributed in reservoir hosts and the environment in England, and rodents are not an important component of that reservoir. In addition, only small numbers of beavers at low density will be released.

Risk Estimation

The likelihood of *M. bovis* infection in a translocated beaver is very low. There is a medium probability that translocated beavers are infected with *M. microti* and MAC. There is a low likelihood of exposure of mammals at the destination and a high probability of dissemination to sympatric species at the destination site(s). The consequences to mammals in England from the translocation of beavers is negligible. The overall risk to mammals in England from beaver translocation is NEGLIGIBLE.

Risk Management

Risk Evaluation

Although the risk from mycobacteria to other mammals in England is negligible, we consider option evaluation.

Risk options

Testing for mycobacterial infection is unlikely to be rewarding. Isolation, culture and spoligotyping of *Mycobacteria* spp. is regarded as the gold standard method of diagnosis but cannot be effectively performed in the live animal as shedding of bacilli is intermittent and bacterial growth is slow, often up to 12 weeks and potentially six months for MAP, and requires specialist laboratory facilities (Gavier-Widén *et al.,* 2012). Serological assays to detect antibodies may be used to test wildlife for *M. bovis* but sensitivity tends to be low and tests may only work reliably in animals with more severe disease (Chambers 2009). In addition, validation of serological tests has not, as far as we are aware, been performed for beavers, while cross-reactivity with non-pathogenic environmental mycobacteria may also be an issue (Gavier-Widén *et al.,* 2012).

The intradermal tuberculin test used in cattle could potentially be used in beavers for detection of *M. bovis* exposure but sensitivity is of variable reliability in wildlife species and a minimum of 72 hours is required before results can be assessed (Chambers 2009). Enzyme immunoassays may offer the greatest promise but would require validation and must be performed on fresh blood samples (*ibid.*) so may have only limited potential for use in beavers. BAL, chest radiographs and abdominal ultrasound could be used in the

anesthetised animal to detect pulmonary infections and gross lesions, but sensitivity and specificity are likely to be unacceptably low.

Given the *M. bovis*-free status of Norway and Scotland, the beavers in these countries represent a good source population from the perspective of risk of mycobacterial disease in domestic and free-living mammals in England.

5.4.9 Disease risk analysis for the carrier hazard *Mycobacterium* spp.

Carrier Hazard

Justification for Hazard Status

Mycobacterium spp. have already been assessed as a hazard to free-living and domestic animals in England, and the risk estimated to be negligible. However, a known case of MAA-associated disease in a beaver (Nolet *et al.,* 1997), and reported prevalence of MAA in other rodent species, suggest that beavers may be susceptible to infection following exposure to *Mycobacteria* spp.. Progress of disease following infection with *Mycobacteria* spp. depends on the ability of the host animal to mount a successful immunological response in order to control the multiplication rate of bacilli and so host immunocompetence may have a major effect on the degree of morbidity experienced (Gavier-Widén *et al.,* 2012). As all translocations are associated with stress (Dickens, Delehanty, and Michael Romero 2010), and stress precipitates reduced immunocompetence, translocated beavers will be predisposed to clinical disease following infection with *Mycobacteria* spp. which should therefore be considered as carrier hazards for the translocation of Eurasian beavers.

Risk Assessment

Release Assessment

Transmission of MTBC species is primarily aerogenous, and faeco-oral for MAC species, but a wide range of transmission routes, including bite-wounds, is possible for all species with the environment a key source of exposure due to the potential for prolonged survival of bacilli in water and soil. The environment, in particular water, is probably the main reservoir of MAA (Percival and Williams 2014). By contrast, animal hosts are probably the primary reservoirs for the other *Mycobacteria* species of interest. Animals that do not develop granulomas following infection may, therefore, have low infection potential but Gavier-Widén *et al.,* (2009) report that microscopic lesions are frequently detectable by histopathology in animals without visible granulomas and that these animals may still present a risk to other animals if predated, scavenged or inadvertently ingested via contaminated foodstuffs.

Beavers may be exposed to *Mycobacteria* spp. in water and soil, and on plant materials. In addition, MAA and MAP probably replicate in soil and water, increasing the environmental reservoir of infectious bacilli (Percival and Williams 2014). *Mycobacteria* spp. are capable of prolonged survival in the environment due to their hydrophobic, lipid-rich cell walls which enable them to withstand desiccation and ultra-violet light (Gavier-Widén *et al.,* 2012).

There is a medium likelihood that translocated beavers are infected with mycobacteria.

Exposure Assessment

An infected beaver could shed *Mycobacteria* bacilli in saliva, urine or faeces, depending on the location of lesions, which could be either inhaled by other animals or ingested from the environment in contaminated soil, water or food items. In addition, animals could become infected by predating or scavenging an infected beaver or through bite wounds from an infected beaver. The likelihood of transmission to conspecifics depends on host density, distribution and behaviour (Gavier-Widén *et al.,* 2012). For example, badgers tend to aggregate in underground setts, use communal latrines, move between family groups and fight frequently, increasing their risk from all routes of transmission: aerogenous, environmental, ingestion and bite-wounds (*ibid.*). The likelihood of conspecific transmission among beavers is unknown but is likely to be low as beavers live in small family groups at low density (Gurnell *et al.,* 2009) and rodents rarely experience extensive granuloma formation.

As beavers inhabit aquatic environments there is potential for widespread dissemination of infectious bacilli within watercourses and in riparian margins to sympatric species. However as rodent species do not appear to be susceptible to severe disease following infection, shedding of bacilli is likely to be low and beavers are unlikely to act as a major source of mycobacteria, and increase the mycobacterial load, in the destination environment. There is a low likelihood that mammals at the destination will be exposed and infected with mycobacteria.

Many different mammalian species have been shown to be susceptible to infection with *Mycobacteria* spp. and bacilli are extremely persistent in the environment and so there is a high probability of dissemination at the destination site(s).

Consequence Assessment

There is a low likelihood of one translocated beaver being infected with mycobacteria.

There has been one recorded case of MAA associated with mortality in a beaver which died just under two years after translocation to the Netherlands (Nolet *et al.,* 1997). The susceptibility of beavers to infection with other *Mycobacteria* spp. is unknown but, given the widespread prevalence of mycobacterial infection in other rodent hosts, it should be

assumed that beavers are similarly susceptible and could, under certain conditions, develop clinical disease following infection. Beavers in England and Scotland have been tested for disease associated with *M. bovis* by BAL and/or chest radiographs (n = 20) and MAP infection by faecal microscopy (n = 70) with no positive results to date (Campbell-Palmer *et al.,* 2015b; Campbell-Palmer and Girling 2019). As diagnostic testing is not very sensitive (see below for further discussion of testing protocols), it is possible that cases of infection have been missed.

Following infection with *Mycobacteria* spp., a cell-mediated immune response may result in the formation of granulomas in organs and lymphatic tissue. Lympho-haematogenous dissemination and granuloma rupture facilitate the spread of infectious bacilli within the host and shedding, for example through nasal secretions, urine or faeces (Gavier-Widén *et al.,* 2012). As a result, shedding is intermittent and may be related to the size and location of granulomas (*ibid.*). The location of mycobacteria lesions is thought to relate to the route of infection: aerogenous infection causing predominantly pulmonary lesions, ingestion causing primarily alimentary lesions and bites causing cutaneous lesions. However, as disease progresses, bacilli may spread by haematogenous distribution to multiple organs. (*ibid*.). Haematogenous dissemination of large numbers of mycobacterial bacilli simultaneously may result in miliary tuberculosis, a fast developing spread of numerous, small white foci of infection. More typically, disease progress is slow, with growth and coalescence of large granulomas ultimately resulting in organ failure and death (*ibid*.).

Recrudescence of latent infection may be triggered by stress following translocation. In addition, beavers may be less resistant to infection and disease progress following exposure at the destination site(s). Infected beavers may therefore develop disseminated granulomas, resulting in organ failure, severe morbidity and death. As disease progress can be slow, these effects on individual beaver health may not be discernible for months or even years following translocation. Infected beavers experiencing severe disease may be more likely to shed bacilli and contribute to dissemination of *Mycobacteria* spp. at the destination site(s) through faeces, urine or saliva as well as constituting an infection risk to predators and scavengers after death. There is a low likelihood of disease in translocated beavers but the probability of failure of the translocation is negligible. The biological, environmental and economic consequences are negligible.

Risk Estimation

There is a medium likelihood that a translocated beaver is exposed to and infected with mycobacteria. There is a low likelihood of exposure of mammals at the destination and a high likelihood of dissemination. There is a low likelihood of disease in translocated beavers. The overall risk is LOW.

Risk Management

Risk Evaluation

Preventative measures should be considered to reduce stress associated with translocation and to reduce the risk of exposure to and infection with *Mycobacteria* spp..

Risk options

In line with previous recommendations, efforts should be made to minimise stress to beavers during capture and transit and to reduce the level of handling and duration of time in transit and captivity to the lowest possible levels.

Consideration could be given to the use of BCG vaccination which has been shown to be effective in wild boar, red deer and badgers against *M. bovis* (Balseiro *et al.,* 2020) and, in humans, has been shown to protect against other *Mycobacteria* spp. (Zimmermann, Finn, and Curtis 2018). Additionally, release sites with reduced access for grazing livestock and low levels of waterfowl presence could be considered.

PCR and/or extended culture of tissues removed during post-mortem examination of beavers found dead before or after release is therefore recommended in order to improve understanding of mycobacterial infection and disease progression and characterisation in beavers.

5.4.10 Disease risk analysis for the carrier hazard *Streptococcus castoreus*

Carrier Hazard

Justification for Hazard Status

Streptococcus spp. are gram-positive cocci of worldwide distribution responsible for a wide range of suppurative conditions and abscess formation in host animals (Quinn *et al.,* 2011). Most *Streptococcus* species are found as commensals in the upper respiratory or urogenital tract of the host and have poor survival in the environment (*ibid*). The genus comprises both highly host-adapted and tissue-trophic species of varying pathogenicity as well as more generalist organisms only capable of causing disease as opportunists (Speck 2012b).

A novel *Streptococcus* spp. was isolated by Lawson *et al.,* (2005) from the carcase of a Eurasian beaver that had died in a wildlife park as a consequence of multiple bite wounds from conspecifics. Gene sequencing confirmed that the novel species was a beta-haemolytic group A *Streptococcus* spp. which exhibited more than 3% diversity from other,

reference streptococcal species and was most closely related to, but phenotypically and phylogenetically distinct from, *S. porcinus* and *S. iniae* (*ibid*.). Lawson *et al.,* (2005) named this novel bacterium *S. castoreus* sp. nov.. *S. castoreus* was subsequently isolated from 44% of beavers (n=16) found dead in Germany and these beavers were co-infected with other gram-positive and gram-negative bacteria (Schulze *et al.,* 2015). *S. castoreus* was cultured from rectal swabs from two of these 16 animals suggesting that it is part of the normal commensal enteric flora in Eurasian beavers (*ibid.*). Schulze *et al.,* (2015) found that, in four of seven cases, *S. castoreus* was associated with suppurative lesions but a mixed bacterial flora was grown from all four suppurative lesions. The other bacteria grown are also associated with pus-forming lesions and therefore the pathogenicity of *S. castoreus* is unclear. A summary of the post-mortem findings is given at Table 5.

Table 5: Post-mortem findings in beavers infected with *S. castoreus* (Source: Schulze *et al.,* (2015))

Isolate Identifier	Animal characteristics, localisation of *Streptococcus castoreus* isolation and significant diseases			
	Sex, age, body condition	Isolated from	Accompanying bacterial flora	Significant concurrent diseases
10UCF 103	Male, juvenile, emaciated	Abscessing gonarthritis	*Fusobacterium necrophorum, Prevotella* sp.	Alveolar echinococcosis, Tibiafracture
11UCF 142	Male, adult, emaciated	Biting wound abscess	Species of the *Actinomycetaceae* family, *Fusobacterium necrophorum,*	Metacarpal fracture
11UCF 216	Male, adult, fair	Incised skin wound, internal organs	*Actinobacillus* sp. *Prevotella* sp.	Septicaemia following wound infection
12UCF 3	Male, adult, good	Suppurative laryngitis	*Yersinia pseudotuberculosis*	Yersiniosis
12UCF 17	Female, adult, good	Suppurative cloaca	Coliform bacteria	Fatty heart muscle degeneration
12UCF 33	Male, adult, fair	Normal cloaca	Coliform bacteria	Tularaemia, Postrenal uraemia
12UCF 94	Female, adult, emaciated	Normal cloaca	Coliform bacteria, *Staphyloccus aureus*	Endocarditis and septicaemia (*Staphylococcus aureus*)

Further evaluation between 2010 and 2017 by Mühldorfer *et al.,* (2019) of 27 *Streptococcus* spp*.* isolates from 18 free-living Eurasian beavers, 17 from Germany, including the seven previously assessed by Schulze *et al.,* (2015), one from the UK, and four captive Canadian beavers, confirmed that all isolates were *S. Castoreus.* Twelve of the 27 isolates were found in the respiratory or intestinal tract in otherwise apparently healthy beavers and so Mühldorfer *et al.,* (2019) concluded that *S. castoreus* is a normal commensal organism in beavers but may, in common with other *Streptococcus* spp., act as an opportunistic pathogen under certain circumstances. It should be noted that, as far as we understand, Mühldorfer *et al.,* (2019) isolates were not grown in pure culture from a lesion in any of the 27 cases and therefore the pathogenicity of this bacterium is uncertain. Additionally, as *S. castoreus* has not been isolated from any other host species, Mühldorfer *et al.,* (2019) proposed that *S. castoreus* is a host-specific bacterium.

Disease surveillance work undertaken in 2021 isolated *S. castoreus* from swabs taken from the brain of a beaver carcase found on a road in Kent. This beaver was suspected to have been involved in a road traffic collision and *S. castoreus* an incidental finding; histopathology of the brain of this beaver did not show signs of disease. (Common, Gerard, and Sainsbury 2022).

Opportunistic pathogens are usually of low pathogenicity under normal circumstances but when host immunity is impaired they may behave as conventional pathogens to cause disease in the host (Shanson 1989). As translocation is a known stressor and stress may reduce host immunocompetence (Dickens, Delehanty, and Michael Romero 2010), on the assumption that *S. castoreus* is an opportunistic pathogen, it should be considered a carrier hazard for the translocation of beavers.

Risk Assessment

Release Assessment

Streptococcus spp. can be isolated from bodily fluids including nasal discharges, pus, milk and exudative infected tissues (Speck 2012b). As *Streptococcus* spp. are of short-lived duration in the environment and are commensal bacteria in the respiratory and intestinal tracts, beavers are exposed to, and infected by, *S. castoreus* bacteria harboured by conspecifics through maternal milk, mutual grooming and bite wounds. Beavers may also transfer infection through licking or chewing lesions (Schulze *et al.,* 2015).

Exposure Assessment

There is a high likelihood that *S. castoreus* will be transmitted between beavers during translocation, or at the destination site, by maternal suckling, mutual grooming or fighting. Other beavers translocated to the destination may already be infected.

Since *S. castoreus* appears to be host-specific and *Streptococcus* spp. do not survive well in the environment, the likelihood of exposure of, and dissemination to, other species at the destination site(s) is very low in the short term, but as a commensal infectious agent there is a high likelihood that it would be transmitted through the reintroduced population in the long term.

Consequence Assessment

There is a high probability that at least one beaver is infected with *S. castoreus* when translocated because this bacterium is a component of the normal commensal flora of beavers.

On the assumption that *S. castoreus* is confirmed as an opportunistic pathogen, there is a high probability that, if beavers are under stress and consequential immunodepression from trauma during capture or transit, or respiratory disease, they will be predisposed to develop *S. castoreus*-associated disease. There is substantial evidence that beavers are prone to severe disease and even fatalities following minor injuries and, in addition, are susceptible to stressors (Campbell-Palmer and Rosell, 2015) and therefore there is a high probability of stressor-associated diseases in general. Mühldorfer *et al.,* (2019) reported that *S. castoreus* was associated with a range of lesions from local suppurative inflammation to systemic infection, but not in pure culture as far as we understand, and therefore its pathogenicity remains unclear. A captive Canadian beaver died at Berne zoo as a result of streptococcosis (*Streptococcus* species not identified), although *Salmonella enteriditis* was also cultured (Dollinger *et al.,* 1999).

There is therefore a high likelihood that the stress of translocation will lead to immunocompromise resulting in severe *S. castoreus*-associated disease in an injured or sick beaver. However, reports appear to show disease incidence is sporadic and therefore there is a very low likelihood of economic and biological consequences due to translocation failure. There is a negligible likelihood of biological or ecological consequences due to dissemination of *S. castoreus* at the destination because *S. castoreus* is a commensal infectious agent, and conservation of infection may be important to the future health of the reintroduced population.

Risk Estimation

There is a high likelihood that beavers will be exposed to and infected with *S. castoreus* at the source site(s) and a high likelihood that other beavers will be exposed to and infected with *S. castoreus* at the destination but a very low likelihood of onward transmission to other species and dissemination at the destination site in the short term and a high likelihood in the long term. There is a high likelihood that translocation acts as a stressor on beavers and, given their known susceptibility to stress, there is a high likelihood of disease associated with *S. castoreus*. There is a very low likelihood of economic and biological consequences due to translocation failure. The overall risk from disease caused

by *S. castoreus* is estimated to be MEDIUM, on the assumption that *S. castoreus* is an opportunistic pathogen.

Risk Management

Risk Evaluation

Risk management should be implemented based on the estimated medium risk level.

Risk options

In addition to measures to minimise stress to beavers during capture and handling, care should be taken to avoid injuries through careful planning and preparation of translocation methods, and to ensure that prompt veterinary attention is given to even apparently minor injuries where veterinary intervention is unlikely to cause further stress to the beaver(s). Particular attention should be taken to minimise the risk of fight injuries and bite wounds by avoiding mixing of non-related beavers and releasing beavers at low density into environments with ample opportunities for dispersal and territory establishment.

It may be important to conserve commensal parasites during translocation because it may be counterproductive to create a population of beavers at the release site without exposure and immunity to these parasites, should a non-immune population be subsequently exposed to them.

5.4.11 Disease risk analysis for the carrier hazard *Yersinia* spp.

Carrier Hazard

Justification for Hazard Status

The genus *Yersinia* comprises twelve species of gram-negative coccobacilli (Martin *et al.,* 2009) of which *Y. enterocolitica* (YE) and *Y. pseudotuberculosis* (YP) are associated with disease in mammals in Europe (Najdenski 2012). Both *Y. enterocolitica* and *Y. pseudotuberculosis* consist of serotypes of varying pathogenicity associated with the disease, yersiniosis, in a wide range of species globally, particularly in northern Europe (Najdenski 2012). Other *Yersinia* spp. have been detected in rodents but, to the best of the authors' knowledge, are not associated with disease in this family. *Y. frederikensii* has been isolated from free-living rodents in Europe including bank voles and Eurasian beavers in Great Britain (Campbell-Palmer *et al.,* 2021; Healing and Greenwood 1991). *Y. pestis*, the agent responsible for human plague, is known to use rodents as a reservoir (Zhou *et al.,* 2004); however, to the best of the authors' knowledge is not currently known to be present in Europe, nor has been detected in captive collections.

Both YE and YP are considered to be ubiquitous in numerous species of wild mammals, including rodents, with birds acting as subclinical carriers (Najdenski 2012). A study in Scandinavia found 8% (n=12/154) prevalence of YE in free-living, small rodents (Kapperud 1975). However, in both Sweden and Norway, domestic pigs are believed to be the primary reservoir of YE (Lindberg *et al.,* 2018; Jorgensen *et al.,* 2016). Additionally, both YE and YP have been confirmed in Sweden in a wide range of birds, including those known to migrate to the UK, for example the barnacle goose *(Branta leucopsis)* (Niskanen, Waldenstro, and Fredriksson-ahomaa 2003).

In the UK, YE was isolated from faecal samples from free-living wild animals in Dorset, including the bank vole, between 1986 and 1989 (Healing and Greenwood 1991) and YP from free-living birds and mammals including, prior to its extirpation, the coypu, mouse and field vole (Mair 1973). Infection with either YE or YP was not found in screening of free-living beavers (n = 65) in Great Britain (Goodman, 2014; Campbell-Palmer *et al.,* 2015b; Girling *et al.,* 2019a). However, a gravid female in good body condition was found to be infected with *Y. frederikensii* following re-release in Devon (Campbell-Palmer *et al.,* 2018).

Both YE and YP have been widely detected in captive collections in numerous species including rodents. In fact, *Yersina* was one of the most common pathogen genera detected in rodents across 207 publications, being identified in 12 papers (Hardgrove *et al.,* 2021). Moreover, it was found to be the most common pathogen genera detected in captive rodents (n=9 studies) in the same review (Hardgrove *et al.,* 2021). For example, YP was responsible for pseudotuberculosis cases in numerous rodents in Antwerp Zoo across a five-year period between 1970 and 1974. Affected species included capybara*,* black-tailed prairie dogs (*Cynomys ludovicianus*), guinea pigs, Brazilian porcupines (*Cynomys ludovicianus*) and agouti (*Dasyprocta aguti*) (Kageruka *et al.,* 1976). YP was also isolated from numerous organs on post-mortem examination of four captive capybara that died of disseminated disease in Ljubljana Zoo, Slovenia (Gombač *et al.,* 2008), as well as in a Paca (*Cuniculus paca*) which died suddenly in a zoo in North America (Fogelson *et al.,* 2015). In England, pseudotuberculosis associated with *Yersinia* spp. has been reported in Patagonian mara in Whipsnade Zoo (Holz 1994), and YP associated disease reported in a North American beaver in London Zoo (Parsons 1991).

Susceptibility to yersiniosis probably varies from species to species but sporadic outbreaks of disease resulting in high mortality have been reported in a wide range of wildlife species (Najdenski, 2012). Additionally, stressful conditions such as cold and wet weather, limited food availability, overcrowding and capture may precipitate clinically-significant disease in sub-clinical carriers (Gasper and Watson 2001). Disease incidence is reported to be higher in winter months (Najdenski 2012).

Yersiniosis has been cited as the cause of deaths in Eurasian beavers, either in isolation or in combination with other diseases, in three studies (Nolet *et al.,* 1997; Platt-Samoraj *et al.,* 2015; Stefen 2018; Parsons 1991). For example, of 57 beavers translocated from

Germany to the Netherlands between 1988 and 1994, four died with yersiniosis associated with either YE or YP, including one which had been vaccinated prior to translocation against YP, in the first three months following release (Nolet *et al.,* 1997). Nolet *et al.,* (1997) suggested that stress from territorial conflict and food shortages contributed to disease susceptibility in these translocated beavers as they had all settled in habitats of poor quality compared to other translocated beavers.

A wild-caught beaver from Norway (M08K33) which died during quarantine in the UK with severe enteritis and focal hepatic necrosis was found to have an *Escherichia coli* bacteraemia; histopathology was reported to be suggestive of yersiniosis (Cranwell 2009b). It was suggested that suspected yersiniosis in this beaver (M08K33) and another (M08K20), might be a result of prolonged confinement in captivity (Cranwell 2009a). The difficulty of monitoring the health and disease of beavers following translocation, either due to the difficulty of finding sick or dead wild animals (Wobeser 2007) or the challenges of trapping free-living beavers (Campbell-Palmer *et al.,* 2015) suggests other cases of yersiniosis in beavers may have been missed.

As all translocations are associated with stress (Dickens, Delehanty, and Michael Romero 2010), and stress precipitates reduced immunocompetence, and YE and YP are ubiquitous at the source and destination, translocated beavers will be predisposed to yersiniosis. Therefore, YE and YE should be considered as carrier hazards for the translocation of Eurasian beavers.

Risk Assessment

Release Assessment

Yersinia spp. are psychrophilic, able to survive and multiply at low temperatures (2-5°C), and capable of surviving for up to 20 days in water and 540 days in soil (Najdenski 2012). Beavers at the source site will be exposed and infected primarily via the faeco-oral route via contaminated food or water. In captive collections, rodents are thought to be exposed to *Yersinia* spp. through food contaminated with faeces from free-living birds and rodents (Obwolo 1976); *Yersinia* spp. have been detected in the faeces of free-living rats caught within zoo grounds (Baskin *et al.,* 1977; Kaneko and Hashimoto 1983). Transmission between different captive species is also possible, for example through fomites, as endemicity has been reported in species such as Patagonian mara (Parsons 1991; Holz 1994).

In both free-living and captive beavers, the likelihood of being exposed to *Yersinia* spp. at the source site is estimated to be high because these bacteria are known to be ubiquitous and persistent for prolonged periods in the environment. If exposed, there is a high likelihood that beavers will be infected because beavers are known to be susceptible to infection.

Exposure Assessment

Mammals, including beavers, at the destination will be exposed to *Yersinia* spp. through the faeco-oral route. Carriers of YE and YP are known to shed these bacteria for prolonged periods (Najdenski 2012) and because YE and YP may survive for prolonged periods in the environment, there is a high probability of direct exposure at the destination site(s). Many mammal species are susceptible to infection and therefore there is a high likelihood that mammals at the destination will be infected.

There is a high likelihood that mammals at the destination will maintain and disseminate these agents at the destination site(s) by shedding infectious *Yersinia* bacteria in their faeces. In addition to faeco-oral transmission, venereal and transplacental routes are possible (*ibid.*).

Consequence Assessment

The clinical presentation of disease in mammals caused by both YE and YP may be similar (Najdenski 2012). Where YE is associated with acute disease, the signs are fulminating septicaemia and enteritis, leading to death within one to three days (Najdenski 2012). Chronic disease typically features necrotising enteritis resulting in weight loss, anorexia and lethargy amongst other clinical signs (Najdenski 2012).

There is a high probability that one translocated beaver becomes infected. Since translocated beavers will be under stress there is a high likelihood that they will be affected by yersiniosis (acute, subacute or chronic disease) as illustrated by reports of disease following translocation (Nolet *et al.,* 1997). As *Yersinia* spp. are psychrophilic, there may also be recrudescence of latent infections during the winter months due to the stresses of cold and hunger, resulting in disease. Therefore, yersiniosis may occur weeks or months following translocation. There is a high probability of biological and economic consequences through failure of the translocation. However, since YE and YP are ubiquitous, the long term environmental and biological consequences are negligible.

Risk Estimation

There is a high likelihood that released beavers will be exposed to, and infected with, YE or YP. The likelihood of exposure, infection and dissemination at the destination is high. There is a high probability that the stress of translocation may precipitate disease in infected beavers and lead to the failure of the translocation. The overall risk of disease in translocated beavers and failure of the translocation from YE- and YP-associated disease is therefore HIGH.

Risk Management

Risk Evaluation

Based on the risk assessment above, preventative measures should be employed to reduce the risks from YE and YP.

Risk options

Measures to reduce the stress from translocation are important. For example, efforts should be made to minimise stress from capture, transport and, in particular, reduce the need for repeated handling and the duration of transit. Consideration should also be given to the timing of releases, avoiding winter months when lower temperatures and food shortages may increase the risk from stressor-associated disease.

Diagnosis is usually by isolation of bacteria from faeces, throat swabs, mesenteric lymph nodes, peritoneal fluid or blood, with faecal culture the usual method in practice, and can be considered in the event that a sick beaver is detected. However, this method is regarded as unreliable as positive cultures may only be achieved in the first two weeks of illness. As a consequence, cases of infection with *Yersinia* spp. may not always be detected.

Diagnostics for this disease should be considered as part of the post-release health surveillance protocol to help inform future decision making on disease risk management regarding this parasite but is not considered necessary ahead of release.

5.4.12 Disease risk analysis for the unclassified hazard *Cryptosporidium parvum*

Unclassified Hazard

Justification for Hazard Status

Cryptosporidium spp. are ubiquitous enteric protozoan parasites that can infect a broad spectrum of vertebrate hosts causing a range of clinical disease from asymptomatic to acute or chronic diarrhoeal disease (Mateo *et al.,* 2017). Infection in healthy humans is usually self-limiting and declines in prevalence with increasing age (European Centre for Disease Prevention and Control 2019b) but disease can be severe in young mammals, especially if malnourished, and persistent in immunodeficient adults (Laurent 2019). Transmission is primarily faeco-oral, either directly or indirectly via the environment in water and food, and respiratory infection via nasal secretions is also reported (Thompson *et al.,* 2005b). Oocysts have been shown experimentally to remain viable in river water for almost six months with prolonged survival in faeces (Robertson, Campbell, and Smith

1992). Water-borne oocysts are resistant to chemical treatment, including chlorine (Chalmers *et al.,* 2019), and ingestion of fewer than 10 oocysts may lead to infection (Ryan, Fayer, and Xiao 2014).

At least 38 species of *Cryptosporidia* have been identified to date, most of which are host-specific (Feng, Ryan, and Xiao 2018). Genotyping, usually using the Gp60 gene, has facilitated understanding of *Cryptosporidium* spp. and epidemiology and transmission between species and the environment (Chalmers *et al.,* 2019). At least 20 *Cryptosporidium* species and genotypes have been identified in humans but not all may be true infections as it is often hard to differentiate patent infections with replicating parasites from the mechanical transmission of ingested oocysts (Feng, Ryan, and Xiao 2018). Humans are commonly infected by *C. parvum* or *C. hominis,* with *C. ubiquitum* regarded as an important emerging zoonosis because of its wide geographic distribution and host range (Mateo *et al.,* 2017).

C. hominis is usually regarded as host-specific to humans but is increasingly reported in animals. However most animal infections with *C. hominis* are probably spillover events from human reservoirs (Feng, Ryan, and Xiao 2018). To date, eight host-adapted sub-families of *C. ubiquitum* have been identified (Feng, Ryan, and Xiao 2018). In the USA, humans are predominantly infected with rodent sub-types XIIb to XIId but in the UK zoonotic infection is predominantly from ruminant-adapted sub-type XIIa (*ibid*.). The broad host range of rodent-adapted *C. ubiquitum* sub-types may indicate a sylvanian transmission cycle with occasional spillover to humans (Tang *et al.,* 2016).

C. parvum is the most important zoonotic *Cryptosporidium* spp. and also the most common cause of cryptosporidial disease in young calves (Brook *et al.,* 2009). Currently nearly 20 sub-types of *C. parvum* are recognised of which the most prevalent, IIa and IId, are adapted to animals and IIc adapted to humans (Feng, Ryan, and Xiao 2018). Of these, IIaA15G2R1 is the dominant IIa subtype in calves and lambs and is also commonly reported in humans (*ibid*.). In addition to ruminants, IIa has been reported in a wide range of species including wild trout (*Salmo trutta*) in northwest Spain (n=47/613) (Couso-Perez, Ares-Mazas, and Gomes-Couso 2019) and rats in Malaysia (n=9/12) (Tan *et al.,* 2019) although cats and dogs do not appear to be susceptible to infection (Thompson *et al.,* 2005b). Historic reports of high levels of *C. parvum* prevalence in wild rodents by Sturdee *et al.,* (2003) and Bajer *et al.,* (2002) may have been overstated due to reliance on diagnosis by morphology alone prior to the advent of molecular genetic tools and the potential for cross-reactivity between *C. parvum* and newly identified *Cryptosporidium* species in voles (Horcickova 2019).

Chalmers *et al.,* (2019) analysed outbreaks of human infections with *Cryptosporidium* spp. between 2009 and 2017 in England and Wales and found that 56% (n=82/178) were caused by contact with recreational waters and 42% (n=74/178) were as a result of animal contact. Of outbreaks where the causative species was identified, 53% were found to be *C. parvum* (n=69/131) and 46% (n=60/131) *C. hominis*. Using gp60 subtyping, Chalmers

et al., (2019) identified that animal contact-based outbreaks predominated in the first half of the year, when incidence in calves and lambs also peaks, and were all caused by *C parvum.* Identical subtypes were isolated from lambs in 12 outbreaks and from calves in two (*ibid.).* The predominant subtype (IIaA15G2R1) was also previously isolated from faecal samples from calves on 14/41 farms in a study in Cheshire in 2004 (Brook *et al.,* 2009). *C hominis* was not isolated from any animals at locations associated with recreational water outbreaks in the study by Chalmers *et al.,* (2019). Following the outbreak of foot and mouth disease in the UK in 2001, and the extensive culling of ruminant livestock and limits on human and animal movements, reported human cases of cryptosporidiosis caused by *C. parvum* were only 35% (n=338/977) of the previous year's level (Smerdon *et al.,* 2003) further suggesting that ruminants are a major reservoir of zoonotic *C. parvum* isolates.

