

Disease Risk Analysis for the Reintroduction of the Pine Marten to Dartmoor and Exmoor National Parks, Southwest England





Report for submission to the Two Moors Partnership Claudia Carraro, Anthony W. Sainsbury July 2023



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## **Executive Summary**

The Two Moors Pine Marten Project is a partnership between Devon Wildlife Trust, Woodland Trust, National Trust, Dartmoor National Park Authority, and Exmoor National Park Authority. The Two Moors Partnership has been investigating the potential conservation translocation of free-living, wild pine martens (*Martes martes*) from the Scottish Highlands to Dartmoor and Exmoor National Parks, southwest England. Expert advice and support for the project is being given by The Vincent Wildlife Trust, Forestry England, Natural England, and NatureScot.

This disease risk analysis (DRA) was adapted from the DRA carried out for the Forest of Dean pine marten reintroduction (Carraro & Sainsbury, 2019). We reviewed background information and added new evidence, as appropriate, on the pine marten's ecology, conservation and legal status, described the proposed translocation pathway, evaluated the geographical and ecological barriers to parasite spread along this translocation pathway, identified, reviewed and evaluated 72 potential hazards (66 infectious and six non-infectious), and carried out a detailed disease risk analysis on sixteen prioritized hazards, followed by a brief discussion on how the risk from these hazards could be mitigated. In so doing, fourteen hazards fully analyzed in Carraro and Sainsbury (2019) and in Carraro and colleagues (2021) have been re-assessed and two newly prioritized hazards fully analyzed (i.e., *Mycobacterium bovis*, High and Low Pathogenic Avian Influenza Virus).

No geographical and/or ecological barriers are likely to be crossed in this translocation pathway thus markedly reducing the probability that translocated or recipient populations will be exposed to novel infectious agents, and markedly reducing the probability of a serious disease outbreak following translocation. Therefore, we focused our analysis primarily on population, carrier, and transport hazards. However, considering its peculiar epidemiology within the UK, *M. bovis* was classified and fully analyzed as a destination hazard.

Of the sixteen hazards prioritized for full disease risk analysis, one, the transport hazard SARS-CoV-2, was assessed as currently being of high risk for specific groups of translocated pine martens but of medium risk for other mammals at the destination; and a further two were assessed as medium risk, Anticoagulant Rodenticides (ARs) and Illegal Persecution, both non-infectious population hazards that represent a threat to a small, newly released pine marten population hazards Canine distemper virus (CDV), High and Low Pathogenic Avian Influenza Virus (HPAIV and LPAIV), *Mucoraceae* spp., *Toxoplasma gondii*, Trauma, and the carrier hazards *Leptospira* spp., *Yersinia* spp., *Hepatozoon* spp., *Toxoplasma gondii*; and four as very low risk: the destination hazard *Mycobacterium bovis*, the population hazards Canine amdoparvovirus 1 (previous Aleutian mink disease virus AMDV), and the carrier hazard *Neospora caninum*.

Our analysis estimated that there is a very low likelihood that transmissions amongst reintroduced pine martens will be sufficient for *M. bovis* to become endemic in the reintroduced pine marten population. The proposed pine marten reintroduction is likely to have negligible biological and economic consequences for the epidemiology of bovine tuberculosis disease in livestock in England.

In the qualitative ZSL DRA method used in this report, risk estimation is made prior to consideration of disease risk management, which is evaluated thereafter. Mitigation measures are employed to reduce the risk level and therefore the risk estimations noted here might be reduced when risk management is implemented. In this report, we briefly outlined some mitigation options for each of the fully assessed hazards and our recommendation is to further develop such measures in a Disease Risk Management and Post-Release Health Surveillance (DRM PRHS) protocol.

Because of the paucity of information on the current parasite and disease status of free-living pine martens in Britain, the presence of unknown, novel parasites affecting this translocation cannot be discounted. Since unknown parasites have caused severe epidemics as a result of translocations, this DRA should be regularly updated as new evidence becomes available. Post-release health surveillance will be extremely important to detect emerging diseases at the reintroduction site and should be integrated with post-release population monitoring. Prompt efforts should be made to incorporate surveillance data back into the DRA and hazards re-evaluated accordingly.

## **1 INTRODUCTION**

### 1.1 PINE MARTEN CONSERVATION STATUS IN THE BRITISH ISLES

Native species.

IUCN Red List: Global: LC; Great Britain: LC (England: [CR]; Scotland: [LC]; Wales: [CR]).

The European pine marten (Martes martes) is a medium-sized mustelid widely distributed in the west and central Palearctic. Because of the pine marten's wide distribution and large population across most of Europe, occurrence in a number of protected areas and tolerance to some degree of habitat modification, the pine marten is listed as Least Concern in the IUCN Red List of Endangered Species (Herrero et al., 2016). However, both population and range have contracted in many parts of its distribution. While the pine marten was formerly widespread in the British Isles, deliberate persecution by gamekeepers on sporting estates, persecution for its fur and the loss and fragmentation of woodland habitats have all contributed to a dramatic decline across much of its former range and by the early 20<sup>th</sup> century it was restricted to the far north-west of Scotland and small areas in north Wales, Cumbria and Yorkshire (Langley & Yalden, 1977; Webster, 2001). Such a major reduction in pine marten range and numbers occurred also, and for the same reasons, in Ireland during the 19<sup>th</sup> and 20<sup>th</sup> centuries (O'Sullivan, 1983). Since the mid-1970s, an increasing use of reared game birds for shooting instead of wild game birds has led to a reduction in trapping effort for predator control by gamekeepers (Tapper, 1992 in McDonald & Harris, 1999; Stringer et al., 2018) which, in association with the full legal protection given to pine martens in Scotland in 1988 and the increase in forest cover, has resulted in the recovery of the species across much of the Scottish Highlands (Balharry et al., 1996; SNH, 2018). Some isolated populations are also found in southern Scotland as a result of the first publicized translocation of pine martens from the Highlands to Galloway Forest in the early 1980s (Croose et al., 2014). Harris and colleagues (1995) estimated a British pine marten population of about 3,650 individuals, with the majority recorded in Scotland (about 3,500) and the few remnants in England (less than 100) and Wales (less than 50). The most recent comprehensive review of the status of British mammal populations, published in 2018 by the Mammal Society (Mathews et al., 2018), has provided a pine marten population estimate of 3,700 individuals in Britain (95%CI = 1,600-8,900). The current (up to 2016) distribution of pine martens in Britain can be seen in Figure 1.

A similar recovery has occurred in Ireland where the core pine marten population appears to be expanding (O'Mahony et al., 2006). In 2016, a Pine Marten Population Assessment produced a population estimate of 3,043 pine martens for the Republic of Ireland (Birks, 2017). In contrast with its Scottish and Irish counterparts, the species remains very rare in England and Wales, failing to recover from its historical decline, and to date there is no convincing evidence of natural recovery south of the Scottish border (Birks & Messenger, 2010; Jordan, 2011; Macpherson et al., 2014). As pointed out by Croose and colleagues in 2014, it is probable that parts of northern England will be re-colonised by the Scottish pine martens will be able to expand their range as far as central/southern England and Wales due to the large conurbations and lack of suitable habitat in some of these areas (MacPherson et al.

al., 2014; Macpherson & Dunman, 2015; Stringer et al., 2018). Evidence of pine marten's presence in Northumberland and Cumbria has been reported by the "Back from the Brink" project that facilitated and monitored the natural recovery of the mustelid in northern England between 2017 and 2020. The information gathered has indeed confirmed a natural southward expansion of the Scottish pine marten population, resulting in parts of northern England being slowly re-colonised (Croose, 2021). Surveys conducted over several years suggested that the scattered Welsh and English populations were functionally extinct, not being large enough to be viable in the long-term (Jordan, 2011), thus the urgent need of preventing extinction and restoring viable pine marten populations in their historical range in England and Wales has resulted in an increase in conservation action over the past few years.

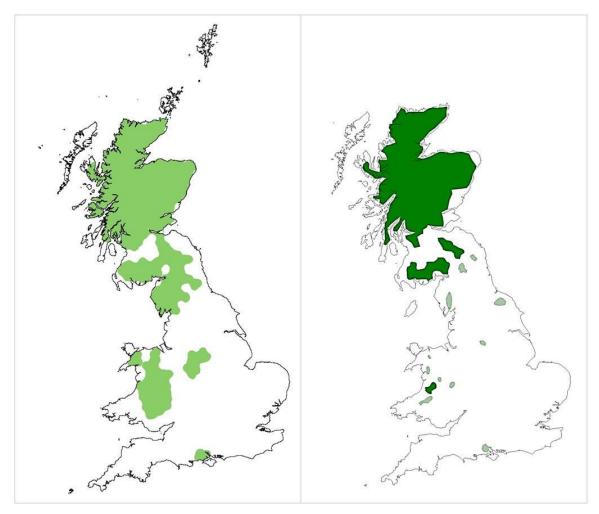


Figure 1. Left panel: Pine marten range in Britain based on presence data collected between 1995 and 2016. Some highlighted areas, particularly those in England and Wales, represent very occasional records rather than established populations. Some areas that contain very isolated records may not have been included in the area of distribution. Right panel: Map produced by the Vincent Wildlife Trust is provided for comparison. This map is mainly the result of two recent expansion zone surveys in Scotland (Croose et al., 2013; Croose et al., 2014), and monitoring following population reinforcement in Wales. The map shows established populations (dark green) and occasional records (light green) up to 2016 (source: Mathews et al., 2018 – courtesy of Katherine Walsh, Natural England).

In Wales, a preliminary strategy for restoring the pine marten (Jordan, 2011) and a feasibility assessment in accordance with IUCN guidelines (MacPherson et al., 2014) resulted in a conservation translocation (reinforcement) of 51 adult pine martens from the Scottish Highlands into central Wales, coordinated by Vincent Wildlife Trust (VWT) between 2015 and 2017. The animals were captured, under license from Scottish Natural Heritage, from areas where there is a healthy pine marten population. The pine martens translocated to Wales have established a population in which breeding has been recorded every year since the project started.

In England, following the successful start of the Welsh reinforcement project, Gloucestershire Wildlife Trust, in partnership with Forestry Commission and VWT, developed a reintroduction project to create a stable pine marten population in the Forest of Dean and lower Wye Valley, Gloucestershire. The feasibility study highlighted how this reintroduced population might in time become connected to the reinforced population in central Wales, thus maximizing the chance of meta-population establishment and successful conservation of pine martens in both Wales and England (Stringer et al., 2018). A total of 35 pine martens were successfully reintroduced in the Forest of Dean between 2019 and 2021. Breeding is occurring and the population was estimated to be just over 40 individuals in 2022. There are no further releases planned in Gloucestershire and the reintroduced population will continue to be monitored as it spreads and expands throughout the region.

In a recently published long-term strategic recovery plan for pine martens in Britain (MacPherson & Wright, 2021), southwest England has been identified as a priority region that merits further consideration as a potential pine marten reintroduction site. A partnership of conservation organizations including Devon Wildlife Trust, Dartmoor National Park Authority, Exmoor National Park Authority, National Trust, and Woodland Trust, is currently developing a conservation project, the Two Moors Pine Marten Project, that aims to reintroduce pine martens to the southwest of the country after about 150 years of absence. Expert advice and support to the project is being given by VWT, Forestry England, NatureScot, with additional economic support from Turnstyle Designs.

## 1.2 PINE MARTEN LEGAL STATUS

Internationally, the pine marten is listed as a species "of community interest whose taking in the wild and exploitation may be subject to management measures" under Annex V of the European Union Habitats and Species Directive of 1992, and is listed as a protected species in Appendix III of the Bern Convention (1982). In the UK, the species is protected under the Wildlife and Countryside Act 1981 – Schedule 5 since 1988. This makes it an offence to intentionally kill, injure or take a pine marten without a license; it is illegal to damage, destroy or obstruct access to their dens which are used by pine marten for shelter or protection; they cannot be disturbed whilst using such dens; it is illegal to possess and/or sell any living or dead pine marten. In 2007, the pine marten was added to the UK List of Priority Species and Habitats as a priority species requiring conservation action under the UK Biodiversity Action Plan (UK BAP). The pine marten was also listed as a species "of principal importance for the purpose of conserving biodiversity" covered firstly under section 41 (England, in 2008) and subsequently also under section 42 (Wales, in 2009) of the Natural Environment and Rural Communities Act 2006.

## **1.3 PINE MARTEN ECOLOGY AND HABITAT REQUIREMENTS**

## 1.3.1 Physical Characteristics, Behaviour and Activity Patterns

The European pine marten is a semi-arboreal mammal, member of the *Mustelidae* family and closely related to the stoat, weasel, mink, polecat, otter, and badger. The pine marten is likely to have arrived in Britain spreading northwards from just one or two central-southern European *refugia* soon after the last glaciation came to an end, before the English Channel formed (Birks, 2017), as suggested by the low level of genetic variability of modern pine martens sampled across Europe (Davison et al., 2001).

Pine martens have a slim body and a long tail which they use to balance themselves when skillfully treetop hunting, racing along branches and leaping from tree to tree. They have a rich brown fur covering most of their bodies with a creamy yellow bib on the throat and chest and large, rounded ears fringed with pale fur. The main body colour varies seasonally: thick, mid-brown with a bushy tail in winter; darker but shorter in summer. Compared to the other members of the *Mustelidae*, pine martens have a more pronounced snout, which together with the rounded ears gives them a distinctive heart-shaped face (Birks, 2017).

Sexual dimorphism is common in mustelids and is seen in pine martens where males (1360-1587g, 69-71cm nose to tail) are larger than females (960-1116g, 62-64cm nose to tail) (Zalewski, 2007; Ruette et al., 2015). In the wild, pine martens can live up to 10-15 years, although the average lifespan is 3-4 years. Pine martens are elusive, solitary for most of the year, however individual territories will often overlap and are not aggressively defended. They are mainly but not strictly nocturnal, with most of their activity occurring between dusk and dawn and spending most of the day sleeping in a den which can be a hollow in a tree, a disused squirrel drey or a crevice amongst rocks. During the summer, however, they are commonly active during the day too. Pine martens greatly reduce their activity levels in winter as a strategy to cope with the cold (Birks, 2017). Like other mustelids, pine martens also use scent-based communication to mark their territories, to ensure males and females successfully meet during breeding season and to enable females to successfully rear their kits. They use both anal and abdominal scent glands, the latter being more actively used during the breeding season.

## 1.3.2 Breeding Ecology

Pine martens produce small litters once a year and sexual maturity is reached at 3 years in males and about 16 months in females, and this breeding rate limits population recovery and range expansion and also increases population vulnerability to elevated mortality levels (Birks, 2017).

Mating occurs between July and August, however the implantation of the blastocysts in the female's uterus is delayed for about 230 days and only takes place between mid-January and mid-March, allowing birth to occur under the most favourable conditions. The length of such embryonic diapause is influenced by metabolic and environmental conditions such as the changing day length; the number of blastocysts implanting may be influenced by food availability (Birks, 2017). The post-implantation gestation period is generally stable (30 days). A single litter of 1-5 kits, with 3 being the most common litter size, is therefore produced

between mid-March and mid-April in the year following mating. This reproductive strategy allows offspring more time to perfect their foraging skills, thus increasing their chances of survival over winter. Kits are born weighing about 30 grams, with eyes and ears closed. They open eyes at 35 days and weaning starts soon afterwards. At seven or eight weeks, kits start exploring the entrance to their natal den and then venturing out of it. Sub-adult pine martens reach adult body size at 6-8 months of age.

Juvenile dispersal in pine martens occurs later than in other mustelids, usually between mid-February and mid-March in the year following their birth, at about 11 months old, and it coincides with the onset of the gestation period in adult females and with a period of testis growth in sub-adult males (Larroque et al., 2015). Dispersal distance is highly variable: a minimum of 2.5 km to a maximum of 214 km has been recorded (Larroque et al., 2015; Johnson et al., 2009) and some juvenile individuals might not disperse at all remaining in the proximity of the dam's home range instead (Larroque et al., 2015).

#### 1.3.3 Habitat and home range

The pine marten is commonly described as a specialist of old-growth forests, preferring mature forests with specific structural features (multilayered tree canopies, a dense and diverse understory vegetation, many old trees as well as tree hollows, standing deadwood and fallen root masses) which can be used as dens for resting, sheltering from bad weather and breeding; increase foraging efficiency and provide protection against predators like foxes and golden eagles (Caryl et al., 2012a; Birks, 2017). However, pine martens are highly adaptable and, where woodland is scarce or unsuitable, they use a range of three-dimensional rocky habitats instead (cliffs, crags, and rocky mountain sides) and use rock crevices as dens if tree hollows are not available. Recent studies in Scotland (Caryl et al., 2012a), France (Mergey et al., 2011) and Italy (Balestrieri et al., 2011), interestingly suggest that pine martens make use of non-wooded habitats as well, challenging the conventional view that they are strictly forestinhabiting mammals which avoid open spaces (Birks, 2017). For example, two open habitats, scrub and tussock grassland, are often selected in Scotland, providing pine martens with fundamental resources (den sites and primary prey, *Microtus agrestis*) otherwise less available within intensively managed plantation forests (Caryl et al., 2012a). Intersexual differences in habitat utilization have been observed, with female pine martens being more reluctant to leave forest habitats to visit open areas than males, typically travelling just 30 m from woodland compared to 75 m typically travelled by males (Caryl et al., 2012a). Males are also more frequently located outside the forest than females. A possible explanation for such intersexual differences could be either the smaller size of female pine martens which may therefore be more vulnerable to open space predators (foxes Vulpes vulpes and golden eagles Aquila chrysaetos), or the females' attitude to avoid risks for their dependent kits (Birks, 2017).

Being a solitary species, pine marten social organization is structured around what is called 'intrasexual territoriality' with adults occupying separate areas (territorial home ranges) without overlapping of individuals of the same gender (Birks, 2017). Males exclude other males from their territories and so females exclude other females, but male home ranges overlap with those of females, especially in spring-summer (Zalewski & Jędrzejewski, 2006; Birks, 2017). There are huge variations in the average size of home ranges occupied by pine martens, directly related to habitat quality and food availability with home range size decreasing with increasing habitat quality (Birks, 2017). Across Europe, mean pine marten

home ranges vary from 2 to 29 km<sup>2</sup> and male home ranges are always bigger than those of females. This is explained by the greater energy needs of males than females and by the reproductive benefits associated with larger ranges in which males can maintain contact with more females (Birks, 2017). Within home ranges, pine martens tend to concentrate their time in the best foraging habitat and such favourite patches, known as core areas, average 1 km<sup>2</sup> in males (40% of the home range) and 0.4 km<sup>2</sup> in females (30% of the home range) (Zalewski & Jędrzejewski, 2006).

Recent evidence has arisen of some sociability among pine martens, especially during winter when fully adult pine martens may tolerate the presence of subdominant youngsters in their ranges, before the latter disperse in early spring to find their own territories (Birks, 2017).

#### 1.3.4 Foraging behaviour and diet

The pine marten is a generalist omnivore, with presumably the broadest food niche compared to other species in the Mustelidae family, eating a broad spectrum of different food according to local availability and showing seasonal patterns in its foraging behaviour (see review in De Marinis & Masseti, 1995). In Scotland, the pine marten diet is essentially dominated by three food groups (Lockie, 1961; Caryl et al., 2012b): small mammals (41%), fruit (30%) and birds (25%), with 93% of the food found in scats accounted for by just a few principal foods: field vole, Microtus agrestis (40%), berries (30%; bilberry, Vaccinium myrtillus 11%, rowan berries Sorbus aucuparia 19%) and small birds (25%) (Caryl et al., 2012b). Large birds and eggs are also taken but only in small proportions. Other food groups are eaten but make little contribution to the pine marten diet: large mammals (1.7%) taken as carrion of deer and sheep; medium mammals like rabbits and squirrels; invertebrates (1.4%, comprised of Hymenoptera – Bombus, Vespa – and Coleoptera – Carabidae, Curculionidae, Scarabidae). Very infrequently, pine marten will also prey upon amphibians and reptiles. Small mammals represent the major component of the pine marten's diet throughout continental Europe, with representatives from three genuses commonly taken: Apodemus, Microtus and Myodes. Among these, continental pine martens mostly prey on *Myodes* voles which, being a typical forest-dwelling species are therefore more commonly found in pine martens' preferred habitat. Contrary and surprisingly, Caryl and colleagues (2012b) found that field vole, a species generally restricted to open grassland and hedgerows, represents the staple food for pine martens in Scotland despite being the least abundant small mammal species within the Scottish marten ranges (accounting for 12±6% of the estimated small mammal population compared to 43±9% accounted for by the bank vole, Myodes glareolus and 30±8% accounted for by the wood mouse, Apodemus sylvaticus), and the field vole dominates the total composition of the pine marten diet for most of the year apart from the autumn when pine martens switch their diet towards fruit. In summer pine martens have a more varied diet, consuming approximately equal proportions of small mammals, as well as small birds and fruit as they become available. Conversely, in winter and spring their diet mainly consists of small mammals and in autumn there is a significant increase in fruit consumption and fewer small mammals are taken despite their increased abundance at that time of the year. Both bilberry and rowan berries become most abundant during autumn, therefore suggesting that fruit foraging preference is frequency-dependent while small mammal foraging preference is frequency-independent (Caryl et al., 2012b). A diet switch from rodents to fruits when both are at peak abundance has been observed in different pine marten populations (Rosellini et al., 2008; Caryl et al., 2012b) and in other Martes species (Zhou et al., 2011), presumably due

to the fact that fruits are easier to obtain than actively hunting for prey (relatively to time and effort invested) and therefore frugivory may represent a more energy-efficient foraging strategy (Zhou et al., 2011; Caryl et al., 2012b). This facultative foraging behaviour means pine martens can adapt to fluctuations in food resource availability, allowing them to benefit from a wide biogeographic range.

### 1.4 GENETIC EVIDENCE: THE ORIGINS OF PINE MARTEN POPULATIONS IN BRITAIN

The origins and persistence of pine marten populations across Britain were investigated through a comparison of the mitochondrial haplotype compositions of contemporary populations (post-1981) with those of historical populations (pre-1981) from the same geographic areas (Jordan et al., 2012). Historically, pine marten populations in Britain appeared to have two haplotypes: haplotype *i* in England and Wales, and both haplotype *i* and a in Scotland, with haplotype a predominating. Scottish contemporary populations still contain both haplotypes, indicating they directly descend from historical populations from the same regions; in contrast, English and Welsh contemporary populations contain primarily, if not exclusively, haplotype a individuals and therefore they appear to be genetically distinct from historical populations from the same geographical areas. This shift might have occurred by rapid genetic drift in the mitochondrial DNA control region or, more likely, is the result of escapes and/or deliberately released animals, or perhaps natural southward migration from a southern Scottish source population, as for example the undergoing natural re-colonization seen in Cumbria and Northumberland (Croose, 2021). If haplotype *a* records found in northern England can be explained by such a natural dispersal from Scotland, the same explanation seems highly unlikely for those haplotype a records from North Wales and central and southern England due to the vast distances involved and the significant barrier (urban conurbations) to dispersal (Jordan et al., 2012). Pine martens are easy to catch where they are common and covert releases between stronghold and other areas have been happening for many years (Birks, 2017). Between the 1980s and 2000s at least seven pine martens were captured from a Scottish shooting estate and released in the west of England, possibly explaining the genetic origin of marten populations in this area (Birks, 2017). Genetic evidence suggests the historical haplotype *i* was lost from England and Wales during the 20<sup>th</sup> century, with the last one found in England from a 1924 specimen and in Wales from a 1950 specimen, increasing the potential conservation value of the rare haplotype *i* individuals from presentday Scottish populations.

Overall, present-day almost exclusive haplotype *a* composition of pine marten populations in England and Wales suggests a Scottish origin, although occasional evidence of continental haplotypes (known to occur in captive pine marten populations; Jordan et al., 2012) suggests release or escape from captivity (Birks, 2017).

## 1.5 THE TWO MOORS PINE MARTEN PROJECT

A potential conservation translocation project to reintroduce wild, free-living pine martens to Dartmoor and Exmoor National Parks, is being investigated by the Two Moors Partnership, a partnership made up of several organizations including Devon Wildlife Trust, Woodland Trust, National Trust, Dartmoor National Park Authority, and Exmoor National Park Authority. Expert advice and support to the project is being given by Vincent Wildlife Trust, Forestry England, Natural England, and NatureScot. The proposed plan involves capturing wild, free-living pine martens from a range of sites across the Highlands of northern Scotland to be transported, in a purpose-fitted and climate-controlled animal transport vehicle, to the release sites in Dartmoor and Exmoor National Parks, southwest England. The exact source sites are yet to be confirmed, nevertheless individuals will not be harvested from those donor populations used for the Forest of Dean reintroduction project, but they will likely be taken from those populations used for the Welsh 2015-2017 reinforcement project (Ed Parris Ferris & Tracey Hamston personal communication, 8<sup>th</sup> March 2023). A minimum of five years gap before harvesting the same donor population will therefore be observed, as per recommendation made in the pine marten long-term recovery plan (MacPherson & Wright, 2021). Potential release sites can be seen in Fig. 2.

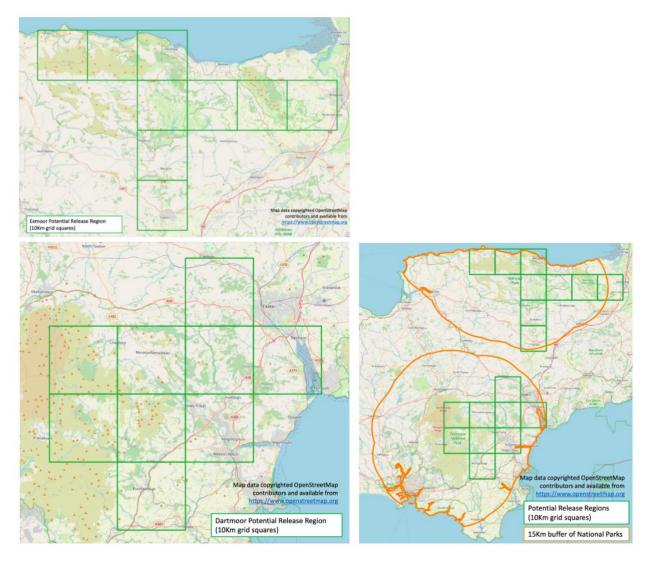


Figure 2. Potential release sites in the Dartmoor and Exmoor regions. Maps provided by Tracey Hamston, DWT.

Pine marten sightings, both confirmed and not, have been reported from the Dartmoor and Exmoor regions in the last few years and they are likely of illegally released individuals (Ed Parr Ferris & Tracey Hamston, personal communication, 8<sup>th</sup> March 2023). Considering the sensitivity of such data, the exact location of sightings is not provided in this report. The

nearest pine marten populations are the one reintroduced in the Forest of Dean, Gloucestershire, and the one recently confirmed in the New Forest, Hampshire.

The capture/translocation and husbandry protocol used for the Forest of Dean reintroduction project will be applied to the Two Moors reintroduction (Jenny MacPherson, personal communication, 13<sup>th</sup> March 2023) and it is outlined below here.