Rodents are considered to be important reservoir hosts of *Cryptosporidium* spp. (Quy *et al.,* 1999; Chalmers *et al.,* 1995) including in the UK (Webster and Macdonald 1995). A review undertaken by Feng (2010) found an overall prevalence of 18.4% (1937/10344) of *Cryptosporidium* spp. in free-living rodents across multiple studies undertaken in Europe. This included several reports of considerable prevalence in rodents which could be sympatric to beavers at riparian margins. For example, they reported an overall prevalence of 26.7% (69/259) in the yellow-necked mouse in studies across Poland and Spain (Bajer *et al.,* 2002; Sinski, Hlebowicz, and Bednarska 1993; Torres *et al.,* 2000; Bednarska *et al.,* 2007), 31.4% (160/510) prevalence in wood mouse populations in studies undertaken in Portugal, UK and Spain (Chalmers *et al.,* 1997; Torres *et al.,* 2000; Hajdušek, Ditrich, and Šlapeta 2004) and a 49.7% (201/405) prevalence in field voles in Finland and Poland (Bajer *et al.,* 2002; Laakkonen, Soveri, and Henttonen 1994).

There has been only limited testing of beavers for infection with *Cryptosporidium* spp. and so susceptibility and reservoir potential is poorly understood in the species. Paziewska *et al.,* (2007) analysed faecal samples from 52 wild caught and farmed Eurasian beavers in Poland using an immunofluorescence assay (MeriFluor IFA) for *Cryptosporidium* antigen. 19.2% (n=10/52) samples were positive with statistically insignificant differences between prevalence and abundance in wild and farmed beavers which Paziewska *et al.,* (2007) proposed as an indication of autogenous rather than environmental infection. The test used in this study is specific for *C. parvum* but is reported to also cross-react with *C. muris* and *C. meleagridis* (Y. Craig, pers. comm). Sroka *et al.,* (2015) tested 79 water samples from 14 watercourses close to beaver habitats between 2010-14 in Poland. 45.6% (n=36/79) of water samples were positive for *Cryptosporidium* spp. by immunomagnetic separation which is not specific for *C. parvum*. There was no statistical difference in the prevalence of oocysts at different distances from the beaver lodge, unlike for *Giardia duodenalis*, also tested in this study, for which prevalences were significantly higher the closer to the lodge the water was sampled. As a result, Sroka *et al.,* (2015) were unable to conclude that beavers were the source of water contamination with *Cryptosporidium* spp..

Human cases of *Cryptosporidium* infection in Norway are reported to be the 4th highest in Europe and to be increasing rapidly, with a 50% increase in 2017 (n=379/255), the last year for which figures are available (European Centre for Disease Prevention and Control 2019b). *Cryptosporidium* oocysts are regularly isolated from surface water in Norway (Rosell, Rosef, and Parker 2001) but were not detected in limited testing of 241 free-living Norwegian beavers in Telemark, Norway between 1997 and 1999 using a microplate immunoassay for *Cryptosporidium* spp. antigen (*ibid.*). Human outbreaks in 2009 and 2012 in Norway have been associated with sub-type IIaA19G1R1, shown to have been caused by contact with infected lambs and kids (Lange *et al.,* 2014). Beavers imported from Norway (n=19) for the Knapdale project in Scotland in 2008 all tested negative for *Cryptosporidium* infection (Goodman *et al.,* 2012).

The UK reported the highest number of *Cryptosporidium* spp. infections in humans (n=5052) of any reporting country in Europe in 2017 (European Centre for Disease Prevention and Control 2019b) with nearly half of cases resulting from animal contact (Chalmers *et al.,* 2019). Testing of free-living beavers by microscopy as part of the River Otter Beaver Trial (n=43) did not identify any infected beavers (Campbell-Palmer and Girling 2019); however a single adult male (n=1/22), shot in Tayside, Scotland (Campbell-Palmer *et al.,* 2015b) and a predated kit, recovered in Knapdale (Mackie 2014), were found to be infected although the *Cryptosporidium* species was not identified in either case. Testing for *Cryptosporidium* spp. infection by microscopy is not regarded as sensitive and it is estimated that about 50% of all cases are missed by this method (Nichols *et al.,* 2006). In addition, oocyst shedding may be intermittent (Ryan, Zahedi, and Paparini 2016) so it is possible that further infected beavers have been missed. In addition, it has been proposed that beavers can amplify and contribute to the environmental reservoir of *Giardia duodenalis*, even if they are not a primary reservoir (Monzingo and Hibler 1987), and there may similarly be potential for beavers to amplify environmental burdens following infection with zoonotic *Cryptosporidium parvum* sub-types. Prior to and following the release of beavers for the Knapdale trial, watercourses were monitored for the presence of *Cryptosporidium* spp. oocysts (Mackie 2014). 4/6 sites in Knapdale were found to contain *Cryptosporidium* spp. oocysts of unknown species prior to the release of beavers but, following release of the beavers, *Cryptosporidium* oocysts were only recovered from one of the four sites (*ibid.*). However, this may indicate that beavers were not susceptible to infection with the particular *Cryptosporidium* species detected. As beavers have been shown by other authors to be susceptible to unidentified *Cryptosporidium* species which may include sub-types that are infectious to livestock and humans*, C. parvum* should be considered as a hazard for humans and livestock following the translocation of beavers from mainland Europe or enclosures and within Great Britain.

Risk Assessment

Release Assessment

Contamination of watercourses by faeces from infected humans and other animals may be sporadic with oocysts remaining infectious for several months following excretion. Beavers sourced from, or released into, contaminated areas may ingest *Cryptosporidium* oocysts in water or on plant material. As the infective dose is low, oocysts can survive for prolonged periods, and *C. parvum* sub-types IIa and IId can infect, and replicate in, a wide range of species which may include beavers, there is a very low likelihood that translocated beavers may be exposed to and infected by sub-types of *Cryptosporidium* spp. infectious to humans and other animals. Following ingestion or inhalation of sporulated oocysts by a suitable host, the oocyst excysts and its four sporozoites rapidly invade epithelial cells and undergo asexual proliferation, ultimately resulting in the formation of large numbers of thick-walled oocysts which are released in either faeces or nasal secretions (Thompson *et al.,* 2005b).

Exposure Assessment

Infected beavers will excrete large numbers of oocysts in their faeces into watercourses close to their lodges. In addition, as beavers are coprophagic they are likely to repeatedly reinfect themselves and to increase the number of infectious oocysts shed in their faeces into water surrounding their lodges (Monzingo and Hibler 1987). Conspecifics, sympatric species and humans and domestic animals drinking or accidentally ingesting water will be exposed to infection. As *Cryptosporidium* oocysts have prolonged survival in water and are resistant to chlorine treatment there is a high likelihood of exposure and infection of people and domestic animals. There is a high likelihood of dissemination to other susceptible species, even at some distance from beaver lodges due to the prolonged survival in water.

Consequence Assessment

There is a very low likelihood that a translocated beaver will be infected with *Cryptosporidium* spp..

No cases of cryptosporidiosis disease have been reported in beavers so it is likely that, in common with many other species, otherwise healthy adult animals do not experience long-lasting clinical disease following infection. However, immunocompromised hosts may develop more severe clinical signs or recurrent and chronic infections and young calves, lambs and kids may die from dehydration and cardiovascular collapse (Thompson *et al.,* 2005b). The economic cost to farmers as a result of impaired weight gain and the cost of treatment may be significant (*ibid*.). There is a medium likelihood of sporadic disease in humans and domestic animals and economic effects from public health control, hospital treatment and veterinary treatment.

In a previous assessment following the Knapdale trial, Boden and Auty (2015) concluded that other, existing sources of contamination such as humans and other animals are likely to be greater contributors to the overall number of oocysts shed into the environment than beavers. It seems likely that beavers may have potential to contribute to and amplify the environmental burden of infectious *Cryptosporidium* spp. oocysts but are likely to cause only a very low increase in the overall burden.

Risk Estimation

There is a very low likelihood that beavers will be exposed to and infected with *Cryptosporidium parvum* sub-types IIa or d, a high risk of exposure and infection of beavers, sympatric animals, humans and domestic animals at the destination, and a high risk of dissemination to other species at the destination site(s). There is a medium likelihood of sporadic disease in humans and domestic animals at the destination. The change in risk at the destination site(s) as a result of beaver translocations is likely to be very low. The overall risk is VERY LOW.

Risk Management

Risk Evaluation

Since the risk is estimated to be higher than negligible, management measures should be implemented.

Risk options

Public health advice, particularly warning of the risks of swimming close to beaver lodges, and regular water testing may prove valuable in management of the risks. Release sites should, ideally, be chosen in consultation with relevant water authorities or private water supply owners, particularly given the likely long-term potential for beavers to disperse away from release sites. Fencing to prevent livestock defecating into water edges may also be advisable in order to reduce the likelihood of infection of beavers and transmission from beavers to domestic animals.

5.4.13 Disease risk analysis for the carrier hazard *Cryptosporidium* spp.

Carrier Hazard

Justification for Hazard Status

At least 38 species of *Cryptosporidium* have been identified to date, most of which are host-specific (Feng, Ryan, and Xiao 2018). Genotyping, usually using the Gp60 gene, has facilitated understanding of *Cryptosporidium* spp. and epidemiology and transmission between species and the environment (Chalmers *et al.,* 2019). *C. parvum* is the most important zoonotic *Cryptosporidium* spp. and has already been evaluated as an unspecified hazard, and the risk estimated to be very low. *Cryptosporidium* spp. may also pose a risk to the beavers themselves as a carrier hazard. As previously discussed, *Cryptosporidium* spp. have been detected in numerous rodent species which are considered to be important reservoir hosts, with a prevalence of between 18.14% and 49.7% detected in Europe (Quy *et al.,* 1999; Chalmers *et al.,* 1995; Feng 2010; Webster and Macdonald 1995; Sinski, Hlebowicz, and Bednarska 1993; Torres *et al.,* 2000; Bajer *et al.,* 2002; Bednarska *et al.,* 2007). Beavers are known to be susceptible to infection with *Cryptosporidium* spp.: Paziewska *et al.,* (2007) analysed faecal samples from 52 wild caught and farmed Eurasian beavers in Poland using an immunofluorescence assay for *Cryptosporidium* antigen and 19.2% (n=10/52) samples were positive.

Unlike in humans and livestock, *Cryptosporidium* spp. are thought to be non-pathogenic in rodents. In all prevalence studies reviewed by the authors, no clinical signs of disease were noted in the free-living rodents examined which were infected with *Cryptosporidium* spp. (Bajer *et al.,* 2002; Bednarska *et al.,* 2007; Sinski, Hlebowicz, and Bednarska 1993; Torres *et al.,* 2000; Chalmers *et al.,* 2011; Hajdušek, Ditrich, and Šlapeta 2004; Laakkonen, Soveri, and Henttonen 1994; Horcickova 2019). However, there have been reports of disease in captive rodents. A prevalence study of *Cryptosporidium* spp. in China across numerous rodents, both free-living and captive, detected faecal matter on the tail (an indicator of diarrhoea) of one captive golden (Syrian) hamster (*Mesocricetus auratus*) infected with *C. parvum*, which could indicate that clinical disease is possible in rodents. This golden hamster was also infected with *Giardia* spp., another diarrhoeal agent, which could have been responsible for the signs seen. Moreover, of the 723 rodents sampled in this study, one golden hamster and two Siberian hamsters (*Phodopus sungorus*) had signs of diarrhoea with no parasites detected, providing further suggestion *Cryptosporidium* spp. may not be related to diarrhoea in rodents (Lv *et al.,* 2009). One gerbil (*Meriones unguiculatus*) is reported to have had diarrhoea associated with infection with *C. hominis* (Widmer, Köster, and Carmena 2020) although no further details are given and so this report must be interpreted with caution.

In rodents, it is possible that, in common with human and livestock hosts, clinical disease associated with infection with *Cryptosporidium* spp. develops during periods of immunocompromise (Thompson *et al.,* 2005a). Translocation is a known stressor (Dickens *et al.,* 2010) which can suppress immune responses. Given the likelihood that beavers will be exposed to and chronically infected with *Cryptosporidium* spp*.* at the time of translocation, this parasite should be considered as a carrier hazard.

Risk Assessment

Release Assessment

Following ingestion or inhalation of sporulated *Cryptosporidium* oocysts by a suitable host, the oocyst excysts and its four sporozoites rapidly invade epithelial cells and undergo asexual proliferation, ultimately resulting in the formation of large numbers of thick-walled oocysts which are released in either faeces or nasal secretions (Thompson *et al.,* 2005b). The most likely route of exposure of beavers to *Cryptosporidium spp.* is through direct ingestion of oocysts shed into the environment by infected humans, livestock or other animals leading to environmental contamination and contamination of water sources. Shedding may be sporadic, but oocysts are likely to remain infectious for several months following excretion. Beavers sourced from, or released into, contaminated areas may ingest *Cryptosporidium* spp. oocysts in water or on plant material.

As previously discussed, *Cryptosporidium* spp. are ubiquitous across Europe, and infection and cross-species transmission in captive collections occurs. *Cryptosporidium muris* has been detected concurrently in free-living mice and bilbies (*Macrotis lagotis*) at a captive breeding facility in Australia (Warren *et al.,* 2003). In zoological institutes, *Cryptosporidium* spp. have been detected in numerous species including reptiles (Xiao *et al.,* 2004), ruminants, carnivores and rodents (Fayer, Santín, and Macarisin 2010; Li *et al.,* 2015; Perrucci *et al.,* 2019).

As the infective dose is low, oocysts can survive for prolonged periods in the environment, and beavers are known to be susceptible to infection with *Cryptosporidium* spp. there is a low likelihood that translocated beavers sourced from free-living populations in mainland Europe and Great Britain, or from captive collections in Great Britain, may be exposed to and infected with *Cryptosporidium* spp..

Exposure Assessment

Infected beavers will excrete large numbers of oocysts in their faeces into watercourses close to their lodges. In addition, as beavers are coprophagic they are likely to repeatedly reinfect themselves and to increase the number of infectious oocysts shed in their faeces into water surrounding their lodges (Monzingo and Hibler 1987). Conspecifics, sympatric species and humans and domestic animals drinking or accidentally ingesting water will be exposed to infection. As *Cryptosporidium* spp. oocysts have prolonged survival in water

and are resistant to chlorine treatment there is a high likelihood of exposure and infection of people and domestic animals. There is a high likelihood of dissemination to other susceptible species, even at some distance from beaver lodges, due to the prolonged survival of *Cryptosporidium* spp. in water.

Consequence Assessment

There is a low likelihood that a translocated beaver will be infected with *Cryptosporidium* spp..

No cases of cryptosporidiosis have been reported in beavers so it is likely that, in common with many other species, otherwise healthy adult animals do not experience clinical disease following infection. However, immunocompromised hosts may develop more severe clinical signs of cryptosporidiosis, or recurrent and chronic infections, and young calves, lambs and kids may die from dehydration and cardiovascular collapse (Thompson *et al.,* 2005b). There is a low likelihood that the conditions of translocation will, as a stressor, lead to immunocompromise and a change in host-parasite dynamics resulting in cryptosporidiosis in translocated beavers infected with *Cryptosporidium* spp. If clinical disease occurs, there is a medium likelihood of severe consequences, including death.

There is a very low likelihood of biological, environmental and economic consequences at the destination as a result of failure of the translocation. The likelihood of ecological consequences at the destination site is negligible.

Risk Estimation

There is a low likelihood that beavers will have been exposed to and infected with *Cryptosporidium* spp. at the time of translocation. There is a high likelihood of dissemination to other susceptible species at the destination site. There is a low likelihood that the conditions of translocation will lead to immunocompromise and a change in host-parasite dynamics resulting in cryptosporidiosis in translocated beavers. If clinical disease occurs, there is a medium likelihood of severe consequences, including death. There is a very low likelihood of biological, environmental and economic consequences at the destination as a result of failure of the translocation. The overall risk is estimated to be LOW.

Risk Management

Risk Evaluation

Since the risk estimation is higher than negligible, mitigation measures should be considered.

Risk options

Due to the nature of a carrier hazard, disease management options will concentrate upon reduction of stress in the translocated population of beavers.

Diagnostics for cryptosporidiosis, for example examination of faecal samples, should be part of the post-release health surveillance protocol to help inform future decision making regarding this parasite but are not necessary ahead of release, given that the parasite is present in England, and the stress of testing and prolonged captivity while test results are pending outweighs the benefits of testing.

5.4.14 Disease risk analysis for the carrier hazard *Eimeria* spp.

Carrier Hazard

Justification for Hazard Status

Coccidia are a subclass of protozoan parasites within the phylum *Apicomplexa,* further divided into four orders including Eucoccidiorida. There are two suborders within Eucoccidiorida, the second being *Eimeriorina* which contains several genera of coccidian parasites known to cause disease in vertebrates.

Eimeria sprehni oocysts have been reported several times as a post-mortem finding in beavers. Demiaszkiewicz *et al.,* (2014) undertook parasitological examinations of 48 free-living Eurasian beaver carcases found between April 2011 and November 2012 in Poland. In one young beaver, oocysts of *E. sprehni* were detected in faeces. A low burden of *Eimeria* spp. oocysts were detected in the faeces of one live-trapped Eurasian beaver in Tayside as part of health screening of this population between 2013 and 2019. The beaver was a juvenile and in good body condition with no signs of associated disease. No analysis was undertaken to determine the species of *Eimeria* (Campbell-Palmer *et al.,* 2021)

E. sprehni has also been detected in free-living North American beavers. A survey was undertaken in Kansas, USA, during the trapping season of 1991, and 63 beaver carcases were analysed to determine their endoparasite fauna. 25% of beavers (n=16) were infected with *E. sprehni*, and a further 5% (n=3) were infected with *E. causeyi*. One of these animals had a mixed infection with both species (Mckown *et al.,* 1995). Two early reports of coccidia in *C. canadensis* exist. Morley (1934) found coccidia oocysts in the faeces of one beaver from Pennsylvania (cited by McKown *et al.,* 1995) and, in the same year, Yakimoff (1934) described a case of *E. sprehni* from a captive North American beaver (cited by McKown *et al.,* 1995). These reports provide evidence that coccidian parasites can be present in beavers, although associated disease has not been reported.

The lack of disease associated with these coccidian infections in beavers concurs with general consensus that these parasites are non-pathogenic in rodents in the absence of underlying disease (Chapman *et al.,* 2013; Schmidt 1995). However, there are several reports which present evidence that some coccidian parasites can lead to disease in rodent species. In guinea pigs, infection with *E. caviae* can lead to severe disease and death. Clinical signs include watery or haemorrhagic diarrhoea, anorexia and a poor quality coat (Brabb *et al.,* 2012; Ellis and Wright, 1961). Gross pathological lesions associated with *Eimeria* spp. include thickening of the colon and petechial hemorrhages alongside white plaques on the colonic mucosa (Schmidt 1995). Virulence of this parasite has been attributed to stress; a group of 12 laboratory guinea pigs died after exhibiting clinical signs of diarrhoea, and the cause of death was attributed to *E. caviae* after lifecycle stages of *E. caviae* were found within the colonic mucosa on histopathological examination. It is thought that disease was triggered after the guinea pigs were exposed to stress including transport, injection and introduction to new surroundings (Ellis and Wright, 1961)

Another *Eimeria* species, *E. falciformis*, has been suggested as a cause of diarrhoea and catarrhal enteritis in European mice when heavy infection occurs (Whary *et al.,* 2015). Mice have been shown to be susceptible to disease from *E. falciformis* in a laboratory setting. In a study by Mesfin *et al.,* (1997), groups of mice were infected orally with different numbers of oocysts to determine if increased parasite burdens lead to increased disease severity. It was found that mortality rates increased as the infective dose increased. The highest mortality rates were seen in mice infected with over 20,000 oocysts (30.8%, n=20), although this mortality rate was not significantly different to mice infected with 5,000 oocysts (27.3%, n=29). No mortalities occurred in the 105 mice infected with 500 oocysts, but disease including diarrhoea, depression, anorexia and weight loss occurred in all experimental groups and histopathology determined that *E. falciformis* was associated with the disease (Mesfin, Bellamy, and Stockdale 1977). Although this study was undertaken in a laboratory setting, it provides indication that rodents can suffer disease and death associated with infection with coccidian parasites under certain conditions, and severity may increase with exposure dose. Although the validity is reduced by the laboratory setting, the increased stress experienced by animals in this environment may have increased the severity of results and this effect may be replicated when undertaking conservation interventions such as translocations. Indeed, stress has been attributed as a cause for increased virulence of coccidian parasites in host species. It is widely understood and accepted that stress can lead to immunocompromise (Dhabhar and McEwen, 1997; Dickens *et al.,* 2010; Glaser and Kiecolt-Glaser, 2005) and stress has been suggested to be an inevitable component of animal translocations, which can occur at multiple stages including capture, transport and captivity (Teixeira *et al.,* 2006; Dickens, Delehanty, and Michael Romero 2010; Dickens, Delehanty, and Romero 2009).

Coccidiosis was suggested to be a common cause of death in red squirrels in the UK after a post-mortem survey was undertaken (Keymer 1983). This finding was further supported

by reports of mortality associated with coccidiosis in red and grey squirrels in the UK (Tittensor 1975, 1977) and red squirrels in Finland (Lampio 1967). However, it is difficult to conclude that coccidiosis was the cause of death of squirrels in these studies as results were not confirmed histopathologically and relied instead on findings of oocysts within the intestines. Pathogenicity of *E. sciurorum* has been confirmed experimentally (Pellérdy 1974), but never in free-living animals. It is likely that stress, infective dose and underlying disease lead to increased virulence of the parasite.

It is known that beavers carry certain coccidian parasites within their intestines, and that rodents can suffer from disease as a result of coccidiosis, particularly under conditions of stress and/or high infective doses. Therefore, since translocation is likely to act as a stressor to the beavers, and there is the possibility that beavers will be exposed to infective doses, coccidiosis could occur.

Risk Assessment

Release Assessment

Eimeria spp. have a direct life cycle. Infected hosts shed unsporulated oocysts in faeces which sporulate in the environment, if conditions are favourable, and become infective. When a new host ingests these oocysts the oocysts migrate to epithelial cells, most often of the intestinal mucosa, where they develop (McDonald and Shirley, 2009; Norton and Chard, 1983). In order to become infected, a beaver must ingest unsporulated oocysts from the environment. Coccidian parasites show a high degree of host specificity, particularly within the *Eimeria* genus (Ellis and Wright 1961; Chapman *et al.,* 2013), and can persist for long periods of time in the environment, particularly soil (Lassen, Lepik, and Bangoura 2013).

There have been no reports of *Eimeria* spp. detection in beavers from Norway, although sporadic cases have been described in beavers across the world, both free-living and captive, including in Scotland. Moreover, coccidian parasites are commonly detected in the faeces of captive animals in zoological collections, including rodents: *Eimeria* spp. were detected in the faeces of seven black-tailed prairie dogs undergoing a period of quarantine in a North American zoo (Gardhouse and Eshar 2015). Moreover, an annual survey of faecal parasites in a Spanish zoo found that *Eimeria* spp. were the most frequently detected parasite, with 17.3% (75/432) of faecal samples collected from animals across multiple taxa positive (Pérez Cordón *et al.,* 2008). This high prevalence of coccidian parasites in captive animals could be explained by the direct lifecycle of these parasites, the intensive husbandry of captive collections, and the fact that stocking densities are higher than would be natural for many species. Moreover, transmission between enclosures is likely to occur in the absence of inappropriate biosecurity measures, or through free-living rodents gaining access to enclosures. Elsheikha *et al.,* (2010) detected *E. muris* in free-living rodents captured around Twycross Zoo suggesting that captive beavers could be exposed to coccidian parasites via this route. However,

Eimeria spp. are relatively host-specific, and it is not clear whether beavers are susceptible to infection with species other than *E. sprehni*.

There is a medium likelihood that beavers will have been exposed to *Eimeria* spp. and a medium likelihood of infection after exposure given that their susceptibility to different *Eimeria* spp. is unknown. Therefore, there is a medium likelihood that beavers will be chronically infected with *Eimeria* spp. when translocated.

Exposure Assessment

Infected beavers will carry the protozoa to the destination and may contribute to the environmental reservoir of these parasites through faecal shedding. Therefore, there is a high likelihood of exposure of other beavers at the destination, especially because the small population will be at relatively high density immediately after translocation.

Since *Eimeria* spp. are host-specific, beaver translocations are unlikely to contribute to infection with *Eimeria* spp. in other rodent species at the destination site. The reintroduction itself is predicted to have a low impact on the host-parasite dynamics at the destination site since *Eimeria* spp. are likely to be prevalent in the environment across Europe. Therefore, the likelihood of dissemination at the destination site because of beaver reintroductions is negligible.

Consequence Assessment

The probability that at least one beaver is infected with *Eimeria* spp. at the time of translocation is medium.

There is a medium likelihood that the conditions of translocation will, as a stressor, lead to immunocompromise and a change in host-parasite dynamics resulting in coccidiosis in translocated beavers. There is a medium likelihood that the conditions of translocation may expose beavers to a higher burden of parasites than would occur naturally, leading to disease.

In cases of acute clinical disease, there is a low likelihood of severe disease in the individual and a low likelihood of death. There is a low likelihood of biological, environmental and economic consequences at the destination as a result of failure of the translocation. The likelihood of ecological consequences at the destination site is negligible because *Eimeria* spp. are already present in the UK.

Risk Estimation

There is a medium probability of beavers being exposed and infected with *Eimeria* spp. at the source site. There is a high likelihood of exposure at the destination but a negligible likelihood of dissemination. There is a medium likelihood that infected beavers will develop

disease as a result of translocation and a low likelihood of biological, economic and environmental consequences through failure of the translocation. Overall, the risk is estimated to be MEDIUM.

Risk Management

Risk Evaluation

Since the risk is estimated to be higher than negligible, mitigation methods should be implemented.

Risk options

Stress reduction and good captive management throughout the translocation process are key in reducing the probability of disease associated with coccidiosis in beavers. In addition, hygiene to reduce environmental burdens of coccidia oocysts will be beneficial.

Faecal sampling during the translocation process would be beneficial in order to detect shedding of oocysts in beavers early, and initiate appropriate treatment and biosecurity measures, given the likely stressors to beavers. There is a need to conserve coccidian parasites where possible following reintroduction to ensure immunity is maintained in the population.

Diagnostics for infection with coccidian parasites should be part of the post-release health surveillance protocol to help inform future decision making regarding this parasite.

5.4.15 Disease risk analysis for the unclassified hazard *Giardia* spp

Unclassified Hazard

Justification for Hazard Status

Giardia spp. are enteric protozoan parasites with marked differences in host specificity, geographic range and host preferences (Mateo *et al.,* 2017). Controversy over nomenclature and species identification has historically hindered investigation into the role of wildlife in the epidemiology of these parasites but is being resolved by the recent application of DNA-based molecular tools which can be used to confirm the identify of species and sub-types, and to differentiate between patent infection and the passage of non-infective oocysts (Thompson and Ash 2019). *Giardia duodenalis* (syn. *lamblia* syn. *intestinalis)* is the only *Giardia* spp. found in humans (Ryan and Cacciò 2013). As well as *G. duodenalis*, two other species can infect mammals: *G. microti* and *G. muris*, and both

can infect small rodents (Helmy *et al.,* 2018). *Giardia* is regarded as a species complex comprising at least eight assemblages, A to H, with each assemblage probably representing a distinct species due to the degree of genetic divergence (Thompson and Ash 2019). A and B, the only assemblages known to infect humans, also infect the largest range of host species, including some domestic livestock, companion animals and wildlife (Horton *et al.,* 2018) and it is proposed that reservoirs may be bi-directional i.e. humans may act as a reservoir of infection to animals and vice versa (Ryan and Cacciò 2013). Recognition of further genetic variation within each assemblage has led to the classification of sub-assemblages, for example, AI, AII, of closely-related isolates (Ryan and Cacciò 2013). It is not known how host-specific sub-assemblages are and it is proposed that minor nucleotide variations between isolates may reduce the potential for inter-specific transmission (Van Keulen *et al.,* 2002).

G. duodenalis assemblage B has a higher prevalence than assemblage A in humans worldwide (Feng and Xiao, 2017) and this pattern has been observed in analysis of faecal samples from 150 human patients in the UK (Minetti *et al.,* 2015) (67% prevalence of assemblage B, and 31% prevalence of assemblage A (all sub-assemblage AII)). However, assemblage B is reported to cause more severe symptoms in human patients than assemblage A and the higher prevalence of assemblage B may therefore be a consequence of reporting bias (*ibid*.). In addition, mixed infections may be under-reported in both humans and animals as PCR testing may only identify the most abundant isolate; this may also lead to missed diagnoses of isolates of relevance in some studies (Ryan and Cacciò 2013).

Giardia spp. have been detected in several rodent species, including the water vole and brown rat (Bajer *et al.,* 2008). Furthermore, a study undertaken in Germany detected *Giardia* spp. in six different free-living small rodent species: *Apodemus flavicollis*, *A. sylvaticus*, *A. agrarius*, *Microtus agrestis*, *M. arvalis* and *Myodes glareolus*, in varying prevalences between 22.9% (8/35) and 90.7% (97/109) (Helmy *et al.,* 2018). Both Canadian and Eurasian beavers have been implicated as the source of infections in humans and domestic animals (Tsui *et al.,* 2018; Paziewska *et al.,* 2007; Sroka *et al.,* 2015). Historic reports based on the presence of beaver colonies upstream from drinking and recreational water sources, and experimental inoculation of humans with *Giardia* spp. isolated from Canadian beavers (Davies and Hibler 1979) have been supported by whole gene sequencing (WGS) which has demonstrated clustering of assemblage A and B isolates in Canadian beavers, humans and domestic animals, supportive of interspecific transmission (Tsui *et al.,* 2018).

We are not aware of similar studies in Eurasian beavers; however, Eurasian beavers have been shown to be susceptible to infection with *Giardia* spp.. Paziewska *et al.,* (2007) isolated *Giardia* spp. from 7.7% (n=4/52) of faecal samples from captive (n=30) and wild (n=22) beavers in a study in Poland. Additionally, PCR and sequencing have been used to identify *G. duodenalis* assemblages A and B in water close to beaver lodges: *Sroka et al.,* (2015*)* analysed 79 water samples from 14 known beaver habitats in north-east Poland.

48.1% of these water samples tested positive by PCR for the presence of *Giardia* spp. DNA (n=38). 11 samples were successfully genotyped and identified as *G. duodenalis* assemblage A (n=3) and *G. duodenalis* assemblage B (n=8). In addition, the density of *Giardia* cysts significantly declined with increasing distance from the beavers' lodges suggesting that beavers rather than other animals were the source of the cysts. *G. duodenalis* assemblage B has been detected in a captive Eurasian beaver in Zhengzhou Zoo, China (J. Li *et al.,* 2015).

No *Giardia* cysts or trophozoites were found by faecal microscopy during testing of beavers from the River Otter Beaver Trial (n=0/43), Tayside, Scotland (n=0/22) (Campbell-Palmer *et al.,* 2015a; Girling *et al.,* 2019a) or Knapdale (n=0/19) by PCR (Goodman *et al.,* 2012). However, microscopy is not a particularly sensitive method of detection of *Giardia* spp. (Fayer *et al.,* 2006) and shedding of cysts is sporadic (Horton *et al.,* 2019) so it is possible that cases of infection with *G. duodenalis* in free-living beavers have been missed. Prior to, and following the introduction of beavers to Knapdale, watercourses were monitored for the presence of *Giardia* cysts (Mackie 2014). *Giardia* spp. were identified at one site prior to release of the beavers by microscopy and, following release, were again found at this site at similar levels but at no new sites. However, neither the species nor the source of the original contamination was identified so it is possible that the beavers were not susceptible to the *Giardia* spp. or assemblages at the site.

Robertson and Gjerde (2001) detected *Giardia* spp. in 29% (n=28/147) of watercourses tested between 1998 and 1999 in Norway using immunofluorescence microscopy. These samples were not genotyped and no association was noted between the presence of beavers at a site and water contamination. In addition, no infected beavers were found in Norway (n=0/241), or beavers imported from Norway for the Knapdale trial (n=0/19), using an immunoassay to detect *Giardia* antigen in faeces (Rosell, Rosef, and Parker 2001; Goodman 2014). It has therefore been proposed that beavers may not be a true reservoir for *G. duodenalis* but may act to maintain and amplify an environmental reservoir once infected (Monzingo and Hibler, 2007). In a previous assessment following the Knapdale trial, Boden and Auty (2015) concluded that existing sources of contamination such as humans and other animals were likely to be greater contributors to the overall number of *Giardia* cysts shed into the environment than beavers but that beavers were likely to make a small additional contribution to the environmental reservoir of *G. duodenalis*.