#### Capture/Transport

At the identified source sites, the translocation team will set up traps at dusk and then check them before dawn. All traps used are Tomahawk 205 live capture traps which will be fitted with a soft wooden bite bar, to prevent dental injuries, and covered with a layer of hay for the animals to pull in for bedding and as a distraction, and a further layer of moss for insulation and camouflage. Traps will be baited with whole egg, peanuts, and jam as well as with high moisture content fruit. An initial assessment of all captured pine martens will be carried out by the translocation team and the potential candidates for translocation will be taken, in their covered trap, to a nearby central point for further assessment (veterinary health examinations). All veterinary equipment is mobile and will be set up in close vicinity of each trap site in the back of a Freelander with a covered awning. One end of the trap will be fitted with a canvas handling cone and the animals will be moved into it using trap combs. Animals will then be lightly anaesthetized with gas anaesthesia for a short period of time to allow health examinations and collection of biometric data and hair samples. Animals will be monitored, both visually and with a real time rectal thermometer and pulse oximeter, throughout the entire duration of anaesthesia. Heat mats will be available if needed. Animals assessed as suitable for translocation (adults in good health, approximately equal sex ratio) will be microchipped and fitted with a radio collar and then placed in a sky kennel, placed in a quiet location away from other martens and fitted with hay bedding, to allow recovering from anaesthetic. An electrolyte solution first and a small amount of food (blueberries) after, will be offered once animals have recovered. Pine martens will then be transported overnight to the release sites in Dartmoor and Exmoor National Parks. The specially fitted transport vehicle is provided with solid divisions between each animal and the drivers. Stops are made every three hours en route and the animals visually checked and offered water and food. The translocation team will be in contact with the drivers while animals are in transit and a transport plan, detailing wildlife vet centers en route able to provide emergency assistance if needed, is provided. Once arrived at the National Parks, sky kennels will be unloaded from the van and transferred into a 4x4 vehicle which is needed to finally access the release sites in the forest. The total time from being trapped to being in their individual, large soft-release pens at the release sites, is no more than 24 hours.

#### Husbandry in the soft-release pens

Once at the release site, each pine marten will be released in a soft-release pen which is provided with a den box and two trail cameras to monitor the animal's movement and behaviour. After arrival, pine martens will be kept in the soft-release pens for five days and supplementary food, consisting of three dead chicks, three eggs and a peanut/jam/raisin mixture, will be provided daily. Chicks and fruit mixture will be placed onto a wooden feeding platform, while the eggs will be hidden around the pen. Water will be provided in a stainless-steel metal bowl. A record of eaten/uneaten food will be kept, and same amount of food will be provided fresh every day. Feeding platforms will be cleared and wiped daily with

moss/damp grass. Any scats will be collected and stored in labelled bags with the animal ID, date, and location. All husbandry will be carried out quietly by one or two people. In case of an animal showing signs of distress (i.e., excessive digging, chewing of wire) at being held inside the soft-release pen, the animal will be allowed to leave the enclosure earlier than five days. On release day, camera trap footage will be checked and if the animal is still in apparent good health and behaving normally then it will be cleared for release. The soft-release pen door will be open at approximately 4pm/early evening, and a camera trap will be set up facing the door. A tracking vehicle will be positioned a distance away to monitor the signal of the radio collar to detect the departure of the pine marten from the pen. Released animals will be radio tracked each day to locate their movements and where they are denning. Footage from the camera trap set in front of the pen's door will be checked to record the exact time the marten left the pen. Supplementary feeding will continue for few nights following release and camera traps will be checked in case of a pine marten returning to the pen. In case of pine martens returning to the pen, an alternative feeding station will be provided off the ground nearby as the soft-release pen will need to be used for the next translocated animal. This alternative feeding station will be camera trapped and will be cleared daily as in the release pens. After pine martens have left their soft-release pen, and before the arrival of the next translocated animal, used pens will firstly be checked using VHF tracking equipment to ensure the animal is not still in the pen. Den boxes will be removed and replaced with new ones (used den boxes will be mounted on a tree elsewhere in the release area). Wooden feeding platforms will be removed and replaced with new ones. Any heavily scratched or chewed wood/foliage within the pen will be removed and replaced. Water bowls will be washed in dishwasher and returned to pens. Pens that contained males will be designated to arriving females and vice versa, avoiding the same sex to be put in the same pen on consecutive release.

# 2 ASSESSING THE RISKS FROM DISEASE IN WILDLIFE TRANSLOCATIONS FOR CONSERVATION PURPOSES

Wildlife translocations for conservation purposes (reintroduction, reinforcement, ecological replacement, and assisted colonization) have become a key conservation tool to help restore species and/or ecosystem functions (IUCN 2013). Any animal should always be seen as a socalled biological package (Davidson & Nettles, 1992), that is a host and its parasites, and so when animals are moved their parasites are moved too and this may result in risks from disease associated with wildlife translocations. The potential impact of infectious disease on the outcome of wildlife conservation interventions has only recently been recognized and detrimental effects may occur in the focus species or in other species within the wider destination ecosystem. Disease associated with translocations can occur because (i) infectious agents or strains of these agents, carried by translocated individuals, and novel to the release site (SOURCE HAZARDS), may be transferred to species at the destination; (ii) infectious agents found at the release site, to which the translocated animals are naïve (DESTINATION HAZARDS), may cause disease in the translocated individuals; (iii) commensal organisms (CARRIER HAZARDS) which may be carried by translocated animals can trigger disease when stressors reduce host immunocompetence and alter the host-parasite relationship; (iv) infectious agents which may be novel to the translocated animals and/or the release site may be encountered during transport (TRANSPORT HAZARDS); (v) non-infectious and infectious agents present during transit or at the release site, but not novel to the population (POPULATION HAZARDS), could potentially have a negative effect at the population level.

The IUCN Guidelines for Reintroduction and other Conservation Translocations (2013) recommended health/disease monitoring of animals at all stages of a translocation and current scientific opinion is that a Disease Risk Analysis (DRA) should be built into every translocation program to address the disease risks of translocation and allow for mitigation measures (Davidson & Nettles, 1992; Leighton, 2002; Miller, 2007; Sainsbury & Vaughan-Higgins, 2012). The OIE and IUCN have indeed produced a guideline for this purpose (OIE and IUCN, 2014; Jacob-Hoff et al., 2014). DRA provides a structured, evidence-based process that can help decision makers understand the risks from disease-causing agents on translocation objectives and make decisions in light of these (Jacob-Hoff et al., 2014). The Department for Environment, Food and Rural Affairs has recently produced the English Code for Conservation Translocations (DEFRA, 2021) that is based on the aforementioned international standard established by the IUCN and puts guidelines into a national context, thus providing guidance for conservation projects arising in England. The English Code is designed to be read and used alongside the IUCN guidelines.

# 3 AIMS OF THIS DISEASE RISK ANALYSIS FOR PINE MARTEN REINTRODUCTION

In this report we used the ZSL disease risk analysis method, as published by Sainsbury and Vaughan-Higgins (2012) and later modified by Bobadilla-Suarez et al. (2017) and Rideout et al. (2017), to 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 wild pine martens from source sites across the Highlands in northern Scotland (exact sites to be confirmed) to release sites in Dartmoor and Exmoor National Parks, southwest England. The ZSL method was originally developed from previous qualitative DRA methods for wildlife (Davidson & Nettles, 1992; Leighton, 2002) and domestic animals (Murray et al., 2004), and to date it has been applied to more than thirty conservation translocations.

The findings from this DRA, in conjunction with other information as detailed in the IUCN Guidelines on Conservation Translocations and in the English Code for Conservation Translocations (e.g., genetic, economic and population viability), will help the steering committee, other key stakeholders, and the regulatory authorities to decide whether to reintroduce free-living wild pine martens to Dartmoor and Exmoor National Parks. If translocation is agreed as a productive way forward, the DRA will guide the steering committee on how best to reduce risks from disease.

## 4 MATERIALS AND METHODS

A qualitative DRA was carried out to determine the risks from disease associated with a reintroduction of free-living wild pine martens from source sites across the Highlands in northern Scotland, to release sites in Dartmoor and Exmoor National Parks, southwest England.

The ZSL DRA method follows a similar structure to the World Organization for Animal Health (formerly OIE, now WOAH) guidelines for DRA in domestic animal movements between countries (Murray et al., 2004) but adapts it for use in wild animals by (i) using a holistic approach to hazard inclusion, including those not known to cause harm; (ii) defining hazards according to the interactions between the translocated host's immunity and the parasites with which the host interacts; (iii) defining hazards according to geographical and ecological barriers crossed; and (iv) considering both infectious and non-infectious hazards. The method involves a six-step process as follows:

- 1) description of the translocation pathway
- 2) consideration of geographical and ecological barriers
- 3) hazard identification including justification of hazard status
- 4) disease risk assessment
- 5) disease risk management
- 6) disease risk communication

## 4.1 TRANSLOCATION PATHWAY AND GEOGRAPHICAL/ECOLOGICAL BARRIERS

A translocation pathway is a visual representation 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., 2017). A translocation pathway has been drawn to identify the exact route pine martens will take from their source to final destination and how they will be captured and transported in order to identify where and when potential hazards might arise during translocation.

A major consideration in any given translocation pathway is whether any geographical (rivers, mountain ranges, seas, waterfalls) or ecological (physical, behavioral, reproductive characteristics) barriers are to be crossed, in which scenario source and destination hazards will come into play with an increased probability of translocated or recipient populations being exposed to novel infectious agents. Such distinction is crucial because empirical, observed evidence shows that the major epidemics of disease associated with translocations have primarily arisen from source hazards (Cunningham, 1996; Dobson & Foufopoulos, 2001; Sainsbury & Vaughan-Higgins, 2012). If source and destination environments are not separated by barriers and the translocated populations, and closely related species and their parasites, are contiguous then it can be assumed that there is a high probability that novel (source and destination) hazards are absent or minimal in a given translocation and therefore managers should be confident that the overall risk from disease is markedly reduced (Bobadilla-Suarez et al., 2017).

## 4.2 HAZARD IDENTIFICATION AND JUSTIFICATION OF HAZARD STATUS

A hazard is defined as a biological, chemical, or physical agent, or a condition of an animal or animal product with the potential to cause disease. Hazards include infectious agents and noninfectious agents (such as toxins and trauma).

In this DRA, we used the list of potential hazards that was created for the DRA we carried out for the GWT reintroduction of pine martens to the Forest of Dean and lower Wye Valley (Carraro & Sainsbury, 2019) and we reviewed the risk associated with each identified hazard in light of the proposed translocation pathway from Scotland to Devon and analyzed the risk from disease from newly identified hazards as appropriate. We also reviewed the risk from SARS-CoV-2 which was firstly assessed in 2021 for the reintroduced Forest of Dean population (Carraro et al, 2021). Both micro and macro parasites known to be present in pine martens and in Mustelidae in general, as well as multi-host parasites, were included in the list of potential hazards. We used the search engines of Google Scholar, Science Direct, PubMed, and the UCL Library service to review the relevant literature. Through consideration of (i) geographic distribution, (ii) occurrence, (iii) pathogenesis, (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 in Box 1 (justification of hazard status). When feasible, we included evidence for susceptibility of translocated pine martens and other species at the destination 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 the 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 that might represent a disease risk, and similarly assigned these to their respective hazard category.

## Box 1. Hazard Categories (Sainsbury & Carraro, 2023)

- SOURCE HAZARDS, those parasites (or strains of those parasites) present at the source site which would be novel at the destination site. Translocated animals are a potential vehicle for introduction of these parasites to the destination site.
- DESTINATION HAZARDS, those parasites (or strains of those parasites) absent at the source but present at the destination site, thus novel to the translocated animals.
- CARRIER HAZARDS, those commensal parasites to which the source population has coadapted and co-evolved and are effectively carried by translocated animals. They may cause disease, in transit or at destination, when the host is under stress associated with translocation or is subjected to factors that affect the host/parasite relationship, such as alterations in host density.
- TRANSPORT HAZARDS, those parasites that may be encountered during transport (between source and destination) which may be novel to the translocated animals and/or the release environment. Translocated animals can be a potential vehicle for these parasites.
- POPULATION HAZARDS, those infectious and non-infectious agents present during transit or at destination, not novel to the translocated animals, which could potentially have a negative impact at the population level.

## 4.3 DISEASE RISK ASSESSMENT

This component of the DRA involves four steps:

- <u>Release assessment</u>: describes the biological pathway through which a translocated animal (free-living, wild pine marten in this case) could become exposed and infected (or contaminated) with a particular hazard at the source site. Estimates the probability of translocated animals being infected or contaminated when translocated, thus potentially 'releasing' the hazard at destination.
- 2) <u>Exposure assessment</u>: describes the biological pathways that might permit individuals of the same or similar species at the destination to be exposed to, and infected with, the hazard. Estimates the probability of this exposure and infection occurring and the probability of dissemination of the hazard through populations at the destination.
- 3) <u>Consequence assessment</u>: estimates the probability of at least one translocated animal becoming infected or contaminated. Identifies the biological, economic, and environmental consequences associated with the release, establishment and dissemination of the hazard and their magnitude. Includes consequences for the translocated individuals, resident population of the same species (if present) and for the wider ecosystem at the destination. Estimates the probability of the occurrence of such consequences.
- 4) <u>Risk estimation</u>: summarises the results and conclusions of the release, exposure, and consequence assessments and provides an overall risk estimation.

Probability categories assigned to events in the release, exposure, and consequence assessments are chosen using the justifications outlined in Table 1. 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 risk estimation.

In the ZSL DRA method, by definition, destination and population hazards are already present at the destination environment and therefore a release assessment is not included for these hazard categories.

PROBABILITY CATEGORY	INTERPRETATION
Negligible	Event is so rare that it does not merit to be considered
Very Low	Event is very rare but cannot be excluded
Low	Event is rare but does occur
Medium	Event occurs regularly
High	Event occurs very often
Very High	Event occurs almost certainly

Table 1. Interpretation of probability categories used in this disease risk assessment (table from EFSA Panel on Animal Health and Welfare 2006, adapted from Murray et al., 2004).

# **5 RESULTS**

## 5.1 Translocation pathway and evaluation of geographical/ecological barriers

The proposed pine marten reintroduction involves the movement of free-living, wild animals between the identified source sites spread across the Highlands in northern Scotland and the release sites in Dartmoor and Exmoor National Parks. The translocation pathway is visually represented in Fig. 3.

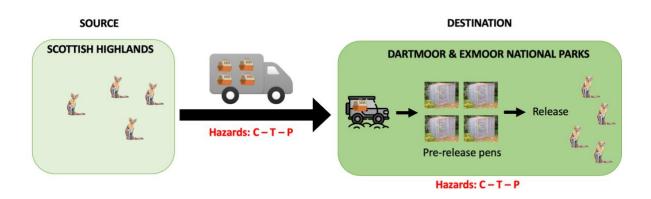


Figure 3. Proposed translocation pathway for pine marten reintroduction to Dartmoor and Exmoor National Parks, southwest England. No barriers are crossed, and it is shown here where and when potential hazards, indicated as C, carrier; T, transport; P, population (see Box 1 for hazard definitions), might arise during translocation.

Both source and destination sites are situated within Great Britain and approximately 600 miles apart from each other. Considering the widespread distribution of pine martens throughout Great Britain before the 19<sup>th</sup> century, and their dispersal behaviour, it would be expected that most populations, at that time, were contiguous and that there was parasite flow between them, resulting in pine marten populations across England, Wales and Scotland having similar parasite complements. When the pine marten population declined in the 19<sup>th</sup> century and populations in Wales and northern England / Scotland became separated, it might be expected that these populations' parasite complements may have developed differences due to a mix of parasite loss due to stochastic processes in small host populations, or possibly through evolutionary divergence.

The pine marten population in the Forest of Dean is the nearest core population to the proposed destination sites in southwest England and the only one for which the origin of animals is known having them all been sourced from the Scottish Highlands. This recovering population has been dispersing since release, with one marten settling as far as the Stroud Valleys, Gloucesterhire. The several pine marten sightings reported from the Dartmoor and Exmoor regions in the last few years are likely to be the result of a covert release of animals, possibly captured from Scotland, although we can only speculate about their origin. Another pine marten population is found in central southern England, the recently established one in the New Forest, Hampshire. The origin of these pine martens is also not certain. It seems likely

that pine marten populations at the identified source sites in Scotland will have similar parasite complement to the recently reintroduced pine marten population in England.

Some species of the *Mustelidae* family are sympatric and can coexist in the same environment. The coexistence of ecologically similar species usually relies on the differentiation of at least one or more aspects of their ecological niches, such as time, space, and resources exploitation (in Wereszczuk & Zalewski, 2015). For example, the study by Wereszczuk and Zalewski (2015) suggests that habitat segregation facilitates the coexistence of pine and stone martens (Martes foina) in the Białowieża Forest. Other studies, however, have found evidence of pine marten having similar patterns of resource exploitation and overlapping habitat use and diet with the weasel (Mustela nivalis), the polecat (Mustela putorius), and the badger (Meles meles) (Sidorovich et al., 2006; Lanszki & Heltai, 2011). Seven species of mustelid live in Britain of which six are native, namely the weasel, the stoat (Mustela erminea), the polecat, the pine marten, the otter (Lutra lutra) and the badger, and one is a non-native that was released from fur farms, the American mink (Mustela vison) (McDonald, 2002). The weasel, the stoat, the badger, and the American mink are widespread and thought to be relatively common across Britain (Harris et al., 1995); the polecat has spread out from its historical stronghold in mid-Wales and has recolonized much of southern and central England, reaching parts of southwest England and East Anglia and some individuals are also present in north-west England and parts of Scotland (VWT 2018). Pine marten populations' contiguity and co-existence with different mustelid species in the same environment may have assisted, and be assisting, parasite transfer between the remnant populations of pine martens in England and Wales, and the population in Scotland and further reduces the likelihood of ecological barriers between these populations. The important role of geographical overlap in pathogen sharing in mammals has been previously demonstrated by Davies & Pedersen (2008).

In view of the above, there is good evidence that ecological and/or geographical barriers will not be crossed during reintroduction of free-living wild pine martens from Scottish Highlands to Dartmoor and Exmoor national Parks, southwest England. Novel source hazards, for which the reintroduced pine martens would act as a vehicle, and destination hazards will both highly likely be absent along the proposed translocation pathway and we have therefore mainly focused our analysis on the risk arising from carrier, transport, and population hazards. We have assumed that pine martens will be taken from a donor site with parasite connectivity, through sympatric mustelids and recent translocations, to pine marten populations in the vicinity of the destination site. However, data are scarce, and the current parasite and disease status of British pine martens is not fully known, therefore the presence of novel parasites in some populations cannot be discounted and we know, from experience, that disease outbreaks due to introduced infectious agents can take many decades to develop and are very difficult to control (Sainsbury et al., 2008). Consequently, post-release health surveillance will be extremely important to detect emerging diseases at the reintroduction sites and will potentially generate crucial information which can be used to revise the disease risk analysis thus improve the outcome of any other future pine marten conservation translocation project.

## 5.2 Hazard identification

The potential hazards identified for the Forest of Dean pine marten reintroduction (Carraro & Sainsbury, 2019; Carraro et al., 2021) have been considered and re-evaluated for this DRA. A list of the 72 potential hazards (66 infectious and 6 non-infectious) is provided in Table 2. Based on scientific review, sixteen of these hazards were prioritised as requiring a full risk analysis in order to determine the risk from disease arising as a consequence of the proposed pine marten reintroduction to Dartmoor and Exmoor National Parks.

Those hazards which were fully assessed are here listed by category:

- Fully assessed CARRIER HAZARDS included *Leptospira* spp.; *Yersinia* spp. (*Yersinia pseudotuberculosis*, *Yersinia enterocolitica*); *Hapatozoon* spp. *Neospora caninum*; *Toxoplasma gondii*.
- Fully assessed POPULATION HAZARDS included Canine Distemper Virus (CDV); Canine parvovirus 2 (CPV2); Carnivore amdoparvovirus 1 (previous Aleutian mink disease virus AMDV); Highly Pathogenic Avian Influenza Virus (HPAIV); Low Pathogenic Avian Influenza Virus (LPAIV); *Mucoraceae* spp.; *Toxoplasma gondii*; Anticoagulant Rodenticides (ARs); Illegal persecution; Trauma.
- Mycobacterium bovis was fully assessed as a DETINATION HAZARD
- SARS-CoV-2 was fully assessed as a TRANSPORT HAZARD

The remaining fifty-six potential hazards received a detailed scientific review as described in Appendix One. These hazards included the viruses Louping ill virus (LIV), Canine adenovirus 1 (CAdV1), Canine coronavirus (CCoV), Ferret coronavirus, Mustelid herpes virus 1 (MusHV1), Canine parainfluenza virus, Feline parvovirus (FPV), Mink enteritis virus (MEV), Rotavirus; the bacteria Anaplasma phagocytophilum, Bartonella spp., Borrelia burgdorferi, Clostridia spp., Coxiella burnetii, Helicobacter spp., Lepromatous mycobacteria, Listeria monocytogenes, Mycobacterium microti, Mycobacterium avium, M. avium subsp. Paratuberculosis, Mycoplasma spp., Respiratory bacteria, Salmonella spp., Streptococcus spp.; the fungus Emmonsia spp.; the protozoa Cryptosporidium spp., Eimeria spp., Isospora spp., Encephalitozoon cuniculi, Giardia intestinalis, Sarcocystis spp.; the endoparasites Angiostrongylus vasorum, Aonchotheca putorii, Biliary trematodes (Pseudamphistomum truncatum; Metorchis albidus), Capillaria hepatica, Crenosoma petrowi, Eucoleus aerophilus, Filaroides martis, Molineus patens, Pearsonema plica, Skrjabingylus petrowi, Strongyloides martis, Taenia martis, Toxocara spp., Trichinella spp., Uncinaria criniformis; the ectoparasites Demodex spp., fleas, lice, Lynxacarus mustelae, Otodectes cyanotis, Sarcoptes scabiei, Ixodes spp.; the non-infectious capture myopathy, environmental pollutants, extreme environmental conditions. According to the scientific review, these hazards are, at least currently, likely to pose very low, if not negligible, risk as a result of the proposed pine marten reintroduction and therefore a full disease risk analysis was not completed. However, these potential hazards should be re-evaluated as further information becomes available and our understanding of disease in pine marten improves.

Table 2. Potential hazards identified for the reintroduction of free-living wild pine martens to Dartmoor and Exmoor National Parks, southwest England. Highlighted in green are those hazards which were prioritized and received a full disease risk analysis. A detailed scientific review is provided in Appendix One for the remaining hazards.

(\*): Because of the paucity of data available on both infectious and non-infectious hazards in free living wild pine martens, a qualitative judgement of pine marten susceptibility to some hazards, based on expert opinion, was used when it could not otherwise be supported by evidence in the scientific literature. Pine martens were considered to be "likely susceptible" to those pathogens isolated in closely phylogenetically related species but also to those multi-host pathogens known to infect many other mammalian families and orders. Similarly, non-infectious hazards known to be associated with morbidity and mortality in other carnivores were considered 'likely susceptible'. D = reported susceptibility to disease; I = reported susceptibility to infection.

POTENTIAL HA	ZARD	Pine marten susceptibility	Other susceptible mustelids / Other reasons for inclusion	Reference	Hazard Category
VIRUSES	Canine distemper virus (CDV)	YES (I, D)		Philippa et al., 2008; Ryser-Degiorgis & Origgi, 2011	POPULATION
	Canine parvovirus 2 (CPV2)	YES (I)	- Stone marten (I)	Frölich et al., 2005	POPULATION
	Carnivore amdoparvovirus 1 (previous Aleutian mink disease virus, AMDV)	YES		Fournier-Chambrillon et al., 2004	POPULATION
	Louping ill virus (LIV)	YES (I, D)		SRUC VS disease Surveillance, August 2018	CARRIER
	SARS-CoV-2	YES Seropositivity reported	- American mink (I, D) - Domestic ferret (I, D)	Oreshkova et al., 2020; Molenaar et al., 2020; Davoust et al., 2022; Shi et al., 2020	TRANSPORT

Canine adenovirus 1 (CAdV1)	No reports,	- Stone marten (I)	Philippa et al., 2008;	Possibly
	LIKELY*	- Fisher (I)	Philippa et al., 2004	POPULATION
Canine coronavirus (CCoV)	No reports, LIKELY*	- Fisher (I)	Philippa et al., 2004	Not assigned
Ferret coronavirus	No reports, LIKELY*	- Domestic ferret (D)	Graham et al., 2012; Thomas et al., 2012	Not assigned
Highly Pathogenic Avian Influenza Virus (HPAIV)	No reports, LIKELY*	- Stone marten (I, D)	Klopfleisch et al., 2007	POPULATION
Low Pathogenic Avian Influenza Virus (LPAIV)	No reports, LIKELY*	- Mink (I)	Reperant et al., 2009	POPULATION
Mustelid herpes virus 1 (MusHV1)	UNKNOWN	- Eurasian badger (I)	Banks et al, 2002; King et al., 2004	Not assigned
Canine parainfluenza virus	UNKNOWN	<ul> <li>European mink (I)</li> <li>American mink (I)</li> <li>Polecat (I)</li> <li>No detection in pine marten</li> </ul>	Philippa et al., 2008	Not assigned
Feline parvovirus (FPV)	UNKNOWN	- Mustelidae spp.	Decaro et al., 2012	Not assigned
Mink enteritis virus (MEV)	UNKNOWN	- Mustelidae spp.	Steinel et al., 2001	Not assigned

	Rotavirus	No reports, LIKELY*	- Domestic ferret (D) - Captive black-footed ferret (D)	Williams & Thorne, 1996	CARRIER
BACTERIA	Leptospira spp.	YES (I)		Moinet et al., 2010	CARRIER
	Yersinia spp. (Y. pseudotuberculosis, Y. Enterocolitica)	YES (D)		Mair, 1973; Najdenski, 2012	CARRIER
	Anaplasma phagocytophilum	No reports, LIKELY*	Multi-host pathogen	Birtles, 2012b	Not assigned
	Bartonella spp.	No reports, LIKELY*	Multi-host pathogen	Birtles, 2012a	CARRIER
	Borrelia burgdorferi	No reports, LIKELY*	Multi-host pathogen	Ytrehus & Vikoren, 2012	Not assigned
	Clostridia spp.	No reports, LIKELY*	- Otter (D) Multi-host pathogen	Simpson et al., 2008	CARRIER
	Coxiella burnetii	No reports, LIKELY*	Multi-host pathogen	Ruiz-Fons, 2012	Not assigned
	Salmonella spp.	No reports, LIKELY*	- Eurasian badger (I)	Wilson et al., 2003; Gaffuri & Holmes, 2012	CARRIER
	Helicobacter spp.	No reports, LIKELY	- Ferret (I, D)	Mcdonald & Larivière, 2001	CARRIER

Lepromatous mycobacteria	UNKNOWN	Multi-host pathogen	Meredith et al., 2014	Not assigned
Listeria monocytogenes	No reports, LIKELY*	Multi-host pathogen	Ferroglio, 2012a	CARRIER
<i>Mycobacterium bovis</i>	No reports, LIKELY*	<ul> <li>Eurasian badger (I)</li> <li>Wild ferret (I)</li> <li>Stone marten (I)</li> <li>Stoat (I)</li> <li>Polecat (I)</li> <li>American mink (I)</li> <li>Otter (D)</li> </ul>	Lugton et al., 1997; Matos et al., 2016; Ragg et al., 1995; Gavier-Widen et al., 2012; Lee et al., 2009	DESTINATION
Mycobacterium microti	No reports, LIKELY*	- Eurasian badger (I) - Ferret (I)	Kipar et al., 2014	Not assigned
Mycobacterium avium	No reports, LIKELY*	- Otter (D)	Gavier-Widen et al., 2012	Not assigned
M. avium subsp. paratuberculosis	No reports, LIKELY*	<ul> <li>Eurasian badger (I)</li> <li>Stoat (I)</li> <li>Weasel (I)</li> <li>Stone marten (I)</li> </ul>	Gavier-Widen et al., 2012; Matos et al., 2014	Not assigned

	<i>Mycoplasma</i> spp.	No reports, LIKELY*	- Captive ferret (D) - Mink (I)	Kiupel et al., 2012	CARRIER
	Respiratory bacteria	No reports, LIKELY*	Multi-host pathogen	Janeczko, 2013; Ferroglio, 2012b	CARRIER
	Streptococcus spp.	No reports, LIKELY*	- Otter Multi-host pathogen	Simpson, 2006	CARRIER
FUNGI	Mucoraceae spp.	YES (I, D)		Simpson, 2016	POPULATION
	Emmonsia spp.	No reports, LIKELY*	- Stoat - Weasel - Polecat - Otter	Simpson & Gavier- Widen, 2000; Simpson et al., 2016	CARRIER
PROTOZOA	Hepatozoon spp.	YES (I)		Criado-Fornelio et al, 2009; Simpson et al., 2005b	CARRIER
	Neospora caninum	YES (I, D)		Sobrino et al., 2008	CARRIER
	Toxoplasma gondii	YES (I)	- American fisher (I) - Mink (D)	Larkin et al., 2011; Jones et al., 2006; Deksne et al., 2017	CARRIER POPULATION

	Cryptosporidium spp.	No reports, LIKELY*	- American mink (I) - Otter (I)	Stuart et al., 2013	CARRIER
	Eimeria spp.	No reports, LIKELY*	- Ferret (D) - Eurasian badger (D)	Sledge et al., 2011; Anwar et al., 2000	CARRIER
	Encephalitozoon cuniculi	YES (I)		Hůrková & Modrý, 2006	CARRIER
	Giardia intestinalis	No reports, LIKELY*	- Captive Eurasian badger cubs Multi-host pathogen	Barlow et al., 2010	CARRIER
	Isospora spp.	No reports, LIKELY*	- Ferret (D) - Eurasian badger (D)	Sledge et al., 2011; Anwar et al., 2000	CARRIER
	Sarcocystis spp.	UNKNOWN	- Mink (D) - Ferret (D)	Dubey et al., 1993; Britton et al., 2010	CARRIER
ENDOPARASITES	Angiostrongylus vasorum	No reports, LIKELY*	- Stoat (I) - Weasel (I) - Polecat	Simpson, 2010; Simpson et al., 2016	CARRIER
	Aonchotheca putorii	YES (I)		Segovia et al., 2007; Zhigileva & Cheboksarova, 2012	CARRIER

Biliary trematodes	No reports,	- Otter	Simpson et al, 2005a;	Not assigned
(Pseudamphistomum truncatum;	UNLIKELY		Simpson et al., 2009;	
Metorchis albidus)	because of	- Mink	Torres et al., 2008	
	parasite life	- Polecat		
	cycle			
Capillaria hepatica	UNKNOWN	Widespread in rodents	Taylor et al., 2007	CARRIER
Crenosoma petrowi	YES (I)		Craig & Borecky, 1976;	CARRIER
			Segovia et al., 2007;	
			Seville & Addison,	
			1995; Zhigileva &	
			Cheboksarova, 2012	
Eucoleus aerophilus	YES (I)		Segovia et al., 2007	CARRIER
Filaroides martis	YES (I)	Likely to be Martes specific	Segovia et al., 2007;	CARRIER
			Seville & Addison,	
			1995; Zhigileva &	
			Cheboksarova, 2012	
Molineus patens	YES (I)	- Eurasian badger	Segovia et al., 2007;	CARRIER
			Jones et al., 1980	
Pearsonema plica	YES (I)		Segovia et al., 2007	CARRIER
Skrjabingylus petrowi	YES (I)	Martes specific	Stuart et al., 2010	CARRIER
Strongyloides martis	YES (I)		Zhigileva &	CARRIER
			Cheboksarova, 2012	

	Taenia martis	YES (I)		Segovia et al., 2007	CARRIER
	<i>Toxocara</i> spp.	No reports, LIKELY*	- Stone marten	Kornaś et al., 2013	CARRIER
	Trichinella spp.	YES (I)		Segovia et al., 2007	CARRIER
	Uncinaria criniformis	YES (I)		Segovia et al., 2007	CARRIER
ECTOPARASITES	Demodex spp.	No reports, LIKELY*	Demodex erminea reported from Irish stoat	Mcdonald & Larivière, 2001	CARRIER
	Fleas	YES (I)		Zielinski, 1984	CARRIER
	Lice	No reports, LIKELY*	Trichodectes retusus reported from stone marten	Visser et al., 2011	CARRIER
	Lynxacarus mustelae	No reports, LIKELY*	- Stone marten	Visser et al., 2011	CARRIER
	Otodectes cyanotis	No reports, LIKELY*	- Feral ferret	Mcdonald & Larivière, 2001	CARRIER
	Sarcoptes scabiei	No reports, LIKELY*	- Stone marten - Japanese marten	Ryser-Degiorgis et al., 2002; Makouloutou et al., 2015	CARRIER
	Ixodes spp.	YES	- Stone marten	Hofmeester & van Wieren, 2014	CARRIER

NON-INFECTIOUS	Anticoagulant Rodenticides (ARs)	No reports, LIKELY*			POPULATION
	Illegal persecution (snaring, hunting, shooting, malicious poisoning)	No reports, LIKELY*			POPULATION
	Trauma (capture/handling related)	No reports, LIKELY*	- River otter - Eurasian otter	Serfass et al., 1996; Fernández-Morán et al., 2002	POPULATION
	Capture myopathy	No reports, LIKELY*	- Otter Common in wild ungulates	Hartup et al., 1999	TRANSPORT
	Environmental pollutants	No reports, LIKELY*	- Otter	Bradshaw & Slater, 2000; Conroy et al., 2000; Simpson, 2003; Pountney et al., 2015	POPULATION
	Extreme environmental conditions	No reports, LIKELY*			POPULATION

### 5.3 Disease risk assessment

Sixteen hazards (13 infectious and 3 non-infectious) received full disease risk analysis and the disease risk assessment and disease risk management for each hazard is presented in tabular form (see Table 4 to Table 19). A summary of the risk estimations for each hazard is presented in Table 3.

Table 3. Summary of the potential risks from disease arising from the reintroduction of free-living wild pine martens to Dartmoor and Exmoor National Parks, southwest England. \* estimated risk for specific groups of translocated pine martens; \*\* estimated risk for other mammals at destination.

HAZARD	CATEGORY	OVERALL ESTIMATED DISEASE RISK
SARS-CoV-2	TRANSPORT	HIGH*
		MEDIUM**
Anticoagulant Rodenticides (ARs)	POPULATION	MEDIUM
Illegal persecution	POPULATION	MEDIUM
Canine distemper virus (CDV)	POPULATION	LOW
Highly Pathogenic Avian Influenza Virus (HPAIV)	POPULATION	LOW
Low Pathogenic Avian Influenza Virus (LPAIV)	POPULATION	LOW
Leptospira spp.	CARRIER	LOW
Yersinia spp.	CARRIER	LOW
Mucoraceae spp.	POPULATION	LOW
Hepatozoon spp.	CARRIER	LOW
Toxoplasma gondii	CARRIER	LOW
	POPULATION	LOW
Trauma (capture/handling related)	POPULATION	LOW
Canine parvovirus type 2 (CPV-2)	POPULATION	VERY LOW
Carnivore amdoparvovirus 1	POPULATION	VERY LOW
(previous Aleutian mink disease virus AMDV)		
Mycobacterium bovis	DESTINATION	VERY LOW
Neospora caninum	CARRIER	VERY LOW

POPULATION	Canine Distemper Virus (CDV)
Justification for Hazard Status	Canine distemper virus (CDV) is an RNA virus belonging to the family <i>Paramyxoviridae</i> , genus <i>Morbillivirus</i> , and is the causative agent of a serious disease in canids and other carnivores. It is a relatively fragile virus, quickly inactivated by ultraviolet light, heat and drying (Frölich, 2012) and rapidly becoming inactive outside the host (Appel & Summers, 1995). All of the common disinfectants are effective against CDV (Appel & Summers, 1995). CDV has a broad host range and within the order <i>Carnivora</i> members of nine families, including <i>Mustelidae</i> , are susceptible to infection (Appel & Summers, 1995; Frölich, 2012). During a serologic survey conducted in France, Philippa and colleagues (2008) found evidence (antibody titers) of CDV infection in the pine marten (5% seroprevalence, n=20). Susceptibility to disease has also been reported in pine martens during a canine distemper epidemic in Switzerland (Ryser-Degiorgis & Origgi, 2011). CDV affects susceptible animals of all ages and morbidity and mortality rates vary greatly among species and age groups, but higher mortality is usually seen in young animals (Appel & Summers, 1995; Frölich, 2012).
	Several dramatic as well as unexpected distemper outbreaks have caused significant mortalities in free-living wildlife species worldwide (black-footed ferrets in Wyoming, Williams et al., 1988; collared peccaries in Arizona, Appel et al., 1991; lions in Tanzania, Roelke Parker et al., 1996), including in Europe (red foxes and Eurasian badgers in Italy and in Switzerland, Martella et al., 2010; Ryser-Degiorgis & Origgi, 2011; red foxes, Eurasian badgers and stone martens in Bavaria, Sekulin et al., 2011). CDV associated disease has recently been confirmed in the deaths of four mustelid species (pine marten, Eurasian badgers, polecat, and stone marten) in Spain (Oleaga et al., 2022). To date and as far as the authors are aware, CDV infections in free-living wild carnivores have not been detected in the UK and serological tests on blood obtained from an intensively studied free-living population of wild badgers at Woodchester Park, Gloucestershire, south-western England suggested no CDV exposure (n = 182) (Delahay & Frölich, 2000). It should be noted, however, that in their study Delahay & Frölich tested for CDV infection in a rural population of badgers but not in suburban populations, for which the seroprevalence is usually higher (Frölich, 2012).
	Regardless, the virus is present in the UK and it has been responsible for a distemper outbreak in captive (domestic) ferrets across England, mainly the Midlands, (Thomas, 2012) and few confirmed cases of the disease in domestic dogs (Adamantos & Warman, 2014). Several studies have suggested domestic dogs may represent a potential reservoir of infection for wild carnivores (Frolich et al., 2000; Philippa et al., 2008; Santos et al., 2009). In the UK, in recent years, there has been a noticeable decrease in the frequency of vaccination of domestic dogs against CDV which could lead to the re-emergence of the disease once

	again (Davies, 2014), as seen in Denmark in 1991 (Blixenkrone-Møller et al., 1993).
	There is a high likelihood that CDV is present at both the source and destination sites and may represent a population hazard for the reintroduced pine martens due to the small number of individuals forming the new population, stressed from translocation, and therefore more susceptible to the effects of epidemic disease.
Risk Assessment	
Exposure Assessment	Transmission of CDV mainly occurs by inhalation of airborne virus but also through contact with oral, respiratory, and ocular fluids and other exudates containing the virus. Transplacental or neonatal infection are other possible routes (Frölich, 2012). Virus shedding begins approximately 7 days post infection and acutely infected carnivores can shed virus in all their body excretions, regardless of whether they show clinical signs or not (Appel & Summer, 1995; Frölich et al., 2000).
	There is a very low likelihood that pine martens translocated to the destination site will be exposed to CDV through direct contact (inhalation of contaminated aerosol) from other infected wild carnivores and/or domestic dogs and free-living feral ferrets because (i) drag hunting dogs and farm dogs are in the area, (ii) since becoming fully legally protected and therefore less persecuted, pine martens have shown an increasing occasional use of roofs of occupied dwellings to breed and/or to rest (Birks, 2017) and this behaviour could lead to interspecific contact with domestic species. The likelihood of this exposure occurring is estimated to be very low because i) to date no distemper outbreaks have been reported in free-living wild carnivores in the UK; ii) according to Savsnet Real Time Data (https://www.liverpool.ac.uk/savsnet/) only one of 30 samples tested positive for CDV in domestic dogs in Devon since 2019; iii) the behavioural ecology of pine martens (elusive and with an overall preference for woodland habitats rather than habitats in proximity to humans) should limit possible interspecific interaction.
	There is a high likelihood of pine martens becoming infected with CDV once exposed because they are known to be susceptible to infection (Philippa et al, 2008; Oleaga et al., 2022).
	There is a low probability of dissemination through the reintroduced pine marten population and other carnivores at the reintroduction site because although infection is highly contagious, pine martens are expected to disperse after release.
Consequence	Canine distemper is an acute or subacute highly contagious febrile
Assessment	disease that may include signs of generalized infection, respiratory disease, gastro-intestinal disease, hyperkeratosis, central nervous system disturbance, or a combination of these (Frölich et al., 2000).

	Depression, mucopurulent oculo-nasal exudates, anorexia, vomiting and diarrhoea are commonly seen. Neurological signs may occur during the acute phase or several weeks or even months later and they will also vary depending on the area of the brain affected (behaviour abnormalities, convulsions, incoordination, paresis or paralysis) (Appel & Summers, 1995; Frölich, 2012).	
	There is a medium likelihood that infection in a pine marten will develop into disease, with a medium likelihood of severe consequences for affected individuals, given the recent case described by Oleaga et al (2022). There is a low likelihood of significant biological, economic or environmental consequences through a failure of the reintroduction programme as a result of an epidemic of distemper in the reintroduced population because i) susceptibility to disease has been reported in pine martens during a distemper outbreak in Switzerland (Ryser-Degiorgis & Origgi, 2011); ii) there have been no reports of disease in pine martens during other outbreaks in European wildlife (Meli et al, 2010; Sekulin et al, 2011); iii) canine distemper has been responsible for significant epidemics in free-living populations of highly susceptible species, including mustelids (Williams et al, 1988; Martella et al, 2010; Ryser- Degiorgis & Origgi, 2011; Sekulin et al, 2011); and iv) mortality caused by CDV has recently been documented in Spain (Oleaga et al., 2022).	
Risk Estimation	There is a very low likelihood that reintroduced pine martens will be exposed to CDV at the destination, a high likelihood of infection and a low likelihood of virus dissemination amongst the reintroduced population and other wild carnivores at the destination. There is a medium likelihood of infected pine martens developing disease and a medium likelihood of significant consequences (death) in affected individuals. There is a low likelihood of failure of the reintroduction through an epidemic of canine distemper.	
	The overall risk is estimated as <b>LOW</b> .	
Risk Management		
Risk Evaluation	Based on the risk assessment above, preventative measures should be employed to reduce the risks from CDV as a population hazard.	
Risk Management Options	It is advisable to build a liaison with veterinary practices at both the source and destination sites for a better knowledge of any CDV infection occurrence in domestic species and of the current immune status in dog populations through vaccination against CDV. The destination site should be evaluated in terms of proximity to houses, farms and of the type of landscape use (hunting with dogs, farming, and recreational activities).	
	Vaccination against CDV in wild carnivores has been problematic and currently there is no safe and efficacious commercially available CDV vaccine for use in non-domestic species (Frölich, 2012). Effective surveillance and prompt gathering, and dissemination of information is	

required to try to adequately control CDV infection in wildlife. We therefore recommend pathological investigation of any wild pine marten or other carnivore found dead within a 20 km radius of the reintroduction site (with average post-release movements from 4.6 to 20.8 km, Stringer et al., 2008).
As in any given translocation, only healthy animals should be released, and stressors should always be kept to a minimum.

Table 4. Disease risk assessment and brief mitigation options for the population hazard Canine Distemper Virus (CDV).

POPULATION	Canine parvovirus type 2 (antigenic variants) CPV-2
Justification for	
Justification for Hazard Status	Canine parvovirus type 2 (CPV-2) is a small, single stranded DNA virus that belongs to the genus <i>Parvovirus</i> within the family <i>Parvoviridae</i> . Species from six families ( <i>Felidae, Canidae, Procyonidae, Mustelidae, Ursidae</i> and <i>Viverridae</i> ) within the order <i>Carnivora</i> are suspected to be susceptible to CPV-2 (Frölich et al., 2005). CPV-2 was recognised as a new virus in 1978 and is thought to have originated from feline panleukopoenia virus (FPLV). A few years after its emergence, it was completely replaced by three antigenic variants, designated CPV-2a, CPV-2b and CPV-2c, which are currently circulating in different dog populations worldwide; CPV-2b is the variant found in the UK (Decaro et al., 2011). Antigenic variants of CPV-2 have also been detected in European wild carnivores, especially wolves ( <i>Canis lupus</i> ) and red foxes ( <i>Vulpes vulpes</i> ) (Decaro et al., 2012), and, within the family <i>Mustelidae</i> , CPV-2a has been first isolated in a stone marten ( <i>Martes foina</i> ) (Steinel et al., 2001) and subsequently evidence of CPV-2 variant exposure has been confirmed in stone martens (Frölich et al., 2005; Santos et al., 2009; Duarte et al., 2013) and described in one pine marten out of two animals screened in Germany (Frölich et al., 2005). During their serological survey in Portugal, however, Santos and colleagues (2009) did not find antibodies against CPV-2 in the two pine martens tested. It must be noted, though, the very limited pine marten sample size available in the studies cited above.
	Canine parvoviruses are found both at the source and destination sites and may constitute a population hazard for the reintroduced pine martens.
Risk Assessment	
Exposure Assessment	CPV-2 is a highly contagious virus that can be transmitted through both direct contact with infected animals or, most frequently, by contact with their faeces or faeces-contaminated environment (oro-faecal route). High titers of virus are shed in the faeces of diseased animals during acute infection and shedding usually lasts a few days. Parvoviruses are very resistant and can remain infectious in the environment for weeks or even months.
	wild carnivores in the UK. The likelihood that pine martens translocated to the destination site will be exposed to CPV-2 is estimated to be very low considering no disease outbreaks in wildlife are known to the authors at the time of writing. The assumed widespread vaccination of domestic dogs against CPV-2 might also contribute to reduce the likelihood of virus circulating at the destination site. There is a medium

[]	
	likelihood of pine martens becoming infected with CPV-2 once exposed because susceptibility has been reported in the species (Frölich et al., 2005). The infection is highly contagious, and the virus is highly stable in the environment, but considering the behavioural ecology of pine martens and the fact that animals will likely disperse after release, the likelihood of the virus being disseminated amongst the reintroduced pine marten population and other carnivores at the destination site is estimated to be low.
Consequence Assessment	The pathogenesis of CPV-2 infection is still unclear in wildlife, but it has been extensively investigated in domestic dogs. Puppies aged between 4 and 12 weeks are the main target within the dog populations, but CPV- 2 disease outbreaks have been reported in adult dogs as well, even in regularly vaccinated subjects. The haemorrhagic enteritis is the predominant clinical form observed during CPV-2 infection in domestic dogs. After an incubation period of 3-7 days symptoms (including loss of appetite, depression, vomiting, diarrhoea, fever, and dehydration) develop. The virus infects white blood cells too, inducing an acute lymphopenia which is responsible for an impairment of the immune system which can predispose to secondary infections. Fatal myocarditis is occasionally reported in 2-3 weeks old puppies because of the ability of the virus to replicate in the cardiac cells. Subclinical, inapparent infections are mainly detected in adult dogs.
	In wildlife, gastroenteritis has been seen in raccoon dogs ( <i>Nyctereutes procyonoides</i> ) while no clinical disease has been noticed in association with seropositivity for CPV-2 in red pandas ( <i>Ailurus fulgens</i> ) (WOAH, 2023). No reports are available on the clinical findings of CPV-2 infection in free-ranging wild carnivores in Europe. Clinical signs observed in affected wild canids in North America were the same as in domestic dogs (Decaro et al., 2012).
	In the absence of reports of CPV-2 disease in pine martens or other mustelids we estimate the likelihood of disease in one pine marten infected with CPV-2 is very low and the likelihood of epidemic disease in reintroduced pine martens is very low. Therefore, the likelihood of significant biological, environmental, and economic consequences through the failure of the reintroduction is estimated to be very low.
Risk Estimation	There is a very low likelihood of reintroduced pine martens being exposed to CPV-2 at the destination, a medium likelihood of infection and a low likelihood that the virus will disseminate through the pine marten population. The likelihood of epidemic disease in reintroduced pine martens and of significant consequences is very low. The overall risk is estimated as VERY LOW.
Risk Management	
Risk Evaluation	Based on the risk assessment above, preventative measures should be employed to reduce the risks from CPV-2 as a population hazard.

Risk Management	It is advisable to build a liaison with veterinary practices at both the
Options	source and destination sites for a better knowledge of any CPV-2 outbreaks in domestic species. CPV-2 related disease should be considered as a differential in investigating pine martens and other carnivores found dead in the vicinity of the reintroduction site, through post-mortem examination.
	As in any given translocation, only healthy animals should be released, and stress should always be kept to a minimum.

Table 5. Disease risk assessment and brief mitigation options for the population hazard Canine parvovirus type 2 (CPV-2).

POPULATION	Carnivore amdoparvovirus 1 (previous Aleutian
	mink disease virus, AMDV)
Justification for Hazard Status	Aleutian Mink Disease Virus (AMDV), is a single-stranded DNA virus which was first reported in the 1940s as responsible for a disease in farmed mink (Canuti et al., 2015). Mink homozygous for the Aleutian (gunmetal grey colour) gene were most severely affected, thus giving the disease its name. The complete genome of the virus was fully sequenced in 1988 and classified in the genus <i>Amdovirus</i> , family <i>Parvoviridae</i> (Bloom et al., 1988). It has recently been reclassified as Carnivore amdoparvovirus 1 within the genus <i>Amdoparvovirus</i> , family <i>Parvoviridae</i> (ICTVdb, 2014). Three distinct lineages can be recognized within Carnivore amdoparvovirus 1: AMDV-1, AMDV-2, AMDV-3. For simplicity, in this risk assessment we will refer to this hazard as AMDV.
	Similar to other parvoviruses, AMDV requires actively dividing cells to replicate. In adult mink AMDV infects macrophages, while in mink kits the virus infects alveolar type II cells of the lungs and causes cell death (Canuti et al., 2015).
	AMDV primarily infects mustelids, however infection has also been reported in striped skunks ( <i>Mephitis mephitis</i> ), raccoons ( <i>Procyon lotor</i> ), bobcats, cats, dogs, mice (see Canuti et al., 2015 for a review). Rare cases of human infections have also been reported, but only in farmers after occupational exposure (Jepsen et al., 2009). Evidence of antibodies against AMDV has been documented in various free-ranging mustelid species in different European countries: in European mink ( <i>Mustela lutreola</i> ), American mink ( <i>Mustela vison</i> ), European polecat ( <i>Mustela putorius</i> ), stone marten ( <i>Martes foina</i> ) and pine marten ( <i>Martes martes</i> ) in France (Fournier-Chambrillon et al., 2004); in European mink, American mink and Eurasian otter ( <i>Lutra lutra</i> ) in Spain (Mañas et al., 2001); in European badger and European polecat in Finland (Knuuttila et al., 2015); in mink species in Denmark and Sweden (Jensen et al., 2015). In the UK, a relatively high seroprevalence for AMDV antibodies has been reported in feral free-living American mink trapped in southern England, upper Thames region (51.8% n=27, Yamaguchi & Macdonald, 2001; 61.5% n=13, Harrington et al., 2012). Evidence of infection has also been reported in domestic ferrets ( <i>Mustela putorius furo</i> ), in which disease was observed too (Welchman et al., 1993).
	AMDV is found in the UK and it is assumed to be present at both the source and destination sites and may constitute a population hazard for the reintroduced pine martens.
Risk Assessment	
Exposure Assessment	AMDV is shed in urine, faeces, saliva and blood of infected animals and transmission can occur horizontally, by direct or indirect (contaminated food, water, environment) contact, and vertically from infected females to kits (Decaro et al., 2012; Canuti et al., 2015). AMDV is highly persistent in the environment and resistant to physical (high

	temperatures) and chemical treatments. AMDV is therefore difficult to eliminate from contaminated surfaces and its stability facilitates viral spreading in the environment (Canuti et al., 2015).
	American mink represents an important source of excretion and spread of the virus and previous studies have found a relatively high AMDV seroprevalence in feral free-living individuals in southern England (Yamaguchi & Macdonald, 2001; Harrington et al., 2012). However, it is unlikely these species will overlap given the American mink is a semi- aquatic mustelid which is primarily found in wetland habitats whereas the pine marten is a semi-arboreal mustelid with a preference for mature forests. The likelihood that reintroduced pine martens will be exposed to AMDV at the destination is estimated to be low. Susceptibility to infection has also been reported in the species (although antibodies were detected in only one pine marten of 16 screened, Fournier-Chambrillon et al., 2004) and we estimate a low likelihood of pine martens become infected upon exposure to the virus.
	Considering the likely low susceptibility to infection, the behavioural ecology of pine martens and the fact that animals will likely disperse after release, the likelihood of the virus being disseminated amongst the reintroduced pine marten population and other carnivores at the destination site is estimated to be low.
Consequence Assessment	Aleutian mink disease is responsible of important economic losses in mink farming but its prevalence and significance in free-ranging carnivores is largely unknown (Decaro et al., 2012). The pathogenesis of AMDV infections has been intensively investigated in farmed mink and varies depending on the age of the animal. In adults, infection is persistent and leads to a chronic immune-complex mediated disease, a progressive wasting syndrome with various organ systems involved, increased mortality and reduced fertility (Decaro et al., 2012; Canuti et al., 2015). In kits, infection is acute and manifests as respiratory distress and fulminant interstitial pneumonia (Decaro et al., 2012; Canuti et al., 2015). Disease severity depends on the species and immune status of the host as well as on the viral strain involved (high or low pathogenic). A wasting syndrome like that seen in farmed mink has been reported in domestic ferrets and skunks (Kenyon et al., 1967; Pennick et al., 2007; Allender et al., 2008) and interstitial pneumonia has also been observed in ferrets (Welchman et al., 1993). Non-persistent, asymptomatic infections with lower antibody response and transient viremia can also occur in both mink and ferrets (Hadlow et al., 1984; Pennick et al., 2005).
	There are no reports of Aleutian mink disease in pine martens, and therefore the likelihood of epidemic disease in reintroduced pine martens is estimated to be very low. The likelihood of significant biological, environmental, and economic consequences through the failure of the reintroduction is therefore estimated to be very low.

Risk Estimation	There is a low likelihood of reintroduced pine martens being exposed to AMDV at the destination site, a low likelihood of infection and a low likelihood that the virus will disseminate amongst reintroduced pine martens and other carnivore species. Pine martens are susceptible to infection but there are no reports on clinical disease in the species, therefore the likelihood of epidemic disease and of significant consequences through failure of the reintroduction are very low. The overall risk is estimated to be <b>VERY LOW</b> .
<b>Risk Management</b>	
Risk Evaluation	Based on the risk assessment above, preventative measures should be employed to reduce the risks from AMDV as a population hazard.
Risk Management Options	It is advisable to build a liaison with veterinary practices at both the source and destination sites for a better knowledge of any AMDV outbreaks in domestic species. Aleutian disease should be considered as a differential diagnosis when released pine martens are examined post-mortem.