A recent review of rodent parasites in zoological institutions across the world found *Giardia* spp. to be the second most commonly reported protozoal agent in rodents, documented in ten publications (Hardgrove *et al.,* 2021). Moreover, *Giardia* spp. were the third most commonly reported endoparasite in captive rodents (Hardgrove *et al.,* 2021). *Giardia* spp. are also a common parasite in other non-rodent species in captive collections (Beck, Sprong, *et al.,* 2011). There is evidence of transmission of *G. duodenalis* between unrelated species in captive collections, including between a Eurasian beaver and a white-cheeked gibbon (*Nomascus leucogenys*): genetic analysis showed the isolates detected from both animals were a 100% match (Li *et al.,* 2015). Other evidence of transmission

between rodents and other species housed separately in captivity is provided by Beck *et al.,* (2011): genetic sequencing identified that *G. duodenalis* isolates from a Malayan sun bear (*Ursus malayanus*), a Prevost's squirrel (*Callosciurus prevostii*), a Patagonianmara, a rock hyrax (*Procavia capensis*), three ring-tailed lemurs (*Lemur catta*), a mantled guereza (*Colobus guereza*), a white-handed gibbon (*Hylobates lar*) and a chimpanzee (*Pan troglodytes*), housed in the same collection, were all assemblage B (Beck, Sprong, *et al.,* 2011). These studies suggest that cross-species transmission is possible and beavers with an unknown history may have been exposed to *Giardia* spp. in captive collections.

Immunosuppression plays an important role in altering host-parasite dynamics. Immunosuppressed humans are more likely to become infected if exposed to *Giardia* spp. and also can suffer from more severe disease after infection (Stark *et al.,* 2009). This has also been suggested in non-human mammals: the prevalence of *G. duodenalis* in dogs undergoing immunosuppressive chemotherapy was higher than in healthy controls, regardless of age (Cervone *et al.,* 2019). Given that Eurasian beavers can be infected with *Giardia* spp., and the possibility for the translocation to alter host-parasite dynamics leading to an increased likelihood of infection after exposure, and the potential for Eurasian beavers to amplify environmental reservoirs once infected thereby increasing the infection potential to humans and livestock and sympatric species, *Giardia* spp. should be considered as a hazard for the translocation of beavers.

Risk Assessment

Exposure Assessment

Beavers living in areas where watercourses have been contaminated by *Giardia* spp. in faeces from infected humans or domestic animals, for example cattle, may ingest *Giardia* cysts in water or on plant material. There is also evidence of cross-species transmission of *G. duodenalis* to a Eurasian beaver in captivity(Li *et al.,* 2015; Beck, Sprong, *et al.,* 2011). Therefore, beavers which have previously spent time in captive collections housing other mammalian species may have been exposed to *G. duodenalis*.

Immunosuppression has been shown to increase the likelihood of infection with *Giardia* spp. after exposure (Cervone *et al.,* 2019; Stark *et al.,* 2009), and therefore beavers undergoing translocation may be at a higher risk of infection with *Giardia* spp. after exposure. As *G. duodenalis* assemblages A and B can infect, and replicate in, a wide range of species, including beavers, the infective dose is low, and cysts survive for prolonged periods in cool water (Tsui *et al.,* 2018), and beavers may have an unknown history, there is a medium likelihood that translocated beavers may be exposed to and infected by *G. duodenalis* assemblages A or B.

Following ingestion, trophozoites are released from the cyst in the duodenum where they undergo repeated mitotic division and formation of infectious cysts which are shed in faeces (Ryan and Cacciò 2013). As beavers are coprophagic they are likely to repeatedly

re-infect themselves and to increase the number of infectious cysts shed in their faeces into water surrounding their lodges (Monzingo and Hibler, 2007). Conspecific, sympatric species and humans and domestic animals drinking or accidentally ingesting water while swimming downstream will be exposed to infection and there is a high likelihood of exposure and infection. In slow-moving water, cysts quickly fall to the bottom of the water course but may spread widely in faster-moving water (*ibid.*).

As *Giardia* cysts have prolonged survival in water and are fairly resistant to chemical treatments (Tsui *et al.,* 2018) the likelihood of dissemination to other susceptible species close to beaver habitat, or at some distance in moderate to fast moving watercourses, is high.

Consequence Assessment

In humans, age, immunocompetence and gut flora determine susceptibility to disease development (Horton *et al.,* 2019) and the same may be true of other species. Young calves, puppies and kittens infected with *G. duodenalis* may experience acute diarrhoea, ill-thrift and even death (Feng and Xiao 2011). Feng and Xiao (2011) report several studies in farm animals demonstrating decreased weight gain and reduced feed efficiency with associated economic loss as a result of giardiasis. The likelihood of a disease outbreak in people or domestic animals as a consequence of beaver translocation and amplification of existing *Giardia* spp. contamination is estimated to be low.

Risk Estimation

There is a medium likelihood that translocated beavers will be exposed to and infected with *G. duodenalis* A or B, a high likelihood of exposure and infection of sympatric species at the destination, and a high likelihood of dissemination to other species in close proximity to beaver lodges or at greater distances in areas of fast-moving water at the destination site(s). There is a low likelihood of a disease outbreak in humans and domestic animals. The change in risk at the destination site(s) as a result of beaver translocations and amplification of *Giardia* spp. is low and the overall risk is LOW.

Risk Management

Risk Evaluation

Since the risk estimation is higher than negligible, risk management options have been suggested.

Risk options

Public health advice, particularly warning of the risks of swimming close to beaver lodges may be valuable in management of the risks from disease to people. Release sites should,

ideally, be chosen in consultation with relevant water authorities or private water supply owners, particularly given the likely long-term potential for beavers to disperse away from release sites. Consultation with local landowners and recommendations to fence grazing areas to prevent livestock defecating into water edges may also be advisable in order to reduce the likelihood of beavers being exposed to and infected with *Giardia* spp..

5.4.16 Disease risk analysis for the carrier hazard *Giardia* spp.

Carrier Hazard

Justification for Hazard Status

Giardia duodenalis has already been evaluated as an 'unclassified' hazard to the Eurasian beaver translocation, given the potential for exposure and infection of humans and animals at the destination site, and the risk from disease classified as LOW. However, disease has been associated with *Giardia spp*. as a result of immunocompromise in humans and dogs (Cervone *et al.,* 2019; Stark *et al.,* 2009)., suggesting that a full DRA should be performed to assess the risk of *Giardia* spp. as a carrier hazard to translocated beavers.

As previously mentioned, *Giardia* spp. have been detected in both Canadian and Eurasian beavers, providing evidence of beavers' susceptibility to *Giardia* spp. infection: *Giardia* spp. have been isolated from the faeces of free-living Canadian beavers in North America (Sulaiman *et al.,* 2003; Fayer *et al.,* 2006), as well as from the faeces of free-living Eurasian beavers in Europe (Paziewska *et al.,* 2007) and from a captive Eurasian beaver in in Zhengzhou Zoo, China (Li *et al.,* 2015). To the best of the authors' knowledge, no cases of giardiasis have been reported in beavers.

Immunosuppression plays an important role in altering host-parasite dynamics which can lead to changes in the disease-causing ability of parasites. Immunosuppressed humans are more likely to become infected if exposed to *Giardia* spp. and also can suffer from more severe disease after infection (Stark *et al.,* 2009). This effect of immunosuppression on susceptibility to infection has also been suggested in non-human mammals: the prevalence of *G. duodenalis* in dogs undergoing immunosuppressive chemotherapy was higher than in healthy controls, regardless of age (Cervone *et al.,* 2019).

A recent review of rodent parasites in zoological institutions across the world found *Giardia* spp. to be the second most commonly reported protozoal agent in rodents, documented in ten publications (Hardgrove *et al.,* 2021). Moreover, *Giardia* spp. were the third most commonly reported endoparasite in captive collections of rodents (Hardgrove *et al.,* 2021). *Giardia* spp. are also a common parasite in other non-rodent species in captive collections (Beck, Sprong, *et al.,* 2011). There is evidence of transmission of *Giardia* spp. between unrelated species in captive collections, including between a Eurasian beaver and a white-cheeked gibbon: genetic analysis showed the *Giardia* spp. isolates detected from both

animals were a 100% match (Li *et al.,* 2015). Other evidence of transmission between rodents and other species housed separately in captivity is provided by Beck *et al.,* (2011): genetic sequencing identified that *G. duodenalis* isolates from a Malayan sun bear, a Prevost's squirrel, a Patagonian mara, a rock hyrax, three ring-tailed lemurs, a mantled guereza , a white-handed gibbon and a chimpanzee, housed in the same collection, were all assemblage B (Beck, Sprong, *et al.,* 2011). These studies suggest that cross-species transmission is possible and beavers with an unknown history may have been exposed to *Giardia* spp. in captive collections.

Infection with *Giardia* spp. may be asymptomatic and, as a consequence, *Giardia* spp. are regarded by some authors as commensal parasites (DuPont 2013). As beavers can be infected with *Giardia* spp., immunosuppression can lead to increased likelihood of infection and disease following exposure, and translocation is a known stressor (Dickens, Delehanty, and Michael Romero 2010), *Giardia* spp. should be considered a carrier hazard for the translocation of Eurasian beavers.

Risk Assessment

Release Assessment

Transmission of *Giardia* spp. is faeco-oral, by ingestion of infective cysts and trophozoites, and may be direct or, more commonly, indirect via contaminated water sources or food (Ryan and Caccio, 2013). Cysts are immediately infectious following excretion and may survive several months in the environment with an infective dose of as few as 10 oocysts (Ryan and Cacciò 2013). Survival of cysts increases with decreases in temperature and a small number of cysts can survive a single freeze-thaw episode (USEPA (UNITED STATES ENVIRONMENTAL PROTECTION AGENCY) 1999).

In order to become infected with *Giardia* spp. a beaver must ingest the infective cysts or trophozoites from the environment, which could be shed by other beavers, or numerous other hosts of *Giardia* spp. (Tsui *et al.,* 2018). In a free-living environment, it is possible that beavers could be exposed through watersources and/or vegetation that have been contaminated by faeces from infected humans or domestic animals, for example cattle, or other free-living species. There is also evidence of cross-species transmission of *Giardia* spp. in captive collections, including of a Eurasian beaver (Li *et al.,* 2015; Beck, Sprong, *et al.,* 2011). Therefore, beavers which have previously spent time in captive collections housing other mammalian species may have been exposed to *Giardia* spp.. Immunosuppression has been shown to increase the likelihood of infection with *Giardia* spp. after exposure (Cervone *et al.,* 2019; Stark *et al.,* 2009), and therefore translocated beavers may be at increased likelihood of infection after exposure.

As *Giardia* spp. can infect, and replicate in, a wide range of species, including beavers, the infective dose is low and cysts survive for prolonged periods in cool water (Tsui *et al.,* 2018), and beavers may have an unknown history, there is a medium likelihood that

translocated beavers may be exposed to and infected with *Giardia* spp. when translocated.

Exposure Assessment

Following ingestion, trophozoites are released from the cyst in the duodenum where they undergo repeated mitotic division and formation of infectious cysts which are shed in faeces (Ryan and Cacciò 2013). As beavers are coprophagic they are likely to repeatedly re-infect themselves and to increase the number of infectious cysts shed in their faeces into water surrounding their lodges (Monzingo and Hibler 1987). As *Giardia* spp. cysts have prolonged survival in water and are fairly resistant to chemical treatments (Tsui *et al.,* 2018) there is a high likelihood of exposure and infection of conspecifics ingesting contaminated water.

Consequence Assessment

No cases of giardiasis have been reported in beavers, to the best of the authors' knowledge, so it is likely that, in common with many other species, otherwise healthy animals do not experience clinical disease following infection. In humans, age, immunocompetence and gut flora determine susceptibility to disease development (Horton *et al.,* 2019) and the same may be true of other species. Young calves, puppies and kittens infected with *G. duodenalis* may experience acute diarrhoea, ill-thrift and even death (Feng and Xiao, 2011). Feng and Xiao (2011) report several studies in farm animals demonstrating decreased weight gain and reduced feed efficiency with associated economic loss as a result of giardiasis.

The probability that at least one beaver is infected with *Giardia* spp*.* at the time of translocation is medium. There is a medium likelihood that the conditions of translocation will, as a stressor, lead to immunocompromise and a change in host-parasite dynamics resulting in giardiasis in translocated beavers. In cases of giardiasis, there is a low likelihood of severe disease in the individual and a low likelihood of death. There is a very low probability of economic consequences as a result of *Giardia* spp. infection and disease in translocated beavers leading to the failure of the translocation. There is a very low likelihood of biological and economic consequences at the destination as a result of failure of the translocation.

Risk Estimation

There is a medium likelihood that beavers will have been exposed to and infected with *Giardia* spp. at the time of translocation. There is a high likelihood of exposure of other beavers and other mammals at the destination and a high likelihood of dissemination. There is a low likelihood that the translocation will lead to a change in host-parasite dynamics and disease. There is a very low likelihood of biological and economic

consequences through failure of the translocation. Overall, the risk is estimated to be LOW.

Risk Management

Risk Evaluation

Since the risk is estimated to be higher than negligible, disease mitigation measures should be implemented.

Risk options

Stress reduction throughout the translocation process is key in reducing the probability of disease associated with *Giardia* spp. in beavers. Diagnostics for *Giardia* spp. in the event that a beaver becomes clinically unwell during captivity may be important to identify shedding individuals and take appropriate action, including preventing the exposure of other captive beavers. Direct visualisation of faecal samples is likely to be the most appropriate method of assessment.

Diagnostics for *Giardia* spp. should be part of the post-release health surveillance protocol to help inform future decision making regarding this parasite.

5.4.17 Disease risk analysis for the carrier hazard *Toxoplasma gondii*

Carrier Hazard

Justification for Hazard Status

Toxoplasma gondii, of the phylum Apicomplexa, is an obligate intracellular protozoan which is ubiquitous worldwide (Tenter, Heckeroth, and Weiss 2000; Herrmann *et al.,* 2013). The parasite has an indirect life cycle: the sexual phase occurs only in felids, but the asexual phase is possible in almost any mammalian intermediate host (Herrmann *et al.,* 2013). In felids, the infectious phase of *Toxoplasma gondii* is the sporozoite, which occurs in oocysts. *Toxoplasma gondii* has two forms in intermediate hosts: tachyzoites and bradyzoites (found in tissue cysts). The initial, acute period of infection occurs when an intermediate host ingests sporozoites from an oocyst, or bradyzoites from a tissue cyst. These then convert to tachyzoites within the intestinal epithelium of the intermediate host and begin to rapidly replicate by asexual reproduction. These tachyzoites spread throughout the body via the bloodstream, leading to systemic infection. At this stage, in most cases, the host immune response leads to clearance before clinical signs develop (Suzuki *et al.,* 1988). However, tachyzoites can convert to dormant bradyzoites within tissue cysts as an immune evasion mechanism. Tissue cysts form more often in muscular

and neural tissue such as the brain, eye and cardiac muscle, but can also be found in the lungs, liver and kidneys (Hill, Chirukandoth, and Dubey 2005). During periods of host immunocompromise, tissue cysts can rupture and bradyzoites can recrudesce to become tachyzoites again. This can lead to acute toxoplasmosis (Skariah, Mcintyre, and Mordue 2010; Shen *et al.,* 2016).

Exposure of American beavers to *T. gondii* has been reported in several studies. A serological survey was undertaken across several free-living mammal species in Missouri, USA, in which 14 American beavers were sampled (Smith and Frenkel 1995). One beaver had a positive antibody titre and *T. gondii* was later isolated from this animal. Several other rodents tested positive using serology, including one woodland white-footed mouse (*Peromyscus leucopus*), seven muskrats and two grey squirrels. *T. gondii* was also isolated from one of these two grey squirrels (Smith and Frenkel, 1995). Furthermore, a *T. gondii* seroprevalence of approximately 10% (n=6/62) was reported in a population of American beavers in Massachusetts, USA (Jordan *et al.,* 2005). American beavers are also susceptible to disease associated with *T. gondii*. A five-month old, free-living beaver found orphaned in Connecticut, USA, died of severe systemic toxoplasmosis, confirmed using immunohistochemistry, after spending 14 weeks at a rehabilitation facility (Forzán and Frasca, 2004). It is unknown whether this animal was exposed before or after admission to this facility, but numerous cysts in the cerebral and cerebellar tissue containing bradyzoites suggest that *T. gondii* infection may have been chronic, and acute infection may have occurred after immunosuppression and reactivation of dormant disease.

Toxoplasmosis has also been reported in Eurasian beavers: Two of six free-living adult beavers found dead around the River Havel, Germany, between 2006 and 2011 tested positive for *T. gondii* by PCR. One of these beavers had histopathological evidence of tissue cysts in the brain along with a moderate to severe inflammatory response which suggested toxoplasma-associated encephalitis as the cause of death (Herrmann *et al.,* 2013)

T. gondii has been shown to be present in Norway. A seroprevalence of 10.9% (n=3907) was found in pregnant women in a survey undertaken in 1992 (Jenum *et al.,* 1998). Another study into prevalence in free-living Norwegian cervids showed a seroprevalence of 33.9% (n= 258) in roe deer, 12.8% (n=270) in moose (*Alces alces*), 7.7% (n= 44) in red deer and 1% (n = 87) reindeer (*Rangifer tarandus)* (Vikøren *et al.,* 2004). More recent data suggest that *T. gondii* is currently prevalent across Europe. Information provided to the European Food Safety Authority and European Centre for Disease Prevention and Control (EFSA and ECDC) by countries including Norway and the UK in 2017 showed seroprevalence across Europe to be between 13 and 30% in small ruminants. A prevalence of 10.5% was reported in cattle, although no data was provided for Norway, and seven cases of congenital toxoplasmosis were described in the UK (European Food Safety Authority and European Centre for Disease Prevention and Control 2018).

T. gondii infection has also been commonly detected in captive collections. Hardgrove *et al.,* (2021) found in a review study that *Toxoplasma* was the most commonly reported pathogen genera in collection rodents globally, and Ippen, Kozojed and Jira (1981) concluded that the prevalence of *Toxoplasma* spp. in captive animals is higher than occurs under natural conditions. Moreover, Bártová *et al.,* (2018) found that the order Rodentia had a 12.5% (3/24) prevalence of *T. gondii* antibodies in Czech zoos. Numerous captive rodents of different species have tested positive for *T. gondii*, including American porcupines (Medway, Skand, and Sarver 1989), Patagonian mara, coypu, Cape springhare (*Podetes capensis*) (Bártová *et al.,* 2018) and red squirrel (Fayyad *et al.,* 2016). Captive Eurasian beavers (number not given) tested positive for *Toxoplasma* spp. antibodies on blood sample in a study by Ippen, Kozojed and Jira (1981). This suggests that beavers originating from captive collections may have an increased likelihood of being infected with *T. gondii* at the time of translocation.

As translocation is a known stressor (Dickens *et al.,* 2010) and, given the prospect of exposure and chronic infection with *T. gondii* in Eurasian beavers from the wild in Norway or Great Britain, or captive collections in Great Britain, it is possible that translocation of beavers could lead to acute toxoplasmosis as a result of resurgence of chronic disease under stressful conditions. Therefore, *T. gondii* should be considered as a carrier hazard for the translocation of beavers.

Risk Assessment

Release Assessment

The most likely route of exposure to *T. gondii* for beavers is direct ingestion of sporulated oocysts shed into the environment by infected felids, for example in drinking water or on vegetation. Toxoplasma oocysts have been reported to be able to survive for between 1.5 and 4.5 years in soil and freshwater environments and in sea water for several months (Aramini *et al.,* 1999; Bowie *et al.,* 1997; Dubey, 1998; Frenkel *et al.,* 1975; Jordan *et al.,* 2005; Lindsay *et al.,* 2003; Tenter *et al.,* 2000. Prolonged survival in freshwater environments suggests that aquatic mammals, such as beavers, may be at particular risk of exposure to *T. gondii* (Herrmann *et al.,* 2013) and there is therefore a medium probability of exposure of free-living beavers. There is estimated to be a high likelihood of exposure of beavers in captive collections in Great Britain given the evidence of high prevalence of *T. gondii* in captive animals including rodents. Vertical transmission is also possible (Parameswaran *et al.,* 2009) and has been shown experimentally in other rodents such as house mice and field mice (Owen and Trees, 1998) suggesting it may also be possible in beavers. There is a high likelihood of infection in beavers after exposure.

Considering the ubiquity of *T. gondii* across Europe, its ability to survive for long periods of time in aquatic environments, and the high likelihood of infection after exposure, the probability of beavers being infected with *T. gondii* at the time of translocation is estimated

to be medium if sourced from the wild in Great Britain or Norway, and high if sourced from captive collections in Great Britain.

Exposure Assessment

Infected beavers will carry the protozoa to the destination but will not contribute to the burden of *T. gondii* sporozoites in the environment at the release site as only felids shed *T. gondii* sporozoites in faeces. However, infected beavers could represent a source of infection for species which prey on rodents such as red foxes (Pavey, Eldridge, and Heywood 2008) or scavenger species. There is therefore a low likelihood of exposure of beavers and other mammals at the reintroduction site.

The reintroduction itself is predicted to have little influence on the host-parasite dynamics at the destination site since *T. gondii* is already prevalent in the environment across Europe. The likelihood of dissemination at the destination site because of beaver reintroductions is negligible.

Consequence Assessment

The probability that at least one beaver is chronically infected with *T. gondii* at the time of translocation is medium if the beaver is sourced from the wild in Norway or Great Britain and high if sourced from captive collections in Great Britain. There is a medium likelihood that the conditions of translocation will lead to an alteration in host-parasite dynamics resulting in immunocompromise and recrudescence of chronic toxoplasmosis leading to acute disease. In cases of acute clinical disease, consequences are likely to be severe for the individual with a high likelihood of death. There is a medium probability of biological and economic consequences as a result of *T. gondii* recrudescence under conditions of translocation stress due to failure of the reintroduction programme. Since *T.a gondii* is widespread in the environment, the likelihood of environmental and ecological consequences at the destination site is negligible.

Risk Estimation

There is a medium likelihood of beavers being exposed to and infected with *T. gondii* in the wild in Norway or Great Britain, and a high likelihood of being exposed to and infected with *T. gondii* in captive collections in Great Britain. There is a medium or high likelihood of beavers being chronically infected when translocated from these respective source sites. The likelihood of exposure and infection of free-living species which prey on rodents is estimated to be low, and negligible in all other free-living species at the destination site. Dissemination of *T. gondii* at the destination is likely to be negligible. There is a medium to high likelihood of at least one translocated beaver being infected and developing disease depending on the source site and a high likelihood of severe consequences for these individuals. There is a negligible likelihood of substantial ecological consequences at the destination, but medium likelihood of negative biological and economic consequences as a

result of translocation failure in the case of recrudescence of disease under stressful conditions. The overall risk is estimated to be MEDIUM if beavers are translocated from the wild in Norway or Great Britain and HIGH if beavers are translocated from captive collections in Great Britain.

Risk Management

Risk Evaluation

Based on the risk assessment above, management methods should be employed to reduce the risk of *T. gondii* to translocated beavers.

Risk options

Disease risk management methods to reduce stress in the translocated population of beavers through good husbandry and management methods are recommended.

Serological testing for *T. gondii* specific IgG antibodies may be a useful tool to gauge the exposure level in the population (Liu *et al.,* 2015), and could be undertaken if beavers are captured and restrained for blood samples during translocation. However, exposure to *T. gondi* is expected and therefore samples for testing are not of sufficient priority to warrant pre-translocation testing for this parasite alone. Where blood samples are being collected for other purposes, and some serum is spare, testing for *T. gondii* specific IgG antibodies will improve our understanding of the prevalence of the parasite in beavers. Currently tests are not validated in beavers but are still likely to provide useful information.

Diagnostics for *T. gondii* should be considered as part of the post-release health surveillance protocol to help inform future decision making on disease risk management regarding this parasite.

5.4.18 Disease risk analysis for the population hazard *Toxoplasma gondii*

Population Hazard

Justification for Hazard Status

Toxoplasma gondii has already been evaluated as a carrier hazard and the risk considered to be medium or high dependent on the source of the beavers. The risk to translocated beavers from road traffic collisions (RTCs) has also been evaluated as a population hazard and considered to be medium. Here we analyse how chronic disease associated with *T. gondii* will affect the risk from road traffic collision and/or predation to beaver reintroduction efforts.

Latent infection with *T. gondii* is known to induce behavioural changes in intermediate hosts as a result of predilection for neural tissue. This is thought to be an evolutionary mechanism of transmission to feline definitive hosts by increasing the likelihood of predation of the intermediate host (Havlícek *et al.,* 2001). In humans, there is evidence to suggest that infection with *T. gondii* leads to slower reaction times (Havlícek *et al.,* 2001) which, as a result, can increase the risk of the host being involved in road traffic collisions (Flegr *et al.,* 2002; Yereli, Balcioğlu, and Özbilgin 2006; Stepanova *et al.,* 2017; Galván-Ramírez *et al.,* 2013; Gohardehi *et al.,* 2018; Kocazeybek *et al.,* 2009). There is evidence to suggest that latent infection with *T. gondi* may affect behaviour in other mammals. An Australian study by Hollings *et al.,* (2013) found a higher seroprevalence of *T. gondii* in road-killed Tasmanian pademelons (*Thylogale billardierii*) (31%, n=16) than in culled individuals (11%, n=212). However, the small sample size of road-killed animals compared to culled animals means that results should be interpreted with caution.

Of particular interest to the beaver reintroduction are the apparent behavioural changes exhibited in rodents as a result of *T. gondii* infection. Berdoy (2000) found that brown rats experimentally infected with *T. gondii* did not exhibit normal predator avoidance when compared to controls. Although the study focused specifically on olfactory queues and avoidance of predator scent, it could be true that avoidance of other dangerous situations, such as road traffic, could also be affected if innate fear is reduced. However, others have suggested that the behavioural effects of *T. gondii* on an intermediate rodent host are likely to be relative to the dose of stimulus and are more likely to be specific to avoidance of feline urine (Vyas, Kim, and Sapolsky 2007).

Positive serology for *T. gondii* has been significantly associated with reduced neophobia (fear of novel objects) in brown rats (Webster, Brunton, and Macdonald 1994). As well as advantageously affecting the parasite by increasing susceptibility to predation by definitive hosts, Webster *et al.,* (1994) suggested that reduced neophobia could lead to an increased risk of trapping and poisoning of infected rats. In addition, rats may be less likely to avoid road traffic. However, causation cannot be established from this observational study and further research is required to deduce whether *T. gondii* infection reduces neophobia. Moreover, the effects of *T. gondii* on rodent behaviour are widely disputed: a study of six mice infected with *T. gondii* found no alterations in cognitive function, anxiety levels, social behaviour or motivation to explore novel objects when compared to controls, although the small sample size reduces the reliability of these results (Gulinello *et al.,* 2010).

Beavers are susceptible to infection with *T. gondii* and a *T. gondii* cyst has been found in the brain of a free-living Eurasian beaver (Herrmann *et al.,* 2012). As discussed in the DRA for *T. gondii* as a carrier hazard, beavers could be exposed to the parasite in the wild in Norway or Great Britain or in captivity in Great Britain. Therefore, *T. gondii* should be considered a population hazard for the translocation of free-living beavers from Norway or Great Britain, or captive beavers from Great Britain to England.

Risk Assessment

Exposure Assessment

Our analysis of *T. gondii* as a carrier hazard estimated a medium likelihood of beavers being infected when translocated if sourced from the wild in Norway or Great Britain, and high if sourced from captivity in Great Britain. Our analysis of RTCs as a population hazard estimated a high likelihood of exposure for translocated beavers. Research suggests that chronic infection with *T. gondii* may cause behavioural changes that increase susceptibility to RTCs or predation which decrease survival. Although evidence is somewhat conflicting, the neurological/behavioural effects of *T. gondii* reported in other rodents as a result of the formation of tissue cysts in the brain, as well as increased likelihood of exposure of RTCs implied in other species, suggests that an increased likelihood of RTCs cannot be ruled out. The probability of these events occurring in an individual beaver chronically infected with *T. gondii* is estimated to be medium.

There is therefore a medium likelihood of reintroduced beavers being exposed to RTCs as a result of chronic toxoplasmosis. There is also a medium probability of reintroduced beavers being exposed to predation as a result of chronic toxoplasmosis.

Consequence Assessment

The probability of severe consequences in the case of predation or RTC is high, as mortality rates as a result of these events are likely to be high. There is a high likelihood of biological and economic consequences due to failure of the reintroduction programme as a result of multiple deaths precipitated by chronic toxoplasmosis and due to predation or RTCs.

Risk Estimation

There is a high likelihood of road traffic collision in reintroduced beavers and a medium to high likelihood of at least one beaver being chronically infected with *T. gondii* when translocated, depending on the source site**.** The likelihood of reintroduced beavers being exposed to road traffic collision or predation as a consequence of chronic toxoplasmosis is estimated to be medium. The likelihood of severe consequences, including death, in individuals involved in these events is high. The probability of biological and economic consequences, and failure of the reintroduction, as a result of RTCs or predation following chronic *T. gondii* infection is high. The overall risk from chronic toxoplasmosis as a population hazard is estimated to be MEDIUM for beavers of free-living origin and HIGH for beavers of captive origin.

Risk Management

Risk Evaluation

Based on the risk assessment, preventative measures should be employed to reduce the risks of RTCs and predation from chronic toxoplasmosis.

Risk options

Mitigation measures against RTCs have been discussed in the individual RTC DRA and also apply to RTCs resulting from chronic toxoplasmosis. This includes taking care when choosing the release site for reintroduced beavers.

Disease surveillance and post-release health surveillance are important tools to identify particular roads of concern for beaver collisions where mitigation measures are required. This could include adding warning signs on stretches of road considered a risk, to encourage safe driving, and the development of beaver corridors for safe passage under roads.

The risk options explained under *T. gondi* as a carrier hazard are also relevant here.

5.4.19 Disease risk analysis for the source hazard *Echinococcus multilocularis*

Source Hazard

Justification for Hazard Status

Echinococcus multilocularis is a tapeworm (cestode) of, primarily, the red fox which can cause morbidity and mortality in intermediate hosts (Barlow, Gottstein, and Mueller 2011b). *E. multilocularis* is endemic in many parts of Europe but is not currently present in Great Britain.

Surveillance of infection in the definitive host, the red fox, is the primary method of assessing distribution and prevalence across Europe. Prevalence of *E. multilocularis* infection in Europe is believed to be increasing, particularly in central Europe, following implementation of rabies vaccination of free-living foxes which has led to an increase in their number and density (Ćirović *et al.,* 2012). In the 1980s *E. multilocularis* was known to be endemic in four European countries and is now found in 24 countries, with prevalence of infection in foxes reported to be as high as 50% (Zancanaro 2019). Studies in Germany since 1995 suggested a prevalence in foxes in Bavaria of 40.4% to 55.5% (numbers tested not reported), the highest of any region in Germany (Deplazes *et al.,* 2017). However, even within regions of low prevalence, or those where infection is non-

endemic, there may be islands of infection as genetic analysis of strains suggest that *E. multilocularis* may have been circulating undetected in some areas for several years (Davidson *et al.,* 2012).

E. multilocularis was first detected in Denmark, in 2000, in a fox hit by a car on the outskirts of Copenhagen (Wahlström *et al.,* 2015). As a result, surveillance in Scandinavia was increased and, in 2011, the first case of infection in a fox was found in Sweden, 80km from the Norwegian border (Wahlström *et al.,* 2015). There is some uncertainty as to whether *E. multilocularis* spread into Sweden via wildlife dispersal or pet dog movements but it is believed that the latter route is more likely (Toth, Frost, and Roberts 2010). Since 2011, prevalence in foxes in Sweden has been detected at levels between 0.1 and 0.9%, with burdens in individual foxes of up to 1235 tapeworms (Wahlström *et al.,* 2015). Knowledge of habitat use and migration behaviour of foxes in Sweden is limited but, given the 1600km shared border with Norway, the probability of *E. multilocularis* being introduced to Norway via infected wildlife is considered high (Zancanaro 2019).

However, *E. multilocularis* has not been detected in mainland Norway or the UK using the European Food Safety Authority (EFSA) threshold of <1% prevalence at the 95% confidence level to date. In 2019, faecal samples from approximately 540 culled foxes were tested in Norway by PCR for *E. multilocularis* DNA. All were negative (Inger Sofie Hamnes, Norwegian Veterinary Institute, pers. comm). Nevertheless, Davidson *et al.,* (2013) reported that *E. multilocularis* is possibly present in Norway already but at a prevalence below the detection level of the surveillance programme. Robertson *et al.,* (2012), reporting on the views of the Norwegian Scientific Committee for Food Safety, have suggested that *E. multilocularis* would probably not be detected on first introduction as up to 1200 foxes could theoretically become infected before the first case was detected based on the 1% prevalence threshold and population estimates of between 70,000 and 120,000 foxes in Norway.

The probability of *E. multilocularis* being introduced to Norway via pet dog movements as a result of poor worming compliance, infrequent border checks and from the illegal pet trade is also considered to be high (Davidson *et al.,* 2012; Davidson and Robertson 2012). In addition, owners may be given incorrect advice on appropriate anthelmintic treatment prior to bringing dogs into Norway: in a phone survey of 90 veterinary practices across Europe in 2011, only 10 gave correct and complete advice on the required treatment (Davidson and Robertson 2012). In 2009, prior to proposed changes in import requirements for pet dogs entering the UK from other EU countries, Torgerson and Craig (2009) predicted that, without compulsory praziquantel treatment, there was a 98% chance for every 10,000 dogs making short trips from the UK to Germany that one would be infected with *E. multilocularis* on return to the UK. The current requirement is that dogs entering the UK from other countries, with the exception of Norway, Finland, Malta and the Republic of Ireland, must receive appropriate tapeworm treatment between 24 and 120 hours (one to five days) prior to entry and again 28 days after entry (DEFRA 2020). The levels of compliance and stringency of border checks is unknown.

E. multilocularis may be spread by wild canids to potential intermediate hosts in captivity: Hardgrove *et al.,* (2021) found that *E. multilocularis* was the most commonly reported helminth in rodents in captive collections. In 2005 a Barbary macaque (*Macaca sylvanus*), recently imported from southern Germany, died in a zoological collection in the UK and was found on post-mortem examination to be infected with *E. multilocularis* (Boufana *et al.,* 2012). The colony which the macaque had been translocated from was in a park from which foxes were rigorously excluded and it was concluded that the source of infection was contaminated foliage (Boufana *et al.,* 2012). Boufana *et al.,* (2012) reported that free-roaming red foxes in zoological gardens in Switzerland have been implicated as the source of infections of captive primates in Switzerland. Additionally, a captive-born coypu and several ring-tailed lemurs died in a wildlife park in France in 2011 from echinococcosis, showing the risks posed to captive wild animals from free-living foxes even in fenced enclosures (Umhang *et al.,* 2016). However, captive intermediate hosts are unlikely to perpetuate the transmission cycle because the probability that their carcases could be scavenged after death is very low.

Reports of infections of beavers suggest the beaver has potential to act as a competent intermediate host for *E. multilocularis* transmission: *E. multilocularis* infestation has been confirmed in free-living beavers in Switzerland (Janovsky *et al.,* 2002), Serbia (Ćirović *et al.,* 2012) and Austria (Posautz and Kübber-Heiss 2015). Additionally, Gottstein *et al.,* (2014) reported that beavers exhibit only limited humoral response to infection which may suggest that they are particularly susceptible. Following the death associated with *E. multilocularis* infection of a captive beaver in England, previously wild-caught in Bavaria, the prevalence of *E. multilocularis* in beavers in Bavaria was estimated to be between 2.5% and 5% (Barlow, Gottstein, and Mueller 2011b). However, this estimate was based on hunters' visual assessment of culled beaver livers, and not on formal testing, so the true prevalence may be higher. Because beavers are intermediate hosts, they cannot transmit *E. multilocularis* to other beavers or intermediate hosts directly or indirectly via the environment (Roberts 2012). It is not known how long beavers can survive following infection with *E. multilocularis*. The case reported by Barlow, Gottstein and Mueller (2011) was of a beaver found dead in England, presumed to be as a result *of E multilocularis*-associated disease, three and a half years after it had been imported. A female beaver, recently imported to England from Bavaria, was euthanised following a positive serological test for *E. multilocularis* in 2017 (Britton and Barlow 2019). The cases reported from Serbia and Switzerland (Janovsky *et al.,* 2002; Ćirović *et al.,* 2012) were of beavers that had died in road traffic accidents. Infection with *E. multilocularis* may have contributed to morbidity in these animals but disease associated with infection was not considered to be the cause of death.

As *E. multilocularis* may now be present in Norway, albeit at low prevalence levels, and beavers are known to be susceptible to infection, *E. multilocularis* should be considered a source hazard for translocation from Norway. Free-living beavers in Great Britain are of uncertain origin, as are several beavers held in enclosures in Great Britain. The limited

genetic testing that has taken place to date has indicated that at least some of the free-living beavers in Great Britain are of Bavarian origin, i.e. from an area known to be endemic for *E. multilocularis*. Moreover, of those beavers known to be held in enclosures in Great Britain, some are known to have been sourced from Bavaria and Poland which are endemic areas for *E. multilocularis*. There is, therefore, a possibility that *E. multilocularis* in beavers infected prior to translocation to Great Britain, and either free-living or in enclosures, is a source hazard to species at the destination site(s). If an infected beaver has been, or will be, predated, or has died and been scavenged by a potential definitive host, the possibility of transmission of *E. multilocularis* to definitive hosts and low-level prevalence of *E. multilocularis* in potential source areas in Great Britain cannot be ruled out.

As a result, *E. multilocularis* in free-living beavers from both Great Britain and Norway, as well as those housed in enclosures which originated from endemic areas, should be considered a potential source hazard.

Risk Assessment

Release Assessment

The lifecycle of *E. multilocularis* in Europe involves two hosts (see Figure 2): a definitive, or primary, canid host, including the red fox, the raccoon dog, grey wolf (*Canis lupus*), golden jackal (*Canis aureus*) and Arctic fox (*Vulpes lagopus*). Pet dogs can also be infected as a definitive host, with increasing prevalence in endemic areas (Karamon *et al.,* 2016). Domestic cats and wild cats (*Felis silvestris*) can be infected but are probably less significant in the transmission cycle because mature adult cestode development and the potential for egg shedding is less likely than in canids (Deplazes *et al.,* 2017; Avcioglu *et al.,* 2018; Knapp *et al.,* 2018). Infection in the definitive host is usually asymptomatic (Davidson *et al.,* 2012). The prepatent period in canids is about 4-5 weeks following infection and then adult tapeworms survive for about 100 days, potentially producing eggs every day (Toth, Frost, and Roberts 2010).

Intermediate hosts in Europe have been shown in metastudies by Oksanen *et al.,* (2016) and Takeuchi-Storm *et al.,* (2015) to be primarily *Cricetidae* spp. (voles) and the muskrat with a distribution of prevalence in most countries similar to that in the definitive host, the red fox, albeit at lower levels of prevalence. However, the role of the muskrat in transmission is still not well understood (Deplazes *et al.,* 2017). The coypu and murids may, in addition, contribute to the transmission cycle in areas with medium to high prevalence in foxes (Oksanen *et al.,* 2016). Infection has also been reported in the

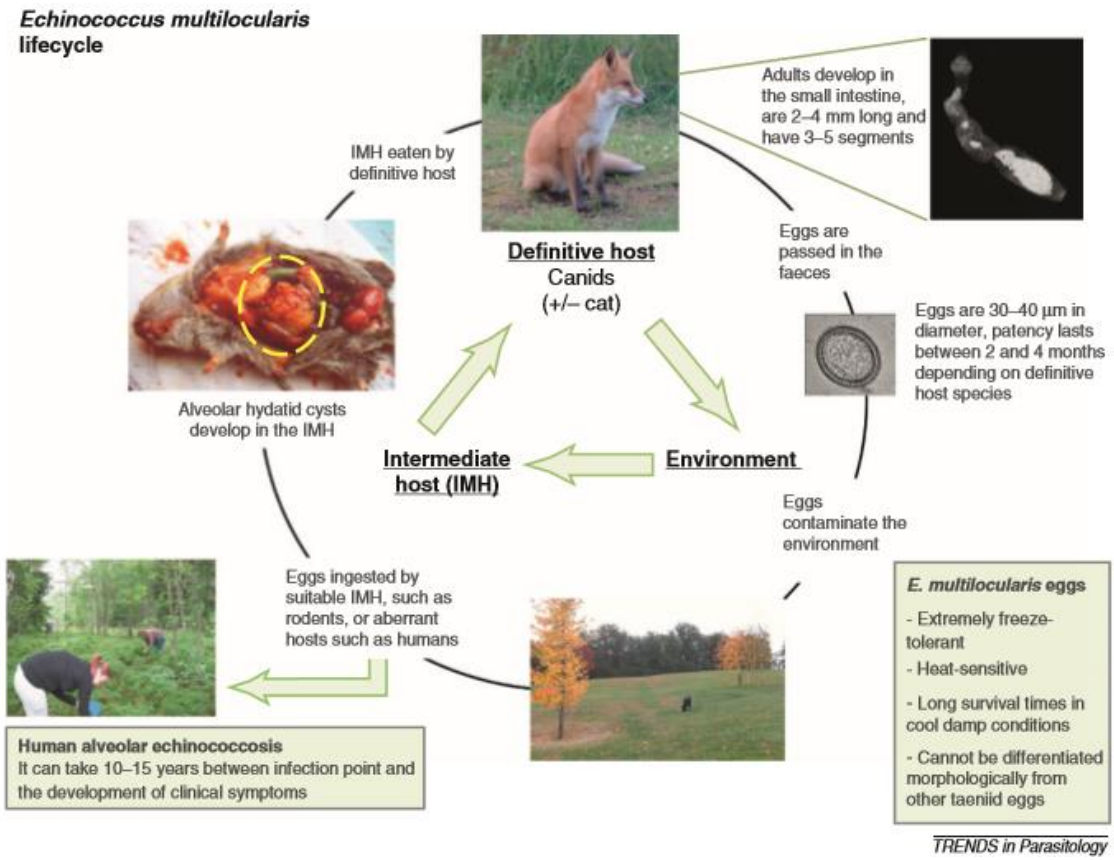

Figure 2: The transmission cycle of *Echinococcus multilocularis* (Source: Davidson *et al.,* 2012)

European brown hare (Chaignat *et al.,* 2015). The main arvicoline hosts in Europe are the common vole and water vole with the bank vole and *Apodemus* spp. of less importance (Miller *et al.,* 2016). Takeuchi-Storm *et al.,* (2015) proposed that this variation may be a consequence of habitat preference, with the bank vole and *Apodemus* spp. preferring wooded environments with reduced predator-prey encounters. However, experimental studies by Woolsey *et al.,* (2016) demonstrated variations in intermediate host susceptibility, suggesting that the transmission capability of the common vole and field vole is high; that the bank vole has limited potential, and that the house mouse probably plays no significant role in transmission. In Sweden, where the common vole is not found, the field vole is believed to act as the main intermediate host (Miller *et al.,* 2017). Unusually, dogs may be infected as both definitive and intermediate hosts (Romig *et al.,* 2017).

E. multilocularis ova are shed in the faeces of infected definitive hosts and ingested in food or water by intermediate hosts. These ova develop in the intermediate host to oncospheres which pass through the intestinal wall and via the bloodstream to organs, primarily the liver, but also, occasionally, the lungs and brain where they develop into encysted larvae (metacestodes) which proliferate by lateral budding into surrounding tissues (Zancanaro 2019). The cysts act in the same way as space-occupying neoplasms with the severity of disease in the intermediate host depending on the location and number

of cysts (Davidson *et al.,* 2012). The parasite lifecycle is completed when the intermediate host is predated or scavenged and the protoscolices are ingested (Davidson *et al.,* 2012).

E. multilocularis ova are persistent in the environment, particularly in cool and damp conditions (Veit *et al.,* 1995). Veit *et al.,* (1995) tested the effect of seasonal conditions on *E. multilocularis* ova survival in south-west Germany and demonstrated that, in the field, *E. multilocularis* ova may be viable for up to 240 days in autumn conditions and 78 days in summer. Additionally, ova stored in vitro in phosphate-buffered saline at 4˚C were viable for at least 478 days (Veit *et al.,* 1995). It is not known how long cysts in the intermediate host remain infectious after the host's death. Survival is likely to be influenced by environmental factors but is considered to be seven to ten days (Roberts 2012).

Beavers are exposed through ingestion of ova in food or water, which are resistant in the environment. The likelihood of exposure of free-living beavers since arrival, or once bred, in Great Britain is very low because (i) although adult beavers may originate from geographic areas with infection (for example, Bavaria), they do not transmit infection to the next generation and (ii) *E. multilocularis* has not been detected in the fox population in Great Britain.

The likelihood that beavers which are known to have originated from endemic areas in continental Europe were exposed to and infected with *E. multilocularis* prior to translocation to Great Britain and are now free-living or in an enclosure and remain infected, is medium. This is because the prevalence of *E. multilocularis* in beavers in Bavaria, an endemic area with reported prevalence in foxes of approximately 50%, was estimated at 2.5 to 5% in 2011.

The likelihood that first generation beavers of uncertain origin held in enclosures in Great Britain have been exposed to and infected with *E. multilocularis* is medium because some of these beavers are known to have been sourced from areas endemic for *E. multilocularis* (including Bavaria and Poland) (Claire Howe, pers. comm. 2021).

The prevalence of *E multilocularis* in definitive hosts in Norway is very low. However, given the absence of a barrier between Sweden and Norway, the presence of *E. multilocularis* in red foxes in Sweden, the possible presence of *E. multilocularis* in Norway without detection, and the large population of red foxes in Norway, the likelihood of a Norwegian beaver being exposed and infected is estimated to be low.

Infection occurs when the oncospheres pass through the intestinal wall and, once exposed, there is a high likelihood of infection. Therefore, we estimate that there is, a medium likelihood that first generation beavers free-living or in enclosures in Great Britain are infected and a low likelihood that free-living beavers in Norway are infected. There is a negligible likelihood that second generation beavers free-living or in enclosures in Great Britain are infected.

Exposure Assessment

Infected released beavers may die and be consumed by potential definitive hosts. There is a high density of foxes throughout England and therefore the likelihood that a dead beaver is ingested by a fox is high. Infection of foxes occurs when they ingest the protoscolices in the beaver intermediate host. Infected foxes will excrete ova in their faeces and these ova may be ingested by intermediate hosts such as voles. There is a high density of intermediate hosts in England and, therefore, a high likelihood of infection of definitive and intermediate hosts at the destination. Dissemination will occur as the life cycle of the parasite repeats and there is a high likelihood of dissemination.

Humans can act as intermediate hosts and fieldworkers, particularly those working in the vicinity of the release location, could be exposed through contact with excreted ova in the environment, in the same way as other intermediate hosts previously described.

Consequence Assessment

There is a very low to medium likelihood of one beaver being infected with *E. multilocularis* at the time of release, depending on the origin of the beaver.

Beavers are intermediate hosts and the effect on their health depends on the location and number of *E. multilocularis* cysts (Davidson *et al.,* 2012). Evidence of *E. multilocularis* infection in beavers suggests that there is a very low likelihood of associated disease in beavers. There is consequently a very low likelihood of biological and economic consequence to the reintroduction programme.

Humans are intermediate hosts and chronic, severe disease occurs as a result of *E. multilocularis* cyst formation which is potentially fatal (WHO 2021). The biological consequences of infection in humans are therefore severe and there is a high likelihood of their occurrence. It is assumed that if *E. multilocularis* were to enter Great Britain it would be very difficult to eradicate due to the high numbers and densities of intermediate and definitive hosts. If the human infection rate were similar to Germany and France, where *E. multilocularis* is endemic, that could equate to 10 to 20 cases per year (DEFRA 2014). There is therefore a high likelihood of economic costs through the diagnosis, treatment, public health awareness, and other medical costs associated with the detection of disease in humans.

Risk Estimation

There is a low probability that free-living beavers in Norway will have been exposed and infected, and a medium probability that first generation beavers in enclosures or free-living in Great Britain will have been exposed and infected with *E. multilocularis*. There is a negligible probability that second generation beavers free-living or in enclosures in Great Britain will have been exposed and infected with *E. multilocularis*.

There is a high likelihood of exposure and infection of definitive hosts, and once the parasite is present in definitive hosts, a high likelihood of exposure and infection of intermediate hosts at the destination and a high likelihood of dissemination.

There is a very low likelihood of disease in beavers and of biological or economic costs to the reintroduction programme. There is a high likelihood of biological consequences from severe disease in people. There is a high likelihood of economic costs from surveillance and monitoring of the human population plus public awareness campaigns.

The risk of disease in people due to *E. multilocularis* disease arising from the translocation of enclosure beavers is highest, followed by Norwegian beavers, and lowest for free-living beavers from Great Britain. The overall risk is HIGH whichever origin of beavers is chosen.

Risk Management

Risk Evaluation

Preventative measures should be considered for any free-living or enclosure beavers being translocated within Great Britain or from Norway.

Risk options

There is no reliable method of screening for *E. multilocularis* infection in intermediate hosts: ante-mortem diagnosis in the intermediate host is considered challenging and in humans is usually based on mixed modalities combining imaging with serology (Campbell-Palmer *et al.,* 2015). Campbell-Palmer *et al.,* (2015) trialed the effectiveness of combined laparoscopy and ultrasonography under general anaesthesia in the field in screening beavers for echinococcosis and achieved reported sensitivity and specificity of 100% (n=45) though the authors acknowledged that the protocol may not be effective in picking up small lesions in early infections. A serological immunoblotting technique has reported sensitivity of 85% and specificity of 100% (Gottstein *et al.,* 2014; 2019) but is not suitable for field use as results are not immediately available (Campbell-Palmer *et al.,* 2015). Blood sampling for serology could be performed on a conscious beaver with restraint and without the need for general anaesthesia, although results should be interpreted with caution given current tests are not validated in beavers.

There will be advantages in using free-living beavers proven to have been born in Great Britain (second generation) for any translocation programme to reduce the risk from *E. multilocularis*.

Treatment for intermediate hosts is limited, and often unsuccessful, requiring surgical resection and prolonged treatment with benzimidazoles (Wen *et al.,* 2019).

5.4.20 Disease risk analysis for the carrier hazard *Neostichorchis subtriquetrus*

Carrier Hazard

Justification for Hazard Status

Neostichorchis subtriquetris (formerly *Stichorchis subtriquetris*), the beaver fluke, is a trematode of both Eurasian and Canadian beavers, not known to infect other species (Demkowska-Kutrzepa *et al.,* 2016). Its life cycle involves infection of the intermediate host, aquatic snails of *Bithinia, Planorbis* and *Lymnaea* spp. (*ibid*.), and ingestion of metacercariae attached to aquatic plants by beavers (Vengušt *et al.,* 2009).

Parasite prevalence, from post-mortem examination analysis of beavers, has been recorded at levels as high as 93.7% (n=45/48) in Poland (Demiaszkiewicz *et al.,* 2014) and 100% (n=30/30) in Sweden (Åhlen, Sjöberg, and Stéen 2021). Such high levels of prevalence may be related to the limited genetic diversity of host animals following a near-extinction bottleneck (*ibid.*) and to a loss of parasite diversity following captive management and reintroduction (Drózdz, Demiaszkiewicz, and Lachowicz 2004). *N. subtriquetrus* ova were found by faecal examination in 70% (n=14/20) of free-living beavers examined alive or post-mortem on Tayside in Scotland, most of which are believed to have originated from, or be descended from, Bavarian beavers (Campbell-Palmer *et al.,* 2015b). However, this may be an underestimate of prevalence because *N. subtriquetrus* ova shedding is likely to be intermittent (*ibid.*). Crucially there has been a confirmed case of *N. subtriquetrus* infection in a British-born beaver from Tayside, confirming that the parasite is able to complete its life cycle through suitable intermediate hosts in Great Britain (Campbell-Palmer *et al.,* 2013). Two adult trematodes were identified in the caecum of an adult male beaver found on a road in Kent which were subsequently confirmed to be *N. subtriquetrus* showing that the parasite is present in England (Common, Gerard, and Sainsbury 2022). The beaver showed signs associated with a road traffic collision and no apparent disease was seen in the caecum associated with the parasites (Common, Gerard, and Sainsbury 2022).

63% (n=10/16) of beavers imported from Norway for the Knapdale trial were found to be infected with *N. subtriquetrus* either pre- or post-release; none were treated with anthelmintics (Goodman *et al.,* 2014). *N. subtriquetrus* burdens are reported to be twice as heavy in young animals under two years old (n=11) compared to adults (n=34) (Demiaszkiewicz *et al.,* 2014): mean *N. subtriquetrus* intensity in young beavers was 201 trematodes (range 5-479) compared to mean intensity in adult beavers of 93 trematodes (range 2-893). This may indicate that immunocompetence to *N. subtriquetrus* infection is increased in the healthy adult animal.

Translocation is a known stressor (Dickens, Delehanty, and Michael Romero 2010). Therefore *N. subtriquetrus* should be considered as a carrier hazard for the translocation of beavers.

Risk Assessment

Release Assessment

Beavers are infected through ingestion of metacercariae attached to aquatic plants which form part of the beaver's diet. These metacercariae complete their life cycle to adult trematodes in the host. (Vengušt *et al.,* 2009). *N. subtriquetrus* trematodes are typically found in the caecum, and with decreasing frequency in the colon and small intestine, and rarely in the stomach of beavers (Sikorowski *et al.,* 2016). Ova are shed in beaver faeces into water and are consumed by the intermediate aquatic snail host. As beavers live in family groups, there is a high likelihood that an infected beaver could disseminate *N. subtriquetrus*, via intermediate hosts, to other beavers in the same habitat which will ingest metacercariae while foraging. Infection appears to be seasonal with highest burdens in the autumn (Drózdz, Demiaszkiewicz, and Lachowicz 2004; Sikorowski *et al.,* 2016). As *N. subtriquetrus* adult infestation is prevalent in beavers in both Great Britain and Norway, there is a very high probability of an infected beaver being released.

Exposure Assessment

As *N. subtriquetrus* has been shown to complete its lifecycle through intermediate hosts in Great Britain, there are likely to be infectious metacercariae present at release sites which will be ingested by beavers when they eat aquatic plants. As a result, there is a high probability that beavers at the destination site(s) will be exposed to and infected with *N. subtriquetrus*. There is a high likelihood of dissemination as a result of animals with *N. subtriquetrus* being released because the lifecycle of the parasite can be completed in Great Britain and beavers will be in relatively high-density family groups

Consequence Assessment

There is a high likelihood of a translocated beaver being infected with *N. subtriquetrus.* Infection is normally asymptomatic (Sager *et al.,* 2005). However, heavy burdens are associated with parasite presence outside the caecum where they may cause clinical signs (Demiaszkiewicz *et al.,* 2014). In histopathological examination of three infected beavers, Niemiec *et al.,* (2016) reported that *N. subtriquetrus* presence in the large intestine was associated with chronic inflammation and Ćirović *et al.,* (2009) reported that, in an earlier study by Romashov and Safonov, (1965) burdens greater than 150 trematodes were observed in association with chronic inflammation and vomiting, diarrhoea, weakness, anorexia, constipation and anaemia in a beaver but did not confirm whether this was an isolated case, nor have we been able to verify the source. Immunocompetent and healthy beavers would be expected to tolerate low levels of

infection with *N. subtriquetrus*. However, beavers undergoing handling, transport, and adjustment to release environments, and therefore stressed, may be more susceptible to disease and, as a result, experience morbidity or mortality. Three beavers (M08K22, M08K29, M08K31) died in captivity in association with *N. subtriquetrus* infection and in one of these, M08K29, the pathologist attributed focal ulceration and haemorrhage in the large intestine and poor body condition to the parasite burden (Deuchande 2009; Howie 2009; Collins 2009). There is a low likelihood of a high proportion of translocated beavers suffering from stressor-initiated trematode-associated-disease and a failure of the reintroduction and the associated economic and biological consequences

Risk Estimation

There is a high likelihood of beavers being exposed to *N. subtriquetrus* and a very high likelihood of an infected beaver being released. There is a high likelihood of exposure and dissemination of the parasite at the release site. There is a low probability that the stress of translocation may precipitate disease in a high proportion of translocated infected beavers and lead to failure of the reintroduction. The overall risk from disease caused by *N. subtriquetrus* is therefore MEDIUM.

Risk Management

Risk Evaluation

Based on the risk assessment above, preventative measures should be employed to reduce the risks from *N. subtriquetrus* as a carrier hazard

Risk options

Measures to reduce the stress from translocation are important. For example, efforts should be made to minimise stress from capture, transport and, in particular, repeated handling, and to reduce transit times. Consideration should also be given to the timing of releases, avoiding winter months as the autumn burden of *N. subtriquetrus* might be high.

5.4.21 Disease risk analysis for the source hazard *Taenia martis*

Source Hazard

Justification for Hazard Status

The family Taeniidae comprises two genera of cestodes: *Echinococcus* (*E. multilocularis* has already evaluated elsewhere within this report) and *Taenia*, both of which are of zoonotic importance globally. Similarly to *E. multilocularis*, *Taenia* spp. have an indirect

lifecycle: adult cestodes parasitise a definitive host and larval forms (metacestodes) require an intermediate host (Miller *et al.,* 2017). Disease in the intermediate host occurs following ingestion of metacestodes. The genus *Taenia* contains several species responsible for human infection: *T. solium* (infection occurs through ingestion of undercooked pork), *T. saginata* (infection occurs through ingestion of undercooked beef) and *T. asiatica* (infection occurs through ingestion of pig liver). *T. solium* is of particular concern as it can lead to cysticercosis which can precipitate seizures and potential deaths in people (World Health Organisation, 2021b).

As well as the more well-known and high-profile *Taenia* spp., many other species exist. Some of these *Taenia* spp. have been associated with cysticercosis in humans but cases are considered to be much rarer than with *T. solium* (Koch *et al.,* 2016). Many *Taenia* species are able to infect wild animals, and rodents often act as intermediate hosts for these parasites (Deplazes *et al.,* 2016). For example, Haukisalmi and Henttonen (1993) reported *T. polyacantha* prevalence as high as 13% (47/359) in female bank voles examined in Finland for presence of metacestodes within the abdominal cavity. Miller *et al.,* (2017) reported a 1.1% (7/655) prevalence of *T. polyacantha* in bank voles, and a 0.5% prevalence (1/206) in wood mice, as well as detecting *T. taeniaeformis* in wood mice, bank voles, field voles, water voles and yellow-necked mice and *T. mustelae* in water voles, bank voles and common voles trapped in Sweden (Miller *et al.,* 2017). *T. martis* was detected in one free-living beaver in Bavaria, Germany. This animal was one of 11 beavers dispatched as part of a routine management programme; post-mortem examination revealed a cystic lesion on the liver and samples of the lesion were tested using PCR confirming the presence of *T. martis* (Campbell-Palmer *et al.,* 2015).

The definitive hosts of *T. martis* are considered to be the stone marten and the pine marten in Europe (*Martes foina* and *M. martes* respectively), although adult *T. martis* parasites have been detected in other mustelids and carnivores across Europe, suggesting a wider variety of possible definitive hosts (Nugaraitė, Mažeika, and Paulauskas 2019; Loos-Frank and Zeyhle 1982; Tylkowska *et al.,* 2019). Rodents are known to be intermediate hosts (Brunet *et al.,* 2014). *T. martis* has been detected in free-living definitive hosts and rodents across Europe, including in Poland, Italy, Germany, Switzerland and France (Ribas *et al.,* 2004; Mathy *et al.,* 2009; Rausch 2003; Kornaś *et al.,* 2013). Prevalence of *T. martis* in rodents across Europe has been reported to vary between 0.95% and 22% (Mathy *et al.,* 2009; Reperant *et al.,* 2009; Ribas *et al.,* 2009). Although several *Taenia* spp. are present and known to be transmitted in Great Britain (e.g. *T. pisiformis*, *T. serialis*, *T. taeniaformis* and *T. hydatigena*) (Boufana *et al.,* 2012), to the best of the authors' knowledge, *T. martis* has not been detected in any species in Great Britain (Global Biodiversity Information Facility 2021).

T. martis has been associated with five human cases of cysticercosis in mainland Europe. Two ocular cases (Koch *et al.,* 2016; Eberwein *et al.,* 2013) and two peritoneal cases have been reported in Germany (Mueller *et al.,* 2020; Rudelius *et al.,* 2017), and a case of *T. martis*-associated cerebral cysticercosis was reported in a patient in France (Brunet *et al.,*

2015). It is not confirmed how these humans were exposed to *T. martis*, but two cases were suggested to have occurred after the patient ate contaminated home-grown vegetables and another is thought to have occurred due to environmental contamination during hiking in the Alps (Eberwein *et al.,* 2013; Rudelius *et al.,* 2017).

A case of peritoneal cysticercosis associated with *T. martis* was also reported in a captive Tonkean macaque (*Macaca tonkeana*) from a captive primate colony in France (Brunet *et al.,* 2014). Free-living martens, the definitive hosts, were known to have access to primate enclosures, and are likely to have contaminated the environment with Taeniid ova, leading to exposure of the macaque (Brunet *et al.,* 2014). This case provides evidence that captive animals can be exposed to *T. martis* in endemic areas where the definitive hosts exist, for example Germany and Poland.

Given that beavers are known to be susceptible to infection with *T. martis* as intermediate hosts, that *T. martis* has never been detected in Great Britain, but species capable of acting as definitive and intermediate hosts exist, and that *T. martis* can cause cysticercosis in humans, this parasite should be considered a source hazard for the translocation of beavers when they have originated from endemic areas in continental Europe, such as Poland and Germany, and have been translocated to be free-living or in enclosures in Great Britain.

Risk Assessment

Release Assessment

The lifecycle of *T. martis* is similar to that of *E. multilocularis* in that it requires an intermediate and definitive host. The definitive hosts are carnivores, commonly a pine marten or stone marten in Europe (Brunet *et al.,* 2015), although *T. martis* has also been detected in red foxes (Loos-Frank and Zeyhle, 1982; Ballek *et al.,* 1992) which could play a role in the transmission cycle (Brunet *et al.,* 2015). Adult *T. martis* cestodes inhabit the small intestine of the definitive host and ova are shed into the environment in faeces. Numerous species can act as intermediate hosts, including humans and beavers (but normally small rodents such as bank voles) which ingest the ova, for example after contamination of vegetation or by grooming of contaminated fur (Brunet *et al.,* 2014). The ova hatch, penetrate the intestinal wall, and migrate to other parts of the body where they become cysticerci. The lifecycle continues when cysts are ingested by definitive hosts; carnivores may prey on small rodent hosts, or carcases could be scavenged by other species (Centers for Disease Control and Prevention 2021d).

It is known that beavers can act as intermediate hosts of *T. martis:* a free-living Bavarian beaver was found to have a liver cyst associated with this parasite (Campbell-Palmer *et al.,* 2015). It is likely that this beaver was exposed to *T. martis* ova through ingestion of vegetation contaminated by definitive hosts. *Taenia* spp. ova can survive for long periods of time in the environment, particularly in temperate environments between 5 and 25

degrees Celsius (Jansen *et al.,* 2021): one study showed that *T. saginata* ova survived for up to a year on pasture (Duthy and van Someren 1948). The likelihood of exposure of beavers to *T. martis* varies from very low in Norway, where this parasite has not been reported in the literature, to medium in endemic areas including Germany and Poland. Moreover, first generation beavers translocated from enclosures in Great Britain have a high likelihood of exposure given that some beavers held in enclosures in Great Britain are known to have been sourced from Bavaria or Poland (Claire Howe, pers. comm), and that others have an unknown history. Beavers in enclosures may have contracted infection when either previously free-living or captive: as demonstrated in France by Brunet *et al.,* (2014), transmission of *T. martis* to captive animals can occur in endemic areas, particularly where enclosures are not secured against free-living definitive hosts. Since a large proportion of beavers in enclosures have originated from endemic areas, and *T. martis* has not been reported from Great Britain since unlicensed and licensed releases of beavers have occurred in Great Britain, beavers released from enclosures are more likely to have been exposed and infected than free-living beavers in Great Britain. Second generation beavers in enclosures are considered to be of very low to negligible risk of exposure.