Table 6. Disease risk assessment and brief mitigation options for the population hazard Carnivore amdoparvovirus 1 (previous Aleutian mink diseaase virus, AMDV).

TRANSPORT	SARS-CoV-2
Justification for	SARS-CoV-2 is the causative viral agent for the COVID-19 pandemic that,
Hazard Status	SARS-COV-2 is the causalive viral agent for the COVID-19 pandemic that, as of 10 March 2023 has led to approximately 676 million confirmed human cases reported worldwide, with more than 6.9 million human deaths (WOAH, 2023). Two major hypotheses on the origin of SARS-COV- 2 have been debated over the last three years: a direct zoonotic origin (i.e., a spill over of the virus into humans from a wild animal reservoir) and the introduction of the virus into humans from a laboratory source (Casadevall et al., 2021). Based on the best existing scientific data, the laboratory leak origin is unlikely while the zoonotic origin cannot be ruled out by current evidence (Alwine et al., 2023). Our understanding of the origin of SARS-CoV-2 will continue to evolve, although it is possible that the origin may never be certain. The virus is currently considered as a human pathogen with people acting as reservoirs and sustaining transmission. Like other coronaviruses, SARS-CoV-2 is capable of infection and replication in numerous mammalian host species in addition to humans and multiple human-to-animal spillovers and transmission to domestic and wild animals has been reported. The first case of SARS-CoV-2 in animals was officially reported to WOAH by Hong- Kong in February 2021 in a dog. Since then, 775 outbreaks in animals have been reported globally, affecting 29 species in 36 countries, although these numbers might represent an underestimate because of the differing levels of surveillance between countries (WOAH, 2023).
	In mustelids, the ability of SARS-CoV-2 to infect and be transmitted has been documented under both experimental and natural settings. Notably, the domestic ferret ( <i>Mustela putorius furo</i> ) has become the best model of Covid-19 disease and several studies have experimentally demonstrated infection and transmission of SARS-CoV-2 in this species (Kim et al., 2020; Richard et al., 2020; Schlottau et al., 2020; Shi et al., 2020). Infections following natural exposure emerged firstly in intensively farmed American mink ( <i>Neovison vison</i> ) in April 2020 at two mink farms in the Netherlands, resulting in signs of respiratory disease and increased mortality (Oreshkova et al., 2020; Molenaar et al., 2020). Since then several other outbreaks have occurred in mink farms throughout Europe (Netherlands, Spain, Denmark, Sweden, Italy) (WHO, 2020a; 2020b) and in the USA (Utah) (Cahan, 2020), with the latest reported in 2023 in Italy (WOAH, 2023) SARS-CoV-2 transmission to mink through infected workers on the farm has been confirmed and spill-over events back to humans have also been reported (Oude Munnink et al., 2021). The transmission of the virus between the human and animal populations is a concern because of the genetic mutations that can occur, and new SARS-CoV-2 variants related to mink transmission were reported in Denmark in December 2020 (ECDPC, 2020; WHO, 2020a; 2020b). Farmed mink might therefore play an important role as reservoirs in the epidemiology of SARS-CoV-2, but great attention and prioritisation of studies have been advocated towards the potential role of wild mustelids as permanent reservoirs and amplifiers of SARS-CoV-2 infection in other animals (Manes et al, 2020). A recent case study of

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	free-living wild mustelids in France found three pine martens and two badgers to be seropositive for SARS-CoV-2, supporting the hypothesis that free-living wild mustelids are good epidemiological sentinels for SARS-CoV-2 (Davoust et al., 2022). In addition, infection in free-living wild American mink has been reported from Utah and Spain (Shriner et al., 2021; Aguiló-Gisbert et al., 2021).
	It is important to prevent human-to-wildlife spill-over to i) protect free- living wild species from disease and ii) avoid establishment of novel SARS-CoV-2 wildlife reservoirs which could interfere with SARS-CoV-2 control efforts. Findings from Tan and colleagues (2022) highlight the 'generalist' nature of SARS-CoV-2 as a mammalian pathogen based on the minimal adaptation that was required for viral onward transmission in novel hosts (mink and deer) following human-to-animal spillover. Even though these animal-adaptive mutations do not appear to result in a significant advantage for viral circulation in humans, surveillance in both humans and animals remains important for a better understanding of the adaptive potential of the virus and its consequences (Tan et al., 2022).
	Because pine martens are more likely to be exposed to SARS-CoV-2 during the translocation procedure, when they are in close contact with people, SARS-CoV-2 is here assessed as a transport hazard. SARS-CoV-2 might indeed represent a novel pathogen for pine martens but, given its widespread distribution within the human population in the UK, translocated pine martens will not act as a vehicle to destination for this hazard (i.e., the virus is already present at the source and destination).
Risk Assessment	
Release Assessment	The total confirmed cases of SARS-CoV-2 infection in humans in the UK are 24,641,348 as of 13 <sup>th</sup> July 2023 (WHO, 2023). Humans are exposed to SARS-CoV-2 directly through aerosol droplets, spread by coughing, sneezing, and talking from an infected individual, or indirectly through touching of contaminated surfaces (Kampf et al. 2020; Rothan & Byrareddy 2020). Since a a new more transmissible variant referred to as SARS-CoV-2 VOC 202012/01 (B.1.1.7) was reported in the UK in December 2020 (WHO, 2020c; PHE, 2021), many other variants of concern, including Omicron, have emerged worldwide and have been circulating in the UK (UK HAS, 2023). According to the latest Covid-19 infection survey delivered by the Office for National Statistics on 24 <sup>th</sup> March 2023, 2.66% (1 in 40 people) of the population in England, 2.41% (1 in 40 people) in Wales, 2.59% (1 in 40 people) in Scotland were testing positive. The level of uncertainty in these estimates is however high because of the low number of samples received. Considering viral circulation continues at the time of writing, that SARS-CoV-2 can be transmitted directly, and that the virus is persistent in the environment (Kampf et al. 2020), the current likelihood of human exposure to SARS-CoV-2 in the UK is medium. Human infection is thought to occur through

contact of viral particles with exposed mucous membranes including the eyes, nose, and oral cavity (Lu et al. 2020, Zheng 2020). There is thus a high likelihood of infection of humans with SARS-CoV-2 upon exposure to the virus.

Pine martens' exposure might occur through i) direct contact, ii) in situations when it is not possible to maintain at least 1 m distance between an infected human and a susceptible animal, and iii) indirectly through fomites (contact with human-contaminated material). Pine martens may therefore become exposed to SARS-CoV-2 during the conservation translocation procedure through respiratory and/or oral routes: i) at the source sites during trapping, health examinations, fitting of tracking harnesses; ii) at the destination sites while held at pre-release pens and at the time of release. Pine martens could also be exposed to SARS-CoV-2 during post-release monitoring when fieldworkers check den boxes and natural den sites to search for their presence. Radio tracking harnesses fitted to pine martens are designed to fall off without human intervention and no routine trapping or health examinations post-release has been, or is, planned. Reports of transmission of SARS-CoV-2 from asymptomatic human carriers, before the onset of clinical signs, have been published (Bai et al., 2020; Rothe et al., 2020; Zou et al., 2020). Therefore, asymptomatic infected fieldworkers are a potential source of exposure to pine martens. Coronaviruses can persist on inanimate surfaces for up to 28 days under the right conditions (Kampf et al., 2020), and there is experimental evidence showing that SARS-CoV-2 can persist for 72 hours on plastic and stainless steel, and for shorter time periods on copper (24 hours) and cardboard (four hours), after which viral titres are greatly reduced (van Doremalen et al., 2020).

Considering that i) conservation translocations involve close contact between field workers and reintroduced wild pine martens and ii) exposure to SARS-CoV-2 can occur indirectly through contaminated surfaces or dust particles in the air, we estimate a medium likelihood of exposure of pine martens to SARS-CoV-2 along the translocation pathway.

Mustelids are the most susceptible animals to SARS-CoV-2 (Shuai et al.,<br/>2020). Like humans, they have the angiotensin-converting enzyme 2<br/>(ACE2) receptor on the cells of the respiratory tract, which facilitates<br/>SARS-CoV-2 penetration into the host cells (via the spike protein) and<br/>infection (Lean et al., 2021). Pine martens are known to be susceptible<br/>considering infection has been detected (Davoust et al., 2022). There is<br/>therefore a high likelihood of pine martens becoming infected after<br/>exposure to SARS-CoV-2.essmentAnimal-to-animal transmission has been demonstrated in domestic

Exposure AssessmentAnimal-to-animal transmission has been demonstrated in domestic<br/>ferrets under experimental conditions and has naturally occurred within<br/>intensively farmed American mink. Onward transmission has also been

	observed in free-living wild white-tailed deer ( <i>Odocoileus virginianus</i> ), with about 30% positive sampled deer in Iowa (Kuchipudi et al., 2022) and Ohio (Hale et al., 2021), and a reported 40% seroprevalence across four U.S.A. states (Chandler et al., 2021). Pine marten's social organisation is likely to reduce onward spread of the virus. Pine martens are elusive, solitary for most of the year, with contact amongst adults being typically confined to the breeding season, thus limiting opportunities for virus transmission and persistence. We estimate a low likelihood of exposure of other pine martens, and other free-living wild mammals following the release of an infected pine marten. There is a high likelihood that other pine martens will be infected with SARS-CoV- 2 and a medium likelihood that other mammals will be infected.
	There is a low probability of further dissemination of SARS-CoV-2 through the reintroduced pine marten population in the Dartmoor and Exmoor National Parks and/or other susceptible mammals because of the social organisation of pine martens noted above.
Consequence Assessment	The duration of clinical disease in farmed mink was similar on each of the Dutch farms (named NB1, NB2, NB3, NB4) affected by the SARS-CoV- 2 outbreaks and lasted approximately 4 weeks during which an increase in mortality was observed. Oreshkova and colleagues (2020) reported an overall mortality rate of 2.4% at farm NB1 and of 1.2% at farm NB2, which are respectively double and triple the expected mortality rate (ca 0.6%, based on observations from previous years in the same period) for the observed period (1 <sup>st</sup> April 2020 to 30 <sup>th</sup> April 2020). Molenaar and colleagues (2020) extended the observation period (1 <sup>st</sup> April 2020 to 27 <sup>th</sup> May 2020) and reported an overall mortality rate of 3.8% at farm NB1, 3.1% at farm NB2, 9.8% at farm NB3, and 2.4% at farm NB4. To note that the exact number of mink that died as a result of SARS-Cov-2 outbreaks is not clearly stated in neither of the two studies.
	Clinical signs included laboured breathing and watery mucoid nasal discharge, with severity ranging from only just noticeable to severe. As noted by farmers, death occurred in all animals with moderate to severe symptoms. Interstitial pneumonia was seen in 77.8% of the mink submitted for necropsy (n=36) (Oreshkova et al., 2020).
	To date and as far as the author are aware, death caused by SARS-CoV-2 has not been reported in pine martens. However, judging by the respiratory disease and increased mortality in naturally infected mink, we estimate a high likelihood of disease and death occurring in pine martens upon exposure and infection with the virus. There is a high likelihood of negative biological, environmental, and economic consequences as a result of disease in a set of pine martens translocated together to the same area and the failure of the specific translocation programme. The likelihood of disease in pine martens, and/or other susceptible mammals, already in the release area is medium because

	there is a low probability of dissemination of the virus in elusive, solitary
	pine martens as stated above.
Risk Estimation	There is currently a medium likelihood of exposure to SARS-CoV-2 in the
RISK ESTIMATION	
	human population, followed by a high likelihood of infection, and viral
	dissemination in the human population. There is a medium likelihood
	that pine martens will be exposed to SARS-CoV-2 as a result of
	conservation translocation activities. There is a high likelihood of
	infection if pine martens are exposed and overall a medium likelihood of
	an infected pine marten being released. The exposure assessment
	estimates a low likelihood of exposure of pine martens and other
	mammals at the release site. There is a high likelihood of infection of
	pine martens, and a medium likelihood of infection of other mammals
	at the release site. The probability of further dissemination amongst the
	reintroduced pine marten population and/or other susceptible wild
	mammals at the destination is low. The likelihood of negative biological,
	environmental, and economic consequences as a result of disease in
	those pine martens translocated simultaneously to the same area and
	the failure of the specific translocation programme is high.
	Currently, the overall risk arising from SARS-CoV-2 is estimated to be
	HIGH in specific groups of translocated pine martens but MEDIUM for
	other mammals at the destination.
Risk Management	
Risk Evaluation	Based on the risk assessment above, preventative measures should be
	employed to reduce the risks from SARS-CoV-2 as a transport hazard.
Risk Management	Biosecurity measures should be employed by all people that will directly
Options	engage with pine marten fieldwork for the proposed reintroduction
	project to Dartmoor and Exmoor National Parks. Detailed
	recommendations will be provided in the DRM protocol, however we
	anticipate here the use of PPE (disposable surgical masks, disposable
	gloves) at all times when handling pine martens; use of dedicated
	fieldwork clothing; a negative lateral flow test prior to fieldwork starting.
L	

Table 7. Disease risk assessment and brief mitigation options for the transport hazard SARS-CoV-2.

POPULATION	Highly Pathogenic Avian Influenza Viruses – HPAIVs Low Pathogenic Avian Influenza Viruses - I PAIVs
POPULATION Justification for Hazard Status	Highly Pathogenic Avian Influenza Viruses – HPAIVs Low Pathogenic Avian Influenza Viruses - LPAIVS Avian influenza (AI) viruses belong to the <i>Orthomyxoviridae</i> family, are placed in the genus Influenzavirus A and are classified into subtypes based on their combination of HA (haemagglutinin) and NA (neuraminidase) surface glycoproteins. At least 16 HA (H1 - H16) and 9 NA (N1 - N9) subtypes circulate in wild aquatic birds, which are considered the natural reservoir for AI viruses (Fouchier et al., 2007; Reperant et al., 2012). Most of the circulating subtypes are geographically widely distributed because of the migratory nature of their reservoir host. AI viruses are classified as low or highly pathogenic based on their pathogenicity in poultry (OIE, 2023). AI viruses, whatever the subtype, are primarily of low pathogenicity (LPAIVS) in wild acquatic birds, inducing a gastrointestinal infection with minimal clinical signs. LPAIVs may be transmitted to domestic poultry in which they also usually cause subclinical infection or a mild clinical disease. However, upon circulation in poultry LPAIVs of the H5 and H7 subtypes may evolve by selection into highly pathogenic avian influenza virus (HPAIVS) subtypes. Infection of poultry with HPAIVs evolves rapidly into a systemic disease with severe clinical signs and potentially high mortality rates (Fouchier et al., 2007; Reperant et al., 2012). HPAIVs pose a serious threat not only to the health of domestic avian species but also to that of mammals, including humans, and to economics in general. As an example, since the late 1990s the highly pathogenic H5N1 has devastated the poultry industry of several countries, spreading from Asia to Europe, Africa, and the Middle East; since its first detection in humans in 2003, 876 cases of infection were reported to the World Health Organization (WHO) from 23 countries, with 458 cases leading to death and resulting in a case fatality rate of 52% (WHO, 2023). Notably, some LPAIVs and HPAIVS (H5 and H7) display virulence in carnivor
	seroprevalence of antibodies reactive to Al viruses in the generic sense (15 positive animals of 689 tested; 2.2%, 1.3-3.6 Cl <sub>95%</sub> ), however none of the seropositive sera were confirmed by haemagglutination inhibition assay as being positive for relevant avian (H5N1, H5N3, H7N1, H7N7, H10N4) Al viruses (Gholipour et al., 2016). During 2021-2022, several

	reports of H5N1 infections in mustelids (precisely otter, badger, polecat,
	and ferret) have been recorded in European countries (EFSA et al., 2022).
	Since 2020, the UK and Europe have experienced annual epizootics of HPAIV. During the first one (winter 2020 to 2021) six H5Nx subtypes were detected, with H5N8 being the predominant one in the UK. In October 2021, an epizootic dominated by H5N1 started and dwarfed the previous one (DEFRA, 2022). According to the latest update provided by DEFRA (2023a), in the UK there have been 188 confirmed cases of H5N1 in kept birds (i.e., all poultry and other captive birds) since October 2022, with most cases reported in England (157; 291 total cases since the H5N1 outbreak started in October 2021), followed by Scotland (23), Wales (8) and Northern Ireland (1). In March 2023, one case of LPAI H7N3 was confirmed in the UK (Scotland). The ongoing routine surveillance for AI in free-living wild (dead) birds has identified 1,637 cases of HPAI virus infection in 73 species during the 2021 to 2022 season (DEFRA, 2022). Where appropriate, free-living wild mammals found dead and submitted to APHA under the Diseases of Wildlife Scheme are tested for influenza viruses of avian origin. Findings of AI virus infection have in a recent report been confirmed in several UK non-avian wildlife including red fox, grey seal, harbour seal, Eurasian otter, harbour porpoise and common dolphin (DEFRA, 2023b).
	Pine martens may be exposed to an HPAIV or LPAIV at either the source or destination sites, and evidence above suggests an epidemic of disease of high mortality may occur in small populations of mustelids including pine marten, and therefore such viruses may represent a population hazard for the reintroduced pine marten population.
Risk Assessment	
Exposure Assessment	In birds, the transmission modes of LPAIVs and HPAIVs may be different: LPAIVs are primarily excreted from the digestive tract of infected birds into the environment and are therefore mainly transmitted via the faecal-oral route, via ingestion of contaminated water (Reperant et al., 2012). As faecal-oral transmission is facilitated in aquatic habitats because AI viruses can persist for extended periods in water (Stallknecht et al., 1990a; Stallknecht et al., 1990b; Brown et al., 2007), surface water contaminated by infected waterfowl is likely to be the main source of LPAIV infection (Stallknecht et al., 2007). By contrast, HPAIVs are mostly excreted from the respiratory tract of infected birds, thus facilitating infection by either direct inhalation of respiratory secretions or ingestion of contaminated water (Reperant et al., 2012). In addition, HPAIVs are likely to be transmitted by predation and ingestion of infected carcasses.
	not totally understood. There have been suggestions that inter-species

transmission may occur through predation and scavenging of infected animals (Reperant et al., 2008; Rimmelzwaan et al., 2006; Songserm et al., 2006a, b) as H5N1 has been isolated from the muscles of infected animals (Tumpey et al., 2002) and ingestion of infected muscle can lead to infection (Kuiken, 2004); or through ingestion of virus-laden water (Root et al., 2014a); or through aerosols (Reperant et al., 2009). Mammals are considered more at risk of acquiring H5N1 at the domestic avian species interface (Kaplan & Webby, 2013) and the tendency of certain mammalian species to commonly exploit a variety of anthropogenically modified habitats, including poultry barns, could facilitate their acquisition of Al viruses.

Pine martens are not typically associated with freshwater ecosystems where natural reservoirs, waterfowl, are found and so the probability of exposure to highly contaminated water is very low. A possible route of exposure could be represented by predation on infected gamebirds, particularly pheasants which are known to be susceptible to AI viruses (Brookes et al., 2021). Gamebirds sport-shooting is a common practice in the UK, with a substantial number of birds (mainly pheasants) being bred and released annually (Mason et al., 2020). The exact number of gamebirds released per estate per year remains difficult to assess as there is no national database comprehensively collecting such data. Data published in the UK Government's Poultry Register by APHA can only be used to get a sense of the situation, an indication of the locations and densities of gamebirds prior to release. In fact, even if it is a legal requirement for all poultry holdings (with 'poultry' including pheasants, red-legged and grey partridges) with more than 50 birds to register, we acknowledge that there might be some lack of compliance in doing so. As per estimates published by APHA in 2019, the majority (85%) of land holdings rearing and/or releasing gamebirds is found in England, with about 13% of reared gamebirds being released in Scotland and 3% in Wales (Mason et al., 2020). As already mentioned in the feasibility study, a volume of pheasants is annually released for shooting on both Exmoor and Dartmoor, thus representing a potential source of infection (in case of an AI outbreak) for translocated pine martens. However, it is important to also note how widely distributed pheasants are in the UK, exception with only the of northwest Scotland (see https://www.bto.org/understanding-birds/birdfacts/pheasant for distribution maps). Pine martens are likely to prey on pheasants, but large birds are usually taken only in small proportions, representing probably the exception rather than the rule, with most of the pine marten's diet mainly made up of fruits (berries), voles, and small birds (Passeriformes) (Caryl et al., 2012; Twining et al., 2019). Pine martens are also known to occasionally use barns for denning. All the above considered, we estimate a low likelihood of at least one pine marten being exposed to AI viruses through ingestion of infected gamebirds in areas of AI outbreaks (either at the source or destination sites), as

	already sporadically seen in other carnivore species that were spatially and temporally connected with avian mass mortalities due to Al (Tammiranta et al., 2023). Given the susceptibility of other mustelids to Al virus infection (as noted above), we estimate a high likelihood of pine martens becoming infected upon exposure. Even though H5N1 has been experimentally shown to acquire the capacity for airborne transmission between ferrets (Herfst et al., 2012), there has been no evidence of sustained intra-species transmission in mammals (Kaplan & Webby, 2013). Also, another more recent experimental study on H5N1 pathogenesis and transmission in ferrets has shown no direct virus transmission from infected ferrets to other co- housed naïve ferrets (Pulit-Penaloza et al., 2022). The presence of wild mammals on farms has been identified as a risk factor for at least one poultry Al outbreak in North America (Vandalen et al., 2009). Root and colleagues (2014b) have demonstrated how striped skunks ( <i>Mephitis mephitis</i> ) have the potential to shed large quantities of viral RNA through the oral and nasal routes following exposure to LPAIV and, considering the species often lives at the human-animal interface and associated with farms, they have hypothesised skunks could play a role in the epidemiology of Al viruses, for example introducing a virus to a naïve poultry flock or acting as a trafficking mechanism of Al viruses to and from an infected poultry flock to naïve flocks or wild bird population. However, striped skunks are in a different family of the Carnivora. Given the primarily woodland adapted, solitary, low density population of pine martens, the probability of dissemination through pine martens and other species at the destination is considered very low. if net pacingingle.
	low, if not negligible.
Consequence	The potential pathogenicity of low pathogenic H9N2 has been
Assessment	experimentally investigated in farmed mink (Zhang et al., 2015). Mink displayed mild clinical signs, including lethargy and initial weight loss. Mild diffuse pneumonia with thickened alveolar walls and inflammatory cellular infiltration was observed. High mortality rates were observed as a result of natural infections with H10N4 in several mink farms in southern Sweden (Klingeborn et al., 1985; Berg et al., 1990). Bronchopneumonia is the predominant lesion at necropsy of ferrets affected by H5N1, although encephalitis has also been described (Govorkova et al., 2005; Maines et al., 2005). Encephalitis caused by H5N1 has also been seen in a stone marten (Klopfleisch et al., 2007). Given the pathological signs and mortality rates in other species of mustelid, primarily mink, the probability of disease in pine martens is high. The likelihood of a biological and economic consequence through a mortality outbreak in reintroduced pine martens is high.
Risk Estimation	We estimate a low likelihood of at least one pine marten being exposed
	to AI viruses in areas of AI viruses' activity (either at the source site in
	Scottish Highlands or following release at Dartmoor and Exmoor
	National Parks). The likelihood of pine martens becoming infected

	following exposure is high. The likelihood of the virus being disseminated amongst the reintroduced pine marten population and other species at the destination is very low, if not negligible. There is a high likelihood of disease in the reintroduced pine marten population and of significant biological and economic consequences. In view of the low probability of exposure of pine martens to AI viruses, and the very low, if not negligible, probability of dissemination of AI viruses from infected pine martens, the overall risk is estimated to be LOW.
Risk Management	
Risk Evaluation	Based on the risk assessment above, preventative measures should be employed to reduce the risks from HPAIVs and LPAIVs as population hazards.
Risk Management Options	We recommend maintaining awareness of possible HPAI and LPAI outbreaks in the vicinity of both the source and release sites. Any dead pine martens found in areas of AI outbreaks should receive detailed pathological examination, with signs of AI virus-associated disease included in differentials, and be tested for AI viruses in order to improve our knowledge on the epidemiology in this species. As the global distribution of HPAIV is constantly changing, it is important to repeatedly reassess this risk category during the reintroduction programme.

Table 8. Disease risk assessment and brief mitigation options for the population hazards High and Low Pathogenic Avian Influenza Viruses (HPAIVs and LPAIVs).