Beavers are known to be susceptible to infection with *T. martis*, so there is a high likelihood of infection after exposure.

Exposure Assessment

Infected, released beavers may die and be consumed by potential definitive hosts. *T. martis* has been detected in red foxes, pine martens, Eurasian badgers (Takács *et al.,* 2012), American mink (Torres *et al.,* 2006), Eurasian otters (Górski *et al.,* 2010; Torres *et al.,* 2004; Nugaraitė, Mažeika, and Paulauskas 2019) and European polecats (Nugaraitė, Mažeika, and Paulauskas 2019), all of which are present in Great Britain providing means for *T. martis* to complete its lifecycle if it were released into England. There is a high density of foxes throughout England and therefore the likelihood of fox exposure to *T. martis*, through ingestion of a first-generation beaver carcass is high. The likelihood of exposure of Eurasian otters is also estimated to be high given that beavers are a sympatric species, otters are carnivorous and the death of a first-generation beaver could lead to contamination of the local environment shared by these animals. Badgers, polecats and mink are widespread across England, may be sympatric to beavers when terrestrial, and the likelihood of exposure of these species through a first-generation beaver carcass contaminating the environment is predicted to be medium. The likelihood of exposure of other mustelids such as polecats is dependent on the release location of the beavers. The likelihood of ingestion by a pine marten is very low given that only small, reintroduced populations exist in specific areas of Great Britain. There is a high likelihood of dissemination of *T. martis* given that many intermediate host species exist in England which would allow completion of the life cycle.

Humans are intermediate hosts, and fieldworkers, particularly those working at the release location, could be exposed through contact with excreted ova in the environment, in the same way as other intermediate hosts above. Moreover, cases of human infection in the general public have been reported in endemic areas. Human exposure is thought to have occurred through environmental contamination such as ingestion of ova on home-grown vegetables. There is a low likelihood of exposure of humans in this manner if the parasite was released into England.

Consequence Assessment

There is a high or medium likelihood that one first-generation beaver will be infected with *T. martis* at the time of translocation, dependent on the origin of the beavers released.

T. martis was detected in a free-living beaver as an incidental finding with no signs of disease noted (Campbell-Palmer *et al.,* 2015). Similarly, disease has not been reported in rodents and therefore there is a very low likelihood of disease occurring in translocated beavers and a very low likelihood of failure of the translocation as a result of beaver mortality.

A growing body of evidence exists documenting cases of cysticercosis in humans associated with *T. martis* infection. Cysticercosis is a severe disease which can lead to serious consequences in humans including blindness, epilepsy and death (World Health Organisation, 2021b). Therefore, if *T. martis* was released into England, currently thought to be free of *T. martis*, and the parasite became established and endemic, the risks from disease in people would be considerable, and the associated medical costs to educate the public and treat cases would be high.

If *T. martis* is released into England, there is a medium likelihood of severe health consequences to the human population and significant associated economic consequences to control and treat cysticercosis. There is a low likelihood of significant environmental or ecological consequences since the parasite is unlikely to cause disease in definitive or intermediate mammalian hosts.

Risk Estimation

There is a very low likelihood of exposure and infection of beavers in Norway, a medium likelihood of first-generation free-living beavers in Great Britain being exposed and infected, and a high likelihood of exposure and infection of first-generation beavers in enclosures in Great Britain. There is a very low to high likelihood of exposure of susceptible mammals at the destination site depending on the species and a high likelihood of dissemination. There is a low likelihood of exposure to humans at the release site, but a medium likelihood of severe disease in people and economic consequences. There is a very low likelihood of beaver mortality and failure of the reintroduction as a result of *T. martis* infection. There is a low likelihood of environmental and ecological

consequences as a result of release. The disease risk is estimated to be HIGH if translocated beavers originate from enclosures and are first generation, MEDIUM if they originate from free-living populations and low if they are translocated from Norway.

Risk Management

Risk Evaluation

Mitigation measures should be implemented to manage this risk.

Risk options

Given that the beaver is an intermediate host for *Taenia martis,* post-mortem identification of liver cysts followed by molecular analysis is the most effective diagnostic measure. Therefore, detailed examination of the liver should be included in the post-mortem examination protocol of the post-release health surveillance (PRHS) of beavers released into England.

Ante-mortem surveillance of *T. martis* poses a challenge given that currently no specific serological tests exist; in fact, in a human case of *T. martis* cysticercosis, an ELISA was positive for *Echinococcus granulosis*, likely due to antigen cross-reactivity within the taeniid family (Rudelius *et al.,* 2017). Ante-mortem diagnosis of *T. martis* in intermediate hosts has previously relied on molecular testing of extracted diseased tissue in patients with clinical signs (Rudelius *et al.,* 2017; Brunet *et al.,* 2015; Eberwein *et al.,* 2013), although in the case of a captive macaque, routine abdominal palpation revealed a mass which was visualised using ultrasonography and later removed via a surgical procedure (Brunet *et al.,* 2014). This case highlights the importance of undertaking a full clinical examination of beavers before translocation; any beavers with clinical signs that could be associated with cysticercosis (e.g. neurological signs) should not be released. Ultrasonography has previously been highlighted as an important tool in the identification of *E. multilocularis* lesions in the livers of beavers (Campbell-Palmer *et al.,* 2015) and could be utilised as part of a pre-release health examination protocol. The sensitivity of ultrasonography is likely to be low given that the *T. martis* cyst identified in the liver of a beaver previously was only 3mm diameter.

Antiparasitic treatment prior to translocation could be useful to treat early infections but is unlikely to be effective at eliminating infection completely when used alone. Antiparasitic therapy was successful in reducing the size of a larval cyst in a human patient; however, the symptoms remained and surgical removal was required (Eberwein *et al.,* 2013). In general, a combination of surgery alongside antiparasitic treatment has shown good success rates in people (Koch *et al.,* 2016).

5.4.22 Disease risk analysis for the source hazard *Trichinella* spp.

Source Hazard

Justification for Hazard Status

Trichinella spp. are parasitic nematodes, currently comprising nine species and four genotypes with variations in host and geographic preferences, and a major historic cause of zoonotic infections and economic losses in Europe (Pozio 2020). *Trichinella* spp. have a broad host range and infections have been reported in over 150 mammalian species, across 12 orders, as well as in birds and reptiles (Pozio 2019). However, humans are thought to be the only mammals to experience clinical disease (trichinellosis); host animals ingesting large numbers of infective larvae have not been reported to exhibit symptoms (Gottstein, Pozio, and Nöckler 2009). Trichinellosis is a disease of varying severity in humans, usually as a result of eating undercooked or raw pork products containing *Trichinella* spp. larvae from both domestic pigs and wild boar (Gottstein, Pozio, and Nöckler 2009). The highest proportion of *Trichinella* spp. infections in humans are of *Trichinella spiralis*, but infections with other *Trichinella* spp., including *T. britovi*, *T. nativa and T. pseudospiralis,* have also been reported (Bronstein and Lukashev 2018; Ranque *et al.,* 2000).

Great Britain is currently considered to be free of *Trichinella* spp. with 6,976,629 farmed pigs, 581 wild boar, 360 red foxes and 2,771 horses screened and found to be negative in 2018 (EFSA 2019). Several isolated wildlife cases of *Trichinella* spp. have been reported in the UK, including a case of *T. spiralis* infection in a red fox from Truro, Cornwall sampled in 1957 (Oldham and Beresford-Jones 1957), and two foxes infected with *T. spiralis* reported in 2007 and 2009 in Northern Ireland (Learmount *et al.,* 2015). More recently, *T. pseudospiralis* was identified by artificial digestion and PCR in a red fox found dead following a road traffic collision near Bristol (Learmount *et al.,* 2015) in 2013. As this was an isolated case (n= 1/6806 red foxes sampled between 1999 and 2013 in Great Britain), Learmount *et al.,* (2015) concluded that the prevalence of *T. pseudospiralis* in Great Britain is extremely low and the associated risk negligible.

Infection with *Trichinella* spp. has been reported from numerous rodent species across the world. For example, the yellow-necked mouse striped field mouse and harvest mouse in Europe (Dick and Pozio 2001); the fox squirrel (*Sciurus niger*), muskrat) and white-footed mouse in North America (Dick and Pozio 2001; Martin *et al.,* 1968); an Indian mole rat (*Bandicota bengalensis*) in India (Shaikenov and Boev 1983); a natal multimammate mouse (*Praomys natalenisis*) in South Africa (Young and Kruger 1967); the gray leaf-eared mouse (*Graomys griseoflavus*) in Argentina (Minoprio, Naves, and Abdon 1967); a wild squirrel (species not specified) in Thailand (Khamboonruang 1991); the brown rat, black rat and little rat (*Rattus exulans*) in Pacific Islands (Alicata 1970).

Infections with *T. britovi* and *T. spiralis* have been reported in Eurasian beavers; 1/182 beavers killed by hunters in Latvia between 2010 and 2014 was positive for *T. britovi* with 148 larvae identified in a muscle tissue sample of approximately 25g (Segliņa *et al.,* 2015). Moreover, a single *T. spiralis* larva was also found in a muscle sample from one of 69 beavers hunted in Poland in 2018 (Różycki *et al.,* 2020). *T. spiralis* larvae were detected in one of 211 free-living Canadian beavers in North America over a 15 year period (Zimmermann and Hubard 1969).

Trichinella spp. have also been detected in animals housed in captive zoological collections. *T. spiralis* was detected in 12% (n= 9/76) of rats trapped and euthanised around Helsinki Zoological Gardens (Tiainen 1966) and infections with *T. spiralis* were historically reported from other species housed in the same zoological collection, including in three polar bears (*Ursus maritimus*) and a wild boar (Tiainen 1966). *Trichinella* spp. infection has also been reported in captive American kestrels (*Falco sparverius*) (Saumier, Rau, and Bird 1988, 1986).

Given that infections with *Trichinella* spp. have been reported in numerous rodent species across the globe, infection has been detected in beavers (including free-living *Castor fiber* from Europe), infections have been reportedly transmitted in captive collections, and the UK is considered to be free from *Trichinella* spp*., Trichinella* spp. should be considered to be a source hazard for the reintroduction of Eurasian beavers sourced from free-living populations in Great Britain or Norway, or from enclosures in Great Britain where the history of the beaver is unknown.

Risk Assessment

Release Assessment

Trichinella spp. are unusual in that they undergo a complete life cycle in a single host animal but require a second host to perpetuate their life cycle (Pozio 2019) (Figure 3). In the domestic environment, pigs are exposed and infected when management and welfare standards are low, for example, by scavenging infected carcases and through tail biting (Pozio 2000). Vertical transmission has also been demonstrated experimentally in ferrets, guinea pigs and mice but not in foxes or pigs (Webster and Kapel 2005).

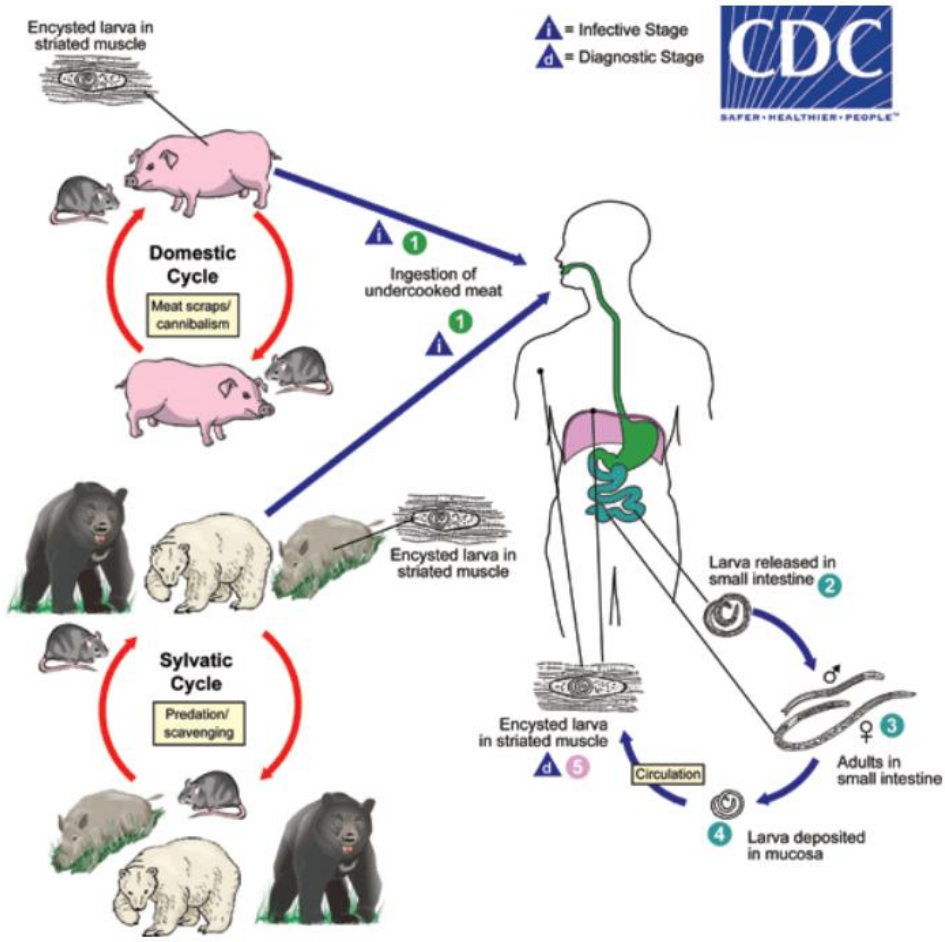

Figure 3: The life cycle of *Trichinella spp. (Source:* (Centers for Disease Control and Prevention 2021e)

The infectious agent in pigs is usually *T. spiralis* which also exists in a sylvanian cycle in Europe in areas where it has been eliminated from domestic livestock (Pozio 2000). There is occasional spillback and spillover between the domestic and sylvanian cycles, probably facilitated by foxes, rats and domestic cats, particularly when pigs are housed outdoors or are fed hunters' scraps (Pozio 2019). Kapel (2001) demonstrated experimentally that wild boar are not particularly susceptible to infection with *T. nativa*, with rapid declines in antibody levels shown to be associated with the disappearance of larvae from muscle tissues and it is believed that domestic pigs are similarly resistant to infection with *T. nativa*. Additionally, infections of *T. britovi* in swine are reported to be short-lived, with larvae surviving for less than one year in pig muscle, but reports of occasional infections of swine in the Baltic states suggest that animals immunosuppressed by stress and hunger or concurrent infection may, on occasions, be susceptible to infection with *T. nativa* (Pozio 2019). *T. pseudospiralis* has been rarely reported in domestic swine in Europe (Pozio 2016b).

T. pseudospiralis is the only *Trichinella* spp. known to infect both birds and mammals and is rarely reported in wildlife: 1.6% (n=63/3925) of isolates in European wildlife were

confirmed as *T. pseudospiralis* between 2007 and 2014 (Pozio 2016b). However, the prevalence of *T. pseudospiralis* in wildlife may be underestimated due to the limited sampling of birds compared to mammals (Learmount *et al.,* 2015). In addition, unlike the other *Trichinella* spp. of interest in Europe, encysted *T. pseudospiralis* larvae lack a surrounding collagen capsule, making visual diagnosis using trichinoscopy almost impossible historically (Pozio 2016b). Although the environmental survival of *T. pseudospiralis* is poor compared to other *Trichinella* spp., its broad host range, and bird migration and dispersal, may perpetuate sylvanian transmission cycles and geographic range expansion (Pozio 2016b). The perpetuation of sylvanian cycles of all *Trichinella* spp. is facilitated in areas where hunters leave animal carcases for other animals to scavenge (Pozio *et al.,* 2009).

As transmission is reliant on ingestion of animal carcases infested with larvae, infections are found primarily in carnivorous or omnivorous animals. However, infection of herbivorous animals, including horses, is also reported (EFSA 2019). Grzybek *et al.,* (2019) screened three free-living populations of bank voles at three intervals between 2002 and 2010 in Poland for *Trichinella* spp. antibodies and found an average prevalence of infection with unspecified *Trichinella* spp. of 1.37% (n=656). Infection probably occurs as herbivores inadvertently ingest larvae while foraging for food near carcases, consume carrion or from cannibalism (Grzybek *et al.,* 2019).

Różycki *et al.,* (2020) hypothesised that the positive beaver found in their study in Poland could have been exposed to *Trichinella* spp. larvae during a fight with infected predators invading beaver settlements, through accidental consumption of contaminated feed, or via intentional consumption of unusual food sources (with a higher potential of contamination such as carcases of other animals) in order to supplement protein or mineral deficits in difficult conditions. The latter has been further supported as the liver parasite, *Capillaria hepatica,* and fish parasite, *Paragonimus westermani,* have been rarely detected in beavers (Bronstein and Lukashev 2018), suggesting that beavers may occasionally feed on meat or fish. Following ingestion, *Trichinella* larvae penetrate the intestinal mucosa where they complete their development to adulthood (Gottstein, Pozio, and Nöckler 2009). Adult nematodes mate and, five days after infection, release larvae which migrate via blood and lymphatic vessels to striated muscle tissue where they complete their development to the infective stage and then enter a dormant state until the host animal is predated or dies (Gottstein, Pozio, and Nöckler 2009). As the first stage of the lifecycle is completed quickly, larvae successfully evade the host's immune system but adult nematodes are expelled and no further reproduction takes place in the host unless further infective larvae are ingested (Gottstein, Pozio, and Nöckler 2009). As a result, an animal ingesting only low numbers of larvae is likely to have only low infectivity potential.

After several years of declining prevalence of *Trichinella* spp. infection in wildlife in the European Union (EU), small increases were reported in 2018 (EFSA 2019). However, surveillance is not standardised across member states and not all member states submit reports. A number of wildlife species are screened for *Trichinella* spp. infection, primarily

the red fox and wild boar, with prevalences in 2018 respectively 1.6% (n=108/6612) and 0.09% (n=1,293/1,465,482) across 14 member states (EFSA 2019). Infections were also reported in a further 10 species, with highest prevalences in the Eurasian lynx (*Lynx lynx*), wolf and raccoon dog (EFSA 2019). As the population levels of these three species are low when compared to the red fox, they are not currently considered to be a significant reservoir of infection in the sylvanian cycles but this may change with increasing population numbers and distribution of these species and the European jackal (Pozio 2019). Since both *T. nativa* and *T. britovi* have been found in red foxes in Norway and Germany and infections with *T. britovi* and *T. spiralis* have been reported in beavers, there is a very low probability that beavers previously imported into Great Britain from these countries were infected with *Trichinella* spp. prior to capture. However, since beavers have been imported, with the exception of an isolated case of *T. pseudospiralis* infection in a red fox in the Bristol region in 2013, infection has not been detected in the red fox population in Britain. There is therefore a very low likelihood that an adult beaver, previously imported from an area with endemic *Trichinella* spp. infection, translocated to England will be infected.

Since *T. nativa* and *T. britovi* have been found in red foxes in Norway, and *T. spiralis* in red foxes in neighbouring Sweden, and infections, of very low prevalence, with *T. britovi* and *T. spiralis* have been reported in beavers in other countries, there is a very low probability that beavers translocated from Norway could be infected with low levels of *Trichinella* spp. larvae.

Trichinella spp. have been reported in numerous exotic rodent species globally (Dick and Pozio 2001; Martin *et al.,* 1968; Shaikenov and Boev 1983; Young and Kruger 1967; Minoprio, Naves, and Abdon 1967; Khamboonruang 1991; Alicata 1970), and also in captive collections (Tiainen, 1966; Saumier, Rau and Bird, 1986; 1988). Therefore, there is a very low likelihood that beavers with an unknown history may have been previously housed in captive collections in contact with exotic species (particularly rodents), exposing them to *Trichinella* spp..

Vertical transmission from parent to foetus has been demonstrated in rodents so there is a very low probability that an infected female beaver could have transmitted *Trichinella* spp. infection to its offspring. However, the maximum larval burden in offspring from experimentally infected guinea pigs was 60 larvae and, in mice, six larvae (Webster and Kapel 2005) so the infective burden, if any, is likely to be very low. There is, therefore, a very low likelihood that a beaver born to an infected dam, previously imported from an area with endemic *Trichinella* spp. infection or exposed to *Trichinella* spp. in a captive collection, translocated to or within England will be infected.

As the number of free-living beavers in Great Britain is low, and some carcases have been retrieved for post-mortem examination, there is a very low probability that a previously imported, infected, free-living beaver in Great Britain has been predated or scavenged, thereby infecting a sympatric carnivore(s) in Great Britain. The probability that an imported

beaver housed in an enclosure in Great Britain is predated and scavenged is considered negligible. There is a negligible likelihood that *Trichinella* spp. larvae from an infected carnivore have been ingested by a sympatric beaver because the prevalence of *Trichinella* spp. infection in red foxes in Great Britain is very low. Additionally, the prevalence in beavers has been shown to be low even in an area with high prevalence in an endemic sylvanian cycle (Grzybek *et al.,* 2019; Bakasejevs *et al.,* 2012). There is therefore a very low likelihood that a free-living beaver in Great Britain, translocated to England, is infected with *Trichinella* spp..

Exposure Assessment

There is a high likelihood that a sympatric carnivore or omnivore will be exposed to *Trichinella* spp. and become infected by predating an infected beaver. In addition, there is a high likelihood that a sympatric carnivore or omnivore would be infected by scavenging the carcase of an infected beaver as the larvae of most *Trichinella* spp. are encapsulated in muscle tissues which facilitates prolonged survival in the environment following the death of the host animal (Pozio 2000). The duration of larval survival is greatest between 0 and -20˚C, and at higher humidity levels, with *T. nativa* shown to remain infective after five years of freezing and *T. britovi* after just less than one year (Pozio 2019). Larvae survive longer in frozen carnivore carcases than in swine and rodents for reasons which are not well understood (Pozio 2016a). In addition, Davidson *et al.,* (2008) demonstrated that *T. nativa* is highly tolerant to repeated freezing and thawing with larval survival after seven events comparable to unthawed larvae. Encapsulated larvae can also survive up to four months after muscle tissue has decayed and so may constitute a source of environmental infection to herbivorous animals (Pozio 2000). There is therefore a very low likelihood that herbivores such as deer and horses, as well as other beavers, at the destination site(s) will become infected through accidental ingestion of infective larvae on plant matter or through deliberate scavenging of infected carcases.

The establishment of sylvanian *Trichinella* spp. cycles in Europe is facilitated by hunter activity and the survival of encapsulated *Trichinella* spp. larvae in carcases is temperature and humidity dependent with optimum survival between 0 and -20˚C . As the average winter temperature low in England is 0.9˚C (Met Office 2020) and sport hunting is less common than in Europe, there is a lower likelihood of *Trichinella* spp. establishing in sylvanian cycles in Great Britain compared with the same cycles on the continent. The probability of dissemination of *Trichinella* spp. through the establishment of a sylvanian cycle is therefore very low.

40% of domestic pigs are kept outdoors in Great Britain (ADHB 2020). A pig could be infected with *Trichinella* spp. if it scavenged the carcase of an infected animal. Small rodents act as vectors between sylvanian and domestic cycles in Europe and a domestic pig could be infected if it scavenged an infected rodent. However, the likelihood of dissemination through the domestic pig population is very low as pigs are not routinely fed hunters' scraps in the UK and are kept in fenced enclosures. In addition, both *T. nativa*

and *T. britovi* appear to have short survival times in swine. The probability of dissemination through the domestic cycle is very low.

There is a very low likelihood that humans are infected by eating undercooked meat from an infected animal if *Trichinella* spp. enters either the domestic or sylvatic cycles through one of the mechanisms above. It is interesting to note that beaver hunting, both licensed and unlicensed, is widespread across Europe and beaver meat is considered healthy and a great delicacy in Eastern Europe (Bronstein and Lukashev 2018). There is therefore a very low probability at release sites of illegal hunting of free-living beavers for consumption.

Consequence Assessment

There is a very low likelihood of one beaver being infected with *Trichinella* spp. at the time of translocation if that beaver is sourced from free-living populations in mainland Europe or Great Britain. There is a low likelihood of one translocated beaver being infected if it is sourced from a captive collection or enclosure with an unknown history.

Following ingestion of *Trichinella* spp. larvae in raw or undercooked meat, disease in humans may range from asymptomatic to more severe illness including fever and gastroenteritis as larvae migrate through the intestinal mucosa. In severe cases, encephalitis and secondary infections may occur (Davidson *et al.,* 2009) and one third of human cases may require hospitalisation (Pozio 2019). The severity of disease in humans is believed to be dependent on the infective dose ingested and may be more severe with *T. spiralis* than with other *Trichinella* spp. (Gottstein, Pozio, and Nöckler 2009). The lowest infective dose associated with disease in humans is not known but is believed to be over 100 larvae (Gottstein, Pozio, and Nöckler 2009). Ingestion of more than 1000 larvae is believed to be associated with severe symptoms in humans (Davidson *et al.,* 2009). There is a high likelihood of severe disease in humans if infected with *Trichinella* spp..

The economic impact of trichinellosis in countries where the parasite is endemic in domestic pigs is considerable due to the cost of control systems in abattoirs (which was estimated at 3USD per pig in the EU in 2000), checks on wildlife, the commercial value of wasted carcases, and medical costs associated with treating human infections (Pozio, 2000). The economic consequence of Great Britain losing its *Trichinella*-free status is therefore high. The biological and economic consequences of disease in humans is high.

Evidence noted above indicates that humans are the only animals which seem to experience clinical signs following infection with *Trichinella* spp.. There is, therefore, a negligible likelihood of clinical disease in infected beavers and a negligible likelihood of translocation failure as a result of *Trichinella* spp. infection of beavers.

Risk Estimation

There is a very low likelihood that a free-living beaver in Great Britain or Norway, translocated to England, is infected with *Trichinella* spp.. There is a very low likelihood that a beaver with an unknown history sourced from an enclosure or captive collection is infected with *Trichinella* spp.. There is a high likelihood of exposure and infection of sympatric carnivores and omnivores at the destination site(s) and a very low likelihood of exposure and infection of herbivores. The likelihood of onward transmission and dissemination into a sylvatic and/or domestic cycle of infection is very low. There is a very low likelihood of exposure and infection of the human population following dissemination into the domestic or sylvatic life cycles. There is a negligible likelihood of translocation failure and biological and economic consequences from that failure. There is a high likelihood of severe disease in humans and of severe economic and biological consequences as a result of disease in humans and domestic livestock. The overall risk is MEDIUM.

Risk Management

Risk Evaluation

Steps should be taken to minimise the risks to humans and livestock from the source hazard *Trichinella* spp.

Risk options

Detection of immature *Trichinella* spp. larvae in carcases by muscle digestion is the gold standard of diagnosis but is time-consuming and costly (Davidson *et al.,* 2009). Serology, in combination with western blot for crude larval antigen, demonstrates comparable sensitivity but may not be a reliable method of diagnosis: seroconversion to detectable levels in animals with low levels of infection may take up to seven weeks and some animals, for example horses, do not appear to seroconvert despite high larval burdens (Davidson *et al.,* 2009). Additionally, haemolysis or contamination of field samples may significantly reduce the sensitivity and specificity of tests (Davidson *et al.,* 2009). Efficacy of serological testing has not, as far as we are aware, been demonstrated in beavers but testing is unlikely to be sufficiently sensitive as the prevalence of *Trichinella* spp. larvae in beavers is low, even in endemic areas.

Sourcing beavers from Great Britain, particularly those proven to have been born in Great Britain, is more likely to be effective in minimising the risk of translocating a beaver infected with *Trichinella* spp..

Post-mortem examination of translocated beavers and sympatric species is strongly recommended to assess for entry of *Trichinella* spp. into the UK. Samples of muscle should be submitted from unfrozen carcases to the Animal and Plant Health Agency

(APHA) for assessment of *Trichinella* spp. through a muscle digestion method. Additionally, farmers and hunters at the destination sites should be reminded of the importance of appropriate carcase removal and disposal following pest control.

5.4.23 Disease risk analysis for the carrier hazard *Emmonsia crescens*

Carrier Hazard

Justification for Hazard Status

Emmonsia spp. (formerly *Chrysosporium* spp.) are saprophytic fungi which can infect a broad range of mammalian hosts, including occasionally domestic animals and humans, leading to adiaspiromycosis, a respiratory disease of variable severity (Danesi *et al.,* 2020). The disease is considered to be one, primarily, of burrowing animals, in particular small rodents and mustelids (Danesi *et al.,* 2020). The two *Emmonsia* species of concern are *Emmonsia crescens, (*syn*. Emmonsia parva var. crescens) and E. parva*, recently reclassified as *Blastomyces parvum*. The two are differentiated primarily on microscopic evaluation of adiaspore size and morphology, with *B. parvum* characterised by thin-walled uninucleate adiaspores of 10 to 40µm and *E. crescens* by multinucleate adiaspores up to 400 µm in diameter (Danesi *et al.,* 2020). *B. parvum* has a narrow host and geographic range and is very rarely found in Europe (Borman *et al.,* 2018). The only reported case of *B. parvum* in Europe is from a red fox in Czechoslovakia in 1975, based on adiaspore appearance prior to the availability of PCR for confirmatory diagnosis (Otcenasek, Krivanec, and Slais 1975).

E. crescens infection has been diagnosed in a broad range of wildlife species in Great Britain. Borman *et al.,* (2009) reported that almost 1/3 (n=27/94) of animals found dead in Great Britain and submitted to the Wildlife Veterinary Investigation Centre, Truro between 2003 and 2005 were positive for *E. crescens* infection on either microscopy or histopathology (Table 6). When both microscopy and histopathology were used together for diagnosis, recorded prevalence was higher at 43% (n=9/21) (Borman *et al.,* 2009). It was also noted that the true prevalence of infection may have been even higher, as low burdens could have been missed as only a small portion of lung tissue was selected for evaluation. Numerous mammal species have been reported to be positive for *E. crescens* in Great Britain, including the American mink, European rabbit and European mole (*Talpa europaea*) (Hughes and Borman 2018; Simpson, Davison, and Dagleish 2019; Harrington *et al.,* 2012). Of particular interest, several free-living rodent species have also tested positive in Great Britain: the water vole, red squirrel, brown rat and unspecified mice (*Mus* spp.) (Borman *et al.,* 2009; Chantrey *et al.,* 2006; Simpson *et al.,* 2013).

Of 562 mammals from 16 species culled for evaluation in Norway in 1959, 40% (n=4/10) of voles (*Microtus* spp.) and 1/1 water vole were positive for *E. crescens* (Table 6) and

infection was reported in museum specimens of two wood mice and six bank voles from a sample of unspecified size (Table 7) (Jellison and Vinson, 1961). High prevalences of infection with *E. crescens* in otherwise healthy animals have also been reported in Europe in the muskrat: 22.3% (n=46/206) of muskrats culled in Sweden (Maciera 2019) and 8/8 culled in Czechoslovakia (Otcenasek *et al.,* 1974).

Table 6: Prevalence of *E. crescens* in British wildlife 2003-5. (Source: Borman *et al.*, 2009)

Mammalian species	Prevalence (%)				
	Immature		Adults		Total
	M	F	M	F	
Lutra lutra (Otter)	4 / 8 (50)	4 / 8 (50)	4 / 19 (21.1)	6 / 20 (30)	18 / 55 (32.7)
Mustela nivalis (Weasel)	-	0 / 3	2 / 5 (40)	0 / 2	2 / 10 (20)
Mustela erminea (Stoat)	-	-	1 / 4 (25)	1 / 3 (33.3)	2 / 7 (28.6)
Mustela vison (Mink)	-	-	0 / 1	0 / 2	0 / 3
Vulpes vulpes (red Fox)	-	1 / 3 (33.3)	0 / 1	0 / 3	1 / 7 (14.3)
Martes martes (Pine marten)	-	1 / 1 (100)	0 / 1	-	1 / 2 (50)
Talpa europaea (Mole)	-	-	-	1 / 3 (33.3)	1 / 3 (33.3)
Mus sp. (Mice)	-	0 / 1	1 / 1 (100)	1 / 2 (50)	
Rattus norvegicus (Rat)	-	-	1 / 2 (50)	-	1 / 2 (50)
Mustela furo (Ferret)	--	-	0 / 1	-	0 / 1 (0)
Sorex sp. (Shrews)	-	-	-	0 / 2	0 / 2
Total	4 / 8 (50)	6 / 15 (40)	8 / 35 (22.9)	9 / 36 (25)	27 / 94 (28.7)

Table 7: Prevalence of *E. crescens* in Norwegian wildlife 1959. (Source: Jellison and Vinson, 1961

Hosts	Number examined	Number infected
Mus musculus (House mouse)	239	0
Apodemus spp, (Wood mice)	102	0
Sus spp. (Domestic pig)	60	0
Sorus sp. (Shrew)	40	0
Rattus norvegicus (Rat)	27	0

Hosts	Number examined	Number infected
Clethrionomys sp. (Red-backed mouse)	22	0
Mustela vison (Mink)	22	0
Vulpes sp. (Fox)	14	0
Microtus sp. (Vole)	10	4
Felis catus (Domestic cat)	9	0
Lepus sp. (Rabbit)	6	0
Sciurus sp. (Squirrel)	4	0
Mustela sp. (Weasel)	4	0
Arvicola terrestris (Water vole)	1	1
Lemmus sp. (Lemming)	1	0
Meles meles (Badger)	1	0
Total	**562**	**5**

Borman *et al.,* (2009) reported that *E. crescens* infection burdens in most animals were low (≤2 adiaspores /cm^3 of lung tissue) and unlikely to have impaired physical health; however, several animals (three otters, one weasel (*Mustela nivalis*) and one mole) had higher infection burdens (range 3-8 adiaspores/cm^3 of lung tissue) with significant areas of lung parenchyma in the weasel infiltrated by granulomata likely to have caused severe respiratory disease. It has been proposed that some species, for example otters and wombats (*Vombatidae* spp.), may be more susceptible to disease following infection than others (Danesi *et al.,* 2020). It is also suggested that immunocompromised animals may be more susceptible to disease: a previously healthy water vole died in captivity one month after capture with widespread adiaspiromycosis and was found on post-mortem examination to be severely emaciated and co-infected with another, unidentified fungus (Chantrey *et al.,* 2006). Large scale die-offs of moles co-infected with *Emmonsia* spp. and other parasites are also reported (Simpson *et al.,* 2016).

Infections with *E. crescens* have been rarely reported in free-living beavers. Mörner *et al.,* (1999) observed macroscopically visual lung lesions, consistent with adiaspiromycosis, with thick-walled adiaspores ranging between 100µm and 200µm noted on histopathology in both the lungs and mediastinal lymph nodes in a beaver shot in 1998 in northern Sweden which was in normal body condition with no signs of clinical disease. However, Mörner *et al.,* (1999) noted that no signs of infection had been noted in 110 previously culled beavers in Sweden. Eight percent of beavers (n=25) culled in Poland were found to

be infected with *Emmonsia* spp. on histopathology and thick-walled adiaspores ranging between 163.4µm and 437.1µm (Dolka *et al.,* 2017). One beaver had severe lesions with extensive granulomata, interstitial inflammation and emphysema, and was in poor physical condition (*ibid*.). In both studies, the causative agent was assumed to be *E. crescens* based on the size and morphology of adiaspores.

As *Emmonsia crescens* is likely to be an ubiquitous organism in the environment, and translocation is a known stressor which may reduce immunocompetence (Dickens, Delehanty, and Michael Romero 2010), *E. crescens* should be considered a carrier hazard for the translocation of beavers.

Risk Assessment

Release Assessment

Infections with *E. crescens* occur when saphrophytic conidia are inadvertently inhaled from the environment, such as soil or nesting materials (Borman *et al.,* 2018). The conidia do not replicate in the lungs, instead enlarging in size to form microscopically visible, dormant adiaspores (Borman *et al.,* 2018). In immunocompetent hosts, granulomata form around the adiaspores and may compress small airways, leading to asymptomatic infection or respiratory disease (Borman *et al.,* 2018) although granulomata without adiaspores may also be observed (Harrington *et al.,* 2012). The severity of disease is believed to be related to the number of spores inhaled (Dolka *et al.,* 2017). Heavy infections, typically in animals that burrow where exposure risk may be higher, are associated with poor body condition, emaciation and occasional mortality (Borman *et al.,* 2009). The lifecycle of the parasite is completed when the host animal dies and spores are released to the environment as the carcase decays where they sporulate on mycelia in decaying plant material (Simpson *et al.,* 2016).

As *E. crescens* is widely present in Europe and Great Britain, there is a medium likelihood that beavers at the source site(s) could be exposed to *E. crescens* in the soil, on bark or in lodges, or from decaying carcases of sympatric infected animals such as otters and muskrats which are reported to frequently share lodges with beavers, particularly in winter (Janiszewski, Hanzal, and Misiukiewicz 2014). In addition, viable adiaspores have been isolated from the digestive tracts of rodents and carnivores that prey on small mammals suggesting that, even if the host is predated, adiaspores may still be returned to the environment and infection reservoir (Borman *et al.,* 2018). If exposed to *E. crescens* conidia, there is a low likelihood that beavers could be infected by inhaling conidia as beavers are known to be susceptible to infection. There is a low likelihood that at least one translocated beaver could be infected with *E. crescens* at the source site(s).

Exposure Assessment

Beavers translocated to the destination may already be infected with *E. crescens*. As *E. crescens* does not replicate in mammalian hosts, the environmental burden of infective *E. crescens* conidia will not be increased as a result of live, infected beavers arriving at the destination. Spores may be released from beavers which die and decompose. As a wide range of mammalian species are susceptible to infection, there is a low likelihood that other beavers and sympatric species will be infected

There is a very low likelihood that spores released from translocated beavers which subsequently die will increase the environmental burden of infective conidia and disseminate infection amongst sympatric mammals including beavers.

Consequence Assessment

There is a low likelihood that one beaver will be infected with *E crescens.*

Infection with *E. crescens* in most mammals is asymptomatic unless the host is immunocompromised, for example by stress, starvation, hunger or concomitant disease (Chantrey *et al.,* 2006; Simpson *et al.,* 2016). Adiaspiromycosis is characterised by compromised respiratory function, loss of body condition and increased susceptibility to secondary infection. Diseased hosts may also be more susceptible to predation if they are weak and slow-moving. There is a low likelihood that at least one beaver will be infected and since translocated beavers will be under stress (Dickens, Delehanty, and Michael Romero 2010) there is a high likelihood that infected beavers will be susceptible to adiaspiromycosis. As disease progression may be slow, clinical disease may not be apparent until weeks or months following translocation.

There is a medium likelihood of economic and biological consequences through failure of the translocation, but the long-term environmental consequences are likely to be negligible.

Risk Estimation

There is a medium likelihood that beavers will be exposed to, and a low likelihood that they will be infected with, *E. crescens*. The likelihood of exposure and infection at the destination site(s) is low and there is a very low likelihood of dissemination. There is a high probability that the stress associated with translocation may precipitate disease in infected beavers. The overall risk is MEDIUM.

Risk Management

Risk Evaluation

Management measures should be implemented to reduce the risk from *E. crescens* to the beaver translocation.

Risk options

The gold standard of diagnosis is histopathological examination of biopsy or necropsy tissues with confirmatory PCR, with no reliable method of testing for infection in the live animal (Borman *et al.,* 2018). Adiaspiromycosis should be considered in the differential diagnosis if sick beavers are found and examined post-translocation. Post-mortem examination of beavers dying following translocation, and of sympatric mammals at the destination, is essential to monitor the effects of the translocation on beaver health.

Given the nature of a carrier hazard, measures to reduce the risk of adiaspiromycosis will focus on stress reduction in the translocated population. For example, efforts should be made to minimise the stress associated with capture, transport and, in particular, to reduce repeated handling, loading and unloading events, and the duration of transit.

5.4.24 Disease risk analysis for the population hazard captivity

Population Hazard

Justification for Hazard Status

It is inevitable that, during the process of translocation of beavers from the source to the destination, a period of captivity will be necessary, as is true for nearly all translocations. Initially, beavers must be trapped, then transported and, depending on quarantine recommendations, held in captivity for a period. There are numerous reports of disease in captive beavers some of which, evidence shows, have resulted from inappropriate husbandry measures and other stressors, and several of these cases have occurred as a result of beaver translocations. Here we consider these cases collectively as a hazard described as ‘captivity during translocation’. We have used some evidence from reports in Canadian beavers because the behaviour of this species and Eurasian beavers in captivity has similarities.

Between 1994 and 1999, 277 Canadian beavers were captured using Hancock traps and snares throughout Wyoming, USA, for the purpose of translocation. Fifteen Canadian beavers (5.4%) died during trapping and 13 (4.7%) died during transport to the release site

(McKinstry and Anderson 2002). Trapping mortality resulted from either predation whilst trapped or entanglement in snares. Diagnoses in those animals which died during transport were unclear. One further case of mortality while trapping using a Hancock trap has been reported in New York, USA (Rosell and Kvinlaug 1998).

Several authors have postulated that a period of time in captivity may reduce the fitness of translocated beavers post-release. For example, 34 beavers in the Wyoming translocation (McKinstry and Anderson 2002), of 114 fitted with radio transmitters, died within 180 days of release as a result of predation. Although beavers in England may not face the scale of predators that Canadian beavers contend with in Wyoming (black bears, coyotes and grizzly bears all contributed to mortality), it is possible that reduced fitness of the beavers as a result of transport resulted in increased predation risk. Translocated beavers may also be more vulnerable to persecution and road traffic collisions: these hazards were responsible for the deaths of 5% (n=14) of released Canadian beavers, a threat too for beavers released into England (McKinstry and Anderson 2002) and evaluated as hazards below.

During trapping and health screening of free-living Eurasian beavers on the river Tay in Scotland, no trap-related mortality was observed in the 17 animals caught. However mild trap-related morbidity was detected in an unspecified number of beavers (Campbell-Palmer *et al.,* 2015). Unusual incisor wear was noted in one individual, thought to have occurred as a result of the beaver biting the metal trap in an attempt to escape. The tooth root was not exposed, and the injury was not believed to be causing pain or feeding problems. Minor abrasions to the oral cavity, nose and forepaws were also noted in some (number not specified) of the 17 beavers, also likely to have resulted from escape attempts from the traps. Elevated creatine kinase levels, a sign of muscular disease, were present in six individuals, and were hypothesised to be to be due to increased activity levels from attempting to escape from the traps (Campbell-Palmer *et al.,* 2015).

Throughout the reintroduction of Eurasian beavers from Norway into Knapdale, Scotland, 20% (n=6) of beavers died during the statutory six-month quarantine period in captivity, despite being housed in purpose-built facilities. Severe parasitism and/or infection contributed to the death of four individuals, and no diagnosis was made with the other two beavers. Goodman *et al.,* (2012) considered that stress-related immunocompromise contributed to the deaths. In general, the beavers' health was compromised during the quarantine period, with most animals losing body weight and "body condition" (Jones and Campbell-Palmer 2014a). Two further animals died following release, one of which was an adult male in poor body condition (Goodman *et al.,* 2012). It was suggested that this male most likely did not feed due to a failure to cope with the stress of the translocation and environmental changes (Harrington, Feber, and MacDonald 2010b).

A reintroduction programme of Eurasian beavers into Hungary was undertaken between 1996 and 2008. Two beavers died during the period of transport and captivity but no diagnosis was made. Moreover, one further beaver was found dead within one month of

release, but a post-mortem examination was not undertaken due to autolysis. Another individual died as a result of intraspecific aggression at the release site (Bajomi 2011).

Captivity-related morbidity was reported when undertaking the Knapdale beaver reintroduction. Similarly, to the case reported by Campbell-Palmer *et al.,* (2015), abnormal tooth wear resulted in postponement of release in a male beaver. Pulp exposure of the upper right incisor and inflammation of the upper lip was reported (Goodman *et al.,* 2012), which may have resulted from escape attempts as reported in Tayside. Other cases of dental disease in captive beavers are reported in the literature. Inadequate wear due to inappropriate feeding resulted in malocclusion of the incisors in a three-year old captive Canadian beaver in Cheong-ju Zoo, South Korea (Kim *et al.,* 2005a; Kim *et al.,* 2005b). A four-year old captive Canadian beaver from National Zoological Gardens of Pretoria, South Africa presented with an infected lesion to the right upper lip resulting from overgrowth of the mandibular incisor. This tooth had elongated due to loss of the upper incisor. Since the beaver arrived at the zoo in this condition, it was unknown how the upper incisor was lost (Steenkamp *et al.,* 2009). In these cases in South Korea and South Africa, the beavers were held in captivity for longer periods of time than would be expected to be necessary during a translocation and the implications for translocations should therefore be drawn carefully.

Trauma from aggressive interactions between beavers, and self-harm by individual beavers, have been reported. Five Eurasian beavers became trapped within a lodge as a result of extreme frosts in Mongolia (Saveljev *et al.,* 2016). It is unclear how long the animals were trapped for but, on release by local residents, the authors concluded that all five beavers had evidence of tail trauma consistent with self-cannibalism. It was hypothesised that this trauma had resulted from the severe stress of the captive environment (Saveljev *et al.,* 2016). A Eurasian beaver kit held in a captive collection in England was found in its enclosure with multiple wounds caused by intraspecific aggression (O'Brien *et al.,*2018). Treatment of this case took several months and complications arose, including abscessation of some wounds and proprioceptive deficits, although the beaver did recover (O'Brien, Meldrum, and Foster 2018). Although this case occurred in a captive collection in which the beavers had been in captivity longer than would be expected to occur during a translocation, it is not possible to rule out this aggression occurring under conditions of stress associated with translocation. Intraspecific aggression has been reported in the wild for beavers (Stefen 2018) and a recent study by Mayer and colleagues demonstrated an inverse density-dependent territorial behaviour pattern in Eurasian beavers; at lower population densities, intraspecific aggression appeared to increase (Mayer *et al.,* 2020). Resource competition in excessively large groups has also been noted to lead to aggression in free-living Eurasian beavers (Kitchener 2001).

It is possible that, in a captive setting, aggression is heightened due to stress and inappropriate husbandry conditions such as lack of space. Post-mortem examination reports from beavers in Scotland provide evidence of aggression in captivity. An adult

female held in a captive collection in Scotland was found on post-mortem examination to have died from blunt trauma. This individual was housed with a male, and it is possible that the death was a result of intraspecific aggression (Brownlow 2011). Moreover, as previously mentioned, one beaver died as a result of intraspecific aggression after reintroduction into Hungary: two animals were released together and one inflicted lethal injuries upon the other. This aggression was thought to have resulted from the stress of translocation and release into a new environment (Bajomi 2011).

Other cases of wounds are reported for captive Eurasian beavers, most likely as a result of inappropriate housing facilities. Injuries and abrasions to the tails and plantar surfaces of feet were found on post-mortem examination of five beavers which died whilst in quarantine as part of the Knapdale reintroduction (Cranwell 2009a, 2009b, Collins 2009, Howie 2009, Deuchande 2009). In one of these cases, a severe tail wound progressed to osteomyelitis of the caudal vertebrae. However, it is unclear whether the original wound was caused by intraspecific aggression (Collins, 2009). Given the nature of the abrasions, lesions in these beavers are likely to have occurred due to unnatural substrate in captive enclosures, such as concrete. Inappropriate use of 'hot wire' fencing has resulted in mortalities of several beavers. The animals bit down on the wire, and their front teeth became locked behind it, trapping them (Campbell-Palmer *et al.,* 2015). This highlights the importance of appropriate husbandry conditions for maximising reintroduction success.

The evidence outlined above indicates that captivity during translocation can result in diseases associated with trapping, stressors and immunosuppression, intraspecific aggression, and housing facilities and, therefore, captivity during translocation is considered a population hazard.

Risk Assessment

Exposure Assessment

Beavers will be required to undergo a period of time in captivity as part of the reintroduction programme, including trapping and transport. Therefore, there will be multiple opportunities for morbidity and mortality to occur as a result, either through wounds and abrasions resulting from inappropriate husbandry measures, stress related immunocompromise, or trauma as a result of aggression. There is a medium likelihood that translocated beavers will be exposed to this hazard, given the numerous previous reports of diseases associated with captivity described above. Beavers originating from a free-living environment may be more prone to stress-related diseases during translocation due to having no previous experience in a captive setting.

Consequence Assessment

The probability of one translocated beaver suffering from morbidity or mortality as a result of captivity during translocation is medium.

The probability of beavers suffering from wounds caused by intraspecific aggression or self-trauma due to stress is medium. The likelihood of severe disease and death from wounds is high as even minor wounds and abrasions can lead to severe consequences in beavers in captivity as a result of infection (Campbell-Palmer and Rosell, 2015). The probability of beavers suffering from injury as a result of inappropriate enclosure conditions, for example inappropriate fencing or substrate, is medium. The probability of dental disease occurring during the period of time held in captivity is predicted to be low as these diseases are likely to take several months to arise. The likelihood of severe dental disease is low. The probability of disease and death occurring as a result of stress-related immunosuppression in captivity is high.

The probability of negative economic consequences occurring due to captivity during translocation is low and there is a very low likelihood of failure of the reintroduction programme due to this hazard. Several other reintroduction programmes of Eurasian beavers have been successful despite numerous deaths occurring in captivity. The probability of environmental or ecological consequences as a result of captivity during translocation is negligible.

Risk Estimation

There is a medium likelihood of the reintroduced population of beavers being exposed to the hazards of captivity during translocation. There is a medium or high likelihood that beavers will exhibit disease (depending on the disease as indicated in the consequence assessment) as a result of captivity and a medium likelihood of severe consequences, such as death, in the case of captivity-associated morbidity occurring. There is a low probability of economic and biological impacts of a failed reintroduction. The overall risk is estimated to be MEDIUM.

Risk Management

Risk Evaluation

It is necessary to implement mitigation measures to reduce the risk from the hazard of captivity.

Risk options

Duration in captivity should be minimised to reduce the propensity to develop stressor-associated disease, dental disease, housing-related injury and aggression-associated injury. Stress reduction should be maximised through appropriate husbandry measures such as good hygiene, appropriate nutrition, appropriate stocking densities and good enclosure design. Naturalistic substrates should be used wherever possible to reduce the risk of abrasion injuries.

5.4.25 Disease risk analysis for the population hazard persecution

Population Hazard

Justification for Hazard Status

The Eurasian beaver is persecuted throughout its range, including through snaring, shooting, hunting and malicious poisoning, and particularly when perceived negatively by local communities. Beavers are important keystone species which undertake landscape modification which benefits numerous other species within the ecosystem (Janiszewski, Hanzal, and Misiukiewicz 2014). Nevertheless, the impacts beavers have to local hydrology and fish stocks as a result of this landscape modification have been perceived negatively by local landowners and angling interests in the past, for example when the Scottish beaver reintroduction trial was proposed (Scottish National Heritage 1998; Halley and Rosell 2002). Although there is substantial evidence confirming the positive effects of beaver populations to ecosystem health and other species populations, there have been conflicting reports on their impacts on fisheries. It was concluded by Scottish National Heritage, in response to concerns voiced about beaver reintroduction, that there may eventually be some areas of conflict between beavers and fishery interests depending upon the management of the beavers (Scottish National Heritage 1998). Reduced fish stocks downstream of beaver dams have been reported in countries such as Lithuania (Kesminas *et al.,* 2013; Virbickas *et al.,* 2015), although a meta-analysis undertaken by Kemp *et al.,* (2012) reported that the majority of experts found beaver populations to have an overall positive impact on fish populations in European and North American fisheries. North American beavers are a different species but their ecological impact is likely to be comparable to the Eurasian beaver's. It is likely that the impact of beavers is highly dependent on specific environmental components and management, and therefore the possibility of negative impacts to local communities after the reintroduction cannot be ruled out.

Pathological findings on free-living beavers following reintroduction into Tayside, Scotland found four animals examined suffered gunshot wounds (Campbell-Palmer *et al.,* 2015b) which clearly indicates that this population was persecuted. It is unclear whether (Campbell-Palmer *et al.,* 2015b undertook toxicological testing to detect malicious poisoning (or poisoning through misuse) and, therefore, it is possible that further persecution has gone unreported. A survey-based study in Eastern Poland found beavers to be one of four species most frequently blamed for reducing yield at commercial fisheries. 21.2% of fish farms (n=29) reported serious, intolerable losses to fish stocks, and a further 46% (n=63) reported tolerable losses. Moreover, despite their protected status in Poland, and notwithstanding the provision of government compensation for losses attributed to beavers, persecution and culling of beavers still occurred (Kloskowski 2011).

Furthermore, in a study by Stefen (2019), 1137 records of beavers found dead in Germany between 1941 and 2009 were analysed. Each case was attributed a probable cause of death and, overall, 41.5% (n=472) of deaths were directly related to anthropogenic impacts. Suspected intoxication accounted for 1.8% (n=21) of deaths, metal traps 0.9% (n=10) and shooting 3.5% (n=40), indicating the potential for population losses to Eurasian beavers as a result of persecution. Other authors have reported beaver shootings across Europe including a further two cases in Germany (MacDonald *et al.,* 1995). Licenses to undertake lethal control were granted after the Tayside beaver population grew to an unmanageable level. However beaver shootings have occurred 'outside of licences' in Scotland/Wales (Roisin Campbell-Palmer, pers. comm, 7th May 2020). Some traps likely to be targeting beavers have also been noted in Great Britain but the target species cannot be proven (Roisin Campbell-Palmer, pers. comm, 7th May 2020).

Historically, Eurasian beavers have been hunted for meat, their coats and castoreum, a urine-based secretion used for scent marking which was considered to have medicinal properties. As well as hunting for consumption that continues across some parts of Europe, persecution for castoreum is thought to still occur in parts of their range, including Mongolia (Batbold *et al.,* 2017). Nonetheless, there is little likelihood of a fur/castoreum market being re-established within the UK and therefore hunting pressures are not likely to affect these populations in the same manner as conflict-related persecution (Scottish National Heritage 1998).

Risk Assessment

Exposure Assessment

There are several opportunities for human-wildlife conflict to occur as a result of the beaver reintroduction. Fisheries, angling and farming interests are widespread in England, and therefore there is a medium likelihood of exposure to persecution through shooting and poisoning. Between April 2018 and March 2019, 955,310 fishing licences were granted in the UK (Environmental Agency 2020). Between 2017 and 2018, 1,191,142 fishing licences were granted across England; highest numbers were in Yorkshire (120,961), Staffordshire, Warwickshire and West Midlands (109,798) and Kent and South London (106,741), showing that angling interests are extensive across England (Environment Agency 2019).

Attitudes towards beavers are mixed across Europe. A telephone survey of pond fisheries in Eastern Poland found a generally negative attitude towards beavers as a 'nuisance species' (Kloskowski 2011). Before the Knapdale beaver reintroduction was undertaken, attitudes towards the scheme from local residents were largely positive with 46% (n=680) of Argyll and Bute residents agreeing that a trial reintroduction of beavers should be undertaken and 21% (n=310) disagreeing. The remainder were indifferent.

Any potential conflict between wildlife and humans may result in persecution and, therefore, we estimate a medium likelihood of sporadic cases of illegal persecution occurring amongst the reintroduced Eurasian beaver population, particularly given the perceived negative impacts of landscape modification on fisheries and farmland. There is a negligible probability that reintroduced beavers will be hunted for their fur or castoreum.

Consequence Assessment

The probability of one beaver being persecuted is high. The consequence could range from severe injury to death. Judging by the infrequent shootings which occurred to beavers during reintroduction in Scotland, cases of persecution are likely to be sporadic. However, the small population size of reintroduced beavers may be significantly affected by even low numbers of persecutions. Notwithstanding, within the Tayside population, the sporadic shootings did not lead to significant population effects. Therefore, there is a low likelihood of a negative impact on the population of reintroduced beavers and a failure of the reintroduction with resultant biological and economic consequences. There is a negligible impact of environmental consequences.

Risk Estimation

There is a medium likelihood of the reintroduced population of beavers being exposed to persecution. There is a high likelihood of severe consequences, such as death, from the persecution of one individual. There is a low probability of economic and biological impacts of a failed reintroduction. The overall risk is estimated to be MEDIUM.

Risk Management

Risk Evaluation

Preventative measures must be employed to reduce the risk and consequences of illegal persecution to reintroduced Eurasian beavers.

Risk options

It is imperative to educate local communities about the reintroduction programme and the benefits of reintroducing Eurasian beavers to the local area.

Eurasian beavers should be closely monitored and detailed pathological examinations performed on any carcases found using pre-determined protocols. Testing should include toxicology to identify cases of accidental/non-targeted/malicious poisoning so that, if necessary, mitigation measures can be implemented.

It would be an advantage to give the Eurasian beaver population protected species status in England, as has been granted by the Scottish government to those beavers

reintroduced into Scotland. Furthermore, licences to alter beaver habitats which result in negative impacts to adjacent agricultural land could be authorised to certain individuals to try to minimise conflict as far as possible. Dam removal or modification has been suggested to mimic natural dam failures which have no significant impact on populations and rarely cause problems to beavers (Jones *et al.,* 2012).

5.4.26 Disease risk analysis for the population hazard road traffic collisions (RTCs)

Population Hazard

Justification for Hazard Status

Road traffic collisions (RTCs) have been reported as a cause of death of beavers across Europe. Stefen (2019) analysed 1137 post-mortem reports of Eurasian beavers in Eastern Germany dating from 1941 to 2009 and found RTCs to account for the highest number of deaths (25.7%, n=292). Train collisions also caused 1.3% (n=15) of deaths. Other authors have similarly reported that RTCs are responsible for as many as 50-86.5% of beaver deaths in Germany (Pokorny *et al.,* 2014., Muller 2014 cited by Grubešić *et al.,* (2015).

RTCs have been suggested to be the main cause of beaver mortalities in Croatia (Sager *et al.,* 2005). Another study carried out across Croatia and Serbia found that 33% (n=50) of beaver carcases analysed had been involved in traffic accidents (Grubešić *et al.,* 2015). Furthermore, a report from France suggests that, of 46 beavers found dead in the Haute-Savoie region, 37% (n=17) died as a result of RTCs (Estève 1988). After translocation to the Netherlands, four beavers were killed by traffic in the Biesbosch (Nolet *et al.,* 1997). Along the Elbe, three beavers were found to have been killed in RTCs in a study by Hinze (1950), and a further 10 in a study by Piechocki (1977). Two free-living beaver carcasses submitted and analysed after the Tayside beaver reintroduction in Scotland had injuries consistent with RTCs (Campbell-Palmer *et al.,* 2015b), as did a beaver carcase submitted to a veterinary practice near Honiton, Devon (Brazier *et al.,* 2020, p97). Post-mortem examinations revealed RTCs to be the cause of death in five out of six beavers found dead in the north-western suburbs of Berlin, Germany between 2006 and 2011 (Herrmann *et al.,* 2013).

In recent disease surveillance of free-living beavers found dead in England three out of four post-mortem examinations concluded the likely cause of death to be RTCs given the beavers' extensive injuries and history of being found by the side of the road (Common, Gerard, and Sainsbury 2022).

Given the considerable evidence of beavers being involved in RTCs, including those free-living in England, RTCs should be considered a population hazard for the translocation of beavers to England.

Risk Assessment

Exposure Assessment

Many factors are likely to contribute to the exposure of beavers to traffic and therefore RTCs. Studies have indicated that elements such as traffic volume and roadside vegetation cover are associated with higher roadside mortality, and mammals are more frequently affected by RTCs than birds or reptiles (Taylor and Goldingay 2010). Moreover, road width has appeared as a broadly important predictor of mammalian road mortality (Barthelmess 2014) as has landscape interconnectivity (Grilo *et al.,* 2011).

It is possible that beaver dispersal at the release site will be high, leading to an increased probability of exposure to roads and thus RTCs. Following reintroduction into the Loire, France, post-release monitoring over a ten-year period identified 13 beavers reproducing in an area 200km upstream of the release site demonstrating that substantial movement is possible in this species. Nevertheless, beavers at release sites surrounded by urban areas did not undergo the same range expansion (MacDonald *et al.,* 1995). Once settled, beavers are also thought to travel up to 1.5km into adjacent territories (Campbell *et al.,* 2005).

Traffic densities at the release site are likely to impact upon incidence of beaver RTCs. In the areas surrounding the river Tay (Perth and Kinross, plus Angus), where two beavers were found dead due to RTCs, the road and traffic density is relatively low. In Perth and Kinross, there are 124 major roads and 12 minor roads, with 1604.4 million vehicles travelling on these roads in 2018. In Angus, there are 59 major roads, 9 minor roads and 715 million vehicles travelled in 2018. In Devon, where one free-living beaver mortality was reported as a result of a RTC, road and traffic density are higher despite Devon being comparable in size to Perth and Kinross/Angus. There are 246 major roads in Devon, 200 minor roads and 5441.8 million vehicles travelled in 2018 (“Road Traffic Statistics - Department for Transport” 2018). These findings suggest that free-living beavers are at risk from RTCs in many areas of Great Britain with road numbers and traffic densities in a similar range in their release area. Notwithstanding, it has been suggested that the natural behaviours of beavers make them less likely to cross roads than other mammals (Jones *et al.,* 2012) and so lower number of roads would therefore probably reduce their exposure to RTCs.

Minor roads have been suggested to have a greater impact on mortality than major roads in some mammalian species such as badgers (which display territorial behaviour patterns similar to that of beavers), particularly if there is a high number of these roads (Taylor and Goldingay 2010; van Langevelde, van Dooremalen, and Jaarsma 2009). Therefore, the traffic densities and road size at the release site of beavers is likely to impact survival, even if the roads are small and traffic density low.

We estimate a medium likelihood that reintroduced beavers will be exposed to a vehicle collision at the release site.

Consequence Assessment

There is a high likelihood that at least one reintroduced beaver will be hit by a vehicle. We found no reports of beavers surviving RTCs and therefore conclude that there is a high likelihood that death of the beaver will result. Economic impacts of a failed reintroduction would be considerable. However, given the general success of the Scottish reintroductions despite two RTCs occurring, as well as numerous other successful reintroduction programmes across Europe in which RTCs have occurred, there is a low probability that sporadic RTCs will lead to sufficient population losses to lead to reintroduction failure.

Risk Estimation

We estimate a high likelihood that at least one beaver will be hit by a vehicle at release sites. The likelihood of death if a beaver is involved in an RTC is high. However, there is a low probability that sporadic RTCs will lead to ecological and economic consequences through failure of the reintroduction programme. The overall risk is estimated to be HIGH.

Risk Management

Risk Evaluation

Mitigation methods should be employed to reduce the risk of reintroduced beavers being involved in RTCs.

Risk Options

Traffic density, road size and road interconnectivity should be considered before choosing the release site and, ideally, areas with low traffic density and smaller numbers of roads should be chosen to reduce the risk from RTCs. Warning signs and fencing could be placed along stretches of road which are considered to be at risk from beaver RTCs to encourage careful driving (Jones *et al.,* 2012). Moreover, post-release health surveillance and disease surveillance could help to identify problem roads where management efforts, such as signage and the development of beaver-safe passages under roads, should be directed.

6. Discussion

In this disease risk analysis for the conservation translocation of (i) free-living Eurasian beavers from Norway or Great Britain, or (ii) beavers held in captivity, in fenced enclosures or captive collections, in Great Britain, to England, we have described the translocation pathway; assessed geographical and ecological barriers to the spread of parasites;

identified, reviewed and evaluated 96 (89 infectious and seven non-infectious) potential hazards; and carried out a full disease risk analysis on 26 selected hazards. In doing so, 22 hazards from the DRA already published (Donald, Common, and Sainsbury 2021), have been reassessed and four new hazards analysed.

All three possible translocation pathways (from Norway, Great Britain, or captive collections/ fenced enclosures in Great Britain) were found to be crossing geographical and/or ecological barriers and, consequently, an in-depth and detailed disease risk analysis was required which included source and destination hazards in addition to carrier and population hazards. No transport hazards have been identified to date but when the specific translocation route has been determined these hazards can be reviewed.

Of the 26 hazards assessed in detail, four were evaluated as being of high risk of precipitating disease in beavers or sympatric mammals, including people (road traffic collisions; *Echinococcus multilocularis*; *Leptospira* spp.; *Yersinia* spp.) and a further 13 were evaluated as medium risk (*Toxoplasma gondii* (as both a carrier and population hazard); *Taenia martis*; Persecution; Captivity; *Eimeria* spp.; *Streptococcus castoreus; Neostichorchis subtriquetrus; Emmonsia crescens; Trichinella* spp.; gram-negative bacteria*; Brucella* spp.; and hantaviruses (Puumala-virus (PUUV) and Saaremaa-virus (SAAV)). Of the 17 medium and high-risk hazards, eight are triggered by stressors and later in this discussion we set out how to minimise the effects of these stressor-related hazards as a group. In detailed review of the literature for this revised disease risk analysis we uncovered evidence that three further infectious hazards (*Baylisascaris procyonis*; *Capillaria hepatica*; *Travassosius rufus*) and one non-infectious hazard (drowning) require further scrutiny through a full DRA (see Appendix 1), and information from a recently started disease surveillance project assisted with the evaluation of this last hazard.