Justification for Hazard Status	Leptospira spp. Leptospires are globally distributed Gram-negative spirochete bacteria belonging to the genus <i>Leptospira</i> that currently comprise 17 validated species and as many as 300 recognized serovars (Birtles, 2012c). Different <i>Leptospira</i> species and serovars have evolved to exploit different mammals as reservoir hosts and it is widely recognized that almost every mammal can serve as a reservoir. These carriers harbour leptospires in their kidneys and excrete the bacteria into the environment with their urine. Rodents are considered among the most
	belonging to the genus <i>Leptospira</i> that currently comprise 17 validated species and as many as 300 recognized serovars (Birtles, 2012c). Different <i>Leptospira</i> species and serovars have evolved to exploit different mammals as reservoir hosts and it is widely recognized that almost every mammal can serve as a reservoir. These carriers harbour leptospires in their kidneys and excrete the bacteria into the
	important reservoirs of some leptospires, including zoonotic serovars. Within the <i>Mustelidae</i> family evidence of infection (leptospires or anti- leptospiral antibodies) has been reported in various species, including European and American mink ( <i>Mustela lutreola</i> and <i>Mustela vison</i> ), polecats ( <i>Mustela putorius</i> ), pine martens ( <i>Martes martes</i> ), stone marten ( <i>Martes foina</i> ) and badgers ( <i>Meles meles</i> ) (Birtles, 2012c). A study conducted in France (Moinet et al., 2010) reported a high prevalence of anti-leptospiral antibodies in pine martens (74%, n=19) but no renal carriage of spirochetes was detected (renal PCR negative), suggesting no epidemiologic role of the species. However, evidence of <i>Leptospira</i> renal carriage in mustelids has been found in a more recent study (20.6% prevalence in weasel, n= 34; 15.4% in pine marten, n=175; 15% in polecat, n=107; 14.1% in stone marten, n=205; 8.3% in stoat, n=24; 8.2% in badger, n=316) indicating their important role as leptospiral carriers (Ayral et al., 2016). In their study, Ayral and colleagues (2016) found that mustelids where infected by three different <i>Leptospira</i> spp. ( <i>L. interrogans, L. borgpetersenii, L. kirschneri</i> ) which they probably were exposed to through the environment and the diet as many small prey mammals often carry these leptospires. A recent study (Piredda et al., 2021) has detected <i>L. interrogans</i> and <i>L. borgpetersenii</i> in pine martens and weasel in Sardinia, Italy. Mustelids have therefore been suggested to act as a sentinel, reflecting the serovars circulating in the environment, or they may act as a maintenance community (Ayral et al., 2016), defined as any set of connected host (sub)populations that together can maintain a pathogen over the long term (Viana et al., 2014).
	Leptospiral infection does not necessarily result in disease and in reservoir host species is frequently only seen as a chronic, sub-clinical infection. However, systemic, potentially life-threatening disease can evolve in accidental hosts or in immunocompromised individuals within a reservoir host population (Birtles, 2012c). The reintroduction, which is likely to act as a stressful event, could therefore trigger disease, leptospirosis, in translocated pine martens.
Risk Assessment	
Release Assessment	Bacteria are shed from infected animals in urine and transmission usually occurs through direct contact with contaminated environments, especially watercourses. Leptospires can enter the body through mucous membranes (eyes, nose, oral cavity) or abraded skin. In carnivores, transmission could also occur through ingestion of infected

prey (Moinet et al., 2010). The high prevalence of infection reported in         the species by Moinet and colleagues (2010) would suggest a medium         likelihood of pine martens being exposed to and infected with         Leptospiro spp. at the source site.         Exposure Assessment         Pine martens that become infected with Leptospiro spp. will become         long term carriers and will therefore carry the bacteria to the         destination site where they will shed bacteria when they urinate, thus         contributing to the maintenance of the pathogen in the environment.         The pine marten reintroduction itself is however predicted to have a low         wipaut and the wide range of reservoir hosts Leptospira spp. have, and         the low population density of the reintroduced pine martens. Therefore,         the likelihood of exposure and infection of other free-living wild animals         at destination because of pine marten behavioural ecology and animal         post-release dispersal, the likelihood of dissemination of infection         amongst reintroduced animals and other free-living wild animals is also         estimated to be very low.         Consequence         Assessment         Assessment         Assessment         Assessment         Assessment         Assessment         Assessment         Assessment     <		many (Mainet at al. 2010). The bish manufacture of the strength of the
likelihood of pine martens being exposed to and infected with Leptospira spp. at the source site.Exposure AssessmentPine martens that become infected with Leptospira spp. will become long term carriers and will therefore carry the bacteria to the destination site where they will shed bacteria when they urinate, thus contributing to the maintenance of the pathogen in the environment. The pine marten reintroduction itself is however predicted to have a low impact on the host-parasite dynamic at the destination, considering the ubiquity and the wide range of reservoir hosts Leptospira spp. have, and the low population density of the reintroducted pine martens. Therefore, the likelihood of exposure and infection of other free-living wild animals at destination because of pine marten behavioural ecology and animal post-release dispersal, the likelihood of dissemination of infection amongst reintroduced animals and other free-living wild animals is also estimated to be very low.Consequence AssessmentLeptospires can rapidly multiply after entering a host and they will disseminate and further replicate in various sites throughout the body. An antibody-mediated response will promptly control and clear infection in those immunocompetent individuals, although bacteria colonizing the renal tubules often persist establishing a chronic infection and a "carrier status" of the host. If the immune system is compromised, systemic infection can result in disease. Our knowledge of the pathology and the clinical signs associated with leptospirosis is primarily limited to observations of the disease in dogs and livestock. Canine acute leptospirosis usually mainfests with fever, shivers, myalgia, vomiting, congested mucous membranes, petechial and ecchymoses. Severe renal dysfunction results in oliguria or even anuria. Icterus (ue he patoi dysfunction) is a c		
Leptospira spp. at the source site.Exposure AssessmentPine martens that become infected with Leptospira spp. will become long term carriers and will therefore carry the bacteria to the destination site where they will shed bacteria when they urinate, thus contributing to the maintenance of the pathogen in the environment. The pine marten reintroduction itself is however predicted to have a low impact on the host-parasite dynamic at the destination, considering the ubiquity and the wide range of reservoir hosts Leptospira spp. have, and the low population density of the reintroduced pine martens. Therefore, the likelihood of exposure and infection of other free-living wild animals at destination because of pine marten reintroduction is estimated to be very low. Considering the pine marten behavioural ecology and animal post-release dispersal, the likelihood of dissemination of infection amongst reintroduced animals and other free-living wild animals is also estimated to be very low.ConsequenceLeptospires can rapidly multiply after entering a host and they will disseminate and further replicate in various sites throughout the body. An antibody-mediated response will promptly control and clear infection in those immunocompetent individuals, although bacteria colonizing the renal tubules often persist establishing a chronic infection and a "carrier status" of the host. If the immune system is compromised, systemic infection si o sign and has been observed in foxes as well as dogs (Birtles, 2012c). In livestock, chronic infection on reservoir hosts infection is usually asymptomatic. The translocation procedure itself could however act as a stressor to the reintroduced pine martens and there's succestinal and ecohymoses. Severe renal dysfunction is a common sign and has been observed in foxes as well as dogs (Birtles, 2012c). In livestock, chronic in		
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and other free-living wild animals at the destination is likely to be very		and other free-living wild animals at the destination is likely to be very

	low. There is a low likelihood of significant biological, environmental, and economic consequences.
	The overall risk is estimated to be <b>LOW</b> .
<b>Risk Management</b>	
Risk Evaluation	Based on the risk assessment above, preventative measures should be employed to reduce the risks from <i>Leptospira</i> spp. as a carrier hazard.
Risk Management Options	It is advisable to minimise the level of stress in translocated pine martens (through good husbandry during the entire translocation procedure) to reduce the probability of disease occurrence at the destination site.

Table 9. Disease risk assessment and brief mitigation options for the carrier hazard Leptospira spp.

DESTINATION	Mycobacterium bovis
Justification for	The Mycobacterium genus is a group of intracellular, rod-shaped, non-
Hazard Status	sporing, Gram-positive bacteria and it comprises about 200 bacterial species. Mycobacteria are highly persistent in the environment due to their lipid-rich waxy cell wall that protects them from desiccation, ultraviolet light, and cold temperature (Converse, 2008). Most mycobacteria exist as free-living saprophytes that live on dead and decaying matter in the environment, while a few are obligate pathogens. Of these pathogens, members of the <i>Mycobacterium tuberculosis</i> complex (MTBC) are the causative agents of human and animal tuberculosis. The MTBC includes <i>M. bovis</i> , the most common cause of tuberculosis, including bovine tuberculosis, (bTB) in domestic livestock and wildlife in the UK; <i>M. caprae</i> , a species closely related but distinct from <i>M. bovis</i> also causes disease in animals; <i>M. tuberculosis</i> , predominant in humans; <i>M. microti</i> , which occurs in voles and other rodents; and <i>M. africanum</i> , <i>M. canetti</i> , and <i>M. pinnipedii</i> .
	Bovine TB has a notoriously complex epidemiology worldwide, involving cattle as the primary host of <i>M. bovis</i> but also multiple other hosts, including humans, most domestic animals, and a large number of wild species (O'Reilly & Daborn, 1995). Several different species hosts can act as natural reservoirs making it difficult to eradicate the pathogen in many countries worldwide because spillback infection from wildlife can spread infection into cattle herds (Nugent, 2011). Examples of wildlife reservoirs considered to be important are the Eurasian badger ( <i>Meles meles</i> ) in the UK (Donnelly et al., 2006), the white-tailed deer ( <i>Odocoileus virginianus</i> ) in Michigan, USA (O'Brien et al., 2002), the African buffalo in South Africa (Palmer, 2013), and the non-native Australian brushtail possum ( <i>Trichosurus vulpecula</i> ) in New Zealand (Livingstone et al., 2015). Estimates of <i>M. bovis</i> prevalence in badgers in the UK vary widely depending on the post-mortem examination and culture methods used and are influenced by the tendency for infection to be spatially aggregated (Delahay et al., 2000; Woodroffe et al., 2005), but may be as high as 24.2% (Allen et al., 2018). There may be some other species, known to be susceptible to infection with <i>M. bovis</i> , that may play a role in the epidemiology of the parasite. For example, wild boar has been suggested to contribute to maintaining infection in the wild, thus increasing the risk of infection in cattle herds in parts of France and Spain (Richomme et al., 2010; Gortazar et al., 2021). In England, disseminated TB has been detected in a wild boar in Herefordshire (Foyle et al., 2010) and a small number of isolations of <i>M. bovis</i> , genotypically identical to those isolated from the local cattle, are made from wild boar each year; however, wild boar in England are
	currently thought to act as spill-over, sentinel hosts for <i>M. bovis</i> and the probability of wild boar to cattle transmission is likely to be very low and localised ( <u>www.tbhub.co.uk</u> ). In the UK and at the time of writing, only deer, most notably red deer ( <i>Cervus elaphus</i> ) and fallow deer ( <i>Dama</i>

*dama*), have been identified as wildlife species, other than badgers, of potential sources of infection to livestock, particularly where deer density is high.

There are no reports of *M. bovis* infection in pine marten as far as we are aware. Other mustelid species are however known to be susceptible. In a survey carried out in TB endemic areas in New Zealand, disease was identified by the presence of macroscopic lesions suggestive of TB during post-mortem examination and the prevalence rates were 17.9% for free-living ferrets (n=548) and 1.6% for free-living stoats (n=62) (Ragg et al., 1995). A survey to determine the prevalence of *M. bovis* in wild mammals in Portugal was conducted on 393 animals representing 11 different species belonging to eight families, including the Mustelidae family (Matos et al., 2016). Post-mortem examination was performed on all animals; tissue and faecal samples were cultured, and acid-fast isolates were identified by PCR; tissues were also screened for M. bovis by directly extracting DNA and testing for the M. bovisspecific sequences. M. bovis was detected (no visible lesions, culture negative, PCR positive) in one stone marten of the four examined and in one otter (visible lesions, culture positive, PCR positive) of the three examined. In Europe M. bovis infection has also been confirmed in freeliving feral ferret, polecat, and American mink (Gavier-Widén et al., 2012). M. bovis associated disease has been reported as the cause of death in an otter in Northern Ireland (Lee et al., 2009). In their survey of M. bovis infection in wildlife other than badgers in endemic areas of southwest England, Delahay and colleagues (2007) cultured and spoligotyped the pathogen in the following mustelids: 3/78 stoats; 1/24 polecats; 0/50 weasels; 0/16 feral ferrets; 0/51 mink; 0/21 otters. In an opportunistic study of respiratory disease in mustelids found dead in southwest England between 1999 and 2004, cases showing numerous granulomas in lungs (n=10) were Ziehl-Neelsen-stain negative for acidfast bacilli; further investigation by PCR and amplification of the hsp65 gene in five of these cases found no evidence of *M. bovis* infection (Simpson et al., 2016).

Scotland was recognised as being Officially TB Free by the European Commission in September 2009. This recognition means that the incidence of bTB is low enough to be declared free of disease, however small sporadic cases of bTB linked to livestock movement still occur in Scotland. On the other hand, *M. bovis* is considered endemic in southwest England which is regarded as a High Risk Area (HRA) for bTB (Fig. 4).

	Area Information         Image: Section 4 List of Mar.
	Figure 4. Bovine TB risk map for Great Britain. Sourced and modified from www.tbhub.co.uk The incidence of bTB is relatively high in HRA when compared with that of the Edge Area and Low Risk Area. Also, in the HRA there is a recognised reservoir of infection in wildlife (badgers) (www.tbhub.co.uk). Considering the different bTB epidemiological situation at the source sites (Scotland) compared to the destination sites (southwest England) for this proposed pine marten reintroduction project, <i>M. bovis</i> is here assessed as a potential destination hazard given Scottish pine martens are unlikely to have encountered the pathogen before and may become exposed to it at the destination.
Risk Assessment	before and may become exposed to it at the destination.
Risk Assessment Exposure Assessment	Pathogen transmission seems particularly complex for <i>M. bovis</i> , with opportunities for both direct and/or indirect transmission between and within livestock and wildlife hosts and potentially a contaminated, shared environment. The main recognised routes of transmission are i) the respiratory one, through inhalation of infected aerosolised droplets into the lungs where infection is then established, and the ii) oral route, through ingestion of contaminated tissues (e.g., through scavenging of infected carcasses) (Palmer, 2013). Transmission via biting (inoculation of infectious saliva at the bite site) has also been suggested in badgers (Corner et al., 2011). Indirect environmental transmission is another possible route of infection and one that may be playing an important and underappreciated epidemiological role in <i>M. bovis</i> maintenance, especially in the bTB cattle/badger system in the UK (Allen et al., 2021). Infected animals shed <i>M. bovis</i> in their respiratory exudates and mucus but also in faeces and urine (the renal system is a common site for tuberculous lesions in badger, [Allen et al., 2021]), thus contaminating the environment that becomes a key source of exposure due to the persistence of the bacterium. <i>M. bovis</i> can indeed survive for long

periods of time on a wide range of matrices and substrates, including soils of several pH range in experimental systems or under natural climate conditions (Fine et al., 2011; Ghodbane et al., 2014).

Devon, where Dartmoor and Exmoor National Parks are found, is in the HRA for bTB and had both the highest population of herds and accounted for the highest proportion of all new bTB incidents in the HRA in 2021 (23% for both) (APHA, 2022). Badgers were identified as the predominant source of infection in Devon, followed by cattle movements as the second likely source (APHA, 2022). The current, as of July 2023, ongoing cattle herd breakdowns are shown in Fig. 5.

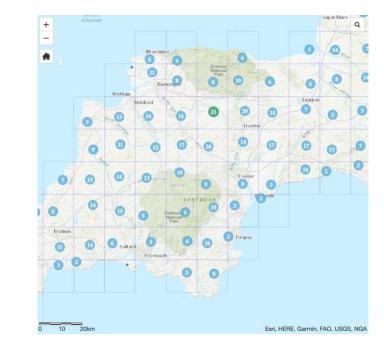


Figure 5. Map showing current, as of July 2023, locations of ongoing bTB breakdowns in cattle herds in southwest England. Generated using the ibTB tool jointly developed by APHA and ERGO and available on <u>www.tbhub.co.uk</u>. Updated maps of the incidences of bTB in individual farms in England are regularly published on the website. Blue dots: 2 – 20 breakdowns; green dot: 21-150 breakdowns.

Recent badger population size estimates for England and Wales, made on the basis of sett density survey and habitat-specific social group sizes derived from genetic analysis, are of 485,000 individuals (391,000-581,000 Cl<sub>95%</sub>), with an overall density of 3.29 badgers/km<sup>2</sup> (Judge et al., 2017). These estimates are similar to those given by Mathews and colleagues (2018). In Exmoor, one of the last remaining herds of freeliving wild red deer in England is also found.

The potential routes of exposure for reintroduced pine martens to *M. bovis* at the destination are i) aerosol inhalation, in case of direct contact with infectious cattle or wildlife, mainly badgers but possibly also deer; ii) bite wound contamination, in case of direct contact with infectious

wildlife; iii) scavenging infected carcasses, most likely dead badgers or deer; iv) environmental exposure, via contaminated substrates, for example feed or pasture. The pine marten is a solitary species, with the most extensive social interaction being between mother and young, and with any aggressive encounters being between males and females during reproduction season. It is a species well adapted to exploit woodland, mainly avoiding open areas such as pasture where environmental contamination might be higher due to faeces deposited by grazing cattle. Given the pine marten's arboreal habits the likelihood of interactions with the badger (and also deer) is low. Its feeding behaviour is that of a generalist, with scavenging on carcasses included. Given these considerations we estimate a very low likelihood of pine marten exposure to *M. bovis* at the destination through scavenging on carcases.

Given the prevalence data on *M. bovis* infection in Mustelidae above, and therefore known susceptibility to infection, we estimate a low likelihood that exposed pine martens will become infected.

Environmental transmission of mycobacteria is yet to be fully understood in the well-studied *M. bovis*/badger disease system, in which it is likely positively associated with badger population densities, prevalence of infection in badger populations and prolonged mycobacterial excretion in badgers (Tomlinson et al., 2013). The pine marten ecological characteristics mentioned above (solitary species, strong woodland affinity, low population density) led us to estimate that infected individuals will likely have minor impact on environmental contamination. Other susceptible species at the destination may become infected by inhaling or ingesting contaminated soil, water, food; by scavenging infected pine marten carcasses; or through bite wounds from an infected pine marten. The likelihood of M. bovis infection spreading from infected reintroduced pine martens to other wildlife species at destination is estimated to be low; and very low, if not negligible, for livestock species because the pine marten's ecology precludes interactions with livestock. The very low prevalence of infection with *M. bovis* in stoats reported above, also a solitary species but with more terrestrial habits than pine martens, supports the above assumptions. Intra-species transmission depends on host density, distribution, and behaviour. Badgers, for example, tend to aggregate in underground setts, use communal latrines, move between family groups and fight frequently, thus increasing the likelihood of intraspecies transmission from all routes (respiratory, environmental, oral and bite wounds). Given the solitary nature of the pine marten combined with a low population density at the destination we estimate a very low likelihood of *M. bovis* dissemination within the reintroduced pine marten population, with direct transmission largely limited to

	contacts during dam/kit interaction and to male/female encounters in
•	the reproductive season.
Consequence Assessment	<ul> <li>There is a low likelihood of one reintroduced pine marten becoming infected with <i>M. bovis</i>.</li> <li>The pathological effects of <i>M. bovis</i> infection vary widely between host species and have not been recorded in pine martens. Insight on the pathogenesis of <i>M. bovis</i> infection can be drawn from the detailed knowledge that has been generated from observations of infected badgers. Infection is usually followed by a cell-mediated immune response that 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</li> </ul>
	host and shedding, for example through nasal secretions, urine, or faeces. As a result, shedding is intermittent and may be related to the size and location of granulomas. The location of mycobacterial lesions is thought to relate to the route of infection: respiratory infection causing predominantly pulmonary lesions, ingestion causing primarily alimentary tract lesions and contamination of bite wounds causing cutaneous lesions. Respiratory infection results in a chronic disease with animals expressing a variety of disease states ranging from latent subclinical most of the time to moderate and to severe overt disease. Rapidly progressing generalized disease in badgers is more commonly seen following bite wound infection (Gormley & Corner, 2018). Given this evidence from badgers, and signs of mycobacterial disease in other Mustelidae, there is a medium likelihood that if pine martens become infected with <i>M. bovis</i> that it will induce severe chronic disease in individuals. There is a medium likelihood of economic and biological consequences through disease in a pine marten affecting the reintroduction programme.
Risk Estimation	The likelihood of at least one pine marten being exposed to <i>M. bovis</i> is very low and there is a low likelihood of pine martens becoming infected upon exposure. There is a low likelihood of infection spreading from infected pine martens to other susceptible wildlife species at destination, and a very low, if not negligible, likelihood that infection will spread from pine martens to livestock. The probability of dissemination amongst the reintroduced pine marten population is very low. There is a medium likelihood of severe chronic disease in pine martens and biological and economic consequences to the reintroduction programme. Bovine TB, as a barrier to local, national, and international trade, has significant and detrimental financial consequences for cattle farmers in the UK. <i>M. bovis</i> transmission across the wildlife-livestock interface represents a significant obstacle to bTB eradication in several countries, including the UK. Concerns have been raised relating to the potential effects of pine marten reintroduction on <i>M. bovis</i> epidemiology in southwest England, especially regarding pine marten being capable of onward transmission. In any given population, maintenance of <i>M. bovis</i> infection can be achieved only when sufficient

	intraspecies transmission exists to sustain disease at a threshold level (i.e., when each infected animal transmits to one or more individuals) (Palmer, 2013). Even though there is a low likelihood of pine martens becoming infected at the destination and a low likelihood of <i>M. bovis</i> dissemination amongst other reintroduced individuals, the likelihood of onward transmission being sufficient to maintain infection in the reintroduced pine marten population is estimated to be very low. There is a very low likelihood that the reintroduced population will play a role in the <i>M. bovis</i> epidemiological situation at the destination sites. The biological and economic consequences to bTB disease in livestock attributable to pine marten reintroduction are likely negligible.
	The overall risk arising from <i>M. bovis</i> in the proposed pine marten project is <b>VERY LOW</b> .
Risk Management	
Risk Evaluation	Based on the risk assessment above, preventative measures should be employed to further reduce the risks from <i>M. bovis</i> as a destination hazard.
Risk Management Options	Bacterial culture is regarded as the 'gold standard' method for TB diagnosis in all wild animals but it cannot be effectively performed in live animals as shedding of bacilli is intermittent and bacterial growth is slow. It is also expensive and requires category 3 level laboratory facilities (Thomas & Chambers, 2021). Serological tests, being economic and easy to perform even to large scale surveillance in both live and dead animals, are instead very useful in wildlife and the species-specific lateral flow tests based on immunochromatography are the most useful currently available (Chambers, 2013). However, accurate estimates for the sensitivity and specificity of these tests are lacking for most wildlife species, including pine martens, and their use in disease surveillance or management still requires a degree of uncertainty and reliance on other ancillary tests (Chambers, 2013).
	PCR and/or extended culture of tissues removed during post-mortem examination of any pine marten found dead after release is recommended to improve understanding of <i>M. bovis</i> pathogenesis (infection and disease course) in pine martens.

Table 10. Disease risk assessment and brief mitigation options for the destination hazard Mycobacterium bovis.

CARRIER	Yersinia spp.
	(Y. pseudotuberculosis and Yersinia enterocolitica)
Justification for Hazard Status	<ul> <li>Yersinia pseudotuberculosis and Yersinia enterocolitica are Gramnegative bacilli belonging to the genus Yersinia within the family Enterobacteriaceae. Yersinia spp. are ubiquitous in the environment, being found in humans, livestock, wild animals, soil, water, and food (meat products, milk, vegetables) (Gasper &amp; Watson, 2001). Various serotypes and biotypes are known, some are avirulent while others are pathogenic and responsible for disease, yersiniosis. In wildlife, yersiniosis is more often associated with Y. pseudotuberculosis. These bacteria have been isolated from a broad range of hosts including artiodactyls, carnivores, rodents, marsupials, swine, and primates (Mair, 1973), but susceptibility to disease vary among species. Among wild animals, hares (<i>Lepus</i> spp.) are probably most susceptible to disease (Mair, 1973; Wuthe et al., 1995). Disease is usually sporadic but epizootics with high mortality have also been documented in captive ungulates (Welsh et al., 1992), captive New- and Old-World monkeys (Chang et al., 1980; Rosenberg et al., 1980), free-ranging wild muskox (<i>Ovibos moschatus</i>) (Blake et al., 1991). Within the <i>Mustelidae</i> family, sporadic disease cases have been recorded in pine martens (<i>Martes martes</i>) and in European mink (<i>Mustela lutreola</i>) in Continental Europe (Mair, 1973).</li> <li>In the UK, Y. pseudotuberculosis strains were isolated, between 1961-1971, from various free-living wild bird species and from six species of free-living wild mammals: coypu (<i>Myocastor coypus</i>), brown hare (<i>Lepus</i>)</li> </ul>
	<ul> <li>europaeus), mouse (Mus musculus), rabbit (Oryctolagus cuniculus), red fox (Vulpes vulpes) and field vole (Microtus agrestis) (Mair, 1973).</li> <li>Rodents and birds have been implicated as the principal reservoirs of Yersinia spp. and, because of their resistance to natural disease, rats and mice may play an important epidemiological role in the spread of infection (Mair, 1973; Obwolo, 1976).</li> <li>Most mammalian species that are susceptible to infection may also become subclinical carriers of Yersinia spp. and disease may be precipitated by stressful conditions such as cold and wet weather, decreases or changes in food availability, overcrowding or capture (Mair, 1973; Gasper &amp; Watson, 2001). Exposure to bacteria concomitant with stressors could also result in yersiniosis occurrence (Gasper &amp; Watson, 2001). There seems to be a seasonal influence on the occurrence of yersiniosis, with increased incidence associated with the colder temperatures of late autumn, winter, and early spring (Mair, 1973; Zwart, 1993). The reintroduction, which will happen during the autumn season, is likely to be a stressful event for translocated pine martens and therefore yersiniosis could occur.</li> </ul>
	Yersiniosis is often considered a zoonosis, however many cases recorded in humans may not be of animal origin, as suggested by the

	fact that the strains which have historically been associated with human
	disease are not those commonly isolated from animal sources
	(Kapperud, 1994). We will therefore not consider <i>Yersinia</i> spp. as of
D'-1 A	zoonotic importance in the following risk assessment.
Risk Assessment	
Release Assessment	Transmission primarily occurs via the oro-faecal route through ingestion of contaminated food sources and water. In carnivores, transmission can also occur through ingestion of infected prey (Mair et al., 1967). Bacteria are shed in faeces of infected individuals as long as clinical signs persist, normally 2-3 weeks, but prolonged sub-clinical carriage has also been observed (Najdenski, 2012). Data on the current distribution of <i>Yersinia</i> spp. in both the environment and in free-living wild species at the source and destination sites are not available. However, the likelihood of pine martens being exposed to <i>Yersinia</i> spp. at the source site is estimated to be medium because i) these bacteria are known to have a widespread geographical distribution and are rather resistant in the environment and ii) field voles ( <i>Microtus agrestis</i> ) represent the staple food for pine martens in Scotland (Caryl et al., 2012b) and like
	other wild rodents (e.g., <i>Microtus</i> spp. and <i>Apodemus</i> spp.) may act as reservoirs of <i>Yersinia</i> spp Outside the host, <i>Yersinia</i> spp. may survive, and even replicate, in soil and water for months to years given their minimal nutritional requirements and broad temperature range tolerance, from 5° C to 42° C (Gasper & Watson, 2001). If exposed, there is a medium likelihood that pine martens will be infected because infection has been reported in the species (Mair, 1973).
Exposure Assessment	Pine martens that become infected with <i>Yersinia</i> spp. at the source site will carry the parasite to the destination site, may remain infected and
	will contaminate the environment by shedding the bacteria in the faeces. The environment at destination is however assumed not to be <i>Yersinia</i> spp. free considering the ubiquity of these bacteria. The likelihood of other free-ranging wild animals becoming exposed and infected at the destination site, through ingestion of contaminated food or water, is estimated to be medium because many species are susceptible to <i>Yersinia</i> spp., however the release of pine martens is
	unlikely to impact the probability of exposure. The likelihood of dissemination of infection amongst reintroduced pine martens and other free-ranging wild animals, because of pine marten reintroduction, will depend on the behavioural interactions of these species in the wild and, considering the pine marten behavioural ecology and animal post-release dispersal, is estimated to be very low.
Consequence	The likelihood of at least one reintroduced pine marten being infected
Assessment	at destination is estimated to be medium.
	Following ingestion, Yersinia spp. attach to the intestinal mucosa and
	can cross the intestinal epithelium, reaching the intestinal lymphoid
	aggregates where they proliferate. Bacteria may then disseminate to
	the mesenteric lymph nodes and eventually to the liver and spleen

	(Gasper & Watson, 2001). Less commonly, bacteria can also infiltrate lungs, kidneys, and bone marrow (Zwart, 1993). Infections can be acute, subacute, or chronic. In the most severe form of acute infection a fulminating septicaemia involving visceral organs is seen together with enteritis, and death occurs within 1 to 3 days. In subacute and chronic forms of infection the common clinical signs are lethargy, anorexia, severe diarrhoea, respiratory distress, incoordination, muscular weakness, weight loss, and emaciation.
	In populations of free-ranging species, yersiniosis is usually of little concern because it tends to manifest as a sporadic disease, affecting only single individuals or few animals. However, the translocation procedure itself could act as a stressor and lead to an increased susceptibility of the released pine martens to disease. There is a low likelihood, however, that stress may precipitate yersiniosis in a large proportion of released pine martens and thereby leading to failure of the translocation. The likelihood of significant biological, environmental, and economic consequences is predicted to be low.
Risk Estimation	There is a medium likelihood of pine martens being exposed to <i>Yersinia</i> spp. at the source site and a medium likelihood of at least one pine marten becoming infected. The likelihood of exposure and infection of other wild animals at the destination site is medium but not affected by the pine marten reintroduction itself. Dissemination of <i>Yersinia</i> spp. amongst reintroduced pine martens and other free-living wild animals at the destination is likely to be very low. There is a low likelihood of significant biological, environmental, and economic consequences.
Risk Management	
Risk Evaluation	Based on the risk assessment above, preventative measures should be employed to reduce the risks from <i>Yersinia</i> spp. as a carrier hazard.
Risk Management Options	Methods to reduce the level of stress in translocated pine martens (through good husbandry) and to reduce the probability of transmission (though good biosecurity), may be effective in reducing the probability of an outbreak of disease at the destination site. Hygiene at the soft release pens should always be kept to a high standard and it might be useful to check prey items that are fed to pine martens for any possible lesion associated with yersiniosis.