One non-infectious hazard was assessed as high risk (road traffic collisions), and the other two assessed as medium risk (captivity and persecution). These population hazards represent a threat to small populations of beavers either during or post-translocation. There is reliable evidence of beaver persecution in free-living populations, including in Scotland, and local community involvement in translocation projects would be beneficial to combat this hazard. Consideration of the release location, including roads and traffic density in the vicinity of release sites, will assist in the mitigation of road traffic collisions.

The origin of some beavers in captivity, including fenced enclosures, has been obscure and they may have been in contact with exotic species, and therefore contracted non-native parasites (see section 6.9). For infectious hazards, the assessments and subsequent risk estimations have been updated based on evidence of the potential for cross-transmission of parasites between species in captive collections given that ecological and environmental barriers are crossed in these artificial situations. New hazards were also assessed based on these principles. If captive beavers which have been in contact with exotic species, and therefore potentially contracted exotic parasites,

are released, they may be a source of exotic parasites to free-living populations of rodents, including beavers, in England. These parasites may be novel to the native rodent populations in England and novel parasites are known to have the potential to cause catastrophic epidemic disease in free-living wildlife populations (Sainsbury *et al.,* 2012; Rideout *et al.,* 2016). This disease risk analysis should be continually updated as new information becomes available, the literature scrutinised, and immediate efforts made to use retrospective sample archives for parasite microarray and multi-organ parasite screens.

6.1 Zoonotic source hazards

The disease risk analysis identified one zoonotic source hazard of high risk (*Echinococcus multilocularis*), two of medium risk (*Trichinella* spp. and *Brucella* spp.). One further hazard, *Taenia martis*, was found to be of either medium or high risk depending on where the translocated beavers originate and one, hantaviruses (specifically PUUV and SAAV) was found to be of medium risk for humans and low for rodents at the destination site.

Echinococcus multilocularis was analysed as of high risk of disease to people and we consider it a high priority, in undertaking beaver translocations, to maintain the UK's infection-free status from this cestode because of the severe biological and economic consequences which would result from its incursion. There remains a possibility that un-licensed imports of beavers in the past have already introduced this parasite to Great Britain and for this reason we recommend that, should this population be used for translocations to England, robust and comprehensive disease surveillance is used to monitor the population post-release; so far, disease surveillance has not revealed any evidence of *E. multilocularis*, although further research into possible exposure of these beavers is ongoing (Common, Gerard, and Sainsbury 2022). Given (i) the further spread of *Echinococcus multilocularis* through Scandinavia since Roberts (2012) carried out their disease risk analysis for the importation of this parasite to the UK with beavers, and (ii) the understanding that *Echinococcus multilocularis* could have evaded detection in foxes in Norway due to sampling statistics, we estimated that the risk of *Echinococcus multilocularis* incursion is greater from the translocation of free-living Norwegian beavers than those from Great Britain. The risk from *Echinococcus multilocularis* is greater still if beavers in captivity (in enclosures) in Great Britain are utilised for translocations. A large proportion of these beavers originate from areas in which *Echinococcus multilocularis* is endemic and it is possible that they harbour the parasite. The use of second-generation beavers for translocations reduces the risk markedly because the lifecycle of this parasite means it cannot be transferred from parent to offspring. Therefore, further reduction in risk can be achieved by prioritising free-living beavers for translocation proven to have been born in Great Britain.

Trichinella spp. and the newly assessed source hazard *Taenia martis* were estimated to be a medium risk for this translocation, and high risk if captive beavers were chosen for reintroduction. Maintaining the UK's infection-free status for these nematode parasites is,

as for *Echinococcus multilocularis,* important given the severity of the disease in people and the high economic costs of disease prevention should particularly *Trichinella* spp. become endemic in the UK. The risk from disease is reduced if a choice is made to translocate free-living beavers from Great Britain rather than from Norway or those living in captivity given that these parasites are endemic across much of Europe including Poland and Germany (which represent the origin of at least one each of the enclosure beavers currently residing in Great Britain).

Brucella spp. were estimated to be of medium risk to the translocation of beavers into England. These bacteria have a worldwide distribution. However, several countries (including the UK and Norway) are considered to be free from those species responsible for causing brucellosis in humans and livestock. Eastern Europe is considered to be a high risk area (Centers for Disease Control and Prevention 2021b) and cases of brucellosis have been reported sporadically in Germany (European Centre for Disease Prevention and Control 2019a; Al Dahouk *et al.,* 2005). Therefore, if captive beavers of unknown origin or from Poland or Germany are chosen for release, there is an increased likelihood of release of zoonotic *Brucella* spp. into the UK.

Puumala orthohantavirus (PUUV) and Saaremaa virus (SAAV), both zoonotic hantaviruses, represent a medium risk source hazard for humans in the UK. PUUV is endemic in bank voles in Scandinavia (including Norway) (Vapalahti *et al.,* 2003) and Germany (including Bavaria) (Mertens *et al.,* 2011) and SAAV also circulates in North-western Europe including Germany (Olsson, Leirs, and Henttonen 2010). Given that beavers held in enclosures in Great Britain currently are known to have originated from Germany, Poland and Norway, (with several others of unknown origin), if captive (enclosures or other captive collections) beavers in Great Britain or beavers free-living in Norway are chosen for conservation translocation this is likely to pose a higher risk from disease than if beavers free-living in Great Britain are released. Pre-translocation screening using stored archive samples would be of value to improve our risk estimation alongside the ongoing disease surveillance work testing samples from beaver carcases found in Great Britain using pan-hantavirus PCR (Common, Gerard, and Sainsbury 2022; Klempa *et al.,* 2006).

Thus, our analysis shows that the risk from five high or medium risk hazards (*E. multilocularis*, *Trichinella* spp., *T. martis*, *Brucella* spp., hantaviruses (PUUV and SAAV)) inducing serious zoonotic disease in people in Great Britain is greater if captive beavers in Great Britain are chosen for conservation translocation. In addition, one low risk hazard, *Francisella tularensis*, is also of higher risk if captive beavers are utilised for translocation. If beavers from Norway are chosen three zoonotic agents are of higher risk (*E. multilocularis*, *Trichinella* spp and PUUV). Risks from all of these zoonotic agents remain, but are reduced, if free-living beavers in Great Britain are moved to England, particularly if second-generation free-living beavers are translocated.

6.2 SARS-CoV-2

The risk from SARS-CoV-2 in inducing disease in translocated beavers was considered of very low risk. This was downgraded from the 2020 DRA, which found the risk to be medium (Donald, Common, and Sainsbury 2021), in light of new evidence published in recent months. The prevalence in humans is likely to fluctuate as control of the pandemic continues, and the distribution of the virus changes temporally and spatially. Disease risk assessment for SARS-CoV-2, and risk management options, may need to be updated if beaver reintroduction is chosen as a course of action.

6.3 Stressor-associated disease and translocation of beavers

In our disease risk analyses, eight of the high and medium risk hazards were precipitated by stressors. Translocation has been shown through detailed research to be a substantial stressor for all animal species (Dickens, Delehanty, and Michael Romero 2010) and therefore detailed planning of disease risk management for beaver translocation is imperative. The risk from disease precipitated by some carrier hazards, for example *Toxoplasma gondii*, is predicted to be greater if beavers from captive collections are chosen for translocation.

Stressors such as translocation may reduce immunocompetence and consequently immunocompromised individuals will be more susceptible to disease if infected, including with commensal organisms that do not ordinarily cause disease in healthy individuals. We have identified nine stressor-related hazards for which we anticipate a risk of disease (seven of which are high or medium risk) based on cases of previous morbidity and mortality in beavers. In previous translocations, beaver fatalities have been attributed to yersiniosis, leptospirosis and mycobacteriosis (Nolet *et al.,* 1997). In addition, enteric disease from *Neotichorchis subtriquetrus* infection (Howie 2009); adiaspiromycosis (Dolka *et al.,* 2017); gram-negative enteric bacteria (Cranwell 2009a); *Toxoplasma gondii* (Herrmann *et al.,* 2013) and *Streptococcus castoreus* (Lawson *et al.,* 2005) may have contributed to mortalities in beavers triggered by stressors.

It is widely understood and accepted that stress can lead to immunocompromise (Dhabhar and McEwen, 1997; Dickens *et al.,* 2010; Glaser and Kiecolt-Glaser, 2005). Stress has been suggested to be an inevitable component of animal translocations, which can occur at multiple stages including capture, transport and captivity (Teixeira *et al.,* 2006; Dickens, Delehanty, and Michael Romero 2010; Dickens, Delehanty, and Romero 2009). Dickens *et al.,* (2010) stated that all translocated animals will be chronically stressed to some extent when released. Further to this, several reintroduction failures, including of rodents, have been attributed to stress. For example, stress was considered to be a key factor in the failure of a reintroduction programme of Vancouver Island marmots (*Marmota vancouverensis*) in Canada, in which all six died within a year of release (Bryant, Schwantje, and de With 2002). Shen *et al.,* (2016) experimentally demonstrated that transportation stress can alter the immunity of chronically infected mice leading to the

reactivation of dormant bradyzoites and acute toxoplasmosis. This process may be similar in other rodents, including beavers. It is therefore essential that measures are taken to minimise stress to beavers at all stages of the translocation process.

6.4 Disease risk management and post-release health surveillance (DRM PRHS)

Principles of good disease risk management in translocations will reduce the risk from disease for a high proportion of the hazards we have analysed. For example, the risk of exposure to parasite hazards will be reduced through good hygiene during the translocation process. Maintaining high standards of biosecurity should be standard practice and substantial knowledge of efficient methods is available from our previous work and reported in Vaughan-Higgins, Masters and Sainsbury (2017). We have provided disease risk management recommendations to reduce the risk from disease in each disease risk analysis. Our standard practice developed over thirty years of monitoring translocations in England is to convert the disease risk analysis recommendations into a comprehensive, evidence-based, practically orientated Disease Risk Management and Post-Release Health Surveillance (DRM PRHS) protocol. If the Steering Committee decides, following a review of evidence, that translocation of beavers to England is warranted, we strongly recommend that an evidence-based DRM PRHS protocol is formulated. The DRM PRHS protocol will benefit from regular update and revision of this DRA.

6.5 DRM PRHS and minimising the effects of stress

Given the evidence that eight stressor-associated hazards are of high or medium risk to this proposed translocation, the DRM PRHS protocol will consider methods to minimise stress in detail. Some preliminary comments are made here.

Contact with humans should be reduced wherever possible and care should be taken to ensure that human scent is not present within beaver crates or enclosures, for example through wearing gloves (Campbell-Palmer and Rosell, 2010, 2013). During the process of trapping beavers, appropriate traps should be used and checked regularly in order to ensure beavers do not remain in traps for long periods of time. When contact with beavers is necessary, for example to move them from traps to transport containers, reduction of surrounding noise, movement and minimal handling times should be implemented (Campbell-Palmer and Rosell, 2015).

Appropriate stocking densities should be observed during any periods of captivity, including transport. Beavers of the same family should be trapped and housed together, and minimal trapping intervals should be present between trapping members of the same family (Campbell-Palmer and Rosell, 2013). It is also important that beavers from different families are not housed together (Campbell-Palmer and Rosell, 2013). During transportation, sufficient absorbent bedding, ventilation, food and water should be

provided. Including used bedding from an individual in transport crates may also help to reduce stress (Campbell-Palmer and Rosell, 2010).

The captive periods for free-living beavers should be kept to a minimum. Access to fresh water deep enough to allow beavers to fully submerge is essential, along with appropriate shelter, space and substrate to allow expression of normal behaviours such as digging (Campbell-Palmer and Rosell, 2010). It is also important that family groups of beavers are housed out of sight of other groups, for example through the addition of visual barriers to closely-positioned enclosures (Campbell-Palmer and Rosell, 2010).

Collection of samples, for example for parasites, should be non-invasive wherever possible to reduce the necessity of repeated handling, general anaesthetic and/or confinement. Consideration should also be given to the timing of releases, avoiding winter months when lower temperatures and food shortages may increase the risk from stressor-associated disease.

Further information on animal stress physiology and its effects can be found in Dickens *et al.,* (2010). Detailed consideration of stress mitigation should be made in the DRM PHRS protocol.

6.6 Parasite conservation and translocation of beavers

Commensal parasites which induce disease in the presence of stressors are an important component of biodiversity and, as such, efforts should be made, if possible, to conserve them at the same time as keeping disease under control. Careful use of therapeutic protocols can allow for disease prevention without parasite elimination while maintaining host immune responses, as we have shown in the conservation of the commensal parasite, *Isospora normanlevinei*, which was associated with stressor-associated disease in reintroducing cirl buntings to Cornwall (McGill *et al.,* 2010). The Eurasian beaver harbours several species-specific parasites: a nematode *Neostichorchis subtriquetrus*, the beaver beetle *Platypsyllus castoris* (see Appendix 2) and the bacterium *Streptococcus castoreus*. The latter two parasites have been detected in free-living beavers in England through disease surveillance (Common, Gerard, and Sainsbury 2022) and parasite conservation should, we argue, be an integral and important component of a DRM PRHS protocol.

6.7 Disease risk analysis method

The disease risk analysis reported here has been completed using the ZSL method described by Sainsbury and Vaughan-Higgins (2012) and deployed in 36 wild animal conservation translocations to date. This ZSL method uses the foundation of the World Organisation for Animal Health's (OIE) disease risk assessment (Murray 2004), a reasoned, logical and transparent approach which adheres to, and contributed to, IUCN guidelines in DRA. Transparency is crucial to make the qualitative judgements of release, exposure and consequence absolutely clear to stakeholders. Transparency of method

and results also ensures that, in each succeeding beaver translocation, the risks from disease can be easily and quickly reassessed ensuring lessons are learned and improvements made. In addition, the disease risk analysis can be utilised by managers of future translocations in the same or closely related species, anywhere in the world. Information from previously published, transparent, evidence-based disease risk analyses, for example Roberts (2012), has been utilised in this disease risk analysis reported here.

6.8 Unidentified and poorly understood hazards in the source populations

Geographical and/or ecological barriers are crossed in this translocation whichever source population is chosen (free-living beavers in Norway or Great Britain or beavers housed in enclosures in Great Britain). Therefore, any of these source populations may harbour non-native parasites and indeed five source hazards of high or medium risk have been identified and analysed. The risk from source hazards requires careful and thorough analysis because empirical evidence shows that the major epidemics of disease associated with translocations have primarily arisen from these hazards (Sainsbury and Vaughan-Higgins, 2012). For example, chytridiomycosis in amphibians arose as a result of transfer of the causal infectious agent, *Batrachochytrium dendrobatidis*, to novel hosts and environments, and the disease has subsequently led to extinctions of many amphibian species (Scheele *et al.,* 2019). Closer to home, squirrelpox viral disease illustrates the same threatening process in decimating populations of red squirrels in Great Britain following the introduction of the squirrelpox virus with grey squirrels in the 19th century. In both examples, the parasites were not known to science at the time the first epidemics of disease occurred. In addition, the squirrelpox epidemic was undetected for decades, and has continued for over a century since the first outbreak, which shows that immediate positive translocation results do not preclude later disease outbreaks. The parasites and diseases of the Eurasian beaver are poorly described and evaluated and it remains a realistic possibility that beaver populations in either Great Britain or Norway, or in captivity, harbour an unidentified, novel parasite capable of inducing an epidemic in naïve rodent populations in Great Britain. In undertaking this disease risk analysis, we have been vigilant to the need to detect source hazards of greatest risk to translocation and have used the criteria set out by Rideout, Sainsbury and Hudson (2016) to scrutinise the potential hazards and assess the likelihood that these parasites would give rise to an epidemic. We searched for recently identified parasites or new virulent strains of known pathogens and will continue to scrutinise the published literature, grey literature and reports before translocation proceeds.

6.9 Contact between captive beavers and non-native parasites

The captive beaver populations in England, those in fenced enclosures or in other captive populations, are, in many cases, of uncertain origin. Incomplete history and location records are available. In many cases, the management history and location of these beavers is unclear. Some may have been housed in zoological collections and therefore

have been in contact with exotic species. Others may have been in contact with beavers which had been in contact with exotic species in a captive collection. Where animals are housed in close proximity in captive collections the propensity for them to contract exotic, non-native parasites is recognised to be high (Kirkwood and Sainsbury 1997; Rideout *et al.,* 2017). If beavers which have contracted non-native parasites are released, they could be a source of novel parasites to native rodent populations and, as noted above, there is a high risk that novel parasites give rise to catastrophic epidemic disease at the destination. Given the uncertainty in how or whether beavers in fenced enclosures and other captive collections have been in contact with exotic species, these beavers represent an increased risk from disease. The level of uncertainty in their possession of exotic parasites is high but the potential negative consequences of their release, if they do possess exotic parasites, is extremely high. Therefore, we recommend that, only where the history of an individual captive beaver can be verified without doubt, the history of all beavers in contact with this individual can be verified without doubt, and reliable documents show that those beavers have never been in contact with exotic mammalian species, either directly or indirectly via fomites, can this individual beaver be released in England.

Risk management options for unknown and other novel hazards

In order to assist in identifying unknown parasites which may present a source hazard for the translocation of beavers, we recommend retrospective screening of stored beaver sample archives, from both healthy and diseased animals, using, for example, DNA microarrays which can rapidly screen samples for genetic sequences from viruses, bacteria, protozoa and fungi. Sequences are cross-referenced against a databank of known organisms to identify the closest match. Screening programmes would be ideally carried out before translocation goes ahead so that disease risk analyses can be reassessed.

In addition, uncertainty as to the origin of many beavers already present in Great Britain, and the risk of parasites yet to be identified and described in beavers, means that sustained post-release health surveillance of beaver populations will be required. A coordinated, methodical and systematic approach to clinical and pathological examination of all beavers found sick or dead is crucial to improve our understanding of beaver parasites and to ensure early detection of parasites which may cause disease outbreaks in other, naïve hosts. Historically, due to technology or time limitations, pathogens may have been missed on screening. For example, PCR testing and microarrays are relatively novel technologies which have greatly improved detection of viruses in particular; however, even nowadays, such techniques are not routinely deployed in standard post-mortem examination.

6.10 Beavers from Great Britain for conservation translocation to England

There is currently support within the beaver conservation community for careful use of the resource offered by the expanding populations of beavers in Great Britain, for example beavers in Tayside and surroundings. Free-living beavers in Great Britain are, in some cases, of uncertain origin, not subject to disease risk analysis prior to importation. If plans are made to utilise these beavers for translocation to England, we strongly recommend that their uncertain origin and potential to harbour non-native parasites is appreciated. Therefore, we advise that, following translocation, substantial resources are placed in health and disease surveillance of beaver and sympatric rodent populations in the vicinity of the release site(s). Assuming the Steering Committee for Beaver Translocation approves reintroduction to England, we would be able to map out this surveillance programme as a component of the DRM PRHS protocol.

6.11 The influence of beaver translocation on the control of mycobacteria in England

There are severe economic costs to the control of mycobacteria in domestic livestock in England. Therefore, we have considered whether there is any additional risk from mycobacteria to livestock as a consequence of beaver translocation. Scientific evidence shows that rodents in the British Isles are not an important reservoir of *Mycobacterium bovis* (Delahay *et al.,* 2007); for example prevalence in the wood mouse was 0.006% (n = 333) and in the yellow-necked mouse 2.78% (n = 36). There are no reported cases of mycobacterial disease in beavers attributable to *M. bovis.* Detailed research in the UK has established the most important hosts for *M. bovis* and they do not include rodents. Therefore, we considered the risk from beaver translocation to the control programme for *M. bovis*-associated tuberculosis in livestock in England is negligible. Notwithstanding this evaluation, we recommended (i) beavers for translocation are selected from areas, such as Scotland and Norway, currently *M. bovis*-free and (ii) stringent biosecurity protocols are adhered to in beaver translocations. We are confident that biosecurity protocols, as we have previously used in DRAHS-led translocations (Vaughan-Higgins, Masters, and Sainsbury 2017), will prevent risk from the translocation process. There is a low risk from *Mycobacteria* spp. as a carrier hazard for beavers, as a consequence of the stress of translocation, and associated with *Mycobacterium avium* complex (MAC) infection.

6.12 The present and future use of this disease risk analysis

This disease risk analysis will require regular review in the light of changes in evidence and knowledge on the diseases of threat to beavers and sympatric species following beaver translocation, if it is to effectively assess the risks from disease in translocation. It should be viewed as a working document which requires continual update as new evidence becomes available, both published evidence and that arising from any future translocations of beavers.

This report is intended as advisory to the Steering Committee of the beaver translocation in England. The risk estimations made in this study are intended for discussion amongst the Steering Committee. Views on acceptable risk can be made collectively by the Committee in the context of the conservation, social and ecological impact of beaver translocation, and the authors are ready to advise the Committee in this regard.

7.0 APPENDIX 1

Additional possible hazards to the beaver translocation are listed. These hazards are probably of low risk. However, we recommend that a detailed DRA should be performed before beavers are translocated.

- *Baylisascaris procyonis* is a zoonotic nematode parasite of racoons (*Procyon lotor*) primarily present across North America, as well as Germany and Luxembourg (Heddergott *et al.,* 2020), but known to also occur in Japan and South America (Kazacos, Jelicks, and Tanowitz 2013). Rodents are common intermediate hosts to *B. procyonis*, and disease can occur as a result of larva migrans in intermediate hosts, which includes humans (Desprez *et al.,* 2017). Disease and mortality associated with *B. procyonis* have been reported in two captive North American beavers (Desprez *et al.,* 2017), indicating that beavers are susceptible to infection and disease. Other captive rodents have also been infected with this parasite including North American porcupines (*Erethizon dorsatum*) (Medway, Skand, and Sarver 1989) and coypu (Dade *et al.,* 1977). It is possible that *B. procyonis* poses a hazard to the translocation of beavers into England based on the new translocation pathway.

- *Capillaria hepatica* is a zoonotic nematode with worldwide distribution described in more than 90 rodent host species (Fuehrer 2014). Adult worms invade the liver of the host (usually rodents) and lay ova in the surrounding parenchyma. Ova are not passed in the faeces of the host, being released in the environment only when the host dies and decomposes (Fuehrer 2014). Although considered non-pathogenic in rodent hosts in most cases, reports of associated disease exist in certain cases, particularly captive rodents; this may be reflective of an alteration in host-parasite dynamics in captive environments which could cause stress in the host. *C. hepatica* has been reported in both North American (Chitwood 1934) and Eurasian beavers (Mészáros and Kemenes 1973; Pavlov 1955). *C. hepatica* hepatitis has been reported in captive Eurasian beavers (Mészáros and Kemenes 1973) as well as black-tailed prairie dogs (*Cynomys ludovicianus*), water voles (*Arvicola amphibius*) (Redrobe and Patterson-Kane 2005) and capybara (*Hydrochoerus hydrochaeris*) (Villada *et al.,* 1998). Free-living rats are considered to be the primary source of infection of captive rodents (Hardgrove *et al.,* 2021). Beavers are susceptible to infection, morbidity and mortality associated with *C. hepatica*, and may have been exposed in the wild or captive collections. Therefore *C. hepatica* may be a carrier hazard for this translocation.

- *Travassosius rufus* is a species-specific nematode that has been reported in numerous studies of both Eurasian and North American beavers (Goodman 2014; Drózdz, Demiaszkiewicz, and Lachowicz 2004; Mckown *et al.,* 1995). The parasite was detected in the stomachs of 68.7% (33/48) of beavers examined through necropsy in Northern Poland and associated inflammatory changes of the gastric mucosa were also noted (Demiaszkiewicz *et al.,* 2014). It is possible that this nematode could be a carrier hazard for the translocation of Eurasian beavers.

- Drowning has been reported in free-living beavers in Europe (Vengušt *et al.,* 2009), for example after being trapped within lodges in changing water levels, or as a result of trapping (Rosell and Kvinlaug 1998). Moreover, within Great Britain, there are several reports of free-living beavers that may have died from drowning, such as an adult male associated with flooding in the River Otter (Duff 2012). Cases have commonly involved salt-water, with beavers found washed up on beaches. A juvenile male (suspected to have been displaced) washed up on a beach in Kent in severe respiratory distress; it did not respond to treatment and was found on post-mortem examination to have respiratory changes consistent with water inhalation (Croucher 2021). Similarly, an adult female beaver was rescued from the sea in Kent, with post-mortem findings indicative of salt water inhalation (Croucher 2020). A beaver examined post-mortem in March 2021 found washed up on a beach in Kent was suspected to have died by drowning in the sea. However, the state of the carcase did not allow histopathology of the respiratory system (Common 2021). The partial remains of a beaver identified to have been part of the River Otter Beaver Trial through an ear tag was found washed up on a beach in Devon; it was not possible to determine from the remains whether the beaver had died at sea, or whether the carcase had washed out to see after death (Elliot and Chant 2019). Although there are reports of beavers using tidal waters for dispersal, and living without issue in brackish waters (Hood 2012), in certain cases of strong tides or when underlying disease is present, drowning may occur.

8.0 APPENDIX 2

Hazards assumed to be of very low, if not negligible risk of disease in translocated beavers and destination populations, and for which, therefore, a detailed disease risk analysis was not completed.

VIRUSES

- Borna disease virus causes severe neurological disease, mainly in horses and sheep but with sporadic cases in several other species (Weissenbock 2012b). The main host is reported to be the bicoloured, white-toothed shrew (*Crocidura leucodon*) but birds may also act as a reservoir (*ibid*.). It has not been reported in beavers but has been found in several species in Germany and Sweden.

- Cowpox virus is an orthopoxvirus endemic in European free-living small rodents, in particular voles, regarded as the natural reservoir, that can infect many species including humans (Hazel *et al.,* 2000). Cowpox virus has been reported in a North American beaver held in a captive collection in Germany (Hentschke *et al.,* 1999). Transmission has also been shown in captivity to have occurred after a free-living rat carrying the virus was in contact with elephants, which in turn spread the virus to humans (Kurth *et al.,* 2008). Beavers could be infected in captive enclosures in this way.

- Encephalomyocarditis virus (EMCV) is a small non-enveloped single-stranded virus associated with encephalitis and myocarditis in a number of species, including humans. Pathogenesis appears to be strain- and host-specific. Fatal outbreaks have been reported in numerous rodents in captive collections; the virus caused mortality in 24 captive crested porcupines (*Hystrix cristata*) in Italy (Cardeti *et al.,* 2016). Captive outbreaks have been attributed to transmission from free-living rats (Canelli *et al.,* 2010). EMCV has not been reported in beavers but is found in sympatric rodent species (Kaplan *et al.,* 1980; Backhans *et al.,* 2013), and beavers could be exposed at the source and destination.

- Louping ill virus is a tick-borne flavivirus associated with disease and, occasionally, acute mortality in sheep, red grouse and humans. Louping ill virus was found on serology from a single wood mouse (n=57) and a single bank vole (n=21) trapped in western Scotland (Kaplan *et al.,* 1980) and is also reported in cervids in Norway (Gao *et al.,* 1993) but has not been reported in beavers.

- Lymphocytic choriomeningitis virus (LCMV) is an arenavirus found in free-living rodents such as the house mouse, yellow-necked mouse and wood mouse, and field voles in the UK (Duh *et al.,* 2014; Ledesma *et al.,* 2009; Tagliapietra *et al.,* 2009; Blasdell *et al.,* 2008; Murphy 2018; Kaplan *et al.,* 1980). It is associated with neurological disease in humans (Duh *et al.,* 2014), but disease has not been reported in rodents. The virus has not been reported in beavers.

- Monkeypox virus is a member of the family Poxviridae, genus Orthopoxvirus. It is a zoonotic virus, causing clinical disease similar to smallpox in humans reported mainly in Central and West African countries (Weaver and Isaacs 2008). The main animal reservoir is suspected to be rodents such as Gambian giant rats (*Cricetomys gambianus*) and rope squirrels (*Funisciurus* spp.) (Khodakevich *et al.,* 1987; Weaver and Isaacs 2008). African dormice (*Graphiurus kelleni*) have been experimentally shown to suffer lethal clinical disease after inoculation with monkeypox virus (Schultz *et al.,* 2009) suggesting that rodents may be susceptible to disease and mortality associated with monkeypox virus. Human cases in the UK have been confirmed, although are considered to be very rare (World Health Organisation, 2021a). As rodents, beavers could be susceptible to infection with monkeypox virus and disease cannot be ruled out, particularly under conditions of immunocompromise.

- Omsk haemorrhagic fever virus is a tick-borne flavivirus carried by a wide range of aquatic rodents, including the water vole and non-native muskrat, in western Siberia, and the cause of haemorrhagic fever and encephalitis in humans (Centers for Disease Control and Prevention 2021c). It has not been reported in beavers and its narrow geographical distribution suggests that the risk from disease in translocated beavers from this virus is currently negligible.

- Parechovirus B, formerly known as Ljungan virus, has been widely reported in small rodents and is believed to be associated with disease in humans (Fevola *et al.,* 2020). There do not appear to be host-specific isolates (*ibid.*) and so infection of beavers from sympatric species is possible.

- Pneumonia virus of mice is a paramyxovirus known to infect a wide range of rodents and lagomorphs. It has not been reported in beavers but is unlikely to cause disease in immunocompetent hosts.

- Porcine herpesvirus 1 (Aujeszky's Disease virus/Pseudorabies virus) is an alphaherpesvirus associated with rapid onset and usually fatal disease in dead-end hosts, including rats, mice and lagomorphs (Ruiz-Fons, 2012). Wild boar are the primary reservoir in parts of north-east Germany but it has not been reported in beavers, and is not currently in Norway or the UK.

- Rabbit haemorrhagic disease virus (RHDV) is a *Lagovirus* within the Caliciviridae family responsible for a severe outbreaks of haemorrhagic disease with up to 100% morbidity and over 90% mortality rate in adult rabbits (*Oryctolagus cuniculus*) (Parra and Prieto 1990). Antibodies to RHDV have been detected in species other than rabbits, including two free-living wood mice (*A. sylvaticus*) and one Algerian mouse (*M. spretus*) in Spain (Merchán *et al.,* 2011). No clinical signs of disease have been reported in species other than rabbits, but it is possible that beavers could be susceptible to infection with this virus given that other rodents are. It is not clear whether changes in host-parasite dynamics, for example due to stress, could lead to disease in infected rodents.

- Rabies lyssavirus causes acute and fatal encephalitis in all mammals and has been eradicated from most of Europe following vaccination of the primary host, red foxes (European Commission 2017). Rabies lyssavirus remains present in focal areas of Eastern Europe. As mammals, beavers are susceptible to infection with rabies virus. Rabies lyssavirus is not present in the UK or Norway, although sporadic cases are found on the island of Svalbard as a result of migrating animals from mainland Russia. As Svalbard is approximately 2000km from mainland Norway, there is considered to be limited likelihood of transmission to humans or animals in Norway.

- Rotavirus infection and associated enteritis has been reported in free-living squirrels, mice and rats including within Great Britain (Meredith 2012; Greenwood and Sanchez 2002) and antibodies detected in captive capybara (*Hydrochoerus hydrochaeris*) (Petric *et al.,* 1981). Infection has not been reported in beavers.

Immune status is important in determining the severity of disease (Meredith 2012) so immunocompromised animals may be expected to experience morbidity. It is not clear whether disease could occur in beavers as a result of the change in host-parasite dynamics associated with a translocation in infected animals.

- Sendai virus (Parainfluenza 1) is found in a wide range of free-living small rodents (Kaplan *et al.,* 1980), including those sympatric with beavers. It is not known if beavers are susceptible to infection.

- Tahyna (Californian encephalitis) virus is endemic throughout Europe where its main reservoir is the mosquito vector, amplified by a broad range of mammalian hosts, and which causes encephalitis in humans (Bennett *et al.,* 2011). It is not known if rodents, including beavers, are susceptible to infection.

- Theiler's murine encephalomyelitis virus has been reported in free-living rodent species (Kaplan *et al.,* 1980) including the bank vole and grey squirrel in Great Britain (Lipton *et al.,* 2001; Greenwood and Sanchez 2002). It is not known if beavers are susceptible to infection, but pathogenicity is likely to be low in immunocompetent hosts.