Table 11. Disease risk assessment and brief mitigation options for the carrier hazard Yersinia spp.

POPULATION	Mucoraceae spp.
Justification for	Saprophytic fungi of the Mucoraceae family in the order Mucorales are
Hazard Status	causative agents of an acute angioinvasive infection that leads to a disease known as mucormycosis, a rare but aggressive and often fatal disease. Within the <i>Mucoraceae</i> , the pathogenic genera are <i>Absidia</i> , <i>Apophysomyces</i> , <i>Mucor</i> , <i>Rhizomucor</i> and <i>Rhizopus</i> (Cafarchia et al., 2012). <i>Mucoraceae</i> spp. fungi are ubiquitous, and they use a variety of substrates as nutrient sources. They are found in decaying fruits and vegetables, food items, soil, plants debris and animal excreta. Most of them can rapidly grow and are thermotolerant in that they are able to grow at temperatures greater than 37°C. Sporangiospores are released into the environment as airborne propagules (Ostrosky-Zeichner et al., 2009).
	Mucormycosis has been reported in various species, both domestic/captive and wild: horses (Guillot et al., 2000), sika deer ( <i>Cervus nippon</i> , Sato & Matsuura, 1998), grey parrots ( <i>Psittacus erithacus</i> , Desmidt et al., 1998), canaries ( <i>Serinus canarius</i> , Mitchell et al., 1986), platypus ( <i>Ornithorynchus anatinus</i> , Gust & Griffiths, 2009), cane toads ( <i>Bufo marinus</i> , Speare et al., 1997), marine mammals (Robeck & Dalton, 2002). Disease occurs in humans as well, especially in diabetes mellitus affected individuals, and is commonly associated with a compromised immune function (Teixeira et al., 2013). In pine martens, as detailed in two necropsy reports (ref: M2334/241115 and M2332/171115-Simpson, 2016), two cases of an acute fatal encephalitis associated with a <i>Mucoraceae</i> spp. have been recorded soon after animals were released in Autumn 2015 as part of the Vincent Wildlife Trust pine marten reinforcement project.
	<i>Mucoraceae</i> spp. have a wide geographic distribution and therefore they are highly likely present at both the source and destination sites. Here we consider these fungi as a potential population hazard, and we assess the risk they may pose on the small, reintroduced pine marten population.
Risk Assessment	
Exposure Assessment	<i>Mucoraceae</i> spp. are present throughout the environment and exposure to these organisms may occur daily. In fact, it is probably impossible to completely avoid contact with the fungal spores. Therefore, there is a high likelihood of reintroduced pine martens being exposed to <i>Mucoraceae</i> spp. at the destination site.
	Infection usually occurs through inhalation of the sporangiospores, but it can also occur by ingestion or percutaneous inoculation (Cafarchia et al., 2012). The risk of infection is increased if (i) animals are exposed to many fungal spores (Cafarchia et al., 2012), and (ii) the immune status is compromised through stressors (Teixeira et al., 2013). The two cases of disease associated with a <i>Mucoraceae</i> spp. that occurred following the reinforcement project in Wales suggest that environmental and host (pine marten) conditions during translocation may be conducive to

	infection and disease. The key factors are unknown but could include pine marten susceptibility to infection and disease, increased exposure to the agent due to pre-release management, and stressors of reintroduction (capture, handling, transport, and release) increasing the susceptibility of pine martens. It is important to note that no further cases have been recorded in any translocated pine martens (n=51 individuals translocated during the 2015-2017 Welsh reinforcement project), including those reintroduced to the Forest of Dean (n=35 individuals translocated between 2019 and 2021). We estimate a medium likelihood of pine martens translocated to the destination site becoming infected with <i>Mucoraceae</i> spp. through inhalation of fungal spores.
	Mucormycosis is an infectious but not contagious disease and therefore the likelihood of dissemination of the fungal infection amongst reintroduced pine martens and other wild animals at the destination site is negligible. However, more than one individual in the reintroduced population could be affected because of exposure to the same environmental conditions and stressors.
Consequence Assessment	Mucormycosis can manifest in four different clinical forms: i) cutaneous or subcutaneous, described in platypus, horses and amphibians (Gust & Griffiths, 2009; Guillot et al., 2000; Speare et al., 1997); ii) gastrointestinal, described in sika deer and canaries (Sato & Matsuura, 1998; Mitchell et al., 1986); iii) pulmonary, described in horses and grey parrots (Carrasco et al., 1997; Guillot et al., 2000; Desmidt et al., 1998); iv) rhino-cerebral, described in humans (Teixeira et al., 2013) and caused by fungal spores that invade the nasal mucosa and then germinate, forming angio-invasive hyphae that cause infarction of the involved tissue, frequently invading orbital and cerebral regions.
	In pine martens, post-mortem examination of one male and one female found dead a week apart, each of them 14 days post release, revealed a devitalised mucosa of the nasal cavities (turbinates and sinuses) and an intense inflammation of the olfactory bulb of the brain (Simpson, 2016). This resembles the rhino-cerebral pathological presentation commonly seen in humans (a necrotic ulcer in the palatal or nasal mucosa, Teixeira et al., 2013).
	Mucormycosis is more often associated with a compromised immune function (Teixeira et al., 2013) and because reintroduction is likely to be a stressful event, there is a medium likelihood of infected reintroduced pine martens developing disease. Mucormycosis is usually considered a sporadic, rare disease and, even though all reintroduced pine martens will be exposed to the same stressors and environment conditions, the likelihood of an epidemic occurring is estimated to be low. There is a low likelihood of significant biological and economic consequences at the destination site through multiple deaths and consequently failure of the

	reintroduction. The environmental consequences due to disease in one
	or more pine martens are considered negligible because Mucoraceae
	spp. are geographically widespread.
Risk Estimation	There is a high likelihood of reintroduced pine martens being exposed to <i>Mucoraceae</i> spp. at the destination, a medium likelihood of infection and a negligible likelihood of dissemination of the hazard amongst reintroduced pine martens and other free-living wild animals at the destination site. As a consequence of the stressors placed on reintroduced pine martens there is a low likelihood of significant biological and economic consequences. The likelihood of significant environmental consequences is negligible. The overall risk for this hazard is estimated to be <b>LOW</b> .
Risk Management	
Risk Evaluation	Based on the risk assessment above, preventative measures should be
	employed to further reduce the risks from <i>Mucoraceae</i> spp. as a population hazard.
Risk Management Options	The best ways to reduce the risks from <i>Mucoraceae</i> spp. is to: a) translocate healthy animals, b) reduce stress during translocation and c) lower the environmental spore count at the release pens. Therefore, pine martens should receive a rigorous health examination before translocation, efforts should be made to reduce the stress of translocation through good husbandry, transport methods, fast, quiet, and gentle translocation. The hygiene at the soft release pens should be kept to a high standard and uneaten food items daily removed to avoid the development of fungus promoting environments.

Table 12. Disease risk assessment and brief mitigation options for the population hazard Mucoraceae spp.

CARRIER	Hepatozoon spp.
CARRIER Justification for Hazard Status	Hepatozoon spp. are apicomplexan protozoa belonging to the family Hepatozoidae and to date 336 species have been described (Baneth, 2011). Hepatozoon spp. are vector-borne parasites with a complex life cycle that includes a sexual phase in an invertebrate final host, and an asexual phase in a vertebrate intermediate host. The final hosts are blood-sucking arthropods, including ticks, mites, sand flies, mosquitoes, fleas, lice, reduviid bugs, and leeches (Baneth, 2011). Vertebrate intermediate hosts include amphibians, reptiles, birds, marsupials, and mammals. Following infection of the intermediate host, asexual reproduction (schizogony) occurs in various host tissues, followed by release of gamonts into the haemolymphatic system (leucocytes in the case of mammals and birds). The life cycle is completed when a final host feed on an infected intermediate host (Smith, 1996).
	<i>Hepatozoon</i> spp. of domestic animals were the first species described: <i>H. canis</i> and <i>H. americanum</i> in dogs and <i>H. felis domestici</i> in cats.
	Infection in free-living wildlife has been reported in several species worldwide: in American mink ( <i>Mustela vison</i> ) in Canada (Presidente & Karstad, 1975); in coyotes ( <i>Canis latrans</i> ) in the USA (Kocan et al., 1999); and in Japanese martens ( <i>Martes melampus</i> ) (Yanai et al., 1995; Kubo et al., 2009). In Europe, <i>H. canis</i> has been recorded in foxes ( <i>Vulpes vulpes</i> ) (Conceicao-Silva et al., 1988; Cardoso et al., 2014; Najm et al., 2014; Duscher et al., 2015). <i>Hepatozoon</i> spp. have also been found in stone martens ( <i>Martes foina</i> ), pine martens and stoats ( <i>Mustela erminea</i> ) in Germany (Geisel et al., 1979; Krampitz & Geisel, 1982 in Simpson et al., 2005) and in pine martens in Spain (Criado-Fornelio et al., 2009). Molecular analyses indicated that the species found in Spanish pine martens was closely related to <i>Hepatozoon</i> isolates from felids ( <i>H. felis</i> ) (Criado-Fornelio et al., 2009). A survey conducted in England (McDonald et al., 2000) found no evidence of <i>Hepatozoon</i> spp. infection in stoats (n=44). In Scotland, Simpson and colleagues (2005) identified <i>Hepatozoon</i> spp. infection in three pine martens which had died as a result of road accidents (n=4). The species of <i>Hepatozoon</i> primarily infecting pine martens has recently been identified in a study by Hodžic and colleagues (2018) who named it <i>Hepatozoon martis</i> . Their BLAST search showed 100% identity to <i>Hepatozoon</i> sp. found in the Scottish pine martens, in badgers from Spain and in <i>Ixodes</i> tick nymphs collected from foxes in Germany. Since its identification, <i>H. martis</i> has also been detected in mustelids from Hungary (pine marten and stone
	marten, Hornok et al., 2022) and Spain (stone marten and badger, Ortuño et al., 2022) and in wild cats from Spain (Ortuño et al., 2022). Moreover, another new <i>Hepatozoon</i> genotype with a significant rRNA sequence divergence from <i>H. martis</i> and clustered separately from it has been detected in pine marten and beech marten in Hungary (Hornok et al., 2022). This variant appears to be closely related to that reported in Asia in Japanese martens (Kubo et al., 2009).

	Immune suppression, induced for example by concurrent infectious
	agents, immature immune system in young animals or other immunodeficient conditions, can influence the pathogenesis of <i>H. canis</i> infections (Baneth et al., 2011). The reintroduction, which is likely to act as a stressful event, could therefore trigger disease in infected translocated pine martens.
Risk Assessment	
Release Assessment	Unlike other vector-borne parasites, <i>Hepatozoon</i> spp. transmission is not achieved by arthropod blood feeding on the vertebrate host. Instead, intermediate hosts become infected by ingesting the definitive host that contains mature, sporulated oocysts (Smith, 1996). However, some other modes of transmission have been described, including predation of one vertebrate upon another infected vertebrate host (through ingestion of tissue forms, cystozoite stages, in the prey [Johnson et al., 2008; Johnson et al., 2009a; 2009b]), and vertical transmission from dam to pup in domestic dogs (Murata et al., 1993).
	The vector species responsible for the transmission of <i>H. martis</i> and the existence of alternative transmission routes remain unknown. The brown dog tick ( <i>Rhipicephalus sanguineus</i> ) is known to be the vector for <i>H. canis</i> and the Gulf Coast tick ( <i>Amblyomma maculatum</i> ) for <i>H. americanum</i> (Baneth, 2011). <i>H. canis</i> has also been isolated from ixodid tick species in Germany (Najm et al., 2014). <i>R. sanguineus</i> is considered exotic to Britain, however it has been reported in association with dog importation and has established itself around quarantine kennels in England (Simpson et al., 2005). <i>Ixodes ricinus</i> and <i>Ixodes hexagonus</i> , which are known to parasitize pine martens (Hofmeester & van Wieren, 2014), are widespread across Britain and if they can act as vectors for the <i>Hepatozoon</i> of pine martens than infection is expected not to be restricted to Scotland (Simpson et al., 2005). Detection of <i>H. canis</i> has recently been reported in <i>D. reticulatus</i> , <i>I. hexagonus</i> , <i>I. ricinus</i> , <i>I. canisuga</i> , and <i>I. ventalloi</i> (Uiterwijk et al., 2023). Interestingly, new tickhost associations (i.e., <i>Dermacentor</i> species-pine marten and <i>Haemaphysalis concinna</i> -pine marten) have been observed, as well as the presence of <i>H. martis</i> DNA in questing nymphs of <i>H. concinna</i> , suggesting that this tick species is a carrier, and possibly a vector, of <i>H. martis</i> (Hornok et al., 2022). Nonetheless, merely parasite detection is not sufficient to confirm the vector competence of the tick and further research is needed.
	(n= 2801 of which 446 were mustelids) and ticks (n=754) sampled across several European countries and tested by PCR, Uiterwijk and colleagues (2023) found a high overall (34.8%) positivity in carnivores, especially canids and mustelids. Pine martens showed the highest prevalence of infection (74%, n=50 sampled from the Netherlands) within the Mustelidae, followed by stone martens (63.6%, n= 66 sampled from

	Croatia; 47.8%, n=67 sampled from the Netherlands and Belgium) and polecats (10%, n=100 sampled from the Netherlands and Belgium). High prevalence of infection has been reported in other studies, most recently in pine marten and stone marten by Hodžic and colleagues (2018) and in stone marten by Ortuño and colleagues (2022). In ticks, an overall prevalence of 4.1% was shown, with <i>Dermacentor</i> showing the highest prevalence (16%), followed by <i>Ixodes</i> (5.3%) and <i>Rhipicephalus</i> (1.4%) (Uiterwijk et al., 2023).
	Considering the high <i>Hepatozoon</i> spp. prevalence in wild mammals and ticks, the knowledge of previously identified cases of <i>Hepatozoon</i> infection in pine martens in Scotland, and the ubiquity of potential vectors we estimate a medium likelihood of pine martens being exposed to and becoming infected with <i>Hepatozoon</i> spp. at the source site.
Exposure Assessment	Pine martens that become infected with <i>Hepatozoon</i> spp. at the source site will carry the protozoa to the destination site where potential vectors are also found, thus contributing to the maintenance of the pathogen in the environment. <i>Hepatozoon</i> spp. seem to have a low host specificity (Ortuño et al., 2022), with <i>H. canis</i> and <i>H. martis</i> being detected in different species of carnivores. Moreover, and surprisingly, <i>H. martis</i> was also detected in chamois ( <i>R. rupicapra</i> ) and roe deer ( <i>C. capreolus</i> ) in Austria (Uiterwijk et al., 2023).
	The pine marten reintroduction itself is predicted to have a very low impact on the host-parasite dynamic at the destination, considering the low population density of the translocated pine martens. The likelihood of exposure and infection of other free-living wild animals at the destination because of pine marten reintroduction is estimated to be very low. Considering the pine marten behavioural ecology and animal post-release dispersal, the likelihood of dissemination of infection amongst reintroduced animals and other free-living wild animals is also estimated to be very low.
Consequence Assessment	In wildlife, the pathogenicity of <i>Hepatozoon</i> spp. seems to be low, with mainly subclinical infections. No evidence suggesting otherwise was found during the post-mortem examination of the infected Scottish pine martens (myocardial lesions of uncertain clinical significance were seen but animals were in good condition with fat reserve) (Simpson et al., 2005). The tropism of <i>H. martis</i> for muscular tissue, especially myocardium, has been recently described and is substantially different from that of <i>H. canis</i> for haematolymphoid tissues (Hodžic et al., 2018). Disease has however been recorded in experimentally infected coyote pups (Kocan et al., 2000) and in the case of co-infections with bacteria (Alić et al., 2021).
	The reintroduction procedure itself could act as a stressor to the translocated pine martens and their susceptibility to disease could increase. There is a very low likelihood, however, that stress may

	precipitate disease in a large proportion of released pine martens and
	cause reintroduction failure. The likelihood of significant biological,
	environmental, and economic consequences is predicted to be low.
Risk Estimation	There is a medium likelihood of pine martens being exposed to <i>Hepatozoon</i> spp. at the source site and a medium likelihood of at least one pine marten becoming infected. The likelihood of exposure and infection of other wild animals at the destination site is very low. Dissemination of <i>Hepatozoon</i> spp. amongst reintroduced pine martens and other free-living wild animals at the destination is likely to be very low. There is a low likelihood of significant biological, environmental, and economic consequences.
	The overall risk is estimated to be <b>LOW</b> .
Risk Management	
Risk Evaluation	Based on the risk assessment above, preventative measures should be
	employed to reduce the risks from <i>Hepatozoon</i> spp. as a carrier hazard.
Risk Management Options	It is advisable to minimise the level of stress in translocated pine martens (through good management) to further reduce the probability of disease occurrence at the destination site.
	Treating animals for ectoparasites post-capture (including manual removal of ticks where possible) may further reduce the risks of exposure of pine martens and/or other animals at the destination site.
	<i>Hepatozoon</i> spp. diagnostic should be considered as part of the post release health surveillance protocol, including pathological examination, for reintroduced pine martens.

Table 13. Disease risk assessment and brief mitigation options for the carrier hazard Hepatozoon spp.

CARRIER	Neospora caninum
Justification for Hazard Status	<ul> <li>Neospora caninum is an obligate intracellular cyst-forming protozoan parasite belonging to the phylum Apicomplexa (Dubey et al., 2007). It has a global distribution and is an important veterinary pathogen, causing severe neuromuscular disease in dogs, and abortion and neonatal mortality in cattle (Dubey et al, 2007). There remains uncertainty whether <i>N. caninum</i> infection poses a substantial health risk to wildlife species, both at an individual and population level (reviewed in Donahoe et al., 2015).</li> <li>Neospora caninum has a complex facultative heteroxenous life cycle (Fig. 6) with members of the Canis genus acting as final hosts in which sexual replication occurs, and a range of intermediate hosts in which asexual replication takes place.</li> </ul>
	Neospora caninum
	Definitive host
	Portical transmission (nother to fetus) Vertical transmission (mother to fetus) Entermediate host (developing clinical disease)
	Figure 6. Life cycle of N. caninum (sourced from Donahoe et al., 2015).
	Domestic dogs ( <i>Canis familiaris</i> ), coyotes ( <i>Canis latrans</i> ), grey wolves ( <i>Canis lupus lupus</i> ), and dingoes ( <i>Canis lupus dingo</i> ) are the only confirmed definitive hosts to date (in Donahoe et al., 2015). Similarly to <i>T. gondii</i> , there are three infectious life stages of the parasite: sporozoites within sporulated oocysts (environmental resistant form of the parasite), rapidly dividing tachyzoites, and slowly multiplying bradyzoites within tissue cysts. <i>N. caninum</i> sexually replicates in the intestinal epithelial cells of the final host which then shed un-sporulated oocysts in the faeces. In the environment, oocysts undergo sporulation within 24-72 hours, thus becoming infectious. Intermediate hosts may

Exposure Assessment	Pine martens that become infected with <i>N. caninum</i> at the source site will carry the protozoa to the destination site. Being an intermediate
	Small rodents and rabbits, in which exposure to the parasite has been detected (field mouse, <i>Apodemus sylvaticus</i> , Ferroglio et al., 2007; common vole, <i>Microtus arvalis</i> , Fuehrer et al., 2010; rabbit, <i>Oryctolagus cuniculus</i> , Ibrahim et al., 2009), could act as a source of infection for pine martens. Small rodents are an important part of pine marten's diet, although in Scotland martens mainly prey on field voles ( <i>Microtus agrestis</i> , Caryl et al., 2012b). Medium-sized mammals like rabbits are also occasionally eaten. There is a low likelihood of at least one pine marten being exposed and infected at the source site. There is a low likelihood of pine martens being chronically infected with <i>N. caninum</i> when translocated.
Risk Assessment Release Assessment	<i>N. caninum</i> infection in intermediate carnivore hosts occurs via horizontal transmission through the ingestion of infected prey containing tissue cysts, or of sporulated oocyst-contaminated food or water (Dubey et al., 2007). Vertical (transplacental) transmission is the main route of infection in cattle.
	Clinical neosporosis has been rarely reported in wildlife with only twelve reports to date (see Danohe et al., 2015 for a summary of species in which disease was documented), of which one was in a free-ranging juvenile pine marten from the Netherlands (van der Hage et al., 2002). Stress may play a role in triggering disease and because the reintroduction is likely to act as a stressful event, clinical neosporosis may develop in translocated pine martens.
	<i>N. caninum</i> antibodies have been detected in two pine martens in Spain (n=3, Sobrino et al., 2008). A study conducted in England found <i>N. caninum</i> seroprevalence of 18.6% in polecats ( <i>Mustela putorius</i> , n=70), 10.9% in badgers ( <i>Meles meles</i> , n=64), 10.1% in ferrets ( <i>Mustela furo</i> , n=99), and 4.6% in mink ( <i>Neovison vison</i> , n=65) (Bartley et al., 2013). Results from these studies indicate that several mustelid species are susceptible to infection with <i>N. caninum</i> when they encounter the parasite in the environment. However, results from a test to determine if they could be definitive hosts of <i>N. caninum</i> indicate that they are not likely to be involved in the spread of oocysts (McAllister et al., 1999).
	become infected upon ingestion of sporulated oocysts. The parasite transforms into tachyzoites which spread throughout the body via the blood stream (acute phase of infection). In immunocompetent hosts, tachyzoites transform into bradyzoites, the quiescent life stage of the parasite that produces tissue cysts (chronic phase of infection). Changes in host immune status may result in reactivation of bradyzoites with conversion to tachyzoites and subsequent recrudescence of the infection.

	host for <i>N. caninum</i> , pine martens will not contribute to the release of the parasite in the environment, however they may represent a source of infection for predator species that prey on pine martens, for example foxes. Considering that i) there is no evidence of wild carnivores being involved in <i>N. caninum</i> epidemiology in the UK other than as intermediate hosts
	and ii) the low population density of the translocated pine martens, the likelihood of pine marten reintroduction itself impacting on the host-parasite dynamic at the destination is estimated to be negligible.
Consequence Assessment	Majority of the neosporosis reports documented in wildlife species were in young animals or foetuses, with only two cases described in adults. Clinical disease and pathological changes associated with <i>N. caninum</i> in wildlife species are similar to that of domestic dogs and cattle (Donahoe et al., 2015). The 7-week old pine marten pup diagnosed with neosporosis showed dehydration, diarrhoea, ataxia, dyspnoea and tachycardia. Myocardial lesions were revealed at post-mortem examination (van der Hage et al., 2002).
	Immunosuppression plays an important role in the recrudescence of latent infections and because the reintroduction is likely to act as a stressor, susceptibility to disease could increase in translocated pine martens. There is a very low likelihood, however, that stress may precipitate disease in a large proportion of released pine martens and cause reintroduction failure. The likelihood of significant biological, environmental and economic consequences is estimated to be very low.
Risk Estimation	There is a low likelihood of pine martens being exposed to <i>Neospora caninum</i> at the source site and a low likelihood of at least one pine marten being chronically infected when translocated. The likelihood of pine marten reintroduction itself impacting on the host-parasite dynamic at the destination is estimated to be negligible. There is a very low likelihood of disease occurring in a large proportion of the reintroduced population and a very low likelihood of significant biological, environmental, and economic consequences.
Risk Management	
Risk Evaluation	Based on the risk assessment above, preventative measures should be employed to reduce the risks from <i>Neospora caninum</i> as a carrier hazard.
Risk Management Options	It is advisable to minimise the level of stress in translocated pine martens (through good management) to reduce the probability of disease occurrence at the destination site.

Table 14. Disease risk assessment and brief mitigation options for the carrier hazard Neospora caninum.

#### CARRIER Toxoplasma gondii Justification for Toxoplasma gondii is an obligate intracellular protozoan (Phylum: **Hazard Status** Apicomplexa) with a worldwide distribution and the associated disease, toxoplasmosis, is of significant economic, veterinary, and medical importance. T. gondii has a complex facultative heteroxenous life cycle (Fig. 7) in which felids (domestic and wild) are the only animals capable of sustaining the sexual phase of the life cycle; however, all warmblooded animals, including humans and felids, can act as intermediate hosts. **Definitive host** Environment Sporulation Fertilization Schizogony Gamogony unsporulated (1) resistance in the environment sporulated sexual development only occurs in the definitive host's intestinal epithelium asexual developme occurs in the environ Intermediate host nt occurs in ous host tissues asexual developme multiple tissues of inte Endodyogeny ٦. tachy oite resistance in the host infection through organ transplant congenital infect Figure 7. Life cycle of T. gondii. The three main phases of development are highlighted in grey dashed lines: in the definitive host, in the environment, and in the intermediate host. Sourced from Delgado et al., 2022.

There are three infectious stages of T. gondii: the tachyzoites, the bradyzoites (found in tissue cysts), and the sporozoites (found in oocysts). When an intermediate host ingests an oocyst or a tissue cyst, the bradyzoites or sporozoites stage convert into tachyzoites upon entering the intestinal epithelium. This represents the initial acute period of infection, with tachyzoites rapidly replicating (asexual reproduction) and spreading throughout the body via the blood stream to establish systemic infection in the host. If not controlled, such infection would develop into severe acute toxoplasmosis, however, in healthy individuals symptoms are rarely developed because of the immune response of the host. In fact, under this immune pressure tachyzoites convert into bradyzoites (the slowly replicating stage of the parasite) which cluster together in host cells to avoid immune surveillance (Shen et al., 2016). These bradyzoites are enclosed within a cyst wall to form tissue cysts which are mainly found in muscular and neural tissues, including the brain, eye, skeletal and cardiac muscle but may also develop in visceral organs, including lungs, liver, and kidneys (Hill et al., 2005). Intact tissue cysts can persist for the lifetime of the host, establishing a life-long chronic infection, and can serve as a source of recrudescence to active infection (Hill et al., 2005; Jones et al., 2006). If the immune system of the host becomes compromised, the tissue cysts may rupture to release bradyzoites that can be reactivated to tachyzoites and cause acute toxoplasmosis. Shen and colleagues (2016) experimentally demonstrated that transportation stress can alter the immunity of chronically infected mice (by reducing the levels of key cytokines critical for controlling *T. gondii* infection), thus leading to the reactivation of chronic infection.

A final felid host primarily acquires infection via consuming infected intermediate-host prey, but direct ingestion of oocysts found in the environment is also possible (Hutchinson et al., 1969). In the final host the parasite undergoes sexual reproduction resulting in oocysts shed in the environment. Oocysts of *T. gondii* are formed only in felids, probably in all members of the Felidae (Hutchinson et al., 1969; Hill et al., 2005). Once shed in the environment, oocysts sporulate (become infectious) within 1-5 days depending upon aeration and temperature (Hill et al., 2005).