- Tick-borne encephalitis virus is one of the main arboriviruses of Eurasia, which is adapted to a broad range of vertebrate host species and, primarily, transmitted via hard ticks (Michelitsch *et al.,* 2019). Small mammals are considered to be the main reservoirs of infection and have been shown to act as hosts for co-feeding ticks (Cull *et al.,* 2017) with wild cervids acting as the main reservoir of the tick vector (*ibid*.). There are no reports of infected beavers but as they share habitat with reservoir species, and can be infected by the vector, they may be susceptible to infection. TBEV has recently been shown to be present in England, in Thetford Forest, East Anglia, and the Hampshire/Dorset border (Holding *et al.,* 2020, 2019). The virus sequences are closest to previously isolated TBEV strains from Norway and the Netherlands respectively and are believed to have been introduced by migratory birds (*ibid*.). As a result, translocation of an infected beaver does not constitute a source hazard but may, if beavers are found to be susceptible to disease following infection, constitute a population hazard and merit further assessment in the future.

BACTERIA

- *Aeromonas hydrophila* is an aquatic gram-negative bacterium of amphibians and fish responsible for skin infections and gastroenteritis and occasional systemic disease in other hosts. It has been found as a suspected opportunist pathogen in a beaver M08K25 (enquire with the authors for further details) associated with fatal myocarditis.

- *Anaplasma phagocytophilum*, a tick-borne rickettsial parasite, is a multi-host pathogen for which infection has been reported in many domestic and wild animals

including rodents (Birtles 2012b). It is the causative agent of tick-borne fever (TBF) in domestic ruminants and zoonotic disease in humans. Infections have been reported in the bank vole, brown rat and other rodents (Obiegala *et al.,* 2019; Birtles 2012b), which may act as asymptomatic reservoirs. It is not known if beavers are susceptible to infection.

- *Arcanobacterium pyogenes* is a commensal bacterium of the upper respiratory and genital tracts and opportunistic pathogen of many domestic animals associated with a wide range of suppurative infections (Jost, Songer, and Billington 1999). It was isolated from an adult male beaver which died in quarantine, associated with osteomyelitis of the coccygeal vertebra (Goodman *et al.,* 2017). It is likely that this infection occurred secondary to a primary trauma given that this is believed to be an opportunistic pathogen.

- *Bartonella* spp. are gram-negative bacteria exploiting a wide range of mammalian species, including humans, domestic animals and wildlife, as reservoir hosts. *Bartonella* spp. are generally species specific, causing chronic but asymptomatic infections in their hosts (Birtles 2012a). No reports of infection of beaver with *Bartonella* spp. have been found; one study tested 27 free-living Eurasian beavers in Norway and none were positive (Cross *et al.,* 2012). Nevertheless, 51% (n=93/183) of water voles were positive for *Bartonella* spp. in a study by Oliver *et al.,* (2009) and free-living brown rats have also been shown to be infected (Obiegala *et al.,* 2019) which could be sympatric to beavers. In captivity, a *Bartonella* sp. has been detected in a squirrel flea (*Orchopeas howardi*) from an eastern grey squirrel (*Sciurus carolinensis*) in a North American zoo (Nelder *et al.,* 2008) suggesting that captive animals can also be exposed.

- *Bordetella bronchiseptica* is a small, gram-negative bacteria that can cause infectious bronchitis in dogs and cats and is occasionally recorded in wildlife. *B. bronchiseptica* has been isolated from the lungs of bank voles and has been associated with mortality in voles in laboratories (Soveri *et al.,* 2000). Jensen and Duncan (1980) found that *Bordetella bronchiseptica* was associated with fatal pneumonia in the wild mountain vole, *Microtus montanus*, in North America. There have also been cases of red squirrels (*Sciurus vulgaris*) that have died from bronchopneumonia associated with *Bordetella bronchiseptica* infection in Great Britain (Simpson *et al.,* 2013). It is not known if beavers are susceptible to infection but in most cases bordetellosis is seen as a secondary or opportunistic infection in stressed or compromised animals.

- *Borrelia burgdorferi* is a bacterium responsible for a tick-borne disease, Lyme borreliosis. Its life cycle is maintained by hard ticks in the genus *Ixodes* and a wide spectrum of mammalian, avian and reptilian hosts (Ytrehus and Vikøren 2012). The bacterium has been detected in the bank vole (*Clethrionomys glareolus*), yellow-necked mouse (*Apodemus flavicollis*) and wood mouse (*Apodemus sylvaticus*) (Richter *et al.,* 2004), but no disease has been reported in association with *Borrelia* spp. in a rodent. Beavers may be susceptible to infection as they may harbour the vector.

- *Chlamydia spp.*- the Chlamydiaceae is a family of obligate intracellular bacteria of the Order *Chlamydiales*. The family consists of a single genus, *Chlamydia*, with several species, each of which naturally infects a select host (Batteiger 2014). Despite the ubiquitous nature of chlamydial pathogens and diseases, only two rodent chlamydial pathogens are known. One of these, *C. muridarum*, is widely used by researchers who apply it as a model for various human chlamydial diseases (Rank *et al.,* 2012). The other, the meningopneumonitis strain of *C. psittaci*, causes systemic disease that for various reasons has not been used extensively in modelling human diseases in mice (Rank *et al.,* 2012). Neither of these rodent pathogens has been detected or isolated from any source other than laboratory mice (*Mus musculus*). Researchers found that the organism isolated from laboratory mice was highly infectious and pathogenic for hamsters and produced pneumonia similar to that seen in mice inoculated with human influenza virus but, it was less infectious for ferrets and not infectious at all for rabbits (Nigg 1942). Most reports of *Chlamydia* spp. in rodents relate to laboratory-reared rodents. However, Stephan *et al.,* (2014) reported chlamydial infection in European shrews but reported only rare positives, indicating very low endemicity of chlamydial infection (Stephan *et al.,* 2014). Ramsey *et al.,* (2016) reported deer mice (*Peromyscus* spp.) to be infected or colonised with a chlamydial agent of some sort but were unable to culture the tissues (Ramsey *et al.,* 2016). There are no reports of infection in beavers but they may be susceptible to infection as is the case with other rodents.

- *Clostridia* spp. are obligate anaerobic bacteria that form spores to survive adverse environmental conditions. They are widely distributed in soil, water, decaying organic matter and on mucosal surfaces or within digestive tracts of humans and animals. They produce toxins which are responsible for their pathogenicity (Neimanis and Speck 2012). *Clostridium botulinum* is the most widely reported *Clostridia* species in wild animals, predominantly in birds, particularly waterfowl, but mammals are also susceptible. Botulism in wildlife occurs following the ingestion of preformed toxin. *Clostridium piliforme* is the causative agent of Tyzzer's disease, an acute disease most commonly seen in laboratory animals and commercially bred rabbits but that has also been described in free-ranging mammals, including in a wild Eurasian otter (*Lutra lutra*) cub on the isle of Harris, Scotland (Simpson *et al.,* 2008). Zoonotic strains of *C. difficile* have been found in small rodents, including the muskrat, in the Netherlands (Krijger *et al.,* 2019). To the best of the authors' knowledge, no reports of any *Clostridia* spp. have been found in beavers.

- *Corynebacterium* spp. are gram-positive bacteria which cause a relatively common disease of laboratory rats and mice (Harkness and Ferguson 1982). Subsequent studies in mice have shown that clinical manifestations are usually related to physiological stressors and active disease is precipitated by immunosuppression and is expressed as a chronic syndrome with low mortality (Harkness and Ferguson 1982). *Corynebacterium kutscheri* has also been isolated from asymptomatic hamsters and guinea pigs (Kohn and Clifford 2002). Corynebacteriaceae have been isolated from the oral and vaginal microbiota in selected field mice of the genus

Apodemus but no disease was found (Matějková *et al.,* 2020). Barrow (1981) found the occurrence of an infection caused by *C. kutscheri* in two populations of wild field voles. *C. ulcerans* was identified as the causative agent of ulcerative skin lesions in a group of eight water rats (*Hydromys chrysogaster*) held in a zoo in Germany (Eisenberg *et al.,* 2015). No reports have been found in beavers but we cannot rule out infection and disease in beavers, particularly if immunocompromised by the stress of the translocation.

- *Coxiella burnetii* is a worldwide-distributed bacterium responsible for Q fever, a disease affecting humans and other animals. Infection is usually subclinical but can produce acute disease in animals (abortion in farmed ruminants) (Ruiz-Fons 2012). Virtually all animals are considered able to harbour *C. burnetii*. Seroprevalence in UK rodents was reported as 17.3% (Meredith, Cleaveland, Denwood, *et al.,* 2015). No reports of infection or disease have been found in beavers.

- *Ehrlichia* spp. are gram-negative obligate intracellular coccobacilli, of the family Anaplasmatacaeae and order Rickettsiales. They are transmitted through tick vectors and have been detected in numerous rodents including yellow-necked mice, wood mice and bank voles (Liz 2002; Tadin *et al.,* 2016). However, these species are considered to be reservoir hosts and no associated disease has been reported. *Ehrlichia* species are not endemic in UK but have been detected (Wilson *et al.,* 2013).

- *Erysipelothrix rhusiopathiae* is an ubiquitous and environmentally persistent facultative gram-positive bacillus found as a commensal or pathogen in at least 50 species of wild mammals, including rodents, and over 30 species of wild birds (Wang, Chang, and Riley 2010). It is recognised as a cause of occupational disease in humans with strains of varying pathogenicity (*ibid*.). *E. rhusiopathiae* has not been found in beavers and it is assumed that it would be of low pathogenicity in otherwise healthy animals.

- *Lawsonia intracellularis* is an obligate intracellular bacterium found worldwide that is capable of infecting a wide range of species but only occasionally causing disease in wildlife hosts (Weissenbock, 2012). Rodent species, including the house mouse and yellow-necked mouse, and the red fox are likely carriers (Weissenbock, 2012). Infection has not been reported in beavers.

- *Listeria monocytogenes* is a gram-positive bacterium found worldwide and responsible for a disease, listeriosis, that can affect humans and other animals (Ferroglio 2012a). *L. monocytogenes* has been shown to be ubiquitously distributed in a variety of environments due to its adaptability and is a widespread microorganism in nature. For example, it can survive at a broad range of pH (4.5–9.2), temperature (0–45 °C) and salt concentrations (up to 10% NaCl) (Hain *et al.,* 2007). A study by Wang *et al.,* (2017) found *L. monocytogenes* in the faeces of many rodent species with a incidence of 9.97% (Wang *et al.,* 2017). The gastrointestinal tract is the most probable route of entrance for these bacteria and invasive listeriosis has been induced in laboratory animals by peroral inoculation

(Audurier *et al.,* 1980)**.** Skaren (1981) described an epidemic of *L. monocytogenes*-associated disease with high mortality in a colony of captive wood lemmings (*Myopus schisticolor*) and listeriosis has also been described in captive maras (*Dolichotis patagonum*) (Rosas-Rosas, Juan-Sallés, and Garner 2006) and an captive Indian crested porcupine (*Hystrix indica*) (Jagtap *et al.,* 2017). Soveri *et al.,* (2000) described a case of a free-living field vole that died from listeriosis in Finland. It is not known if beavers are susceptible to infection with *L. monocytogenes* but it is not possible to rule out listeriosis in infected beavers if immunocompromised due to translocation stress.

- *Micrococcus* spp. are environmental gram-positive bacteria that have been isolated from the eyes of 5/16 Canadian beavers with no signs of ocular disease (Cullen 2003). *M. luteus* and *M. roseus* were isolated from the faeces of a Balkan snow vole (*Dinaromys bogdanovi*) in Zagreb zoo, Croatia (Lukac *et al.,* 2017). *Micrococcus* spp. are not considered pathogenic in otherwise healthy hosts and are likely to be opportunistic pathogens.

- *Mycoplasma* spp. are a numerous class of wall-less bacteria, mainly non-pathogenic, although some species are responsible for respiratory disease. Pneumonia associated with *Mycoplasma* spp. infection has been reported in a colony of captive spinifex hopping mice (*Notomys alexis*) in an Australian zoo (Mackie *et al.,* 2001)*;* the bacteria may cause disease when host immunocompetence is reduced (Nicholas and Giacometti 2012). *Mycoplasma* spp. have been isolated from free living bank and common voles in Europe (Pawelczyk *et al.,* 2004; Bajer *et al.,* 2001) and identified in free-living and captive capybaras in Brazil (Vieira *et al.,* 2009). There have been no reports in beavers but, given that related rodents can be infected and disease is often associated with immunocompromise, beavers may be infected and suffer disease as a result of the translocation.

- *Pasteurella* spp. are worldwide multi-host pathogens, often found as commensal organisms in a wide range of hosts but reported as associated with pneumonia and septicaemia in several rodent species including the brown rat, coypu and chipmunk (Astorga *et al.,* 1996; Ferroglio 2012b). Stressors such as weather changes and poor body condition are associated with an increased likelihood of mortality in wildlife species; death associated with *P. haemolytica*-associated pneumonia was reported in captive chipmunks (*Tamias sibiricus*) recently translocated overseas (Astorga *et al.,* 1996)*.* There have been no reports in beavers, but infection and disease associated with translocation stress cannot be ruled out.

- *Pseudomonas* spp. are gram-negative rod bacteria of which the most common, *P. aeruginosa*, is found in the environment and as a commensal organism, occasionally causing abscesses in rodents (Rosas-Rosas, Juan-Sallés, and Garner 2006) It has been detected in numerous captive rodent species including porcupines (*Erethizon dorsatum*) (Barigye *et al.,* 2007) and maras (*Dolichotis patagonum*) (Rosas-Rosas, Juan-Sallés, and Garner 2006). Swabs of ocular disease in a captive guinea pig (*Cavia cavia*) and a captive chipmunk (*Tamias*

tamias) cultured *Pseudomonas* spp. (Williams, Macgregor, and Sainsbury 2000). *Pseudomonas* spp. have been reported in the eye of an otherwise healthy Canadian beaver (Cullen, 2003). It is possible that *Pseudomonas* spp. could cause disease in immunocompromised beavers.

- *Staphylococcus* spp. are gram-positive facultative bacteria commonly associated with suppurative infections and abscess formation but which may also cause septicaemia and toxic shock syndrome (Speck 2012a). Different *Staphylococcus* spp. are associated with different animal species but most diseases of wildlife associated with these bacterial species are attributed to *S. aureus* (Speck 2012a). *S. aureus* was cultured in pure growth from the liver and lung and in mixed growth with a non-haemolytic *Staphylococcus* spp. from a beaver carcase (Common, Gerard, and Sainsbury 2022) which had injuries and history consistent with a road traffic collision, and therefore it is possible that the *Staphylococcus* spp. were associated with a secondary septicaemia (Common, Gerard, and Sainsbury 2022). *S. stephanovicii* has been found in the bank vole and the field mouse in association with enteric and skin disease (Speck 2012a), and *Staphylococcus* spp. have been isolated from ocular lesions in rodent species (Williams, Macgregor, and Sainsbury 2000). *Staphylococcus* spp. have also been isolated from spinifex hopping-mice (*Notomys alexis*) with pneumonia (Mackie *et al.,* 2001), captive Patagonian maras (*Dolichotis patagonum*) (Rosas-Rosas, Juan-Sallés, and Garner 2006) and captive Balkan snow voles (*Dinaromys bogdanovi*) (Lukac *et al.,* 2017). *Staphylococcus* spp. were found in the eyes of 3/10 otherwise healthy free-living Canadian beavers (Cullen, 2003). It is possible that these bacteria could cause secondary disease in immunocompromised translocated beavers.

FUNGI

- *Candida albicans* is an opportunistic yeast which has been reported in association with a cutaneous infection in a Canadian beaver (Sáez and Lagunas 1976). It has also been detected in captive rodents including 6/20 Balkan snow voles in a zoo in Croatia (Lukac *et al.,* 2017). It is unlikely to be pathogenic in an otherwise healthy animal.

- *Cryptococcus neoformans* is a widespread invasive fungal parasite which has the ability to infect numerous mammalian species including humans (May *et al.,* 2016). Rodents, including rats and mice, are susceptible to experimental infection through nasal inoculation (Kuttin *et al.,* 1988), and free-living rodents have also been found to be infected (Iatta *et al.,* 2015). Cryptococcosis was diagnosed post-mortem in a striped grass mouse (*Lemniscomys barbarus*) with other mice and one degu (*Octodon degus*) from the same collection testing positive for antibody titres and/or lung samples (Bauwens *et al.,* 2004). Moreover, *Cryptococcus* spp. were identified associated with 34% (11/32) of mortalities in slender-tailed cloud rats (*Phleomys pallidus*) in Bronx Zoo, and a significant co-morbidity in an additional three cases (Sykes, Wilson, and McAloose 2019). Infection has not been reported in beavers but, given that *C. neoformans* is widespread in the environment and infection and disease has been reported in rodents, beaver infection and disease with *C. neoformans* cannot be ruled out.

- *Enterocytozoon* spp. are intracellular microsporidial parasites of enterocytes associated with chronic disease and potential mortality in humans and animals (Perec-Matysiak *et al.,* 2015). *Enterocytozoon* spp. have been detected in numerous species across multiple taxa in captive collections (Li *et al.,* 2015). Rodents may act as reservoir species (Perec-Matysiak *et al.,* 2015), and infection has been shown to be possible in several experimentally immunosuppressed rodent species (Feng *et al.,* 2006). No reports are available documenting infection in beavers but this cannot be ruled out.

- *Microsporum* is one of three dermatophyte genera. Dermatophytes are keratinophilic fungae; some are responsible for skin disease (including 'ringworm') in humans and animals (Marks and Miller 2013) including rodents: a study by Hardgrove *et al.,* (2021) found that *Microsporum gypseum* was the most commonly reported fungal agent detected in captive rodents; it has been detected in Mexican prairie dogs (Porter 1979), guinea pigs (*Cavae cavae*) (Kraemer *et al.,* 2012) and associated with disease in a North American porcupine (*Erethizon dorsatum*) (Hackworth *et al.,* 2017). *Microsporum* spp. are ubiquitous in the environment. Cases in beavers have not been reported but given the ubiquity and multi-host potential of the agent, beavers may be susceptible.

- *Pneumocystis* is a genus of fungal parasites which have the ability to infect numerous animal species including humans (Tasaka 2015). *P. murina* is an opportunistic pathogen of mice which has been associated with outbreaks of pneumonia in immunodeficient mice housed with immunocompetent individuals harbouring the fungus as a commensal(Percy and Barta 1993; Dagnæs-Hansen, Kilian, and Fuursted 2004). *Pneumocystis* infection has also been detected in field and bank voles (Soveri *et al.,* 2000). There have been no reports of *Pneumocystis* spp. infection in beavers but susceptibility to infection and associated disease during translocation stress cannot be ruled out.

- *Trichophyton* is another of the three dermatophyte genera. *T. mentagrophytes* has been documented in diseased and healthy guinea pigs (Drouot *et al.,* 2009; Kraemer *et al.,* 2012). In a zoological collection *T. mentagrophytes* was isolated from 19 of 20 guinea pigs (Hiruma *et al.,* 2014) indicating a high prevalence in this rodent species in captivity. Given this finding, infection in beavers cannot be ruled out, particularly if beavers are sourced from zoological collections.

PROTOZOA

- *Babesia* spp. are the causative agent of zoonotic babesiosis with widespread prevalence in Europe. Rodents are regarded as an important reservoir with transmission via the tick vector *Ixodes ricinus* but there are no reports of associated disease in rodents (Beck, Vojta, *et al.,* 2011; Paziewska *et al.,* 2007; Cross *et al.,* 2012). *Babesia* spp. have not, to date, been identified in beavers but beavers may have potential to act as a reservoir. *Babesia* spp. has been detected in the South of England in *Dermacentor reticulatus* ticks (De Marco *et al.,* 2017), where free-living

beavers already reside, so post-release health surveillance is recommended to inform future risk analyses.

- *Encephalitozoon cuniculi* is an obligate intracellular spore-forming protozoan which is the causative agent of encephalitozoonosis, an important emerging disease of humans and animals which, in addition to its main hosts of rabbits and hares, has been found in several species of small rodent (Meredith, Cleaveland, Brown, *et al.,* 2015). Infection in rodents is usually asymptomatic but infected animals can exhibit neurological signs and renal failure (Meredith, Cleaveland, Brown, *et al.,* 2015). The parasite was found associated with mortality in captive barbary striped grass mice (*Lemniscomys barbarus*) (Kitz *et al.,* 2018) as well as a captive Vancouver Island marmot (*Marmota vancouverensis*) (Milnes *et al.,* 2018). A strain previously isolated from small rodents has been reported in farmed Arctic foxes and mink in Norway (Akerstedt 2006). Infection in beavers at the time of translocation cannot be ruled out.

- *Entamoeba* spp. are commensal intestinal parasites ubiquitous in species including rodents, rarely associated with dysentery (Cox 1987). Pathogenicity is assumed to be low in otherwise healthy adult animals and *Entamoeba* spp. have been detected in the faeces of guinea pigs and rats without disease (Aviruppola, Rajapakse, and Rajakaruna 2016; Chagas *et al.,* 2017). Mathews *et al.,* (2006) found poorer survival but no significant difference in body weight or body condition in water voles infected with *Entamoeba* spp.. However, sample numbers were small (n=5/54). *Entamoeba* spp. have been detected in the faeces of garden dormice (*Eliomys quercinus*) but not associated with disease (Fischer *et al.,* 2018). Infection and disease in other rodents, including beavers, cannot be ruled out.

- *Hepatozoon* spp. are obligate intra-erythrocytic parasites found in numerous rodent species including several free-living rodent species in Europe (*Clethrionomys* spp., *Apodemus* spp., *Microtus* spp.) (Laakkonen *et al.,* 2001; Craig 2001). *H. erhardovae* has been detected in free-living bank voles in the UK and a higher prevalence has been shown in this species in juveniles (Turner, 1986). Disease associated with *Hepatozoon* spp. has also been documented in rodents: Miller, (1908) reported that domestic rats with high burdens of *H. perniciosum* developed anaemia, anorexia and mortality. Beavers are likely to be sympatric to voles and mice at riparian margins and could be susceptible to infection with *Hepatozoon* spp..

- *Neospora caninum* is a coccidian species closely related to *Toxoplasma gondii* that is a recognised pathogen of dogs and cattle (Fuehrer *et al.,* 2010). Rodents may play a role as intermediate hosts in the sylvatic cycle and *N. caninum* DNA has been detected in free-living house mice (*Mus musculus*), brown rats (*R. norvegicus*), and field mice (*A. sylvaticus*) in Italy using PCR (Ferroglio *et al.,* 2007). Moreover, a prevalence of 10% (10/105) has been reported in free-living house mice and 30% (72/242) of brown rats in North America (Jenkins *et al.,* 2007). Given that rodents are susceptible to infection, beavers could be susceptible to infection with *Neospora caninum.*

- *Sarcocystis* spp. are obligate intracellular protozoa with a complex indirect life cycle in which rodents within the genera *Echimyidae*, *Muridae*, *Sciuridae*, and *Erethizontidae* have been reported as intermediate hosts (Dubey and Odening 2001). Infection is usually asymptomatic in the final host, while disease may be seen in intermediate hosts (Formisano *et al.,* 2013). A *Sarcocystis* spp. cyst was reported in the myocardium of a beaver in Great Britain, M08K20, as an incidental finding with no associated inflammatory reaction on histopathology (Cranwell 2009a), suggesting that beavers could be a reservoir species. It is not known whether beavers are susceptible to disease as an intermediate host of *Sarcocystis* spp..

- *Trypanosoma* spp. are protozoan parasites which can infect all vertebrate classes (Cayla *et al.,* 2019). *Trypanosoma* spp. have been detected in bank voles and wood mice in England (Turner, 1986) and other exotic rodents such as Patagonian mara and agouti (*Dasyprocta aguti*) in captive collections (Porteous and Pankhurst 1998). It is not clear whether beavers are susceptible to infection with *Trypanosoma* spp..

ALGAE

- *Prototheca* is a genus of parasitic algae within the family Chlorellaceae, species of which have been associated with disease in numerous species including humans (Sileo and Palmer 1973). Nodular, exudative skin lesions were detected in the pelt of a North American beaver; *Prototheca* spp. were the only micro-organisms detected within the lesions (Sileo and Palmer 1973), suggesting that they are associated with disease in beavers, but this is possibly a rare occurrence.

PRIONS

- Chronic wasting disease (CWD) is a contagious, neurogenerative and fatal transmissible spongiform encephalopathy of cervids (Williams and Young 1980), including in Northern Europe (Vikøren *et al.,* 2019). Recent experimental evidence suggests that beavers are at risk of CWD pathogen transfer and spillover, due to the fact that the beaver prion protein is an excellent substrate for replication (Herbst *et al.,* 2022). Given that CWD is prevalent in Northern Europe, it is possible that it could be a hazard for beavers, although evidence is limited at this stage.

ENDOPARASITES

- *Fasciola hepatica* is a trematode found worldwide that colonises the bile ducts of its definitive host, most commonly domestic ruminants, with aquatic lymnaeid snails as its intermediate host. It is the cause of considerable economic losses from livestock morbidity and occasional mortality. *F. hepatica* was found in the livers of 2/20 free-living Eurasian beavers necropsied in Belarus, and ova were also detected in the faeces of beavers in the same area, indicating that they can be infected (Shimalov and Shimalov 2000). Coypu (*Myocastor coypu*) have been identified as epidemiologically important for this parasite in Uruguay (Gayo *et al.,* 2011). It is possible that beavers may also be important as possible reservoirs for *F. hepatica*.

- *Hymenolepis* spp. are cestode parasites found in humans and rodents which have been detected in water voles in Great Britain (Gelling *et al.,* 2012). There are no reports of infection in beavers but, as sympatric species, they may be susceptible. There are reports in captive rodents including red squirrels (Dollfus 1951).

- *Mesocestoides lineatus* is a cestode parasite with final hosts consisting of numerous carnivores such as domestic and wild canids (Schirò *et al.,* 2021). The parasite has been commonly detected in free-living rodents which act as intermediate hosts. Although not normally associated with disease in rodents, the parasite was detected on post-mortem examination of an edible dormouse found dead in Italy, in which numerous parasitic cysts were detected throughout the peritoneum and liver surface, along with congestion of the liver and signs of emaciation (Schirò *et al.,* 2021). To the best of the authors' knowledge no reports of this parasite exist in beavers. However, it is possible that, since other rodents are susceptible to disease and infection, beavers are too.

- *Notocotylus* is a genus of trematode parasites infecting several mammalian species, including rodents. *Notocotylus* spp. have been detected in field and bank voles in England and other parts of Europe (Boyce *et al.,* 2012; Mažeika, Paulauskas, and Balčiauskas 2003). Although, to the best of the authors' knowledge, not reported in beavers, infection cannot be ruled out.

- *Renifer ellipticus* is a trematode parasite which has been detected in the caecum of a North American beaver (Canavan 1934). No associated disease was reported in this case and no other cases have been reported to the best of the authors' knowledge.

- *Rodentolepis* spp. are cestode parasites common in many rodent species. They have been detected in brown rats (*Rattus norvegicus*) (Chagas *et al.,* 2017; Obiegala *et al.,* 2019) and captive Patagonian mara (Porteous and Pankhurst 1998). *Rodentolepis* spp. have also been detected in captive bred dormice (Flach *et al.,* 2000). No reports exist in beavers, but infection cannot be ruled out.

ECTOPARASITES

- *Demodex* spp. are arachnid mites, with a worldwide distribution and likely to be host specific. *D. castoris* has been detected on the skin of free-living beavers in Poland (Izdebska, Fryderyk, and Rolbiecki 2016). *Demodex* spp. are not normally pathogenic in immunocompetent hosts. However, disease associated with *Demodex musculi* has been shown in immunodeficient laboratory mice (Smith *et al.,* 2016). It is possible that this parasite could cause disease in beavers suffering immunocompromise as a result of translocation.

- Fleas (Siphonaptera) can have negative effects on mammals and birds and pose an important problem for certain rodent species (Romeo *et al.,* 2013). Negative effects of flea parasitism on the host are varied and include host blood loss, skin damage, irritating bites, response to saliva injected into the wound, and transmission of pathogens (Hawlena *et al.,* 2006). Knowledge of the fleas in

beavers is relatively poor. A flea of the genus *Ceratophyllus* was detected on a free-living Eurasian beaver in Sweden (Åhlen, Sjöberg, and Stéen 2021). Disease associated with flea parasitism is likely to be intensity-dependent and related to irritation and anaemia in immunocompromised animals.

- *Ixodes* spp. are ticks endemic to the UK, with many avian and mammalian species involved in the life cycle. Both *Ixodes ricinus and I. hexagonus* have been reported on free-living beavers (Wodecka and Skotarczak 2016; Haitlinger 1991). *Ixodes* spp. are vectors for a number of parasites to which beavers may be susceptible. Disease associated with tick parasitism (excluding tick-borne pathogens) is likely to be intensity-dependent and related to irritation and anaemia.

- *Platypsyllus castoris*, the beaver beetle, is a species-specific obligate ectoparasite of beavers which has been widely found in free-living beavers, including in Great Britain (Duff, Campbell-Palmer, and Needham 2013). It is not believed to be associated with disease in otherwise healthy animals.

- *Schizocarpus* spp. are fur mites of the Chirodiscidae family. Many species have been detected in Eurasian beavers (Haitlinger 1991; Åhlen, Sjöberg, and Stéen 2021; Bochkov and Saveljev 2012) and, although not known to be associated with primary disease in beavers, infestations in other species can cause pruritus and anaemia and could lead to irritation and secondary infections as a result of self-trauma (Campbell-Palmer and Rosell 2013).

- *Trombicula* spp., also known as harvest mites, are a genus of mite of the family Trombiculidae. Their larvae live parasitically and they infect most mammals, humans and some ground-nesting birds (Wall and Shearer 2008). In the UK, the most prevalent harvest mite is *Trombicula autumnalis. Trombicula* spp. have been found in free-living rodents in Europe (Kirillova, Kirillov, and Ivashkina 2006) and, although there are no reports in beavers to the best of the authors' knowledge, *Trombicula* spp. present in the UK may be able to infect them. Heavy parasitism with *Trombicula* spp. is unlikely in immunocompetent hosts.

NON-INFECTIOUS

- Anticoagulant rodenticides – accidental poisoning of non-target animals via anticoagulant rodenticides can occur and the effects of secondary poisoning can affect animals that eat dead or dying rodents. Anticoagulant poisoning is by far the most common means of rodent control, being the basis of about 95% of rat and mouse control in the USA and 92% of rodent control on UK arable farms (Mcdonald and Harris 2000). Accumulated anticoagulants have been found in the stomachs and livers of many wild species including polecats, barn owls and red kites, and fatal secondary anticoagulant poisoning has been implicated in the deaths of red foxes, owls, buzzards, red kites, corvids and many other species (Newton, Wyllie, and Freestone 1990; Molenaar *et al.,* 2017; Shore, Birks, and Freestone 1999). Exposure to anticoagulant rodenticide in beavers has not been reported and their ecology, behaviour and diet suggest they are unlikely to be affected by these toxins.

- Environmental contaminants such as organochlorine pesticides, polychlorinated biphenyls(PCBs) and heavy metals that are known to be toxic and persistent (Fowler 1993) are ubiquitous environmental contaminants. They have been widely introduced into the environment as agricultural and industrial by-products. Because of their high lipophilicity and persistence, they accumulate in fatty tissue and biomagnify within food webs (Newton 1979). At relatively low concentrations, organochlorine compounds and heavy metals have been implicated in reproductive impairment and population declines as well as behavioural alteration in many species including mammals and birds (Fox and Donald 1980; Colborn, Short, and Gilbertson 1998). Although most organochlorine compounds have been banned from the UK, the few data available indicate the persistence of pesticides and PCBs in several areas (Vane *et al.,* 2014). Heavy metal traces including cadmium, lead, copper, mercury and zinc have been found in tissues from beavers, including those free-living in agricultural areas in Poland, remote from industrial centres, without associated disease (Giżejewska *et al.,* 2015; Peterson and Schulte 2016). Beavers may be susceptible to toxicity from bioaccumulation of pathogenic elements.

- Reports of beavers dying during general anaesthesia (Helen Roberts, pers. comm.) suggest that the species may be susceptible to side effects associated with anaesthetic drugs or stressors associated with anaesthesia. Canadian beavers exhibit bradycardia when diving and also when threatened on land (Swain, Gilbert, and Robinette 1988).

9.0 References

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