In mustelids, *T. gondii* has been isolated in several species including pine marten (Hejlícek et al., 1997, Sobrino et al., 2007; Lopes et al., 2011; Turceková et al., 2014). High T. gondii seroprevalence was found in pine martens (62.5%, n=64; 100%, n=4) and in stone martens (85%, n=20) in Germany and Spain (Weiland & Geisel, 1981; Sobrino et al., 2007). Serological evidence of T. gondii exposure has been reported in freeliving, non-native American mink from southern England (50% seroprevalence [Harrington et al., 2012]) and in wild free-living European polecats (*Mustela putorius*) from Britain (71.8% seroprevalence [Heald, 2017]). Seropositivity for T. gondii has been detected in Eurasian otter (Lutra lutra) across England and Wales (40% seroprevalence [Chadwick et al., 2013]). Similar high seroprevalence has been reported in a reintroduced fisher (Martes pennanti) population in Pennsylvania (Larkin et al., 2011). Also, high prevalence has been documented in pine martens (67.8%, n=152) and in stone martens (52.9%, n=34) in Latvia and Lithuania (Deksne et al., 2017).

The pathogenicity of *T. gondii* varies depending on the virulence of the strain and the susceptibility of the host species, with certain species being more resistant to clinical toxoplasmosis (Hill et al., 2005). Acute toxoplasmosis has been reported in farmed American mink (*Neovison vison*) from Europe and the USA (Frank, 2001; Smielewska- Łoś & Turniak, 2004). Toxoplasmosis was diagnosed in a young free-living mink found in Michigan (USA) with signs of left hind limb lameness, ataxia, head tremors, and bilateral blindness (Jones et al., 2006). Black-footed

and chronic disease considering the epizootic of toxoplasmosis which occurred in a captive-breeding population in Kentucky, USA (Burns et al., 2003). Disease has also been reported in ferrets from New Zealand (Thornton & Cook, 1986; Thornton, 1990). As far as the authors are aware, there are no reports of toxoplasmosis in pine martens.In healthy animals with a competent immune system, clinical signs of disease are generally rare; however, severe illness may develop in immunocompromised individuals. The reintroduction, which is likely to act as a stressful event, could therefore trigger disease, toxoplasmosis, in translocated pine martens.Risk AssessmentThe most common transmission routes for infection of pine martens are through i) ingestion of sporulated oocysts excreted by felids in the environment - vegetation, soil, water - where, under favourable
disease are generally rare; however, severe illness may develop in immunocompromised individuals. The reintroduction, which is likely to act as a stressful event, could therefore trigger disease, toxoplasmosis, in translocated pine martens.Risk AssessmentThe most common transmission routes for infection of pine martens are through i) ingestion of sporulated oocysts excreted by felids in the
Release AssessmentThe most common transmission routes for infection of pine martens are through i) ingestion of sporulated oocysts excreted by felids in the
through i) ingestion of sporulated oocysts excreted by felids in the
conditions, they may remain viable for up to a year (Dubey, 1991; Hill et al., 2005); ii) through ingestion of tissue cysts upon eating infected prey (Hill et al., 2005). Vertical transmission from mother to offspring has been documented in Australian marsupials (Parameswaran et al., 2009).
Considering the ubiquity of the parasite and the high seroprevalence exhibited by several Mustelidae species in the UK, the likelihood of pine martens being exposed and infected with <i>T. gondii</i> at the source site is estimated to be medium. There is a medium likelihood of pine martens being chronically infected with <i>T. gondii</i> when translocated.
<b>Exposure Assessment</b> Pine martens that become infected with <i>T. gondii</i> at the source site will carry the protozoa to the destination site. Being an intermediate host for <i>T. gondii</i> , pine martens will not contribute to the release of the parasite in the environment (only felids are known to shed oocysts in their faeces), however they could represent a source of infection for predator species that prey on martens, for examples red foxes or scavenger species.
Considering the low population density of the translocated pine martens, the reintroduction itself is predicted to have a low impact on the host-parasite dynamic at the destination. The likelihood of exposure and infection is estimated to be low in those free-living species known to predate on pine martens and negligible in all other free-living species.
The hazard is already widespread in the environment and the likelihood of its dissemination at the destination site because of pine marten reintroduction is very low.
<b>Consequence</b> The probability that at least one pine marten is chronically infected with
AssessmentT. gondii at the time of translocation is medium. Infection is often asymptomatic and severe clinical manifestations are rarely seen. However, severe clinical toxoplasmosis (with lethargy, anorexia, ataxia,

	disorientation, circling, incontinence, and death) has been recorded in several Mustelidae species (Frank, 2001; Burns et al., 2003; Smielewska- Łoś & Turniak, 2004). Disease has not been reported in pine martens. Nonetheless, we estimate a medium likelihood that the translocation procedures themselves will lead to an alteration in host-parasite dynamics resulting in immunocompromise and recrudescence of chronic infection leading to acute disease in some individuals. In case of acute clinical disease, consequences are likely to be severe for the individual with a high probability of death. There is a low likelihood, however, that stress may precipitate disease in a large proportion of released pine martens and cause reintroduction failure. No cases of disease have been detected in previous pine marten conservation translocations (n=51 animals reintroduced in the Welsh reinforcement; n=35 animals reintroduced in the Forest of Dean reintroduction). The likelihood of significant biological and economic consequences for the
	reintroduction project itself is predicted to be low. Since <i>T. gondii</i> is widespread in the environment, the likelihood of significant environmental consequences at the destination site is negligible.
Risk Estimation	There is a medium likelihood of pine martens being exposed to <i>T. gondii</i> at the source site and a medium likelihood of at least one pine marten being chronically infected when translocated. The likelihood of exposure and infection of other wild animals at the destination site is low for predator species of pine martens and negligible for all other species. Dissemination of <i>T. gondii</i> at the destination is likely to be very low. There is a medium likelihood of at least one infected pine marten developing acute disease and a high likelihood of significant consequences for such individuals. However, the likelihood of stress precipitating disease in multiple individuals is estimated to be low. There is therefore a low likelihood of significant biological and economic consequences for the reintroduction project itself. The likelihood of significant environmental consequences at the destination site is negligible.
	The overall risk is estimated to be <b>LOW</b> .
Risk Management	
Risk Evaluation	Based on the risk assessment above, preventative measures should be employed to further reduce the risks from <i>Toxoplasma gondii</i> as a carrier hazard.
Risk Management Options	It is advisable to minimise the level of stress in translocated pine martens (through good management) to reduce the probability of disease occurrence at the destination site.
	Serology post capture is possible and the presence of IgG in the absence of IgM has been used as an indicator of <i>T. gondii</i> chronic infection in humans (Stepanova et al., 2017). However, since the proportion of seropositive pine martens is expected to be high, and that there are no records of post-release disease outbreaks due to toxoplasmosis in any

mammal, we do not envisage preventing seropositive pine martens from being translocated.
<i>Toxoplasma gondii</i> diagnostic should be considered as part of the post release health surveillance protocol and pathological examination method for reintroduced pine martens.

Table 15. Disease risk assessment and brief mitigation options for the carrier hazard Toxoplasma gondii.

POPULATION	Toxoplasma gondii
Justification for Hazard Status	<i>Toxoplasma gondii</i> has already been evaluated as a carrier hazard. Here we consider it as a potential population hazard, and we analyse how chronic <i>T. gondii</i> infection may affect the risk from road traffic collision or predation to pine marten reintroduction efforts.
	Latent infection with <i>T. gondii</i> is known to induce behavioural changes in intermediate hosts as a result of predilection to 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). Slower reaction times have been linked to chronic infection with <i>T. gondii</i> in humans (Havlícek et al., 2001) and, as a result, may increase the risk of the host being involved in road traffic collisions (Flegr et al., 2002; Yereli et al., 2006; Kocazeybek et al., 2009; Galván-Ramírez et al., 2013; Stepanova et al., 2017; Gohardehi et al., 2018). There is evidence to suggest that latent infection with <i>T. gondii</i> may affect behaviour in other mammals. An Australian study by Hollings and colleagues (2013) found a significantly higher seroprevalence to <i>T. gondii</i> in road-killed Tasmanian pademelons ( <i>Thylogale billarderii</i> , 31%, n=16) than in culled individuals (11%, n=212), suggesting that infected animals. However, some caution should be used interpreting these data because of the low number of road-killed samples relative to the culling samples. A few studies have been carried out in rodents. In brown rats, Berdoy (2000) found that individuals experimentally infected with <i>T. gondii</i> 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 <i>T. gondii</i> 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 et al., 2007). Positive serology for <i>T. gondii</i> has been significantly associated with reduced neophobia (fear of novel objects) in brown rats (Webster et al., 1994). As well as advantageously aff

Risk Assessment	
Exposure Assessment	Our analysis of <i>T. gondii</i> as a carrier hazard has estimated a medium likelihood of pine martens being infected when translocated (chronic subclinical infection – tissue cyst stage of the parasite). Research suggests that chronic infection with T. gondii may cause behavioural changes that increase susceptibility to road traffic collision or predation which decrease survival. Although evidence is somewhat conflicting, the neurological/behavioural effects of <i>T. gondii</i> reported in rodent species as a result of the formation of tissue cysts in the brain, as well as increased likelihood of exposure of road traffic collision implied in other species, suggests that an increased likelihood of road traffic collision implied in other species, suggests that an increased likelihood of road traffic collision in pine martens is known to be high.
	We estimate a medium likelihood of at least one reintroduced pine marten suffering from road traffic collision or predation because of chronic infection with <i>T. gondii</i> .
Consequence Assessment	<i>T. gondii</i> may cause behavioural changes that decrease survival, and chronically infected individuals could be more susceptible to road mortality or predation. Mortality rates as a result of these events are likely to be high. The likelihood of severe consequences is estimated to be high in those chronically infected individuals; however, cases are expected to be sporadic, and we estimate a low likelihood of significant biological and economic consequences, through failure of the reintroduction programme itself.
Risk Estimation	There is a medium likelihood of at least one pine marten being chronically infected with <i>T. gondii</i> when translocated. The likelihood of at least one reintroduced pine marten suffering from road traffic collision or predation because of chronic <i>T. gondii</i> infection is estimated to be medium. The likelihood of severe consequences is estimated to be high in those chronically infected individuals, but cases are likely to be sporadic. The likelihood of significant biological and economic consequences, through failure of the reintroduction programme itself, is estimated to be low.
Risk Management	
Risk Evaluation	Based on the risk assessment above, preventative measures should be employed to further reduce the risks from <i>T. gondii</i> as a population hazard.
Risk Management Options	Road mitigation measures may include arboreal crossings, improved warning signage of potential wildlife-vehicle collisions, realization of dedicated wildlife underpasses/crossings, reducing habitat quality along roadsides. The risk options discussed in the assessment of <i>T. gondii</i> as a carrier hazard are also relevant here.

Table 16. Disease risk assessment and brief mitigation options for the population hazard Toxoplasma gondii.

The control of commensal rodent populations is a critical component of modern agricultural practice and anticoagulant rodenticides (ARs) are commonly used (Stenseth et al., 2003). First generation ARs, FGARs (coumarin compounds, coumatetralyl, warfarin, 1,3-indandiones, chlorophacinone and diphacinone) have been replaced by the more potent second generation SGARs (brodifacoum, bromadiolone, difenacoum, difethialone and flocoumafen) which display a greater acute toxicity, accumulation, and persistence (Tosh et al., 2011). ARs are not target-specific, and their use can therefore lead to unintended
commonly used (Stenseth et al., 2003). First generation ARs, FGARs (coumarin compounds, coumatetralyl, warfarin, 1,3-indandiones, chlorophacinone and diphacinone) have been replaced by the more potent second generation SGARs (brodifacoum, bromadiolone, difenacoum, difethialone and flocoumafen) which display a greater acute toxicity, accumulation, and persistence (Tosh et al., 2011). ARs are
exposure of non-target species. Exposure can either be primary (direct consumption of bait) or secondary (when predators or scavengers consume an animal that has already been exposed). In the UK, evidence of secondary exposure to ARs has been reported in several predatory mammalian species (22.5% (n=45) of stoats <i>Mustela erminea</i> ; 30% (n=10) of weasels <i>Mustela nivalis</i> ; 36% (n=50) of polecats <i>Mustela putorius</i> , McDonald et al., 1998; Shore et al., 2003; Tosh et al., 2012). An extensive study carried out in northern Scotland (Ruiz-Súarez et al., 2016), focused on the rate of exposure of the American mink which, given its generalist diet, can be considered a sentinel species to ARs for other sympatric mustelid carnivores including pine marten. Overall, 79% of mink (n=99) had detectable residues of ARs compounds in their livers, with over half exposed to two or more compounds, and a fifth to three or more compounds. The most common SGARs found were bromadiolone and difenacoum. The most toxic, single-feed SGARs (brodifacoum, flocoumafen) were found in 10% of mink. The study also showed that mink were increasingly likely to have acquired ARs as they aged, with virtually all two year-old individuals contaminated to 0.2 mg/kg, which has been previously suggested as a potential toxicity threshold associated with mortality in mustelids and other mammals (Grolleau et al., 1989; Newton et al., 1999). The rate of accumulation was found to be 1.7 times higher in locations with proportionally more farms compared to those with fewer farms: nonetheless, these results
farms compared to those with fewer farms; nonetheless, these results do not rule out the contribution of other sources of poisoning (e.g., rodent control by gamekeepers, domestic rodent control). The high levels of exposure and the high concentrations of ARs documented by Ruiz-Súarez and colleagues (2016) suggest possible risk to other non- target species, especially native mustelid of high conservation status. Because of their foraging ecology and diet, pine martens are expected
to be susceptible to AR secondary exposure; in fact, a recent study in the Parc National des Pyrénées, France, has found a 41% (n=17) prevalence of AR exposure in the species (Lestrade et al., 2021). Cases of poisoning may have a significant impact on a newly reintroduced small population of pine martens.
High AR residues have been found in field mice ( <i>Apodemus</i> spp.) followed by voles ( <i>Microtus</i> and <i>Myodes</i> spp.) and shrews (Sorex and Crocidura spp.) sampled in Germany (Gedhun et al., 2015; 2016), strongly supporting previous reports of secondary exposure risk via non-

	targets in the UK (Brakes & Smith, 2005). Pine martens predate small mammals and are known to eat carrion and therefore they have a medium probability of being exposed to ARs. Factors that are likely to contribute to the risk of non-target exposure include the persistence and toxicity of the rodenticides, level of usage, and how and when they are used (Shore et al., 2003; Shore et al., 2006). In the UK, voluntary and regulatory measures relating to ARs usage (best practice guidelines and confinement of the more toxic and persistent flocoumafen and brodifacoum to use indoors) have been introduced to reduce the level of risk to non-target wildlife (Tosh et al., 2011). However, the uptake of best practice guidelines appears to be heterogeneous amongst farmers, leading to a potential increased probability of exposure of non-target wildlife species (Tosh et al., 2011). For example, the incidence of the most toxic, single-feed SGARs brodifacoum (9%) and flocoumafen (2%) in mink in Scotland was of concern considering the chemicals were only approved for indoor use at the time of sampling, thus suggesting unapproved use outside of buildings cannot be ruled out (Ruiz-Súarez et al., 2016).
	Pine marten exposure to ARs is likely to be sporadic. The probability of exposure of reintroduced pine martens to ARs is medium because pine martens feed on rodents which are the target of ARs. In fact, the field vole <i>Microtus agrestis</i> accounts for 40% of the pine marten diet (Caryl et al., 2012b).
Consequence Assessment	ARs work by blocking the vitamin K cycle which is essential to produce blood-clotting factors, thus resulting in haemorrhage (Valchev et al., 2008). Clinical symptoms are initially general and non-specific, including somnolence, weakness, pale mucosa, anorexia, polyuria, polydipsia, decreased locomotion and perception, rapid and easy exhaustion (Valchev et al., 2008). Death results from diffused haemorrhages.
	In the eventuality of pine martens being exposed to ARs, there is a high likelihood of severe consequences due to debility or mortality associated with toxicity. Direct mortality from ARs has been documented in fishers ( <i>Martes pennati</i> - Gabriel et al., 2012). In addition to the risks from lethal toxicosis, sub-lethal AR exposure can produce significant clotting abnormalities and some haemorrhaging (Eason & Murphy, 2000), and such effects may be detrimental if combined with other stressors that have additive or synergistic effects (Erickson & Urban, 2004). Minor wounds or trauma, not normally life-threatening, may cause prolonged bleeding and mortality if blood-clotting mechanisms are disrupted (Erickson & Urban, 2004). Capture and transport of animals could cause minor trauma and the likelihood of this happening is estimated to be low. The likelihood of significant economic and biological impact is estimated to be low, linked to the possibility of sporadic poisoning cases in some reintroduced pine martens.

Risk Estimation Risk Management	There is a medium likelihood of pine martens being exposed to anticoagulant rodenticides. If an animal is exposed, there is a high likelihood of severe consequences to the individual. Cases of poisoning are likely to be sporadic and to not involve the whole reintroduced population. There is a low likelihood of significant economic and biological impact on the reintroduction programme itself. The overall risk is estimated to be <b>MEDIUM</b> .
Risk Evaluation	Based on the risk assessment above, preventative measures should be
	employed to reduce the risks from anticoagulant rodenticides.
Risk Management	Education campaign (in the vicinity of the pine martens' release site) to
Options	raise landowner awareness about the threats posed by anticoagulant rodenticides on non-target wildlife species should be considered. The campaign should promote best practice guidelines relating to a responsible use of anticoagulant rodenticides for rodent control to reduce the risk of non-target exposure.
	The likelihood of mechanical trauma during translocations should be minimised to prevent haemorrhage.
	It is advisable to integrate testing for ARs into the PRHS protocol. Any pine marten found dead will be submitted for post-mortem examination, samples will be submitted for toxicological screening, and if poisoning by anticoagulant rodenticides is diagnosed the appropriate authorities will be notified.

Table 17. Disease risk assessment and brief mitigation options for the population hazard Anticoagulant rodenticides.

POPULATION	Illegal persecution
Justification for Hazard Status	Reintroduced pine martens may be vulnerable to illegal persecution (through snaring, hunting, shooting or malicious poisoning) at the release site if their reintroduction is negatively perceived by local communities. For example, the possibility that reintroduced pine martens would predate captive poultry or pheasants was identified as a potential source of human-wildlife conflict in the feasibility study for the reintroduction of pine martens to the Forest of Dean, Gloucestershire (Stringer et al, 2018). As reported in the feasibility study carried out for the proposed Two Moors Partnership project, active predator control programmes are already in place at shooting estates and poultry farms found at destination. Also, lethal control via trapping for grey squirrels by foresters is known to be operational. Other predator control methods targeting different species might also be in place. Toxic compounds which are reported as a cause of intoxications are insecticides (organophosphate and carbamate), rodenticides (anticoagulant rodenticides, zinc phosphide, bromethalin, strychnine), avicides (alpha-chloralose), and molluscicides (metaldehyde, methiocarb) (Berny, 2007). Although sporadically, wildlife incident cases, including cases where toxic compounds were used in breach of their authorisation with the deliberate intent of harming or attempting to harm wildlife or other animals, have been referred to Wildlife Incident Investigation Scheme in the county of Devon in the last few years.
	reintroduced small population of pine martens.
Risk Assessment	
	Any potential conflict between wildlife and humans may result in illegal persecution. Reintroduced pine martens could be perceived as a potential threat by landowners at destination and therefore become a target of deliberated harming. Also, reintroduced pine martens could accidentally become exposed to lethal control methods intended for other species. We therefore estimate a low likelihood of sporadic cases of illegal persecution occurring amongst the reintroduced pine marten population.
Consequence Assessment	In the eventuality of illegal persecution occurring, there is a high likelihood of severe consequences with death of the persecuted individual. The slow reproduction rate of pine martens means that slow rates of additional mortality can significantly increase the likelihood of local extinctions (MacPherson, 2014). The likelihood of economic and biological impact is therefore estimated to be medium because cases of illegal persecution, even if sporadic, may impact a newly establishing pine marten population.
Risk Estimation	There is a low likelihood of sporadic cases of illegal persecution occurring within the reintroduced pine marten population. There is a high likelihood of severe consequences to the persecuted individual.

Risk Management	There is a medium likelihood of economic and biological impact on the reintroduction programme itself. The overall risk is estimated to be <b>MEDIUM</b> .
Risk Evaluation	Based on the risk assessment above, preventative measures should be employed to reduce the risks from illegal persecution.
Risk Management Options	Engagement with all landowners close to the release areas is crucial and is best done through direct communication so that clear messages are conveyed, thus ensuring that measures are taken to address this risk. A primary stakeholder group to engage with is represented by the shooting estates and poultry farms found at destination, to understand any concern they might have regarding the proposed pine marten reintroduction project and to develop a detailed mitigation plan to address their concerns and mitigate any negative impacts reintroduced pine martens might be responsible for. It is advisable to integrate testing for toxic compounds into the DRM PRHS protocol. Any pine marten found dead will be submitted for post- mortem examination and if poisoning (or other signs of illegal persecution) is diagnosed the appropriate authorities will be notified and mitigation will be implemented.

Table 18. Disease risk assessment and brief mitigation options for the population hazard Illegal persecution.

Justification for Hazard Status	Trauma (capture/handling related) Physical trauma may arise at any point during the reintroduction
Hazard Status	This feat that that any alloc at any point during the reintroduction
	process (capture, handling, transportation) and is usually associated
	with an animal's acute stress response and escape behaviour, poorly
	designed equipment, inadequate planning, and procedures.
Risk Assessment	
Exposure Assessment	There is a medium likelihood of at least one reintroduced pine marten
	suffering from a capture/handling related trauma, especially if capture
	and handling protocols have not thoroughly been discussed and agreed
-	in advance.
Consequence	Oedema, lacerations, foot pad abrasions, fractures of the limbs are all
Assessment	possible capture-related injuries. Dental injuries (tooth damage or loss)
	can also be a consequence of escape behaviour and has been seen in
	reintroduced otters (Serfass et al, 1996; Fernández-Morán et al, 2002). There is a medium likelihood that at least one reintroduced pine marten
	will be affected. The significance of the injury on the health of the animal
	is likely to be multifactorial and influenced by the presence of underlying
	disease and environmental conditions. There is a low likelihood of the
	hazard threatening the reintroduction project itself.
Risk Estimation	There is a medium likelihood of at least one reintroduced pine marten
	reintroduction project itself.
	The overall risk is estimated to be <b>LOW</b> .
Risk Management	
<b>Risk Evaluation</b>	Based on the risk assessment above, preventative measures should be
	employed to reduce the risks from capture/handling related trauma as
Risk Management	•
Options	
	· · · ·
	will be fulfitier discussed in the DRM PRHS protocol.
	All equipment for catching/transport (trans holding pens hoves crates)
	Any abrasion, laceration or wound that might occur should be
	Any abrasion, laceration or wound that might occur should be adequately dealt with (topical cleansing and disinfection, suturing if
Risk Evaluation Risk Management	The overall risk is estimated to be LOW. Based on the risk assessment above, preventative measures should b

Table 19. Disease risk assessment and brief mitigation options for the population hazard Trauma.

## **6 DISCUSSION**

In this disease risk analysis for the reintroduction of free-living, wild pine martens from source sites across the Highlands in northern Scotland, to destination sites in Dartmoor and Exmoor National Parks, southwest England, we have described the translocation pathway; assessed geographical and ecological barriers to the spread of parasites; identified, reviewed and evaluated 72 potential hazards (66 infectious and six non-infectious); and carried out a detailed disease risk analysis on sixteen prioritized hazards, followed by a brief discussion on how the risk from these hazards could be mitigated. In so doing, fourteen hazards which received detailed disease risk analysis for the Forest of Dean reintroduction project (Carraro & Sainsbury, 2019; Carraro et al., 2021) have been re-assessed and two newly prioritized hazards analyzed.

The proposed translocation pathway does not cross geographical and/or ecological barriers and, consequently, we have mainly focused our analysis on the risk arising from carrier, transport, and population hazards, reducing the necessity to consider novel source and destination hazards given the likely parasite connectivity between source and destination sites. One hazard, *Mycobacterium bovis*, was classified and analyzed as a destination hazard considering the peculiar epidemiological situation within the UK which entails Scotland recognised as Officially TB Free by the European Commission since September 2009 while *M. bovis* is endemic in the southwest of England, an area classified as a High Risk Area for bTB.

Of the sixteen hazards prioritized for full disease risk analysis, the transport hazard SARS-CoV-2 was assessed as currently being of high risk for specific groups of translocated pine martens but of medium risk for other mammals at the destination; and a further two were assessed as medium risk, Anticoagulant Rodenticides (ARs) and Illegal Persecution, both non-infectious population hazards that represent a threat to a small, newly released pine marten population. Engagement with landowners as well as hunting estates at the destination is crucial to mitigate the risk from these two population hazards. Of the remaining fully analyzed hazards, nine were assessed as low risk: the population hazards Canine distemper virus (CDV), High and Low Pathogenic Avian Influenza Virus (HPAIV and LPAIV), *Mucoraceae* spp., *Toxoplasma gondii*; and four as very low risk: the destination hazard *Mycobacterium bovis*, the population hazards Canine parvovirus type 2 (CPV-2), Canine amdoparvovirus 1 (previous Aleutian mink disease virus AMDV), and the carrier hazard *Neospora caninum*. Following detailed literature review, the remaining fifty-six identified potential hazards were assessed as of very low, if not negligible, risk and are summarized in Appendix One.

#### SARS-CoV-2

The risk arising from SARS-CoV-2 was identified to be high for specific groups of translocated pine martens and medium for other mammals at the destination. Preventing human-to-wildlife spillover is important to protect free-living wildlife from SARS-CoV-2-associated disease and to avoid establishment of new wildlife reservoirs. Humans are currently the source of ongoing SARS-CoV-2 onward transmission and pine martens are more likely to be exposed to SARS-CoV-2 at the human-animal interface, during the translocation procedure, when in close contact with staff involved in the reintroduction process. Good biosecurity

measures will help mitigate the risk from SARS-CoV-2: the key advice, which conforms to guidelines from the OIE on working with free-ranging wildlife during the Covid-19 pandemic (2020), is to reduce handling of pine martens to the minimum and to employ and correctly use PPE (face masks, gloves, disinfection procedures) when handling these animals so that exposure to SARS-CoV-2 can be prevented.

# Potential effect of pine marten reintroduction on the epidemiology of *M. bovis* and on the control of bovine TB in England

Severe economic costs are associated with the control of *M. bovis* in domestic livestock in England. The presence of a wildlife reservoir may reduce the efficacy of eradication programs in livestock. Concerns have been raised about a potential role of reintroduced pine martens in the epidemiology of this pathogen at the release site, especially regarding the capability of mustelids for onward transmission. Currently in the UK, of wildlife species, only badgers and deer have been identified as playing a role as an actual or potential source of *M. bovis* infection to domestic livestock. For the infection to become endemic in free-living wild populations, interactions between many factors such as density and social behaviour, frequency of contact with domestic animals and availability of trophic resources is required. Sufficient intraspecies transmission is needed to maintain infection in any given population and, even though we have assessed reintroduced pine martens becoming infected with M. bovis once released as of low likelihood, and dissemination of *M. bovis* amongst reintroduced individuals as of low probability, we estimate a very low likelihood that onward transmission will be sufficient for *M. bovis* to become endemic in the reintroduced pine marten population. The proposed pine marten reintroduction is likely to have negligible biological and economic consequences to the epidemiology of bTB disease in livestock in England.

#### Stressor-associated disease and reintroduction of pine martens

In this disease risk analysis, five of the fully assessed hazards, and most of the hazards addressed in Appendix One are precipitated by stressors. Translocation has been shown through detailed research to be a substantial stressor for all wild animal species (Dickens et al., 2010) and therefore detailed planning of stress management to mitigate the risk from stressor-associated disease is crucial for the proposed pine marten reintroduction. It is widely understood and accepted that stress can occur at many stages of the translocation procedure (e.g., capture, handling, transport, release) and stressors may result in reduced immunocompetence, and immunocompromised individuals will be more susceptible to disease if infected. In addition, the pathogenicity of commensal organisms, which do not ordinarily precipitate disease in healthy individuals, may be increased. Stress management is advised for all those infectious agents classified as carrier hazards in this disease risk analysis.

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

In our report we make disease risk management recommendations, to reduce the risk from disease from the sixteen fully assessed hazards. Two principles of good disease risk management in translocations, will be used to reduce the risk from disease for all hazards (both the fully assessed hazards above and those estimated as of very low risk in Appendix One). Firstly, we make recommendations for translocation protocols that minimize stress to

individuals because immunocompromised individuals will be more susceptible to disease, and stress induced by the translocation process is a likely cause of immunosuppression. Information on animal stress physiology in translocations can be found in Dickens et al. (2010). Secondly, the probability of exposure to many hazards will be reduced through good hygiene and biosecurity during the translocation process (e.g., ensuring that all equipment used is dedicated to the project and is routinely cleaned and disinfected prior to use). Maintaining high standards of quarantine and clean environments should be standard practice and some information and case studies on how this can be achieved is available in Vaughan-Higgins et al., 2017. Our standard practice, developed over thirty years of monitoring translocations in England, is to convert the mitigation recommendations given in the DRA into a comprehensive, evidence-based, practically oriented Disease Risk Management and Post-Release Health Surveillance Protocol (DRM PRHS) and we recommend that such a protocol is formulated for any conservation translocation, including the proposed one discussed here.

#### Parasite conservation

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 prevention of disease without elimination of parasites, while maintaining host immune responses, as we have shown in the conservation of the commensal parasite, *lsospora normanlevinei*, which was associated with stressor-associated disease in reintroduced cirl buntings to Cornwall (McGill et al., 2010). Pine martens may harbour species-specific parasites and parasite conservation should therefore be an integral and important component of a DRM PRHS protocol.

#### Current use and future updates to this disease risk analysis

This disease risk analysis was written for the proposed pine marten reintroduction from Scotland to the Dartmoor and Exmoor National Parks, southwest England, and it is intended as advisory to the steering committee of the project. The risk estimations made in this analysis are intended for discussion amongst the steering committee and other relevant stakeholders, and decisions on the level of acceptable risk can be made in the context of the conservation, ecological and social impact of pine marten reintroduction, and the other feasibility studies being carried out. This report should be viewed as a living document which requires continual update as new evidence becomes available, both published evidence and that arising from long-term monitoring of previously translocated pine marten populations. Moreover, this DRA can be readily adapted to any future pine marten translocation within mainland UK, particularly if the pine martens follow a translocation pathway similar to the one described in this report.

# **7 APPENDIX ONE**

Our initial assessment of identified potential hazards indicated that some are likely to pose very low, if not negligible, risk to the proposed pine marten reintroduction to Dartmoor and Exmoor National Parks, southwest England. For these potential hazards a detailed risk assessment was therefore not completed, and no mitigation strategies are suggested. A description of each hazard, to include their geographic distribution, occurrence, species susceptibility, pathogenicity, associated disease, and impact on population numbers is provided in this Appendix instead. The risk posed by these potential hazards requires regular review as our understanding of disease in pine marten improves.

#### VIRUSES

- Canine adenovirus 1 (CAdV1) is the causative agent of infectious canine hepatitis, a • highly contagious and often fatal disease mainly of domestic dogs (Decaro & Buonavoglia, 2012). Closely related CAdV2 is less pathogenic and is responsible of tracheo-bronchitis. In red foxes, CAdV1 can be responsible for neurological manifestations and CAdV1 seroprevalence of 19% to 64.4% has been indicated for the species in the UK (Thompson et al. 2010; Walker et al., 2016). Red foxes are believed to play a major role as wildlife reservoir of CAdV1 in UK and Italy (Walker et al., 2016; Balboni et al., 2013). Antibodies to CAdV1 have also been detected in free-ranging mustelids in south-western France, including European mink, polecats, American mink, and stone martens (Philippa et al., 2008). Detection of antibodies in wildlife may reflect the widespread circulation of CAdV1 in wildlife, which is only sporadically associated with clinical disease, therefore suggesting a lower pathogenicity in freeranging carnivores compared to domestic species (Decaro & Buonavoglia, 2012). CAdV1 is likely to have a very low, if not negligible impact on reintroduced pine martens, however a liaison with local veterinary practices at both source and release sites would be advisable to have a better knowledge of the disease status reported in domestic species. Interestingly, a recent study has detected at least three novel adenoviral sequences in tissues and faeces of pine martens and otters (Walker et al., 2017). However, further research is needed to prove if these represent true mustelid adenoviruses able to replicate and transmit within their host species.
- **Canine coronavirus (CCoV)** antibodies have been detected in fishers in Canada (Philippa et al., 2004) but no other data on infection and/or associated disease in wildlife are currently available. Disease associated with coronavirus infections (*ferret corona virus*) has been reported in domestic ferrets in the UK (Graham et al., 2012; Thomas et al., 2012). No data are available on pine marten's susceptibility to infection and/or disease.
- **Canine parainfluenza virus** causes infectious respiratory disease complex, a common problem in domestic dogs in kennels. Philippa and colleagues (2008) have detected

antibodies in European mink, American mink, and polecats, but not in *Martes* spp. No reports of disease in wildlife have been found. The hazard is considered to have a negligible impact on the pine marten reintroduction.

- Feline parvovirus (FPV) and mink enteritis virus (MEV) are responsible of haemorrhagic enteritis associated with leukopenia in canines and felines. Both have been identified in a wide array of hosts, including *Mustelidae* spp. (Decaro et al., 2012; Steinel et al., 2001). As far as the authors are aware, the only species of martens in which to date these viruses have been identified is the Pacific marten (*Martes caurina*) (Canuti et al., 2020). These hazards are considered to have a negligible impact on the proposed pine marten reintroduction.
- Louping ill virus (LIV) is a tick-borne RNA virus (genus Flavivirus, family Flaviviridae) • closely related to the tick-borne encephalitis virus (TBEV). TBEV is not found in the British Isles, while LIV is present instead and it is mainly detected in sheep, cattle, and red grouse in upland areas of the British Isles, particularly in Scotland, Cumbria, Wales, Devon, and Ireland (Jeffries et al., 2014). Disease (febrile illness that can progress to fatal encephalitis) is relatively common in domestic livestock as well as wild red grouse exposed to the virus (Reid, 2012). Seroprevalence studies suggest that infection in wildlife species do happen, however majority of wild species do not show overt disease (Jeffries et al., 2014). Disease has also been reported in humans in the UK since 1934 (44 published reports to date), with infection mainly occurring through occupational exposure to infected livestock (Jeffries et al., 2014). No human cases of LIV encephalitis have been diagnosed in the UK over the past 20 years, however numerous cases of encephalitis of unknown origin are reported annually, some of which may be due to LIV infection (Jeffries et al., 2014). In August 2018 a case of polioencephalitis due to LIV infection was reported in a pine marten in Scotland (SRUC VS disease Surveillance, August 2018) and it represents the first report of LIV infection and disease in a mustelid species. There have been several experimental studies to determine which mammal species, other than sheep, might contribute to the maintenance of LIV in the environment. However, results suggest that domestic sheep are the only essential reservoirs for LIV, while infection of other species only makes a minor contribution to virus maintenance (Reid, 2012). It is highly unlikely that infected pine martens would play any role in the epidemiology of LIV. Immunosuppression may enhance pathogenicity, as suggested by experimental studies of concurrent infection of LIV with A. phagocytophilum in sheep (Reid et al, 1986), however LIV is considered to have a very low, if not negligible, impact on the proposed pine marten reintroduction.
- Mustelid herpes virus 1 (MusHV1) has been frequently reported in badgers in the UK (Banks et al., 2002; King et al., 2004) but it has not yet been associated with lesions or clinical disease (Widén et al., 2012). Herpesviruses are generally well adapted to their natural host, but there are several that can cross the species barrier and infect other animals (Widén et al., 2012). No reports of herpesvirus infection and/or disease have been found in pine martens.
- **Rotavirus** infection and associated enteritis has been reported in domestic ferrets and captive black-footed ferrets (Williams & Thorne, 1996). No reports have been found in

free-ranging wild mustelids. *Rotavirus* is expected to have a negligible impact on the pine marten reintroduction.

#### BACTERIA

- **Anaplasma phagocytophilum**, a tick-borne rickettsial parasite, is a multi-host pathogen for which infection has been reported in many domestic and wild animals (Birtles, 2012b). It is the causative agent of tick-borne fever (TBF) in domestic ruminants. However, apart from infection being reported in American fishers (Brown et al., 2008) no reports of infection or disease have been found for other *Mustelidae* species.
- **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 on pine marten infection with *Bartonella* spp. have been found. The hazard is considered to have a negligible impact on the pine marten reintroduction.
- 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 & Vikøren, 2012). The role of mustelids in the perpetuation of the bacterium is still unknown. *B. burgdorferi* generally establishes persistent infections with minimal harm to its natural hosts, with clinical disease usually developing only in aberrant hosts such as humans and domestic animals (Ytrehus & Vikøren, 2012).
- *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 & Speck, 2012). Clostridium botulinum is the most significant and widely reported species; it produces a neurotoxin responsible for the disease, botulism, which in the wild predominantly affects birds, particularly waterfowl, but mammals are also susceptible. Botulism in wildlife occurs following the ingestion of preformed toxin (food poisoning), therefore it is advisable to keep hygiene at maximum standards at the release pens to prevent potential toxin production in decaying organic matter. *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). No reports have been found in pine martens and/or other Mustelidae species.
- **Coxiella burnetii** is a worldwide distributed bacterium, responsible for Q fever, a disease affecting humans and 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*. No reports of infection or disease have been found in any *Mustelidae* species.

- *Helicobacter* spp. are Gram-negative bacteria that mainly infect the gastrointestinal tract. Most species are carried by animals and humans without causing apparent disease. Their pathogenicity in wild animals is poorly understood and clinical signs have not been described in wildlife (Speck, 2012a).
- Lepromatous mycobacteria (*M. leprae* and *M. lepromatosis*) are capable of infecting humans, red squirrels, and armadillos (Meredith et al., 2014; Avanzi et al., 2016). No other hosts are currently known. Naturally acquired murine leprosy, caused by *M. lepraemurium*, has been observed in rats, mice and cats, but not in any other species (Rojas-Espinosa & Løvic, 2001).
- Listeria monocytogenes is a Gram-positive bacterium found worldwide and responsible for a disease, listeriosis, that can affect both animals and humans. It is found in soil, decomposing matter but also in the gastrointestinal tract of healthy animals. To date infection and/or disease has not been reported in pine martens.
- Salmonella spp. are Gram-negative bacteria with a worldwide distribution which are responsible of zoonoses of public health significance, causing acute enteric disorders in humans. Infection has been reported from free-ranging wild badgers (Wilson et al., 2003; Gaffuri & Holmes, 2012). Salmonella spp. infection and/or disease have not been reported in pine martens.
- **Mycobacterium microti** is responsible for the disease vole tuberculosis in its main host, the field vole (*Microtus agrestis*). Infection has been reported to be endemic with high prevalence in field voles in the UK (Burthe et al., 2008). Infection has only occasionally been reported in badgers (Gavier-Widen et al., 2012). Simpson and colleagues (2014) have not detected mycobacteria in small mustelids in the UK (polecats, stoats, weasels). Pine marten's susceptibility to infection and/or disease is unknown. Lesions are mainly cutaneous and systemic disease is rarely seen.
- Mycobacterium avium is a ubiquitous environmental bacterium which is the principal cause of tuberculosis in wild, domestic, and captive birds (Gavier-Widen et al, 2012). Infection has also been reported in free-living European mammals including the Eurasian otter (Simpson, 2000). No reports have been found in pine martens.
- Mycobacterium avium subsp. paratuberculosis is responsible for paratuberculosis, a chronic granulomatous enteritis, in ruminants worldwide. It is mainly a disease of ruminants, but bacteria have been isolated from other species including some species of mustelids (badgers, stoats, weasels, and stone martens) (Gavier-Widen et al., 2012; Matos et al., 2014). Carnivores are considered dead-end hosts which may contract infection but do not play a role in the epidemiology of the pathogen. Only mild lesions are seen in carnivores (Gavier-Widen et al., 2012).

- Mycoplasma spp. are widespread bacteria, mainly non-pathogenic, although some species are responsible for respiratory disease. In mustelids, Mycoplasma spp. have been isolated from the respiratory tract of healthy animals (ferrets, Koshimizu et al., 1982; M. mustelae from mink, Salih et al., 1983) and the only known report of disease associated with Mycoplasma infection occurred in captive ferrets, probably related to stress (Kiupel et al., 2012). There are no records of Mycoplasma spp. in pine martens.
- Respiratory bacteria (Bordetella bronchiseptica, Streptococcus equi sub. zooepidemicus, Klebsiella pneumoniae, Pasteurella spp.) are multi-host pathogen widespread in the environment. No reports of severe disease outbreaks associated with such bacteria have been found in pine martens. The risk is estimated to be very low.
- **Streptococcus spp.** are Gram-positive bacteria which are commonly associated with suppurative infections and abscess formation. While some species are highly host-adapted, some are not and can cause disease only as opportunists concurrent with pre-existing tissue damage or impaired immune function (Speck, 2012b). Severe subcutaneous infection has been reported in otters (*Lutra lutra*) because of wound contamination (Simpson, 2006). The likelihood of streptococcal disease in reintroduced pine martens is estimated to be negligible, assuming only animals in good clinical conditions, without pre-existing wounds, are to be translocated.

#### FUNGI

**Emmonsia 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 et al., 1975). E. crescens infection has been diagnosed in a broad range of wildlife species in Great Britain including stoats, weasels, Eurasian otters, foxes and one pine marten (Borman et al., 2009). Fatal disease in freeliving wild animals is rarely seen (one fatal case reported from an otter, Simpson & Gavier-Widen, 2000).

#### PROTOZOA

- Cryptosporidium spp., Eimeria spp., and Isospora spp. are ubiquitous intestinal coccidian parasites. Cryptosporidium has been isolated from American mink and otter in Ireland (Stuart et al., 2013), but pine martens (n=48) examined during the same study were found negative. No reports of pine marten infection with either Eimeria spp. or Isospora spp. have been found. Pathogenicity is assumed to be low in otherwise healthy adult animals. The risk is estimated to be very low.
- **Encephalitozoon cuniculi** is an obligate intracellular spore-forming protozoan with a worldwide distribution (Taylor et al., 2007). It has been isolated from pine marten's brain tissue in Czechoslovakia (Hůrková & Modrý, 2006) but no associated disease has been reported. We assume *E. cuniculi* therefore has low pathogenicity in pine martens. No data is available on prevalence in the UK. The risk is estimated to be very low.
- Giardia intestinalis is a ubiquitous intestinal protozoan known to infect a wide range of hosts, including humans (common cause of waterborne enteric disease). Barlow and colleagues (2010) reported a case of *Giardia* associated enteritis in captive badger cubs. No reports of pine marten infection and/or associated disease have been found, and we assume there will be a low pathogenicity in otherwise healthy adult animals. The risk is estimated to be very low.
- Sarcocystis spp. are obligate intracellular protozoa with a complex indirect life cycle. Infection is usually asymptomatic in the final host, while disease may be seen in intermediate hosts (neurological symptoms reported in sheep in the UK [Formisano et al., 2013]). Carnivores usually act as final hosts, but some species may also act as intermediate hosts. Meningo-encephalitis associated with *S. neurona* has been reported in mink and ferrets in North America (Dubey et al., 1993; Britton et al., 2010), however the parasite is not found in the UK. To date, *Sarcocystis* spp. have not been reported in pine martens, and the species possible role as either intermediate or final host is unknown.

#### **ENDOPARASITES**

• Angiostrongylus vasorum is a nematode of the circulatory system, also known as 'French Heartworm', which is possibly the most significant parasite of dogs in Great Britain. Infection in dogs was first recorded in Cornwall in the early 1980s and since then the parasite has spread northwards. Its range now extends to all Great Britain except for the northern part of Scotland (in Simpson et al., 2016). In British wildlife infection occurs in free-ranging wild foxes, which are also known to play a role in the epidemiology of the parasite by maintaining local reservoirs of infection, and it has also been reported in two stoats and one weasel (Simpson et al., 2016). No associated disease has been recorded in wildlife. No data on pine marten's susceptibility to infection and/or disease are available.

- Aonchotheca putorii is a common nematode of the stomach and small intestine of many wild mammals, including mustelids. It has been reported in pine martens (Segovia et al., 2007; Zhigileva & Cheboksarova, 2012) yet has little clinical significance.
- Biliary trematodes (*Pseudamphistomum truncatum* and *Metorchis albidus*) have been reported from otter, mink, and polecat (Simpson et al., 2005a; Simpson et al, 2009; Torres et al, 2008). Considering their life cycle includes a gastropod and a freshwater fish acting as first and second stage intermediate hosts (Sherrard-Smith et al, 2009), we believe these parasites are more likely to be found in piscivore species and hence unlikely to infect pine martens.
- **Capillaria hepatica** is a parasitic nematode with a direct life cycle that requires only one host. Adult worms invade the liver of the host (usually rodents) and lay eggs in the surrounding parenchyma. Eggs are not passed in the faeces of the host, being released in the environment only when the host dies and decomposes. Upon eating an infected host, predators and scavengers do not become infected because the eggs ingested are not embryonated (not infectious), but they will release such eggs in the environment. Eggs embryonate within 30 days in the environment. *Capillaria hepatica* is considered of low pathogenicity.
- Crenosoma petrowi is a metastrongylid nematode of the respiratory tract which has been identified in pine martens in Spain (Segovia et al., 2007) and in western Siberia (Zhigileva & Cheboksarova, 2012). No data are available about the prevalence of the nematode in the UK. Pathogenicity of *C. petrowi* is unknown, however *Crenosoma vulpis*, isolated from foxes (Morgan et al., 2008), is considered low-moderately pathogenic and has been associated with pulmonary pathology.
- **Eucoleus aerophilus** is a widespread nematode with a wide host range. It has an indirect life cycle in which earthworms act as intermediate hosts. It has been identified in pine martens in Spain (Segovia et al., 2007). In the UK it has been found in foxes (Morgan et al., 2008), and light infection has also been reported during post-mortem examination of four pine martens found dead after being translocated during the first year of the Vincent Wildlife reinforcement project (Tomlinson & Lewis, 2016). It is considered of low pathogenicity, being sometimes associated with pathological changes in the lungs and airways (Morgan et al., 2008).
- **Filaroides martis** is a nematode of the respiratory tract, likely to be *Martes* species specific. It has been identified in pine martens in Spain and in western Siberia (Segovia et al., 2007; Zhigileva & Cheboksarova, 2012). Simpson and colleagues (2005) have reported *Filaroides* like nematodes in Scottish pine martens. We assume *F. martis* has a low pathogenicity.
- Molineus patens is a nematode of the small intestine which has been identified in mustelids (badgers in Cornwall, Jones et al., 1980; stone martens in Italy, Millán & Ferroglio, 2001; pine martens in Spain, Segovia et al., 2007). We assume *M. patens* to have very low pathogenicity as no reports of associated disease have been found.

- **Pearsonema plica** is a nematode of the urinary tract of domestic dogs and wild carnivores. It has an indirect life cycle, with earthworms acting as intermediate hosts. It is considered of low pathogenic significance, mainly establishing only asymptomatic infections. Occasionally, cystitis and secondary bacterial infections have been described in domestic dogs and in a Fennoscandian arctic fox (Fernández-Aguilar et al., 2010). The parasite has been reported in pine martens in Spain (Segovia et al., 2007) but no reports are available of associated disease.
- Skrjabingylus spp. are nematodes which are found within the frontal sinuses of mustelids, their final hosts. S. petrowi has been identified in pine martens in Germany (Heddergott, 2009) and in Ireland (Stuart et al., 2010), S. petrowi has a host preference for Martes species (Stuart et al., 2010). Infection with S. petrowi has been confirmed (but was not the cause of death) in two pine martens found dead during the first year of the Vincent Wildlife reinforcement project (Tomlinson & Lewis, personal communication). Skrjabingylus spp. infection can result in abscesses, perforations, and other damage to the skull (Hansson, 1968). There seems to be a relation between the size of the skull and the frequency of damage, with smaller mustelids (M. nivalis and M. erminea) being more affected than bigger mustelids like the badger and the pine marten (Hansson, 1968). S. petrowi is assumed to have low pathogenicity in otherwise healthy animals.
- **Strongyloides spp.** are worldwide distributed nematodes known to parasitize the small intestine of a wide range of mammals. *S. martis* may be mustelid specific and it has been identified in pine martens in western Siberia (Zhigileva & Cheboksarova, 2012; Zhigileva & Uslamina, 2017). No data are available on its prevalence in the UK. There are no reports of associated disease, and we assume pathogenicity to be low.
- *Taenia* spp. are small intestine cestodes with a worldwide distribution. The life cycle is . indirect, with small mammals/herbivores acting as intermediate hosts and carnivore being the final hosts. Pathogenicity is likely to be very low in the final host (unless there is a high burden of parasite) (Taylor et al., 2007). Segovia and colleagues (2007) have identified T. martis in pine martens in Spain. The definitive hosts of T. martis are considered to be the stone marten and the pine marten in Europe, although adult parasites have been detected in other mustelids and carnivores across Europe, suggesting a wider variety of possible definitive hosts (Loos-Frank & Zeyhle, 1982; Nugaraité et al., 2019; 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 (Rausch, 2003; Ribas et al., 2004; Mathy et al., 2009; 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 author's knowledge, T. martis has not been detected in any species in Great Britain (Global Biodiversity Information Facility, 2021).

- **Toxocara spp.** are ascarid nematodes known to parasitize the small intestine of canids and felids. *Toxocara* spp. have been reported from stone martens (Kornaś et al., 2013) but no data are available on pine marten's susceptibility to infection.
- The intestinal nematodes *Trichinella* **spp**. have been isolated from pine martens in Spain (Segovia et al., 2007), but no reports of associated disease are available. Based on results from routine tests in wild foxes as a sentinel species, prevalence of *Trichinella* spp. in the UK is very low (Learmount et al., 2015).
- Uncinaria criniformis is an intestinal nematode which has been identified in pine martens in Spain (Segovia et al., 2007). Associated disease has not been reported in pine martens, pathogenicity is unknown but assumed to be very low.

#### **ECTOPARASITES**

- **Demodex spp.** are arachnid mites, worldwide distributed and likely to be host-specific. *D. erminea* has been reported from Irish stoat (Mcdonald & Larivière, 2001). No reports have been found in pine martens.
- Fleas are also relatively host specific, but predators can also acquire fleas from their prey. Chipmunk fleas were found on pine martens in the USA (Zielinski, 1984). Heavy infestations can result in irritation, self-excoriation, and are often associated with debility of the individual. Risks arising from this hazard are negligible assuming that fleas will be spotted during clinical examination and therefore only animals not heavily infested will be translocated.
- Lice are highly host-specific ectoparasites. Heavy *Mallophaga* spp. (chewing lice) infestations cause pruritus and self-excoriation. Anaemia can be seen in *Anoplura* spp. (sucking lice) infestations. It is likely that there will be a louse species specific to pine martens, however no data have been found to date.
- Lynxacarus mustelae has been reported from stone martens in Austria (Visser et al., 2011) and is likely to be host-specific.
- **Otodectes cyanotis** is widely distributed and has been reported in feral ferrets (Mcdonald & Larivière, 2001). It is assumed to be low pathogenic in healthy adult animals.
- **Sarcoptes scabiei** is an arachnid mite responsible of sarcoptic mange, a highly contagious skin disease. In Europe mange is recorded in several species, being widespread in carnivores, wild ruminants, wild boar, and lagomorphs. However, epidemics of sarcoptic mange have been reported only in social carnivores like red fox, while there have been only few isolated cases reported in solitary species like pine marten, badger, and stone marten (in Ryser-Degiorgis et al., 2002).

Ixodes spp. are ticks endemic to the UK, with many avian and mammalian species involved in the life cycle. Hofmeester & van Wieren (2014) found that 95% of the pine martens they examined (n=20) were parasitized by *Ixodes* spp. The average tick burden reported was 3.8 for *I. ricinus* and 6.9 for *I. hexagonus*. Disease associated with tick parasitism (excluding tick-borne pathogens) is likely to be intensity-dependent and related to irritation and anaemia.

#### NON-INFECTIOUS

- Capture myopathy is a non-infectious, metabolic disease of wild and domestic animals, mainly documented in cervids (Wallace et al., 1987). In wild animals it is most often associated with capture or restraint procedures involving significant muscle exertion and stress. All those techniques that involve long periods of restraint, unnatural positions of the captured individual, or include lengthy pursuit during capture are major factors in the development of capture myopathy in wildlife. Lesions consistent with capture myopathy were documented post-mortem in four North American river otters (*Lutra canadensis*) during translocation for a population restoration project (Hartup et al., 1999). However, capture myopathy was not identified as the sole cause of death but appeared to be one of many pathological processes that presumably contributed to the otters' clinical deterioration (Hartup et al., 1999). Capture myopathy is likely to develop during translocation process but is often mild or clinically inapparent (Hartup et al., 1999).
- Bioaccumulation of persistent environmental pollutants such as organochlorine • pesticides (OCs) and industrial polychlorinated biphenyls (PCBs) has been associated with the historical decline of Eurasian otter (Lutra lutra) populations throughout the UK and Europe in the 1950s-1970s (Conroy et al., 2000; Roos et al., 2001). Since then, legislative bans on their production and use have led to a gradual improvement in environment quality, and otter populations are now recovering (Kean et al., 2013). The Otter Project run by the University of Cardiff, in collaboration with the UK Environment Agency, has been collecting otter carcasses for post-mortem examination and screening of a range of pollutants since 1992. Data from their analyses show a decline in the concentrations of OCs and PCBs measured in otter livers (Kean & Chadwick, 2012). Recent analyses have highlighted the occurrence of polybrominated diphenyl ethers (PBDEs, used as flame retardants) in otter populations in England and Wales (Pountney et al., 2015). PBDEs, like OCs and PCBs, are resistant to breakdown and therefore, despite recent bans on their production and use, their concentrations in otters are likely to be sustained for some time. PBDEs are similar in structure and mode of action to PCBs but are generally less toxic. Our review of the most recent quarterly reports published by the Wildlife Incident Investigation Scheme between 2015 and 2018, has found no evidence of OCs or PCBs associated with disease in those wild animals found dead and submitted for analysis (including five pine martens). Only low levels of DDE-pp, the breakdown product of the persistent organochlorine pesticide DDT, were identified in some animals (mainly bird species) but were considered consistent with background exposure and not to have contributed to the animals' death.

• Environmental conditions such as extreme temperatures, precipitation, and humidity have been linked to increased likelihood of capture myopathy (Paterson, 2007). Risks to the reintroduced pine martens are evaluated to be very low and can be further mitigated with good planning of the whole reintroduction process (i.e., avoiding capture when temperatures or weather conditions are expected to be extreme).

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